2018 UF Metabolomics Workshop: *In-vivo* NMR Spectroscopy

Dr. James Collins
Why *In-vivo* NMR Spectroscopy?

- Subject
- Body Fluids
- Limited sample preparation
- Solution state NMR
- Tissue Samples
- Extraction
- HR-MAS NMR

*In-vivo* NMR
Why *In-vivo* NMR Spectroscopy?

- Example NMR spectra of rat brain tissue

**Polar Extract: 600 MHz**
- 150 – 200 Peaks
- 20 – 25 Metabolites
- Heavily processed

**HR-MAS: 600 MHz**
- 50 – 100 Peaks
- 15 – 20 Metabolites
- Dead tissue

**In-vivo: 470 MHz**
- 10 – 15 Peaks
- 5 – 10 Metabolites
- Living subject
Why *In-vivo* NMR Spectroscopy?

- *In-vivo* spectroscopy trades resolution for relevance

**Spectra Quality**

- Polar Extract
- HR-MAS
- *In-vivo*

**Biological Relevance**

*Part of the Metabolomic Toolbox*

- **1H Chemical Shift (PPM)**
  - NAA
  - Creatine
  - Lactate

\[
\text{Polar Extract} \rightarrow \text{HR-MAS} \rightarrow \text{In-vivo} \rightarrow \text{Part of the Metabolomic Toolbox}
\]
Why Spatial Localization?

- Acquire data from a specific organ/region of the subject
- Evolution does not optimize for NMR!
  - $B_0$ variations across an animal are large, typically we can achieve $\sim$1 PPM linewidth.
  - Local $B_0$ variations are much smaller, and we can achieve $< 0.1$ PPM linewidths

![Chemical Shift Graph](image)
Spatial Localization Strategies

**Imaging**
- Adapt MR Imaging methods to include spectral data
- Chemical Shift Imaging
  - Single Point Imaging
  - EPSI

**Spectroscopy**
- Use RF and field gradients to localize NMR spectra
- Localized Spectroscopy
  - PRESS, STEAM
  - LASER, semi-LASER
  - ISIS
Spatial Localization Strategies

**Imaging**
- Slower
- Lower SNR
- Hard to shim well enough
- Larger area
- Higher spatial resolution

**Spectroscopy**
- Lower spatial resolution
- Single arbitrary voxel
- Higher SNR
- Easier to shim
- Complex MT experiments
Magnetic Field Gradients: Basics

- **NMR** has (ideally) a homogenous magnetic field such that the recorded frequencies correspond to **Chemical Shift**
- **MRI** deliberately alters the magnetic field such that frequency is now also dependent on **Spatial Position**
Slice Selective Excitations

No slice selection

Slice selection

Frequency dependent on position

\[ \Delta r_s = \frac{\Delta \omega}{\gamma G_{slice}} \]

Shaped or ‘soft’ pulses excite only a narrow range of frequencies

Sinc pulse

Gaussian pulse

Gradient

R.F. coil

Frequency

Time
Single Voxel Spectroscopy

- Localization requires a minimum of three RF pulses
PRESS

- Point REsolved Spectroscopic Sequence
- Double Spin Echo Sequence

Minimum TE when $T_E_1 = T_E_2$
Typically > 30 ms

Signal contrast due to $T_2$

$$\frac{M_z}{M_0} = e^{-\frac{T_E}{T_2}}$$

**Spoiler gradients** to remove signal from imperfect pulses
STEAM

- STimulated Echo Acquisition Mode

Minimum TE < 10 ms
T₁ relaxation during TM

Stimulated echos produce only half maximum signal compared to a spin echo

\[
\frac{M_Z}{M_0} = \frac{1}{2} e^{-\left(\frac{TE}{T_2} + \frac{TM}{T_1}\right)}
\]

**Spoiler gradients** to remove signal from imperfect pulses and unwanted coherences
Stimulated Echo

90° pulse

complete T2* dephasing

M

a
b
c
d

T1 relaxation

a + b increases

c + d decreases

90° pulse

90° pulse

rephasing and echo formation
PRESS vs. STEAM: SNR

- In principle SNR for a given volume dependent on relaxation properties and sequence timings
- If $T_1 >> T_2$ and $T_2 \approx T_{E\text{PRESS}}$, then STEAM gives better SNR

\[
\frac{M_Z}{M_0} = e^{-\frac{T_E}{T_2}} \quad \text{PRESS} \\
\frac{M_Z}{M_0} = \frac{1}{2} e^{-\left(\frac{T_E}{T_2} + \frac{T_M}{T_1}\right)} \quad \text{STEAM}
\]

- However there are other considerations....
Partial Excitation of Slices

Refocusing pulses in PRESS cause partial slice excitation

Excitation pulses also have partial slice excitation

Refocusing Pulse

$$B_1(t)/B_{1\text{max}}$$

Pulse length

$$M_z/M_0$$

Frequency

Bandwidth

$$\Delta r_s = \frac{\Delta \omega}{\gamma G_{\text{slice}}}$$

$$M_{xy}/M_0$$

Frequency

Excitation Pulse

$$B_1(t)/B_{1\text{max}}$$

Pulse length

$$M_z/M_0$$

Frequency

$$M_{xy}/M_0$$

Frequency
Slice Signal Profile

- RF pulse shapes affect signal profile across slice, as well as unwanted excitation outside of slice selected regions.
- For a given pulse shape, excitations (hence, STEAM) have a ‘better’ slice signal profile.

- In a gaussian pulse the difference in SNR from one slice is approx. 30%.
- The SNR difference due to partial slice excitation may cancel the ½ SNR penalty from the stimulated echo.
- ‘Better’ pulse shapes reduces the difference in signal profiles.
Typical RF Pulse Shapes

- Pulses such as sinc3, and sinc7 give improved slice profiles, at the expense of pulse length.
- Shinar-LeRoux (SLR) pulses are often used, PV6 calculates them on the fly (default ‘calculated’ pulse), and give good slice excitation & refocusing performance.
- Gaussian is less used, but has a few advantages. It is one of the shortest shaped pulses for a given power & bandwidth.

Sinc3 vs. Gaussian
Importance of Pulse Bandwidth

- To decrease voxel size, either increase gradient strength or decrease slice bandwidth.
- Pulse bandwidth inversely proportional to pulse length for a given pulse shape.
- Minimum pulse time (max bandwidth) dictated by the maximum RF power available.
- RF bandwidth is proportional to the chemical shift offset...

\[ \Delta r_s = \frac{\Delta \omega}{\gamma G_{slice}} \]

\[ \Delta \omega \propto \frac{1}{\text{Pulse length}} \]
Chemical Shift Offset

- Position of slice is dependent on frequency of excitation
- $^{13}$C example

$12 \text{ ppm} = 600 \text{ Hz @ 4.7T}$

\[ \frac{\Delta r_{\text{Chemical Shift}}}{\Delta r_{\text{slice}}} = \frac{\Delta \omega_{\text{Chemical Shift}}}{\Delta \omega_{\text{slice}}} \]

\[ \frac{\Delta r_{\text{Chemical Shift}}}{15 \text{ mm}} = \frac{600 \text{ Hz}}{3000 \text{ Hz}} \]

\[ \Delta r_{\text{Chemical Shift}} = 3 \text{ mm} \]

- Only 51% of coincident volume between
- High bandwidth r.f pulses reduce the issue
Chemical Shift Offset

- Position of slice is dependent on frequency of excitation
- $^1$H example

\[ \Delta r_{\text{Chemical Shift}} = \Delta \omega_{\text{Chemical Shift}} \]

\[ \frac{\Delta r_{\text{Chemical Shift}}}{\Delta r_{\text{Slice}}} = \frac{\Delta \omega_{\text{Chemical Shift}}}{\Delta \omega_{\text{Slice}}} \]

\[ \frac{\Delta r_{\text{Chemical Shift}}}{2 \text{ mm}} = \frac{2550 \text{ Hz}}{6000 \text{ Hz}} \]

\[ \Delta r_{\text{Chemical Shift}} = 0.85 \text{ mm} \]

- <20% of coincident volume
- High bandwidth r.f pulses reduce the issue
Expectation vs. Reality

- Chemical shift offset is always present, worse at higher fields and for nuclei with large chemical shift ranges
- Can generate higher excitation bandwidths than refocusing ones, thus STEAM can reduce CSO compared to PRESS
- Not the sharply defined region we would like!
Outer Volume Suppression

- Localisation sequences are ‘modular’, allows the easy use of signal ‘preconditioning’ such as OVS and water suppression
- OVS suppresses signal from bands near the desired voxel

- Saturating a slice of the sample
- Simplest is a train of pulses

\[
\text{r.f} \quad \alpha \quad \alpha \quad \alpha \quad \alpha \quad \alpha \quad \alpha \quad \alpha \quad \alpha \quad \alpha
\]

\[
G_{XYZ}
\]

- Reduces signal from outside desired region....
- However, we compromise SNR and therefore quantitation of metabolites based on their chemical shift.
Water Suppression

- Water suppression is required, *in-vivo* approx. 40 M
- PV6 has CHESS and VAPOR as default
- Other derivatives include WET, SWAMP, MOIST
- Need to match suppression bandwidth to water linewidth
- Sequences can be further modified with intra-sequence WS, but PV6 doesn’t as standard

Basic CHESS Sequence

[Diagram of Basic CHESS Sequence]

**RF-pulse** | **RF-pulse** | **RF-pulse** | **Spectroscopy Localization Sequence (STEAM/PRESS)**
STEAM vs. PRESS

- Which is ‘better’? \(_\(\_\(\_\)\_\)_/¯\) it depends...
- PRESS is preferred at lower fields, STEAM at higher fields
- Many sequences based on these such as DRYSTEAM and MEGA-PRESS, which introduce spectral editing
- Can we do ‘better’?

<table>
<thead>
<tr>
<th></th>
<th>STEAM</th>
<th>PRESS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Echo Time</strong></td>
<td>Short (&lt; 10ms)</td>
<td>Long (&gt;30 ms)</td>
</tr>
<tr>
<td><strong>CSO</strong></td>
<td>OK</td>
<td>Bad at high fields</td>
</tr>
<tr>
<td><strong>SNR</strong></td>
<td>Factor of ½ due to STE</td>
<td>OVS can affect it</td>
</tr>
<tr>
<td><strong>Slice Profile</strong></td>
<td>Excitations are better</td>
<td>SLR reduces difference</td>
</tr>
<tr>
<td><strong>Flow Effects</strong></td>
<td>Spoilers can cause issues</td>
<td>Usually better</td>
</tr>
</tbody>
</table>
LASER & SEMI-LASER

• Maybe....
• Paired adiabatic pulses help correct for phase dispersion across the slice that can occur with certain adiabatic pulses
• LASER excites whole sample, using refocusing to localise

Adiabatic Pulses: SECH pulse

Hyperbolic Secant Refocusing Pulse

- High bandwidth > 5 kHz
- Reduced chemical shift offset
- Reduced in slice excitation
LASER & SEMI-LASER

- Better slice refocusing than PRESS
- Long echo time due to adiabatic pulses
- Relatively high bandwidth excitations reduce CSO issues
- High RF deposition, which is an issue for human subjects

$^{13}\text{C} \text{ In-vivo Spectroscopy}$

- $^{13}\text{C}$ is too low abundance to obtain good spectra
  - Inject labelled metabolites, such as $^{13}\text{C}$ glucose
    - Use of slice selective excitation and OVS bands to select regions
    - Requires decoupling, and CSO artefacts are a major issues
  - Hyperpolarized metabolites such as $^{13}\text{C}$ pyruvate
    - Typically done using CSI type image sequences including EPSI
    - Direct images of metabolites using bSSFP or Spectral-Spatial pulses

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Eichhorn T., et al., PNAS., 45, 18064-18069,(2013)
$^{31}$P In-vivo Spectroscopy

- $^{31}$P spectra provide useful metabolic data
- Naturally 100% abundant spin-$\frac{1}{2}$ nuclei
  - ATP, NAD, PCr, and inorganic phosphates at >mM concentrations
- ATP $T_2$ is short, < 20 ms, and decrease with $B_0$
- Short echo time needed!
**ISIS**

- Image Selected In-vivo Spectroscopy

No Echo Time, as only FID’s are acquired

No $T_2$ contrast

8 Step ‘Phase cycle’ to localize a voxel, hence 8 times slower than STEAM or PRESS

Minimize $\tau$ to reduce $T_1$ contrast

Motion during acquisition significantly affects the spectra
Another Potential method is to use multiple OVS bands to ‘select’ a localized region.

Rather than directly select the region of interest, we suppress all of the signal from outside this region.

Quicker than ISIS and also acquires an FID, hence no $T_2$ issues.
$^{31}$P Spectroscopy: pH Estimate

- pH of tissue is related to the chemical shift of the inorganic phosphate compared to phosphocreatine.

$\Delta \delta \propto \text{pH}$
Saturating the γ-ATP resonance causes a change in signal intensity of the PCr peak (as well as in the other ATP peaks).

The change in PCr signal is related to conversion rate of ATP to PCr.

Narrow bandwidth RF pulses used to saturate γ-ATP resonance.
In-vivo measures of Cocaine effects

- Changes in spectra can be subtle, but statistically significant
- Data collected at 11.1 T
RF Coil Choices: Surface Coil

- Coil choice is important and can dictate sequence decisions:
  - Surface coils vs. volume coils

- Surface coils give better SNR at the expense of poor RF homogeneity. Adiabatic pulses help with this
- Provide some signal localization, as they have a limited RF range
- Practically they can be hard to tune accurately, can be susceptible to animal motion depended how they are mounted, and must be accurately placed on the animal
RF Coil Choice: Volume Coil

- Coil choice is important and can dictate sequence decisions:
  - Surface coils vs. volume coils

- Volume coils give much better RF homogeneity, but sacrifice SNR
- Can be paired with a heteronuclear surface coil relatively easily
- Usually can be tuned in situ, less coil positioning issues
RF Coil Choice: VT/SR

- Coil choice is important and can dictate sequence decisions:
  - Surface coils vs. volume coils

- Volume transmit/ Surface receive combines the higher SNR with the good RF performance, but require more complex coil designs and are harder to pair with heteronuclear coils.
Practical Aspects

- So far covered a lot of theory, but there are a number of practical aspects that need to be considered

- Animal cradle design:
  - Needs to keep animal still, and prevent motion from breathing

- Shimming is much harder *in-vivo!* Automatic shimming algorithms do exist.
  - PV6 uses a mapshim technique, that maps the $B_0$ field and then attempts to calculate the required shims. It works great... in a phantom.
  - Iterative local shimming of 1$^{\text{st}}$ order shims is also required
  - Manual shimming is harder in PV6 then previous versions or Varian systems, but can be done to a limited extent
Additional Slides
Phase and Frequency Encoding

- MR imaging uses a combination of ‘Phase’ and ‘Frequency’ (also called ‘Read’) encoding magnetic field gradients.

‘spins’ precess at the Larmor frequency

\[ \nu = \gamma (B_0 + xG_x) \]

\[ \phi = \gamma (xG_x) t_\phi \]

Spectral-Spatial: K-t space

- How do we image spectral & spatial information?

Both Frequency spatial encoding and Spectral information are in time domain!
Single Point Imaging: Basic CSI

• How do we image spectral & spatial information?

Image recorded in k-space

Spectra recorded in time domain

Phase Encoding

Frequency Encoding

Digitally sampled in time
Single Point Imaging

- Using a combination of ‘Phase’ and ‘Frequency’ (also called ‘Read’) is problematic for CSI
- The simplest method is perform Single Point Imaging, where both dimensions are phase encoded
- The time (frequency) dimension is then reserved for spectral information
Single Point Imaging

- Each point in k-space will require a separate acquisition
- Each voxel will have a full spectra associated with it
- A 64x64 image is thus 4096 acquisitions, with a fairly short acquisition time of 1 s, that is still > 1hr for a single average
- Many methods to speed up acquisition, but often at the expense of spectral quality

![Diagram showing k-space with spectral, time, phase, and encoding dimensions]
Echo Planar Spectroscopic Imaging

- EPSI is a modification of Echo Planar Imaging to give spectroscopic as well as spatial information.
- A sequence of gradient echoes are recorded from a single excitation.
- These are used to deconvolve spectral information.
EPSI: Spectral/Spatial Compromise

- In EPSI the spectral sweepwidth is limited by the spatial resolution and field of view
- Increasing the sweepwidth requires either a bigger FOV or less points acquired in the frequency encoding direction
- On 11T we are using a sweepwidth of 2100 Hz (18 ppm)

\[ \text{FOV} \propto \frac{1}{t_{DI}} \]
\[ \text{SW} \propto \frac{1}{K_x t_{DI}} \]
UCSF EPSI Data

- Data presented as spatially resolved spectra


Single Compound Images

- Instead of Spectral/Spatial images, directly image each metabolite in interleaved acquisitions
- balanced Steady State Free Precession

Imaging time ≈ 1 s

Eichhorn T., et al., PNAS., 45, 18064-18069,(2013)