# Magnetic Resonance Spectroscopy: It's maddeningly sensitive!

Med Physics 710
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# Biology Riddle: How do I measure multiple chemicals longitudinally?

(Especially brain, but any organ will do)

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# Biology Riddle: How do I measure multiple chemicals longitudinally?

- PET
  - **Mostly indirect** (displacement)
  - Relative (mean scaled)
  - Rarely >2 tracers
- Microcanulation + HPLC
  - Extracellular only
  - Bad temporal resolution
- Histology/flourescence
  - Obviously not longitudinal
  - Rarely >3 targets
- In situ hybridization/gene chip
  - Low sensitivity and specificity
  - Not longitudinal

**Longitudinal Brain Biopsy?**

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# Magnetic Resonance Spectroscopy

- Measures many chemicals and cellular processes directly (or indirectly)
- Non-invasive and no radiation exposure
- No contrast agents or chemistry*
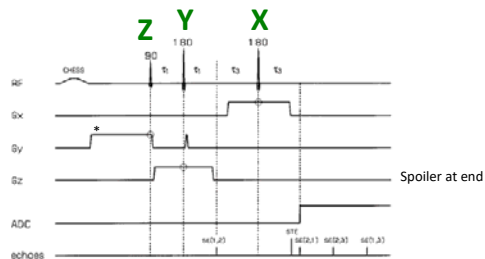- Reliable for longitudinal study

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# Outline

- Basics of single voxel MRS
- Major sources error/variation
- Other nuclei 13C and 31P
- Specialized sequences for coupled peaks
- **Emerging applications & improvements**

Please ask questions throughout!
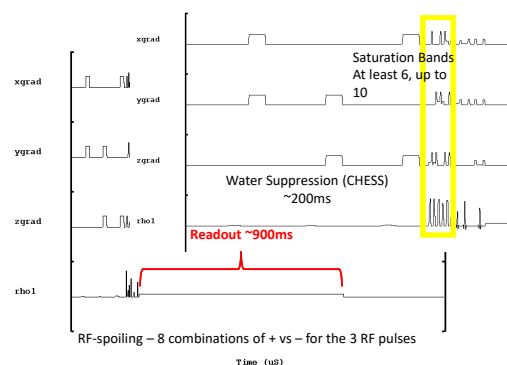
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# Localization & Sequence

## Textbook PRESS Sequence (STEAM rarely used now)



Z  Y  X

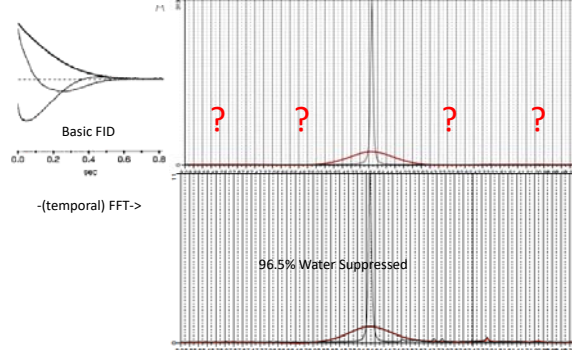Spoiler at end

Drost, Riddle & Clarke 2002

*For 2D/3D phase encoding goes here

## Real PRESS pulse sequence



Saturation Bands
At least 6, up to 10
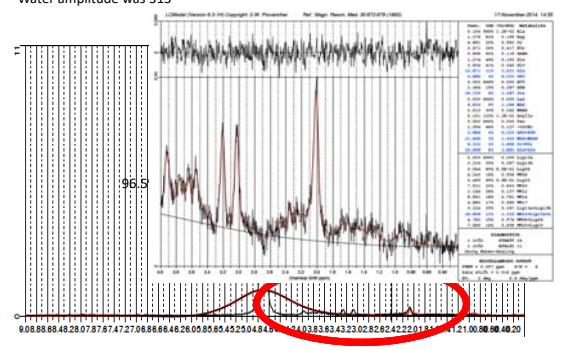
Water Suppression (CHESS) ~200ms

Readout ~900ms

RF-spoiling – 8 combinations of + vs – for the 3 RF pulses

## What does it look like if we take a long, 1-dimensional picture of K space?



Basic FID

-(temporal) FFT->

96.5% Water Suppressed

## Spectroscopy is inherently low SNR!

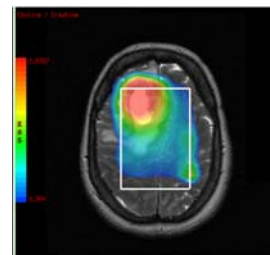Water amplitude was 313



96.5

## Implications of SNR



gamemoir.com

What is this?

## This is terribly misleading!



Kousi et al in *Novel Frontiers of Advanced Neuroimaging* 2013

Keep in mind – Different Chemical Shifts => Different Chemical Shift Artifacts!
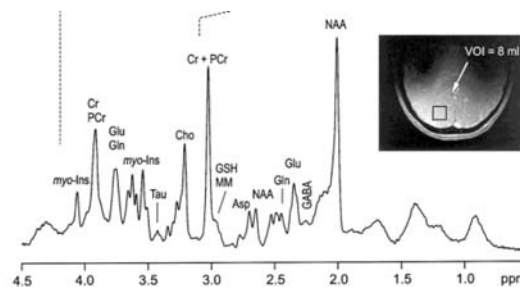
## Resolution

- Classic brain voxel is 8cc (2cm isotropic)
- Small voxel is 3.38cc (1.5cm isotropic)
- With anatomical considerations – push 2.5cc
- Group averaged – 0.5cc
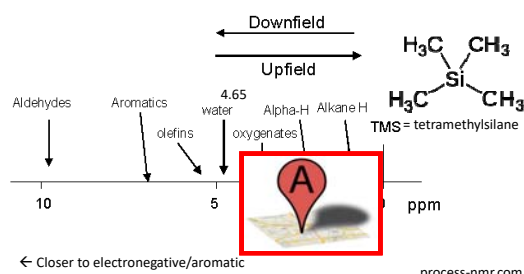- This makes fMRI and DTI look like hi-def!



## Why bother then?

**\*7T – wider chemical shifts, but harder to shim**



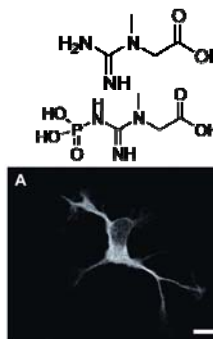Tkac et al *Mag Res Medicine* 2001 In Vivo 1H NMR Spectroscopy of the Human Brain at 7T

## Anyone Remember NMR Spectroscopy?



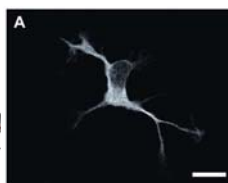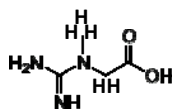← Closer to electronegative/aromatic
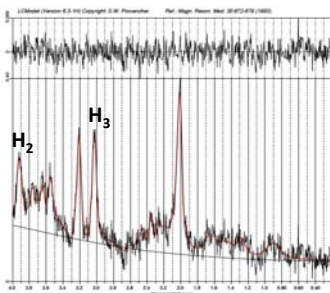
process-nmr.com

## Meet the Family - Creatine

- Storage molecule for high energy phosphates
- Present in all cells (assumed to be uniformly distributed)
- Many studies use ratios to Cr
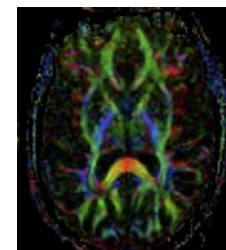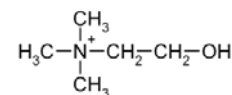- Simple peaks at 3.0 and 3.9ppm



Kuiper et al *BMC Neurosci* 2008

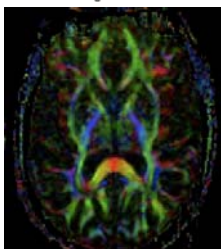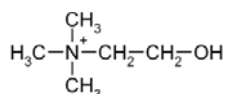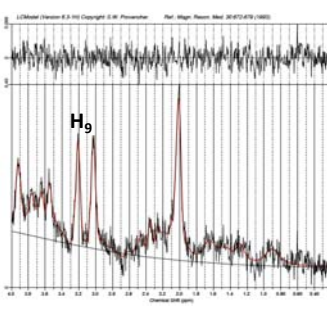## Meet the Family - Creatine



## Meet the Family - Choline

- NOT the neurotransmitter
- Cell membranes
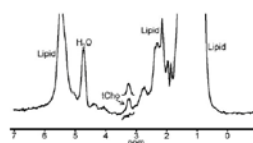- Increased in "turnover"

## Meet the Family - Choline

Almost always tracks with GM/WM partial volume!

$$H_3C - \overset{\overset{\displaystyle CH_3}{|}}{\underset{\underset{\displaystyle CH_3}{|}}{N^+}} - CH_2 - CH_2 - OH$$

H_9

## Test your knowledge!

Breast Mass with Uncertain Appearance
On Typical Imaging

Has a large Choline peak. What do you think?
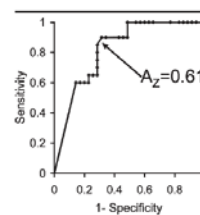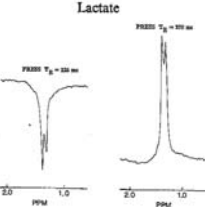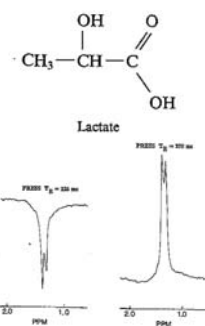
Cancer?      Not Cancer?

$A_z = 0.61$

Figure 2. ROC curve generated from the tCho concentration measured in the 55 lesions evaluated in the observer performance study. With a tCho concentration cutoff point of 1.05 mmol/kg, the accuracy—expressed as the area under the ROC curve ($A_z$)—was 83%; the sensitivity, 69%; and the specificity, 90%.

tCho => total since 31PMRS can see different phosphocholine pools

Meisamy et al *Radiology* 2005

## Meet the Family - Lactate

- Product of anaerobic metabolism
- Tumors with cell death/necrosis
- Bacterial abscesses
- Exercising muscle

$$CH_3 - \overset{\overset{\displaystyle OH}{|}}{CH} - \overset{\overset{\displaystyle O}{\|}}{C} \diagdown OH$$

Lactate

## Meet the Family - Lactate

$$CH_3 - \overset{\overset{\displaystyle OH}{|}}{CH} - \overset{\overset{\displaystyle O}{\|}}{C} \diagdown OH$$ **

Lactate

**                          H_3
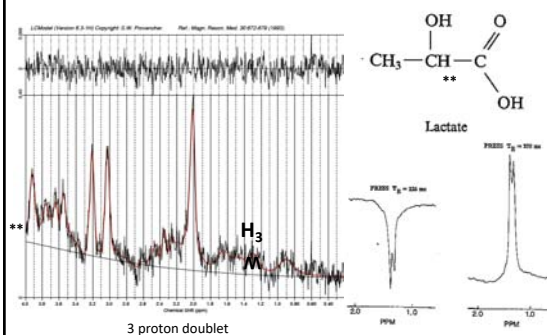
3 proton doublet

## Meet the Family – n-acetylaspartate (NAA)

- Brain specific
- Made in neurons by mitochondria
- Healthy neuropil density
- Canavan's disease:
  – Cannot breakdown NAA
  – Myelin fails
  – ? Acetate transport to oligodendrocytes
- Neurotransmitter role?
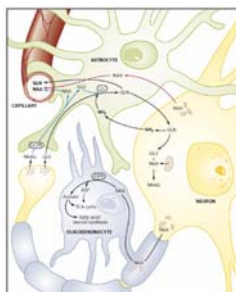
Great review Moffett et al *Progr Neurobiol* 2007

## Meet the Family - Inositol

mI = myo-Inositol, cleavage product of major intracellular 2nd messenger pathway IP_3

NAA
Cr
Cho
mI    Glx
Lactate
Lipid

PEAK HEIGHT (CONCENTRATION)

FREQUENCY (position)
PPM
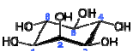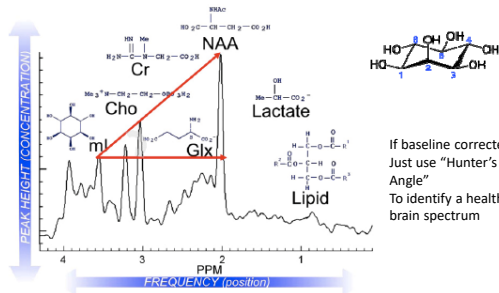
If baseline corrected, Just use "Hunter's Angle" To identify a healthy brain spectrum

Moffett et al *Progr Neurobiol* 2007

## You now know all the major MRS used clinically

Giant peaks, averaged over large regions
Choline, Creatine, Lactate and NAA

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## What makes for a reliable estimate?

- 3 main characteristics of spectral quality
  - Signal to noise ratio (SNR)
  - Frequency resolution (linewidth or full-width at half max)
  - Baseline fit (short echo only)
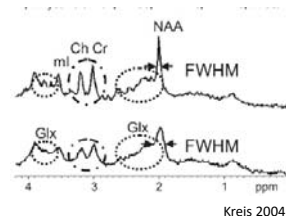


Kreis 2004

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## Paradox of SNR vs Age



Why is spectroscopy easier in young children???

Nacewicz et al *NeuroImage* 2012

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## Good Shim – line width and 1-week reliability

Morning 1 vs Morning 2



ICC=0.89

Note: 2-D and 3-D Spectroscopic Imaging (CSI) always has worse shim*

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## Disturbingly Insensitive to Motion



- Left magnet halfway through scan!
- Only hint is lower SNR!
- Many people do not trust MRS

Kreis 2004

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## Disturbingly Insensitive to Motion



Kreis 2004

## Motion/Frequency Drift Compensation

- Prospective Motion Correction
  - (Navigators vs Camera)
- Frequency & Phase Adjustment



Post-hoc can make spectrum pretty but signal from slightly different area!

Near et al *MRM* 2014

## Coil heating -> Frequency Drift

- Worse w/small volumes
- Worse if far from isocenter
- "Hot" topic right now



**3X WORSE!**

Fig 3. Histogram of calculated frequency drifts. Magnetic resonance spectroscopy (MRS) without preceding diffusion tensor imaging (DTI) is shown in pink and exams following DTI are in gray.

Rowland et al, J Neuroimaging 2016

## Concentrations are I.U. or A.U.!

- T2 and T1 of each metabolite vary by region
- T2 and T1 of water varies
- Even modeling both of these
  - Contribution of peak shape at different echo times!
- Absolute quantitation only if same Rx on phantom immediately
- Ex-vivo T1 greatly affects estimates



Super short echo 9.4T

Xin et al *NMR Biomed* 2008

## Promising New NMR Approaches



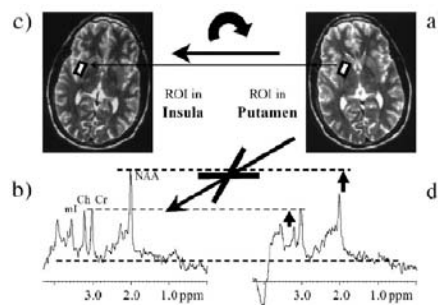Fig. 2 500 MHz $^1H$ $T_2$ measurements on 75 mM clarithromycin in dimethylsulfoxide-$d_6$. (a) and (b), spectra obtained using the sequences of Fig. 1a and b respectively with a delay τ = 8 ms and a total echo time of 4πτ = 128 ms (n = 4). (c) and (d), corresponding results for the two doublets between 4.3 and 4.5 ppm as a function of total echo time 4πτ.

812 | *Chem. Commun.*, 2012, 48, 811–813

- "Perfect Echo" or PROJECT
- Ex Vivo NMR only right now
- Broad decoupling
- Discovered 2010
  - Not ported yet though

Torres et al, *Magn Reson Chem* 2010
Aguilar et al *Chem Comm* 2012
Castanar et al *J Mag Res* 2014

## New Technique Taking over the Field

Adiabatic pulses: see http://triton.iqfr.csic.es/guide/eNMR/eNMRcomp/adiabatic.html
Or http://www.mri.jhmi.edu/~rouwerke/adiabatics.html#Anchor-The-47857



Suddenly can use all portions of excitation region and phasing will be consistent!

Scheenen et al *MRM* 2008

## Even with good spectral quality

- NAA, choline, and creatine test-retest reliability is decent but not great
- Glutamate+Glutamine (Glx, since inseparable) not reliable
- (myo)Inositol not reliable
- Lactate not reliable
- GABA not reliable

## BUT Studies of neurotransmitters and lactate have shown sensitivity to

- Smoking (smoker vs nonsmoker & recency)
- Drinking (EtOH peak also massively visible)
- Exercise (increases lactate and "Glx")
- Hypoventilation
- Migraine
- Anxiety disorders
- Depression
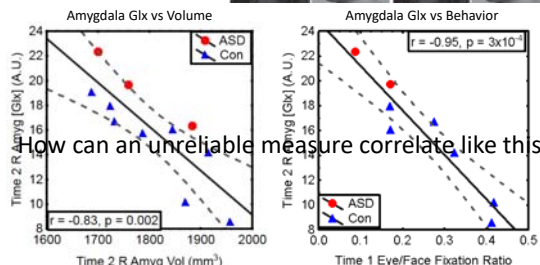- So how can it be unreliable in test-retest?
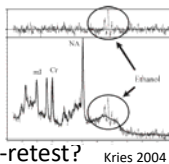
Kries 2004

Too sensitive to physiology?

## For example: Data from our study

Nacewicz et al Arch Gen Psych 2006

Used behavioral data from 3-5 years prior to spectroscopy
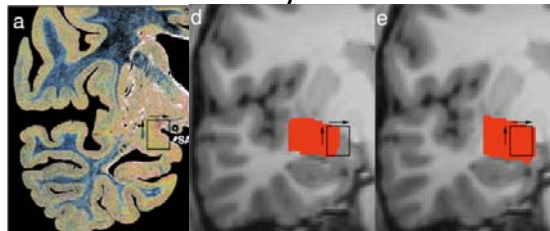Had shown that small amygdala volume=> worse autism
Predicted excess glutamate



Amygdala Glx vs Volume

Amygdala Glx vs Behavior

How can an unreliable measure correlate like this?

$r = -0.83, p = 0.002$

$r = -0.95, p = 3 \times 10^{-6}$

## Anatomy Rules!

- Previous work showed some GM/WM effects and anatomical specificity
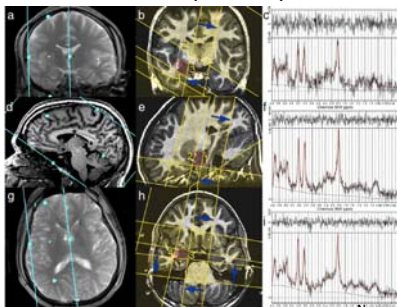


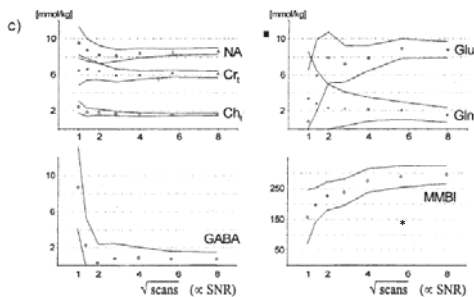Nacewicz et al *NeuroImage* 2012

## Anatomy Rules!



- We found **every millimeter** of medial and inferior amygdala included affected estimates **more than all other factors**
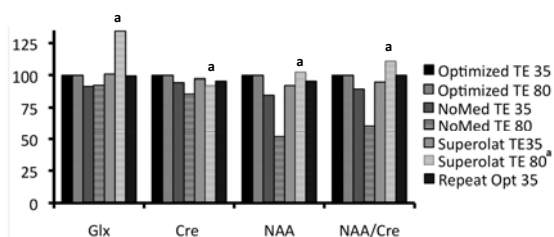
Nacewicz et al *NeuroImage* 2012

## Low SNR – cannot find the baseline so massive errors in everything!



Kreis 2004

## Low SNR/Baseline Fit



As long as all baseline macromolecules were fit, peaks were stable
Repeated 3y later and still within 5-10% for major peaks (25% for GABA)

## The future of MRS?

**Automated Prescription of Oblique Brain 3D Magnetic Resonance Spectroscopic Imaging**

Eugene Ozhinsky,[1,2*] Daniel B. Vigneron,[1,3] Susan M. Chang,[4] and Sarah J. Nelson[1,3]
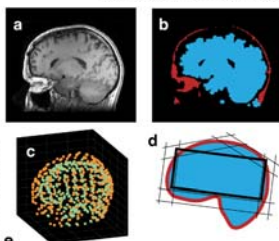
Magnetic Resonance in Medicine 69:920–930 (2013)



FIG. 1. Technique overview: (a) anatomical MRI image, (b) segmented lipid and brain tissue masks, (c) sets of points, defining the brain and lipid surfaces, (d) calculated OVS saturation band and PRESS box configuration, and (e) saturation band and PRESS box parameters loaded into the MRSI pulse sequence. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

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## A brief foray into non-1H MRS

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## Paradox of measuring cellular energy

- Major interest in sleep research: does sleep affect intracellular energy efficiency?
  – Problem: If you kill mouse, cells use energy
  – Neurotransmitters are chewed up
  – Have gone to great lengths to stop this process



wikipedia

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## 31P MRS allows direct measurement of cellular energy molecules

- 31P more abundant than 13C but still much weaker than 1H
- Sparser spectrum
- Can measure cellular energy in terms of ATP vs AMP and phospho-creatine vs creatine
- Can also measure specific phospholipids
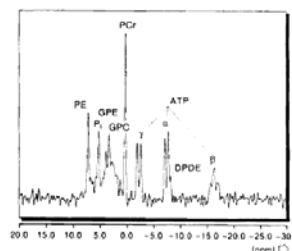


Luyten et al *NMR Biomed* 1989

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## What about 13C NMR?

- Remember from organic chemistry?
- Too rare for good signal H2O>>1HMRS>>12C(nospin)>>13C
- Ex-vivo excite 1H->13C->1H readout
- Run in as 13C label on glucose or TCA intermediate and track metabolism
- No absolute/baseline
- Similar to PET but more chemicals

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## 13C MRS reveals that 80% of resting brain metabolism is glutamate recycling
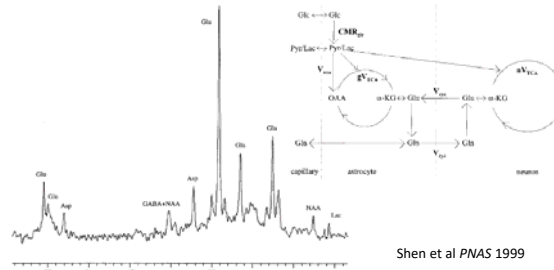


Shen et al *PNAS* 1999

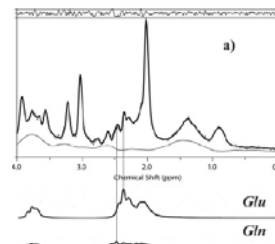Emerging studies: Hot debate as to whether Lactate is normal brain messenger

## Wouldn't it be great to see neurotransmitters in vivo with 1HMRS?

With reasonable sensitivity…
In a short acquisition…

## Glutamate

- Main neurotransmitter in brain
- Most abundant brain chemical
- Higher concentration than NAA
- Why can't we see it?



Hancu & Port *NMR Biomed* 2011

## J-coupled peaks: Glutamate, Glutamine, GABA and Inositol

- Remember AUC of peak:
  [metabolite]*(# of protons)
- But peaks split by neighboring protons
- Multiplets look like noise and overlap!
- This is why analysis package cost $14k – basis sets for each TE on each scanner!
- Even these are incomplete!
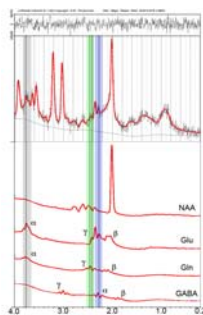


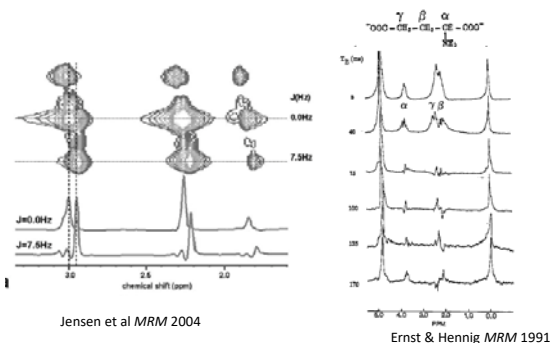## Solution 1: Spectral editing w/Adiabatic Pulse

- Frequency specific inversion at 3.0ppm GABA



ON – OFF = DIFF
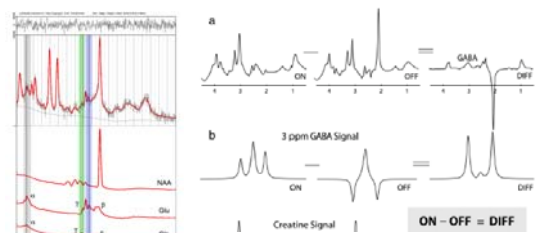
Only possible with TE = 68 ms due to coupling frequency
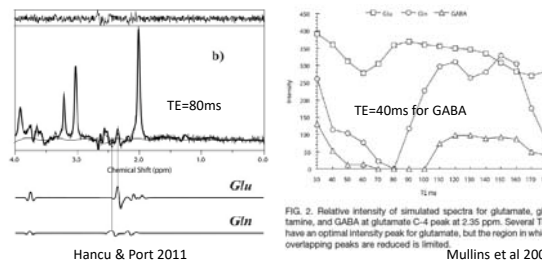
Mullins et al *NeuroImage* 2013

## Solution #2 Multi-Echo Imaging



Jensen et al *MRM* 2004
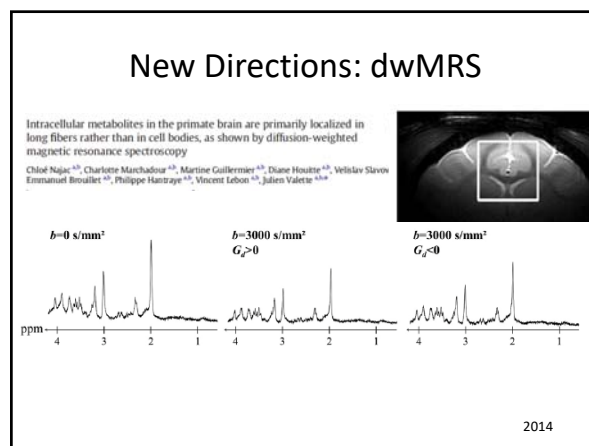
Ernst & Hennig *MRM* 1991

## Over last decade, the field finally moved away from peak isolation

- Finally applied fMRI logic
  – Find best parameters for DECONVOLUTION



TE=80ms

TE=40ms for GABA

FIG. 2. Relative intensity of simulated spectra for glutamate, glutamine, and GABA at glutamate C-4 peak at 2.35 ppm. Several TEs have an optimal intensity peak for glutamate, but the region in which overlapping peaks are reduced is limited.

Hancu & Port 2011

Mullins et al 2008

9

## New Directions at 7T GluCEST



- Cai et al Nat Med 2012
- Cai et al NMR Biomed 2013
- Showed Amygdala has highest glutamate in brain

## In Vivo Glutamate Dynamics

- Mike Dash (Cirelli Lab)
  - High temporal resolution amperometry



Preliminary analyses suggested linear increase Amygdala [Glu]

Error Bars: 95%CIs

## In Vivo GABA Dynamics



Can we prospectively measure infant amygdala maturation?

Error Bars: 95%CIs

## Controversial: Dopamine?



OPEN ACCESS Freely available online

Dopamine Reduction in the Substantia Nigra of Parkinson's Disease Patients Confirmed by In Vivo Magnetic Resonance Spectroscopic Imaging

Adriane Gröger, Rupert Kolb, Rita Schäfer, Uwe Klose

Highly criticized in the field, no replication yet (2014 paper)

## Our own testing: Striatum (2 scans)



## New Directions: dwMRS

Intracellular metabolites in the primate brain are primarily localized in long fibers rather than in cell bodies, as shown by diffusion-weighted magnetic resonance spectroscopy

Chloé Najac, Charlotte Marchadour, Martine Guillermier, Diane Houitte, Velislav Slavov, Emmanuel Brouillet, Philippe Hantraye, Vincent Lebon, Julien Valette
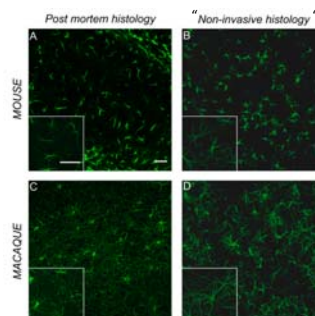


2014

## New paradigm to assess brain cell morphology by diffusion-weighted MR spectroscopy in vivo

Marco Palombo[a,b,1], Clémence Ligneul[a,b], Chloé Najac[a,b], Juliette Le Douce[a,b], Julien Flament[a,c], Carole Escartin[a,b], Philippe Hantraye[a,b,c], Emmanuel Brouillet[a,b], Gilles Bonvento[a,b], and Julien Valette[a,b,1]



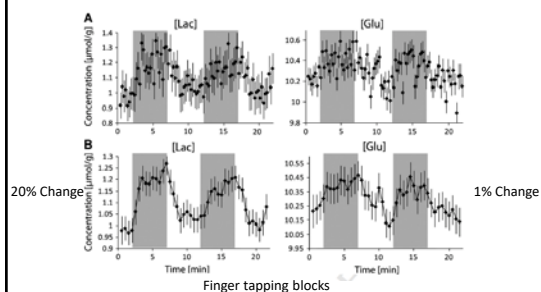PNAS | June 14, 2016 | vol. 113 | no. 24 | 6671–6676

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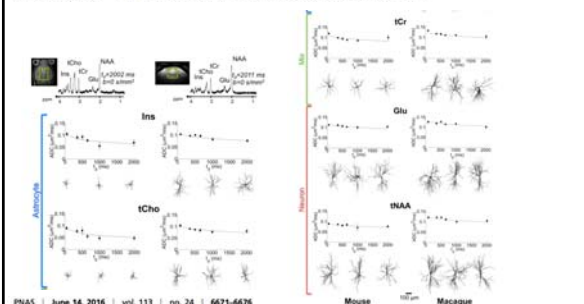
## Pseudo-colored to look like real cells



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## Favorite new area: fMRS!

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## Super short echo fMRS

- Adaptation of STEAM sequence for 12ms echo at 7T



20% Change
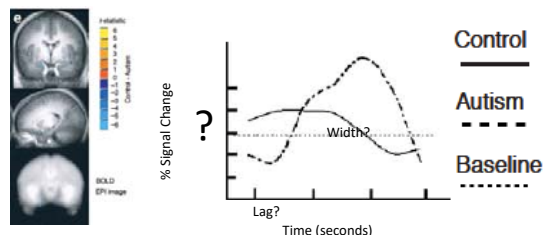
1% Change

Finger tapping blocks

Schaller et al *NeuroImage* 2014

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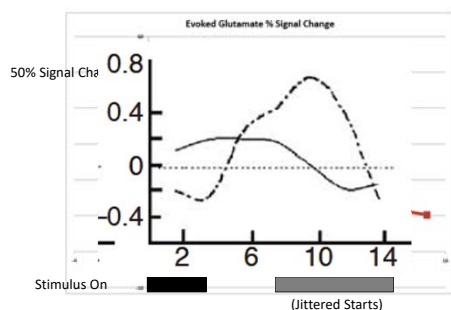## Block design is useless in regions that habituate rapidly

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## Event Related fMRS (4 Ss)

- Used our task from fMRI study
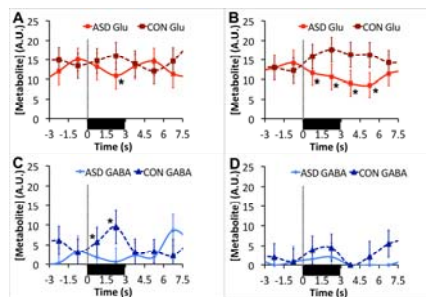- Faces presented for 3s in jittered, event-related design
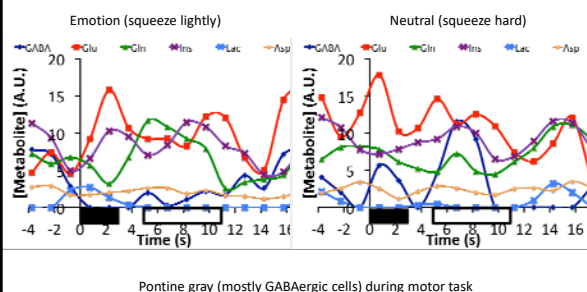


Dalton et al *Nat Neurosci* 2005
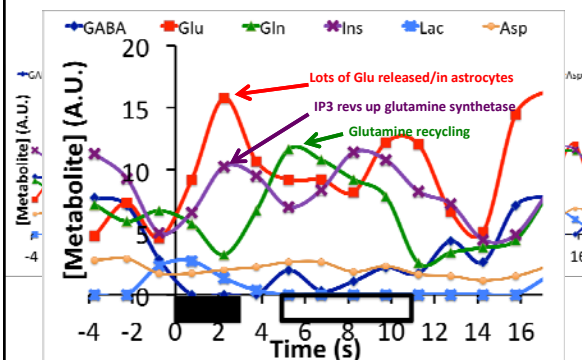
## Same task fMRS (1.5s TR X 2 NEX)



Evoked Glutamate % Signal Change

50% Signal Change

Stimulus On

(Jittered Starts)

## Opposite glutamate dynamics in ASD in Amygdala & PONS (startle regions)



## 2X temporal resolution
## Many more chemicals



Emotion (squeeze lightly)

Neutral (squeeze hard)

Pontine gray (mostly GABAergic cells) during motor task

## Realtime neurotransmitter recycling



Lots of Glu released/in astrocytes

IP3 revs up glutamine synthetase

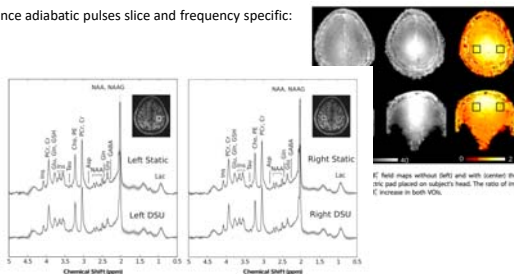Glutamine recycling

## Now to combine with semi-LASER for multiple connected regions
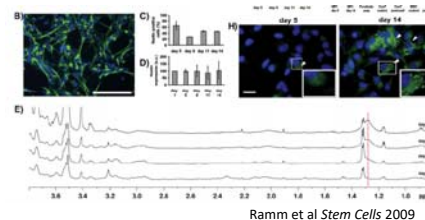
Since adiabatic pulses slice and frequency specific:



Lemke, et al *NMR Biomed* 2015

## New Directions: Biomarker of Neurogenesis/Apoptosis

- 1.28 ppm lipid initially thought neurogenesis
- In vitro – neurogenesis then growth arrest
- 50X increase in inositol in growing cells



Ramm et al *Stem Cells* 2009

## Conclusions

- IF you can use specific anatomical boundaries
- AND get a good shim (or use semi-LASER)
- AND your patient can hold still long enough
- AND you use a deconvolution model
- Magnetic resonance spectroscopy can measure cells and subcellular processes in ~realtime
- If interested, I recommend biochem 800

**800 PRACTICAL NUCLEAR MAGNETIC RESONANCE THEORY**

Fall; 2 cr. Lectures. Multiple pulse NMR, off–resonance effects, composite and shaped pulses, product operators, coherence transfer, multi–dimensional NMR, phase cycling, multiple quantum coherence, and cross relaxation. Prerequisite: Consent of instructor. [Web site] Milo Westler

UNIVERSITY OF WISCONSIN–MADISON

National Magnetic Resonance Facility at Madison