ICANA: Glutamate in Chronic Hepatic Encephalopathy

Direct or Indirect? Distinquishing Hepatic From Alcoholic Encephalopathy

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Huntington Medical Research Institutes
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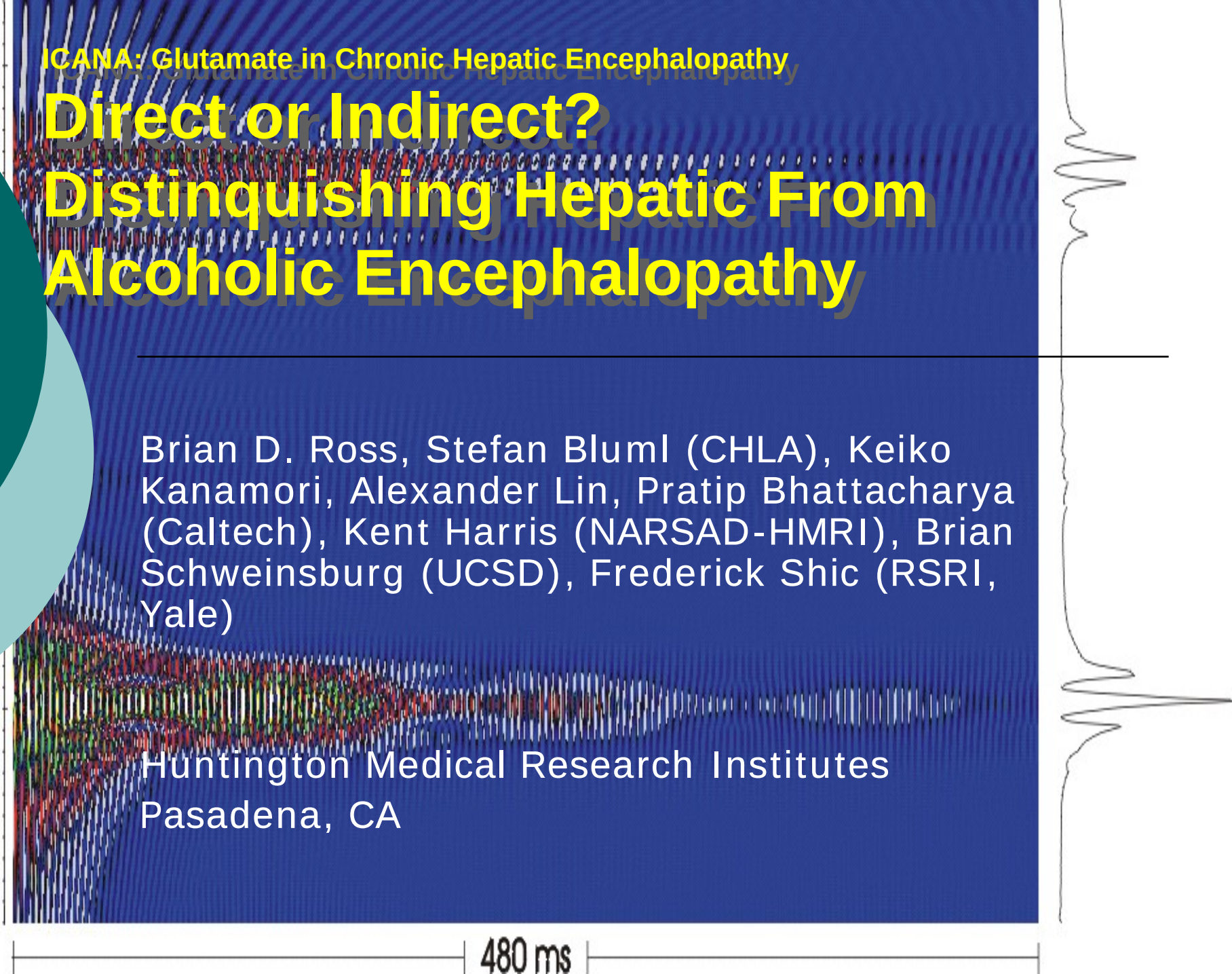
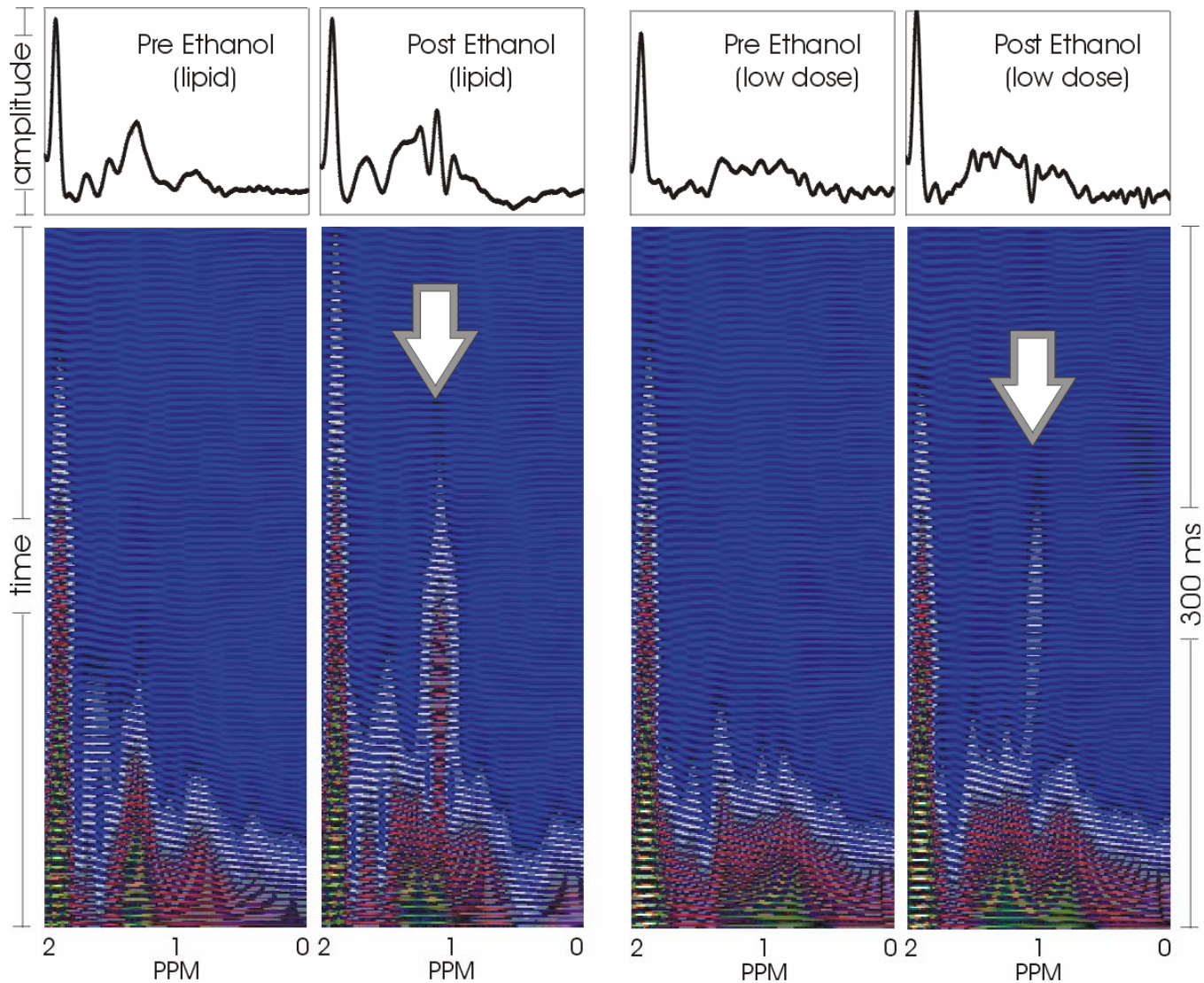
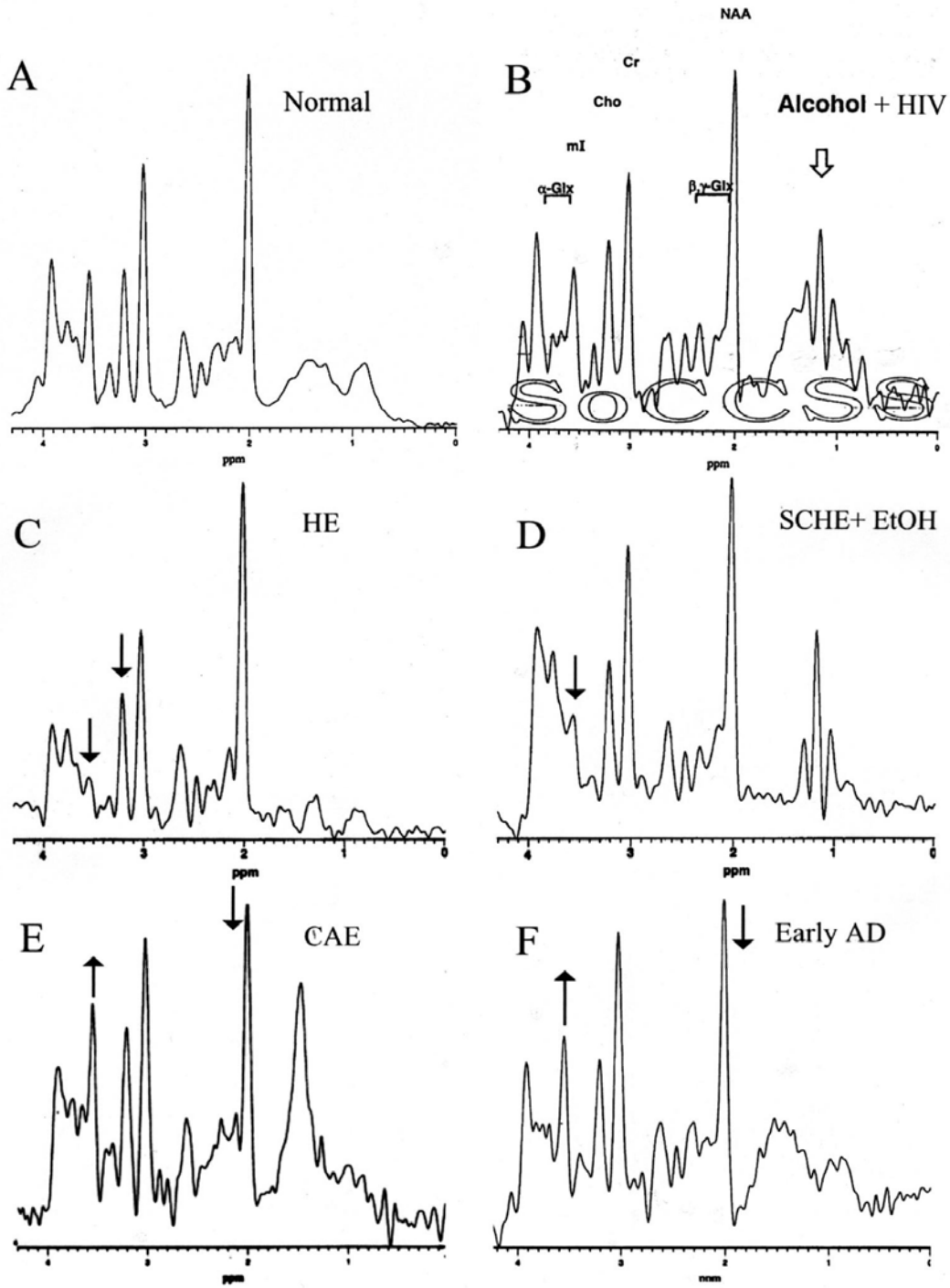


Alcohol enters the brain, and can be directly detected by ^{1}H MRS (FFT or Morlet Wavelet Analysis)



Proton MRS also reveals three or more distinct neurochemical patterns of brain injury after alcohol



Introduction

Alcoholic Brain Diseases

- Multiple neurologic syndromes
- Two distinct etiologies
- Several postulated mechanisms

Mechanisms of Alcoholic Brain Disease

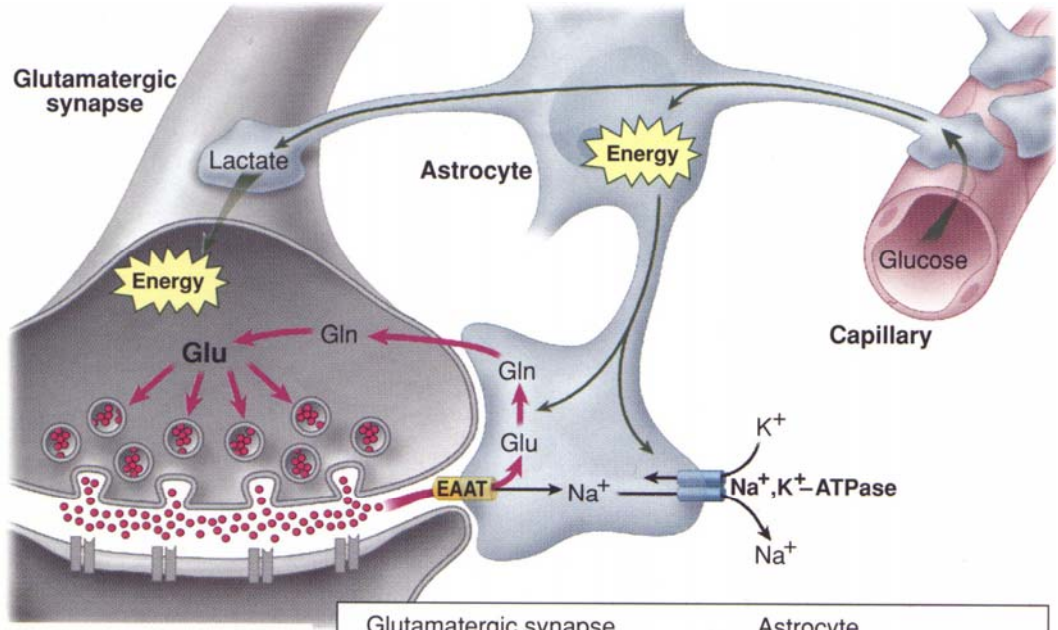
Experience	Transmitter
Activation	↑ Noradrenaline: ↑ dopamine
Euphoria	↑ Dopamine: ↑ opioids
Anxiolysis/ataxia	↑ GABA
Sedation/amnesia	↑ GABA + ↓ NMDA
Nausea	Stimulation 5-HT_3 receptors
Withdrawal	↑ Calcium flux:
	↑ L-type channels; ↑ NMDA receptors
	↓ Magnesium
	↓ *x*-2-adrenoceptor inhibition

Focus of this talk will be glutamate

I. Glutamate neurotoxicity (NMDA receptor defect)
II. Glutamate – Glutamine – GABA cycle defect

Two Current Views of Glutamate-Glutamine Cycle

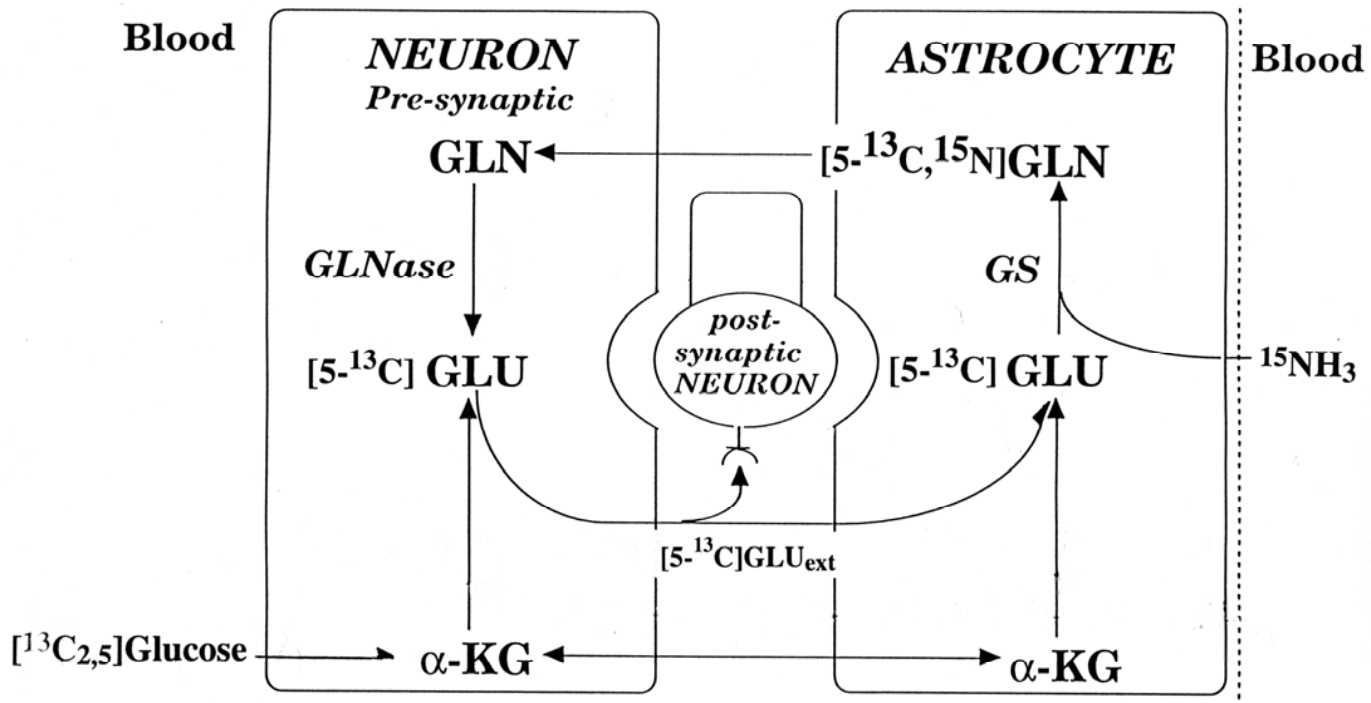
(1) Magestretti - Yale



Food for thought. Upper panel: The mechanisms by which synaptic activity is coupled to glucose usage. Lower panel: Stoichiometry of glutamate-mediated synaptic transmission and glucose usage. V_{cycle}, rate of TCA cycle; V_{gln}, rate of neurotransmitter cycle.

Two Current Views of Glutamate-Glutamine Cycle

(1) Neuroscience (H. Bradford and others)

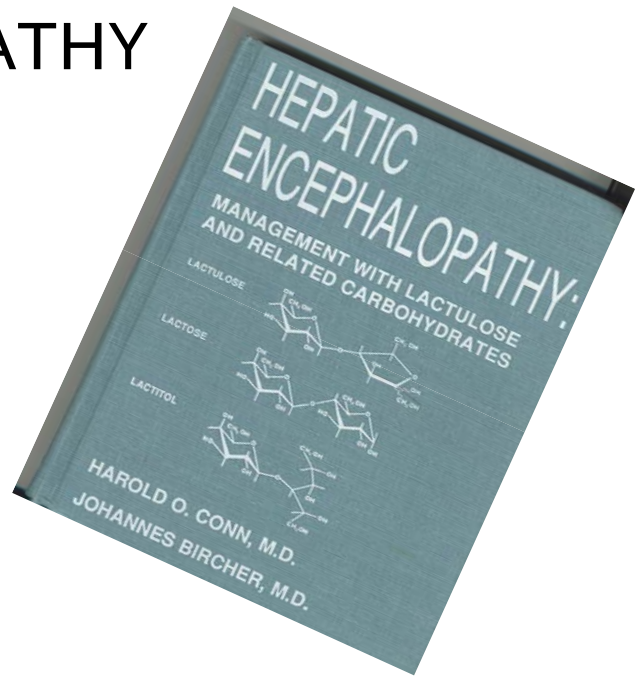


Both are highly simplified versions ot true physiology which is an active area of research world-wide.

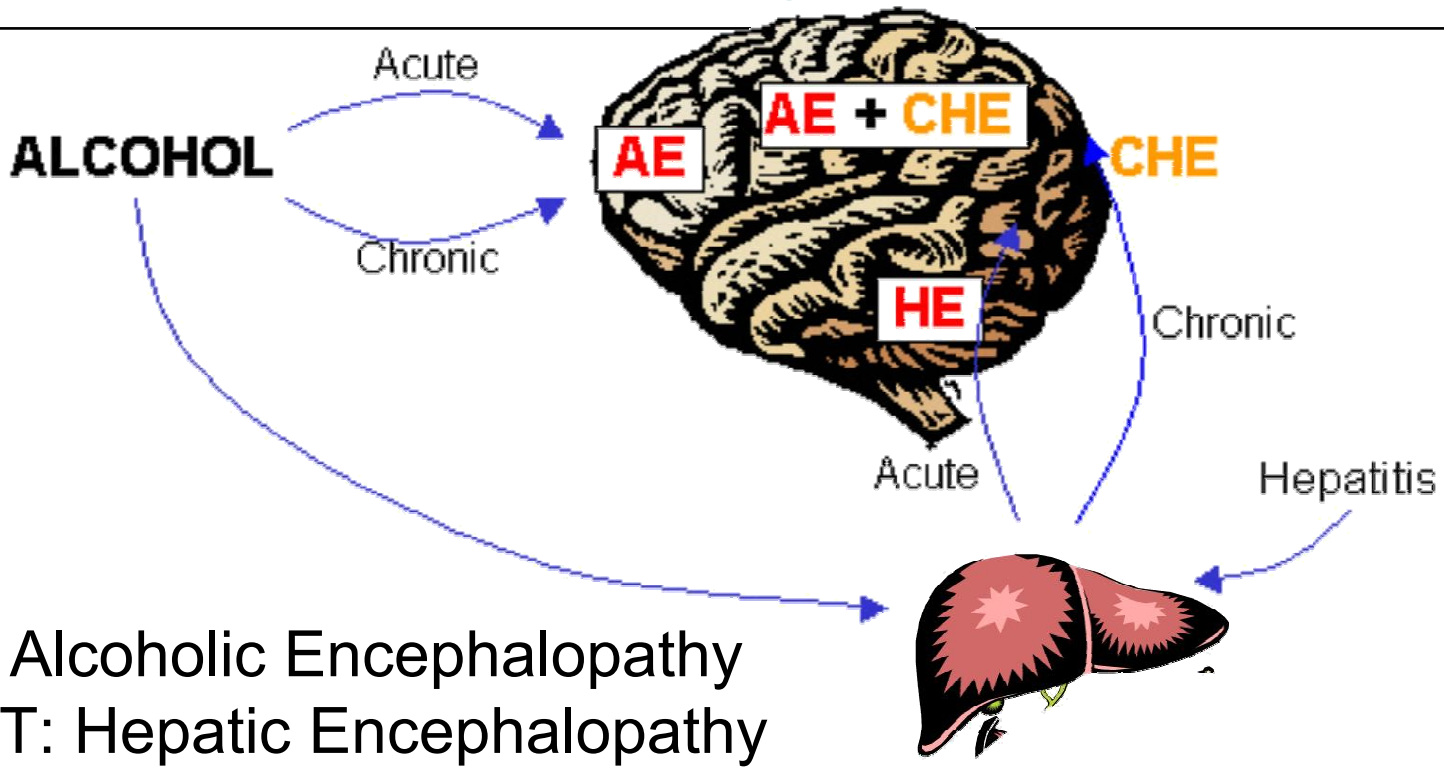
Multiple Neurologic Syndromes

- Korsakov, Wernicke, Marchiafava-Bignami
- Central pontine myelinolysis; cerebellar degeneration
- HEPATIC ENCEPHALOPATHY

H.O. Conn
Yale

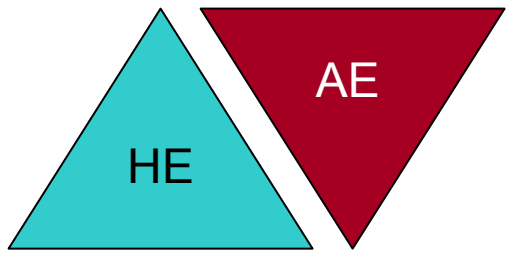


Two Distinct Etiologies



DIRECT: Alcoholic Encephalopathy
INDIRECT: Hepatic Encephalopathy

What is the relationship between them?



Investigational Methods

Neuroradiology

Brian D. Ross, MD, PhD • Sandra Jacobson, MD • Federico Villamil, MD • Jacob Korula, MD
Roland Kreis, PhD • Thomas Ernst, PhD • Truda Shonk, BS • Rex A. Moats, PhD

Subclinical
Proton MR

[1-^{13}
Ence

Stefan

JOURNAL OF NEUROSCIENCE METHODS

ELSEVIER

Journal of Neuroscience Methods 120 (2002) 179–182

Journal of Neurochemistry
Lippincott-Raven Publishers, Philadelphia

In vivo
^{13}C-en

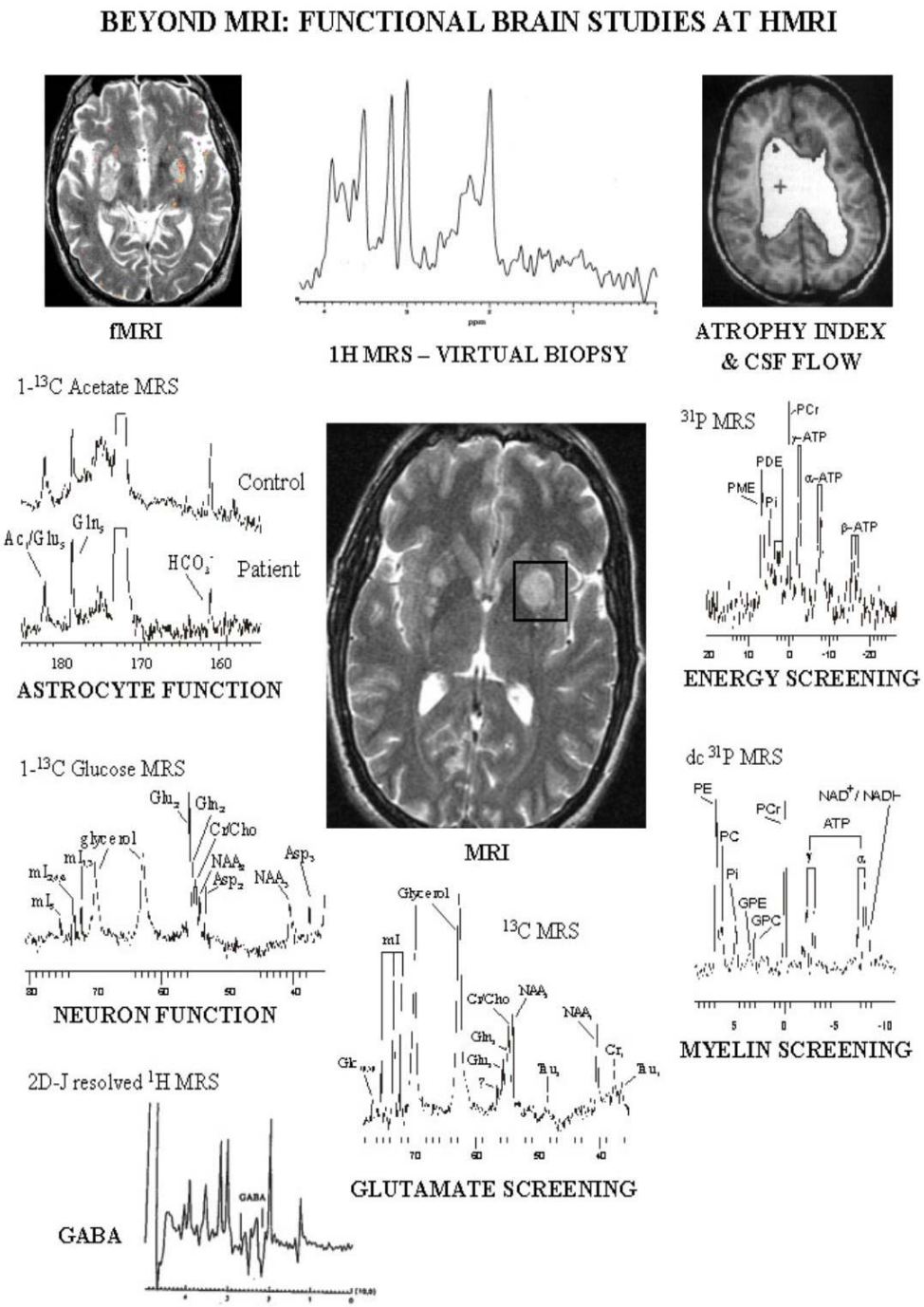
[b] Magne

Proton-Decoupled ^{31}P Magnetic Resonance Spectroscopy Reveals Osmotic and Metabolic Disturbances in Human Hepatic Encephalopathy

*†Stefan Bluml, ‡Eli Zuckerman, *†Jeannie Tan, and *†Brian D. Ross

**Magnetic Resonance Spectroscopy Unit, Huntington Medical Research Institutes, Pasadena; †Rudi Schulte Research Institute, Santa Barbara; and ‡Liver Unit, University of Southern California School of Medicine, Los Angeles, California, U.S.A.*

MRS offers a remarkable series of non-invasive tools



Modern Metabolism

Genome – Proteome – Metabolome

(Enzymes)

Receptors Transporters Concentrations

Flux

Most studies are of cells, membrane preps, etc.

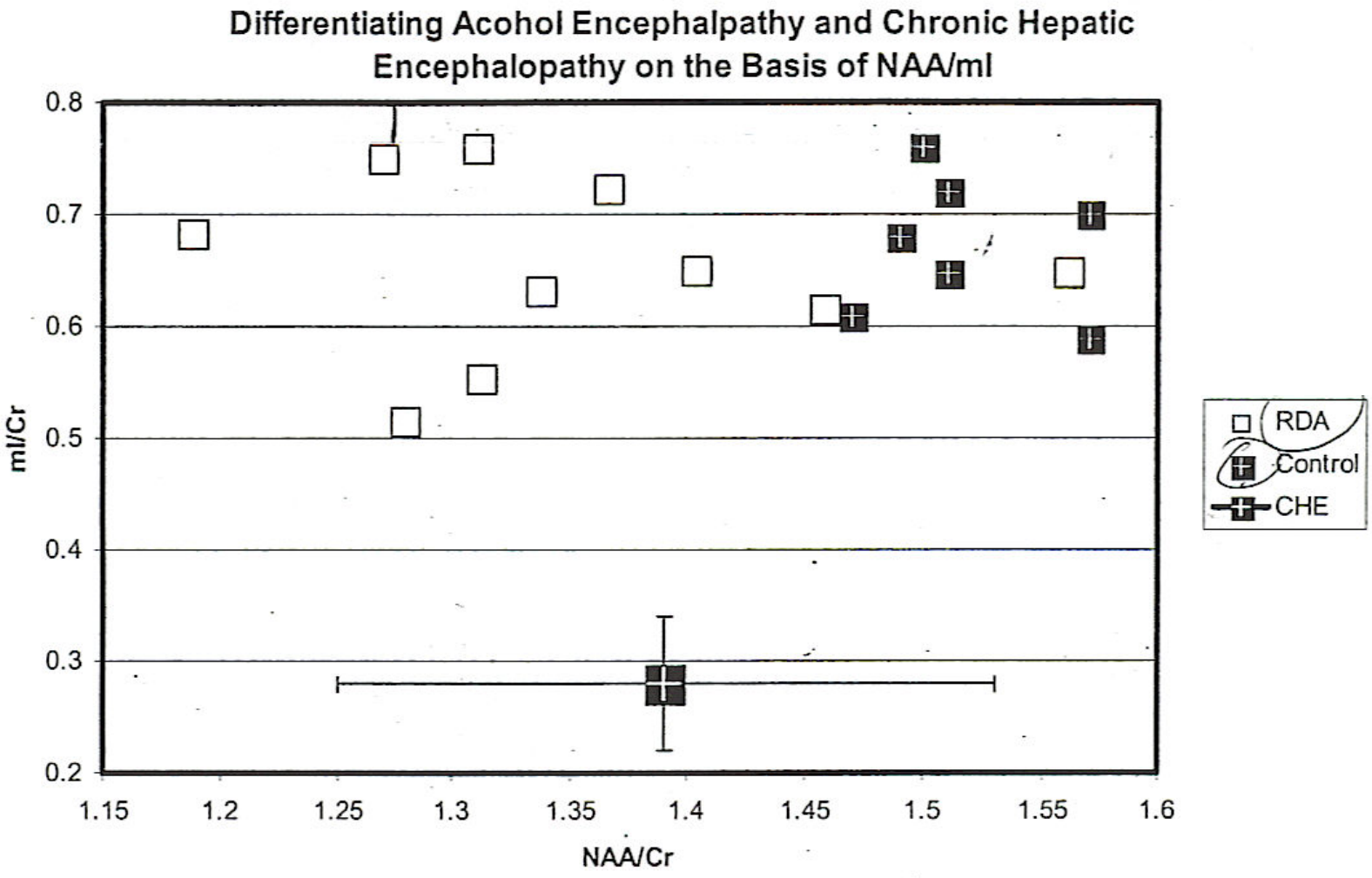
INTEGRATED *IN VIVO* STUDY WITH NMR IS A VITAL TOOL

Human Brain Metabolome

isoleucine	^{1}H	±1mM
lactate	^{1}H	±0.5mM
leucine	^{1}H	±1mM
lipid	^{1}H; ^{13}C	±1mM
lithium	^{7}Li	±0.1mM
macromolecules	^{1}H; 1R	±1.0mM
magnesium (Mg^{++})	^{31}P	±200µM
mannitol	^{1}H	±2mM
myoinositol	^{1}H; ^{13}C	±1mM
NAA	^{1}H	±0.7mM
NAAG	^{1}H	±0.3mM
oxidized hemoglobin	fMRI (1H)	±0.2%
phenyl-alanine	^{1}H	±2mM
phospho-choline	$dc^{31}P$	±0.2mM
phosphocreatine	^{31}P	±0.2mM
phosphodiesters	^{31}P	±2mM
phosphoethanolamine	$dc^{31}P$	±0.1mm
phospholipid (membrane)	$dc^{31}P$	±30%
phosphomonoesters	^{31}P	±2mM
propane-diol	^{1}H	±1mM
pyridine nucleolide(s) (NAD, NADP)	$dc^{31}P$	±1mM
scylloinositol	^{1}H	±0.2mM
sodium	^{23}Na	±
taurine* (*see also scylloinositol)	^{1}H	±1mM
TCA-cycle rate	enriched ^{13}C	± 0.1µmole/min/g
transaminase rate	enriched ^{13}C	± 10µmole/min/g
triglyceride	^{13}C	±5mM
valine	^{1}H	±1mM
water content	^{1}H	±3%

HE ≠ AE

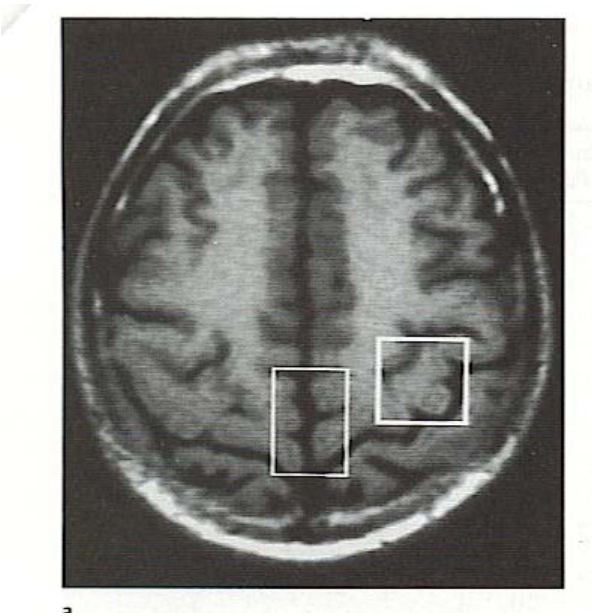
(Partly established by Taylor and others at UCSD)

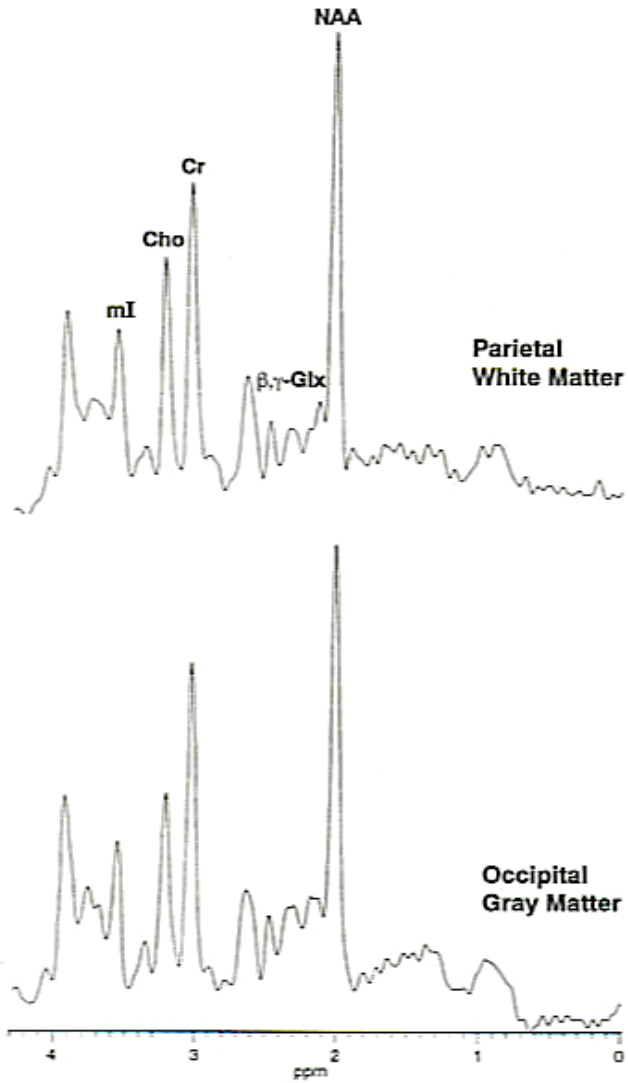


HE is a neuropsychiatric disorder

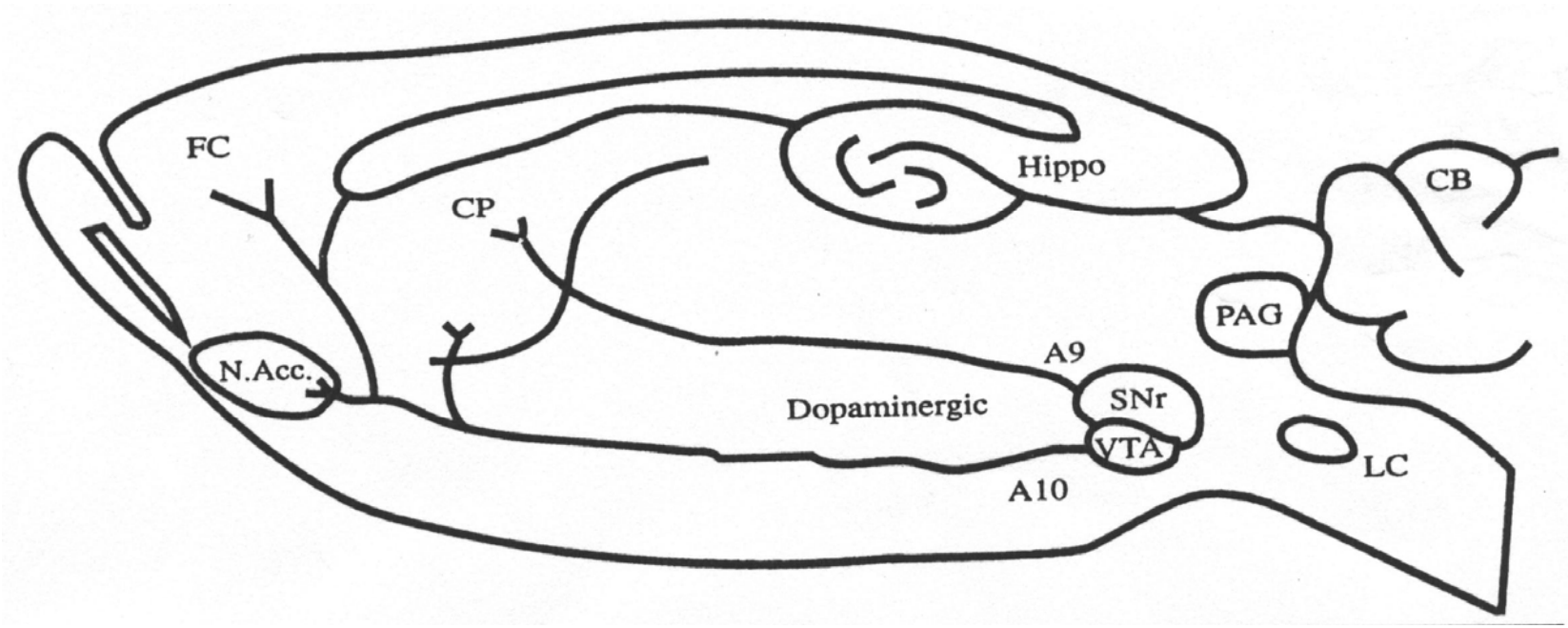
Test*	Normal Range	Results in Liver Disease Patients†	No. of Patients Outside Normal Limits
MMSE	24–30	27.4 ± 3.7	2
Trails A		65.2 ± 69.3	12
<58 y	24.6 ± 7.0		
58–65 y	41.5 ± 7.4		
66–70 y	43.2 ± 14.9		
Trails B		138.5 ± 76.6	16
<58 y	50.8 ± 12.7		
58–65 y	84.4 ± 24.6		
66–70 y	105.2 ± 43.4		
FAS		37.6 ± 7.8	15
30–39 y	49.19 ± 9.11		
50–59 y	46.05 ± 9.41		
60–69 y	45.33 ± 11.56		
Block Design	. . .‡	22.2 ± 9.2§	15
Digit Symbol	. . .‡	36.8 ± 11.3§	17
Rey AVLT trial 1		5.2 ± 2.2	12
Men			
30–39 y	6.0 ± 1.8		
40–49 y	6.4 ± 1.8		
50–59 y	6.5 ± 2.0		
60–69 y	4.9 ± 1.1		
Women			
30–39 y	8.0 ± 2.0		
40–49 y	6.8 ± 1.5		
50–59 y	6.4 ± 1.5		
60–69 y	6.0 ± 2.2		
Grooved Pegboard		97.4 ± 31.8	14
30–39 y	62.95 ± 8.40		
40–49 y	63.50 ± 7.20		
50–59 y	68.10 ± 9.42		
60–69 y	82.70 ± 18.70		

Simple localized ^{1}H MRS shows the neurochemical disorder of HE to be diffuse:

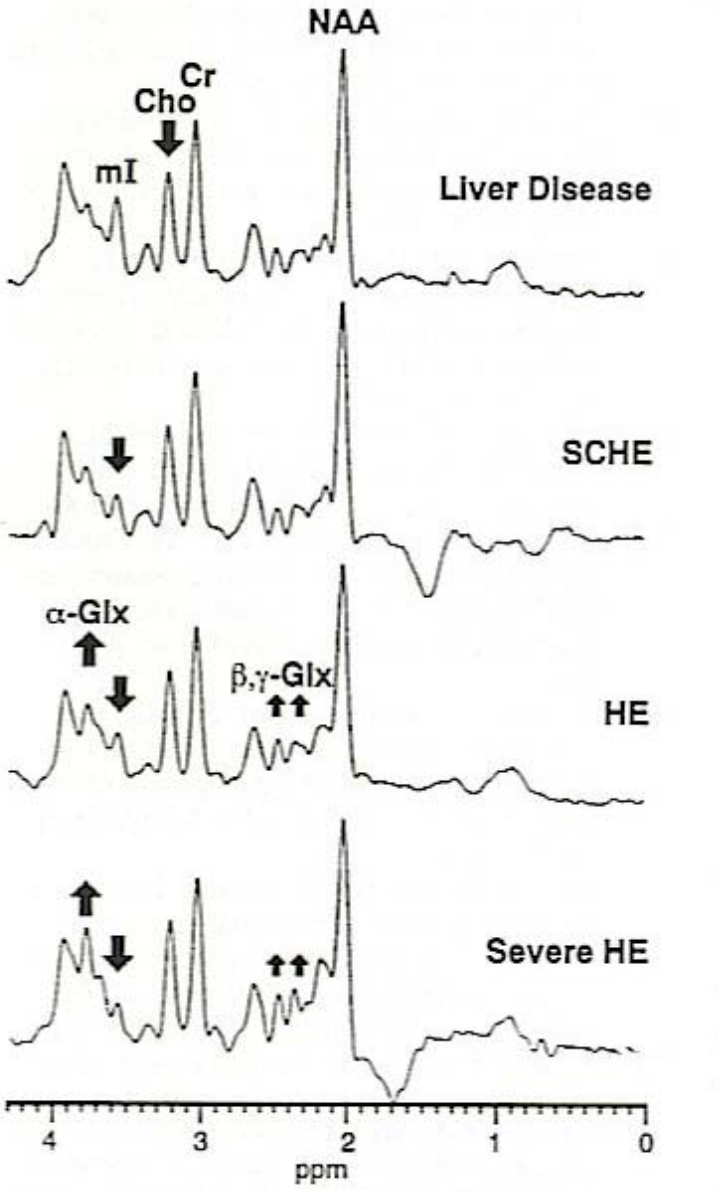




Therefore, NOT essential to perform multivoxel, whole brain, or difficult "keyhole" *in vivo* MRS


FC
CP
Hippo
CB
PAG
A9
N.Acc.
Dopaminergic
SNr
VTA
LC
A10

HE is a graded disease



^{1}H MRS is more specific than neuropsych

Comparison of Results of Neuropsychologic and MR Spectroscopic Evaluation in Identification of HE in 20 Cases

Patient	Criteria of Parsons-Smith et al	Neuropsychologic Tests	MR Spectroscopy	Glx Values
1	+ (3)	+	+	++
2	–	+	+	++
3	+ (4)	+	+	++
4	–	+	+	++
5	+ (1)	+	+	++
6	–	+	+	0
7	+ (1)	+	+	0
8	+ (1)	+	+	++
9	+ (1)	+	+	+
10	–	+	+	++
11	–	+	+	+
12	–	–	–	0
13	–	–	+	+
14	–	+	+	++
15	–	+	+	+
16	–	+	+	+
17	–	+	+	0
18	+ (1)	+	–	0
19	+ (1)	+	+	+
20	–	–	–	0

- And 2-3 times more sensitive than clinical criteria

Results of Stepwise Discriminant Analysis of MR Spectroscopic Variables for Identifying HE in 20 Cases

Variable by Location*	Trails A and B Tests	Criteria of Parsons-Smith et al
Occipital GM		
mI	85	35
Cho	50	30
β-Glx	75	30
mI + Cho	85	35
mI + Glx	85	35
mI + Cho + Glx	85	30
Parietal WM		
mI	80	35
Cho	35	0
β-Glx	65	30
mI + Cho	85	15
mI + Glx	80	15
mI + Cho + Glx	90	10

- Glutamine is involved, ? Glutamate
- Myoinositol… no mere osmolyte
- But mechanism NOT defined by steady state concentrations

Dynamic Study is Required ^{13}C MRS *in vivo*

1. Cumbersome; costly; research only?
2. New methods
   - FDA_IND Protocols
   - Patient Friendly, Cost Effective Protocols
   - Clinical MR Scanners
   - Multi-site trials

Data Analysis

Bluml

In vivo quantitation of cerebral metabolite concentrations using natural abundance 13C MRS at 1.5 T.

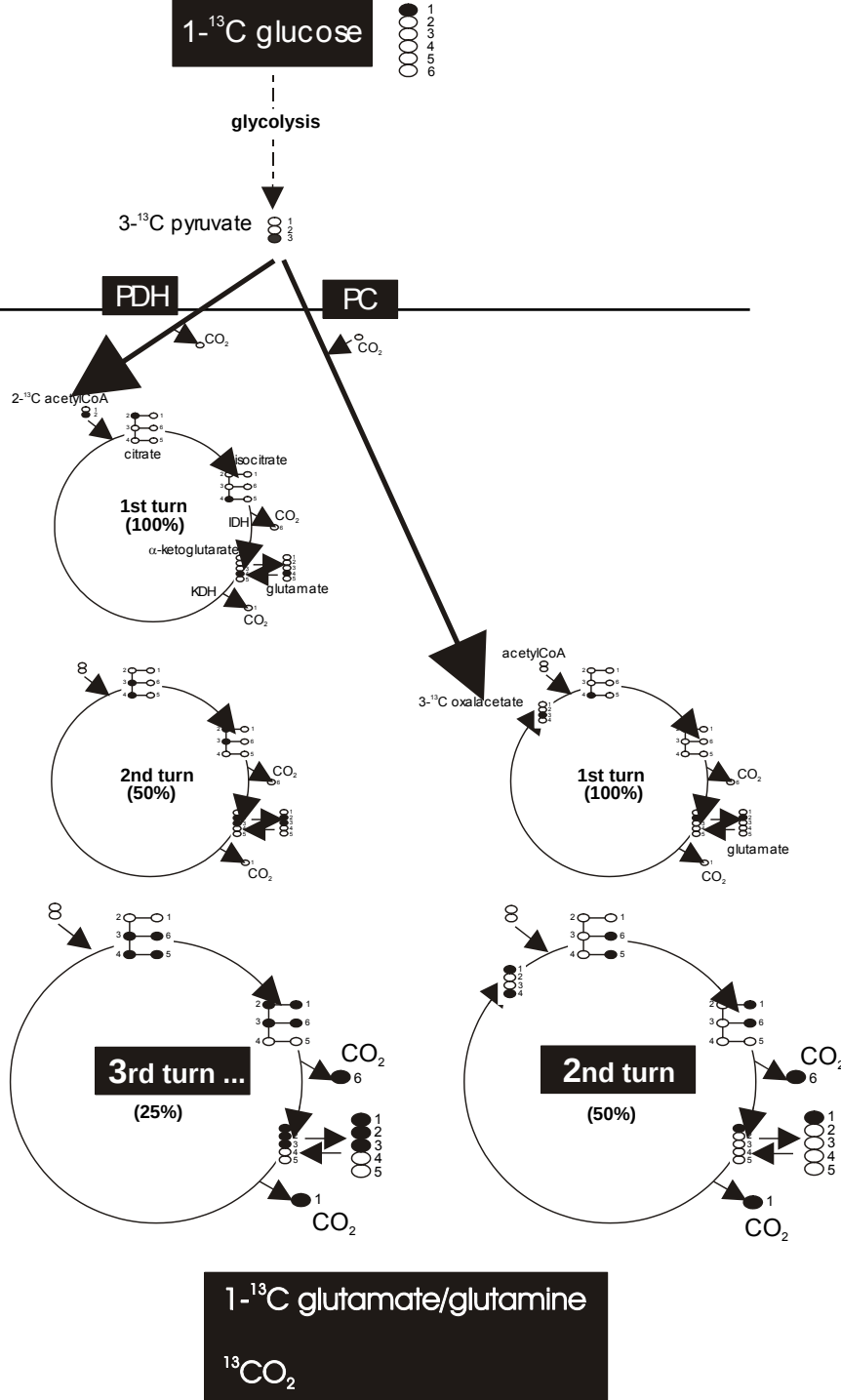
J Magn Reson. 1999 Feb; 136(2): 219-25

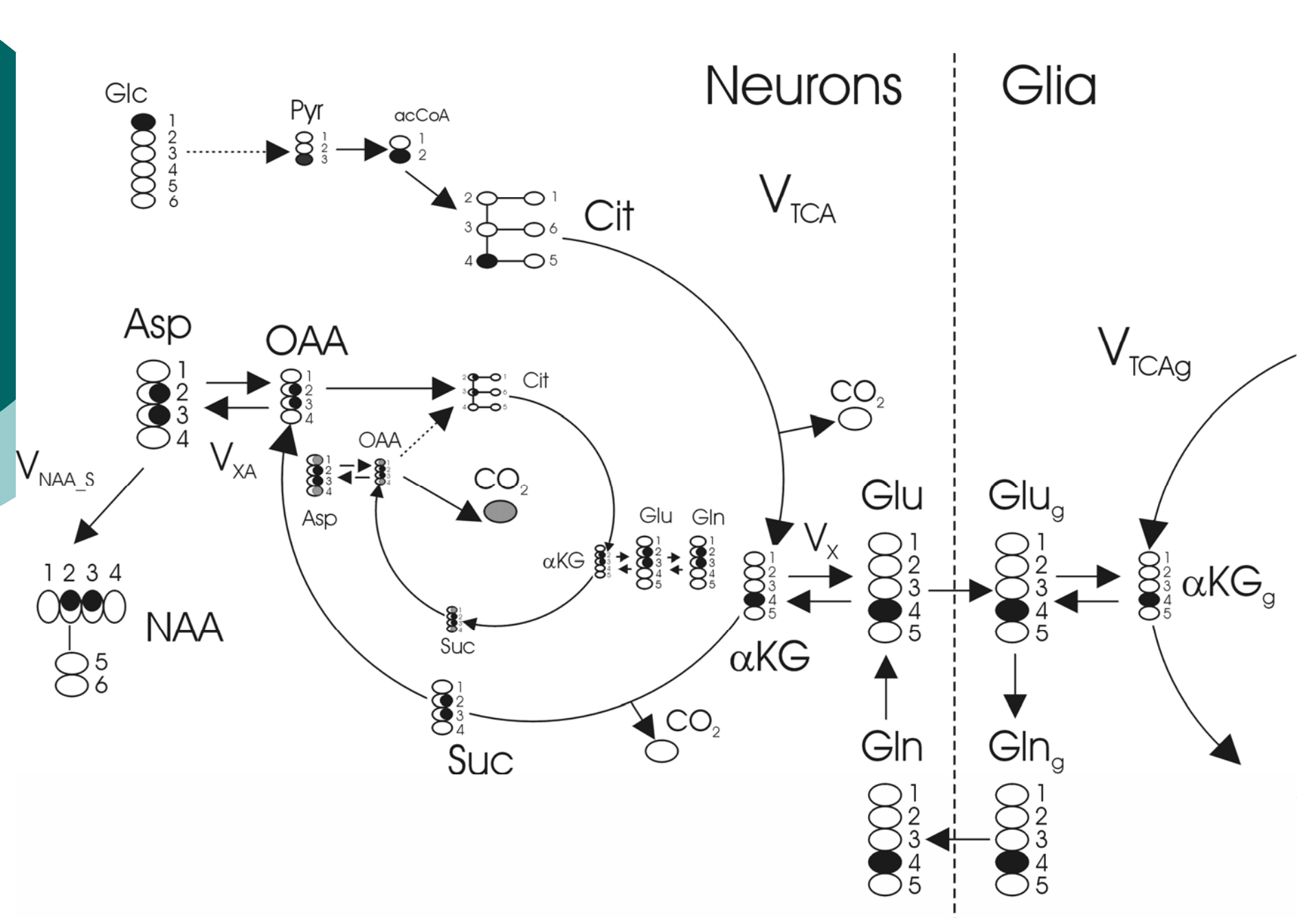
Or

Shic JAUYANG

Automated data processing of [1H-decoupled] 13C MR spectra acquired from human brain in vivo.

J Magn Reson. 2003 Jun; 162(2): 259-68.




Neurons
Glia
Glc
Pyr
acCoA
Cit
V_{TCA}
Asp
OAA
V_{XA}
V_{NAA_S}
NAA
CO_2
Suc
αKG
Glu
Gln
V_X
V_{TCAg}
Glu_g
Gln_g
$αKG_g$

Magnetic Resonance in Medicine 45:981–993 (2001)

[1-^{13}C] Glucose MRS in Chronic Hepatic Encephalopathy in Man

Stefan Blüml,[1,2*] Angel Moreno-Torres,[1,2] and Brian D. Ross[1]

- [1-13C]-labeled glucose was infused intravenously in a single dose of 0.2 g/kg body weight over 15 min in six patients with chronic hepatic encephalopathy, and three controls.

- Serial 13C MR spectra of the brain were acquired.

Patients exhibited the following characteristics relative to normal controls:

1) Cerebral glutamine concentration was increased (12.6 +/- 3.8 vs. 6.5 +/- 1.9 mmol/kg, $P < 0.006$) and
2) glutamate was reduced (8.2 +/- 1.0 vs. 9.9 +/- 0.6 mmol/kg, $P < 0.02$).

3) 13C incorporation into glutamate C4 and C2 positions was reduced in patients (80 min after start of infusion

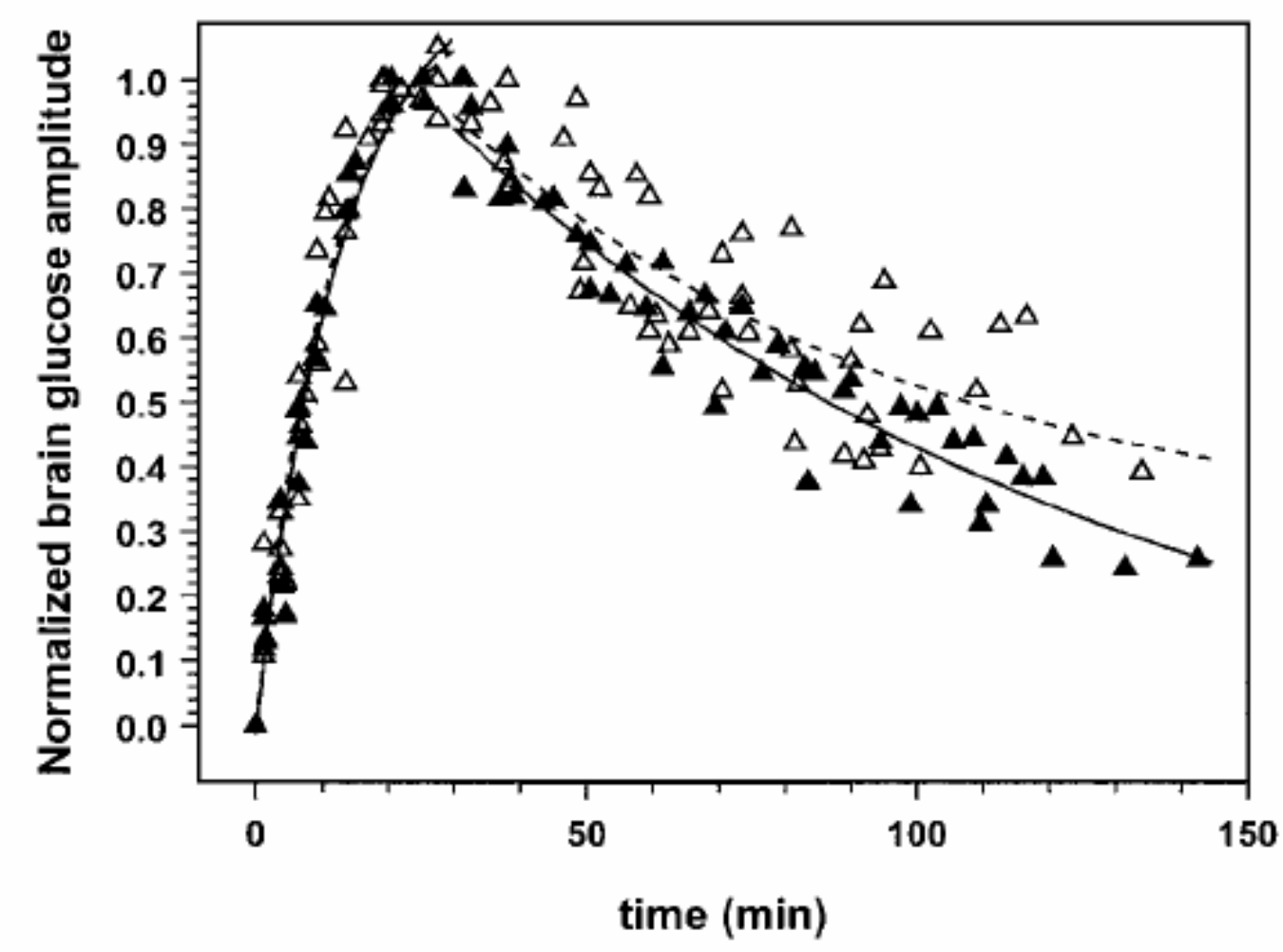
- C4: 0.43 +/- 0.09 vs. 0.84 +/- 0.15 mmol/kg, $P < 0.001$;
- C2: 0.20 +/- 0.03 vs. 0.45 +/- 0.07 mmol/kg, $P < 0.0001$).

4) 13C incorporation into bicarbonate was delayed (90 +/- 21 vs. 40 +/- 10 min, $P < 0.003$), and
5) the time interval between detection of glutamate C4 and C2 labeling was longer in patients (22 +/- 8 vs. 12 +/- 3 min, $P < 0.03$).
6) Glutamate C2 turnover time was reduced in chronic hepatic encephalopathy (17.1 +/- 6.8 vs. 49.6 +/- 8.7 min, $P < 0.0002$).

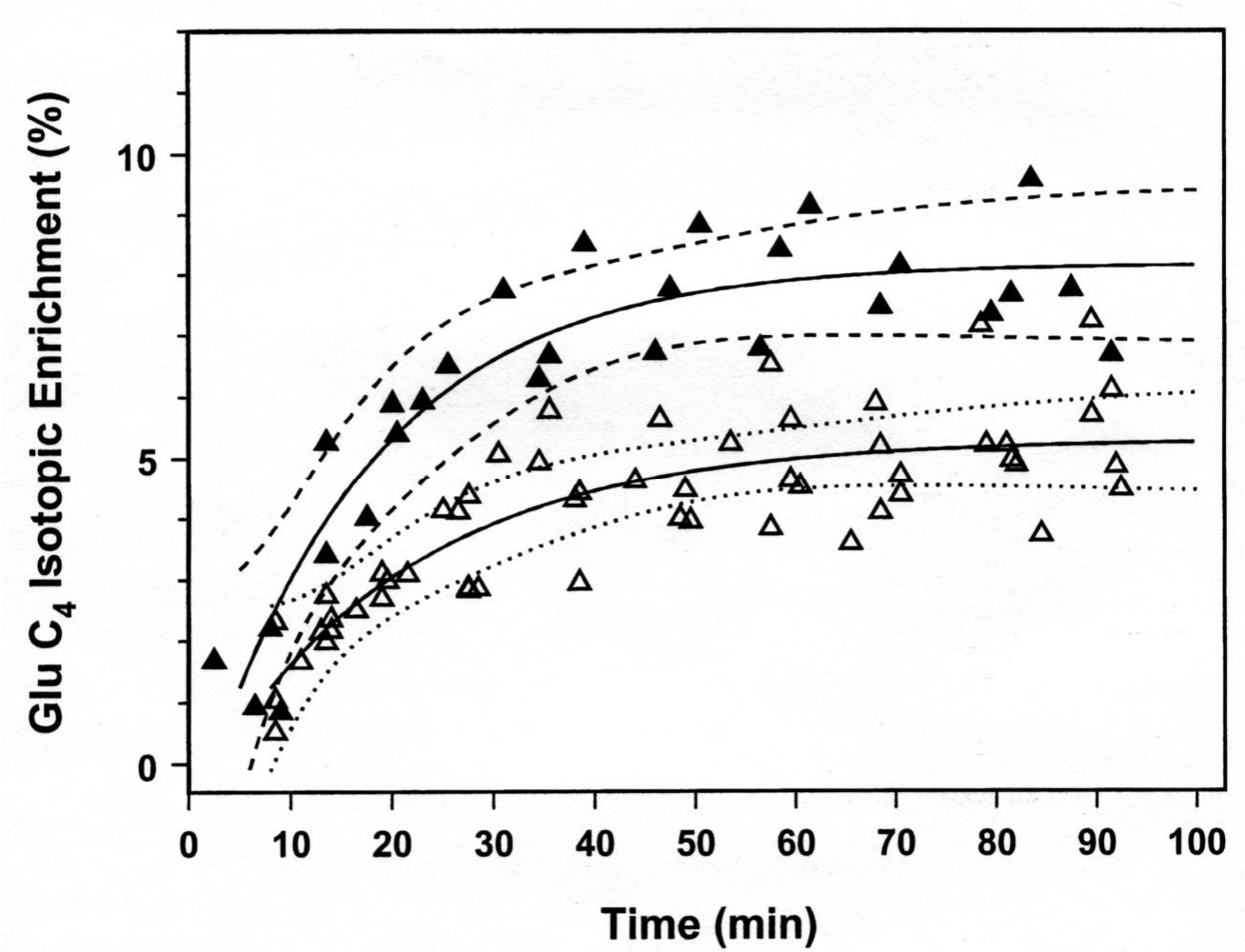
3) 13C accumulation into glutamine C2 relative to its substrate glutamate C2 increased progressively with the severity of clinical symptoms ($r = 0.96$, $P < 0.01$).

- These data indicate
  - disturbed neurotransmitter glutamate/glutamine cycling and
  - reduced glucose oxidation in chronic hepatic encephalopathy. [1-13C] glucose MRS
  - provides novel insights into disease progression and the pathophysiology of chronic hepatic encephalopathy.

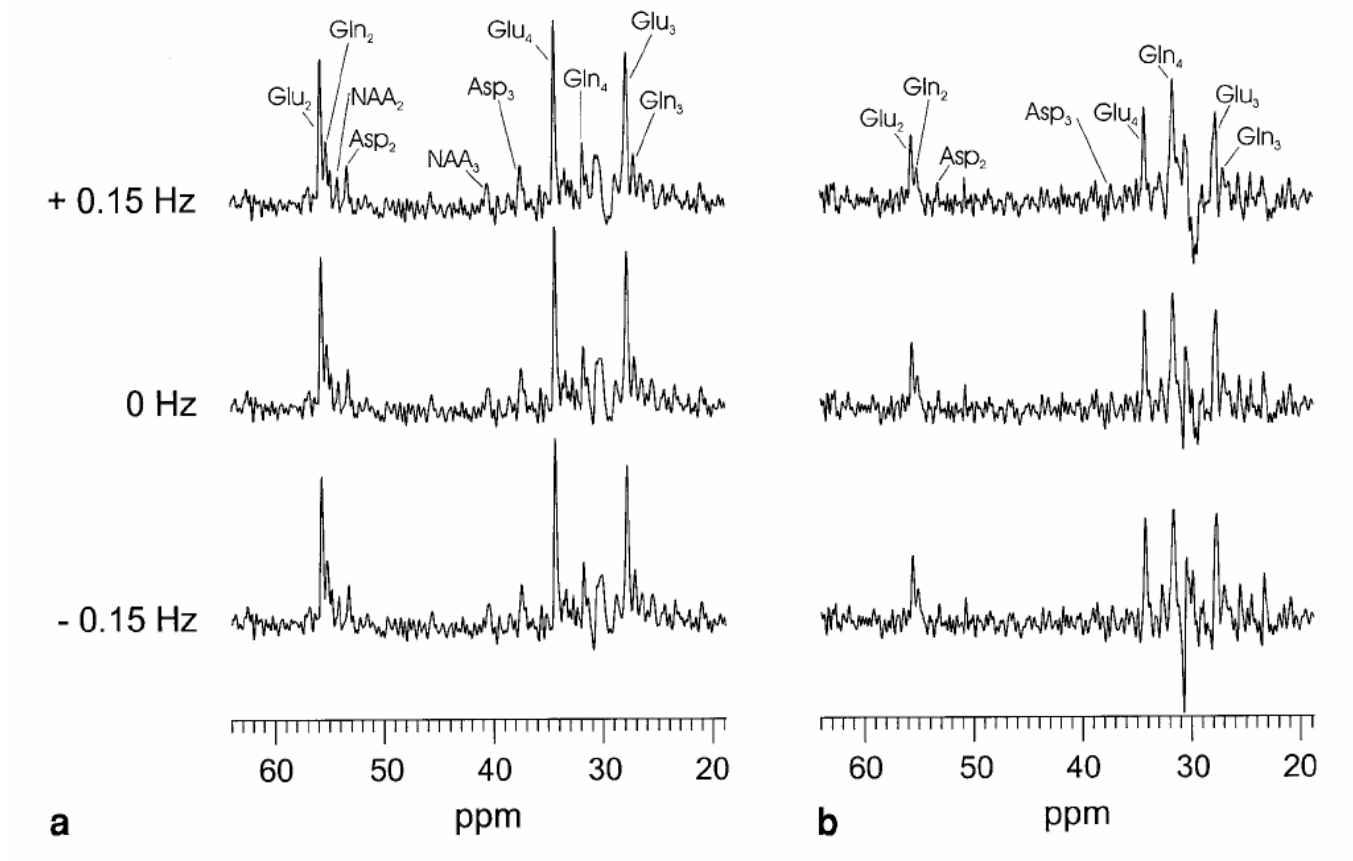
Glucose Time Courses



Glu_4 Time Course



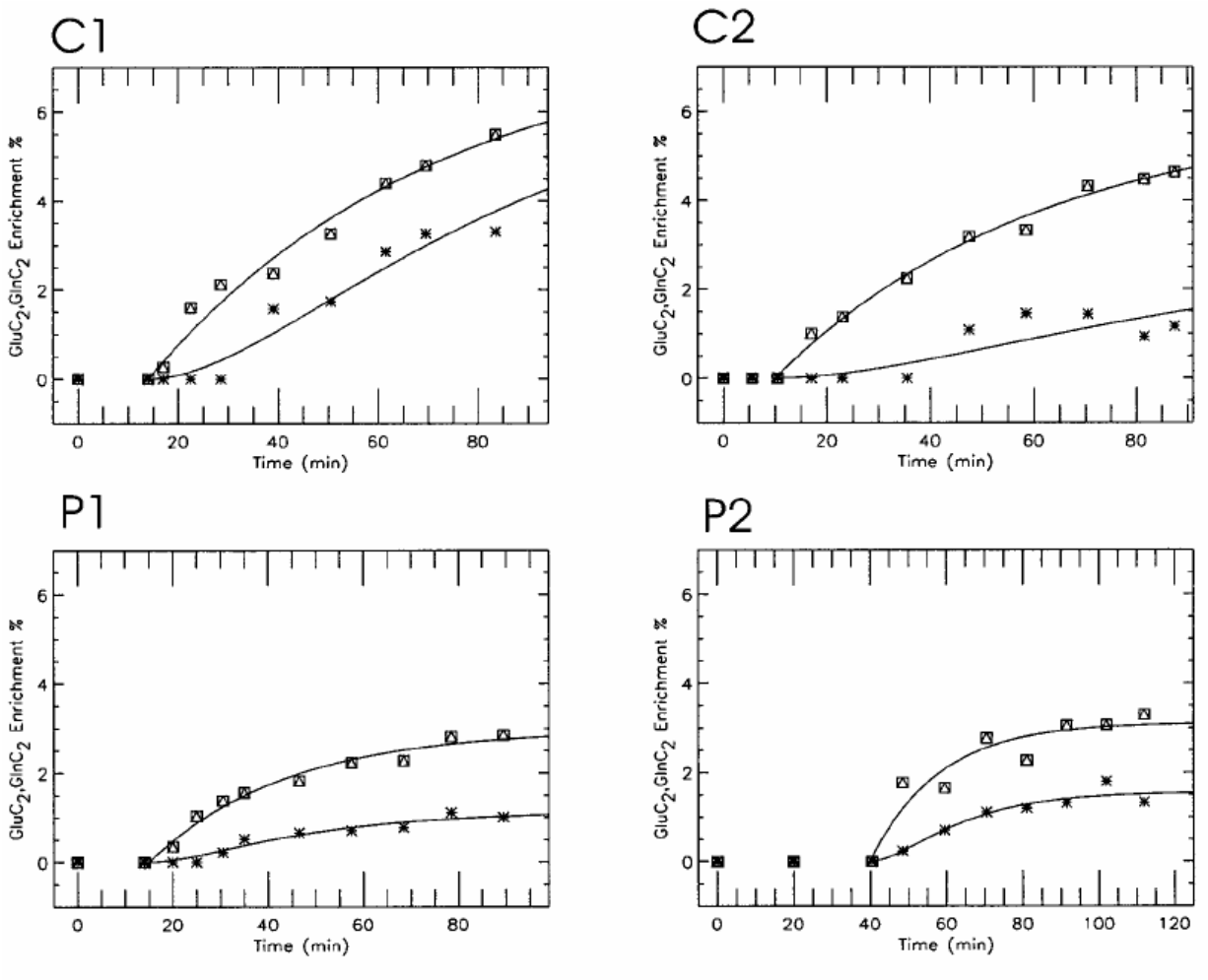
Glutamate and Aspartate Enrichment Severely Restricted in HE Brain *In Vivo*



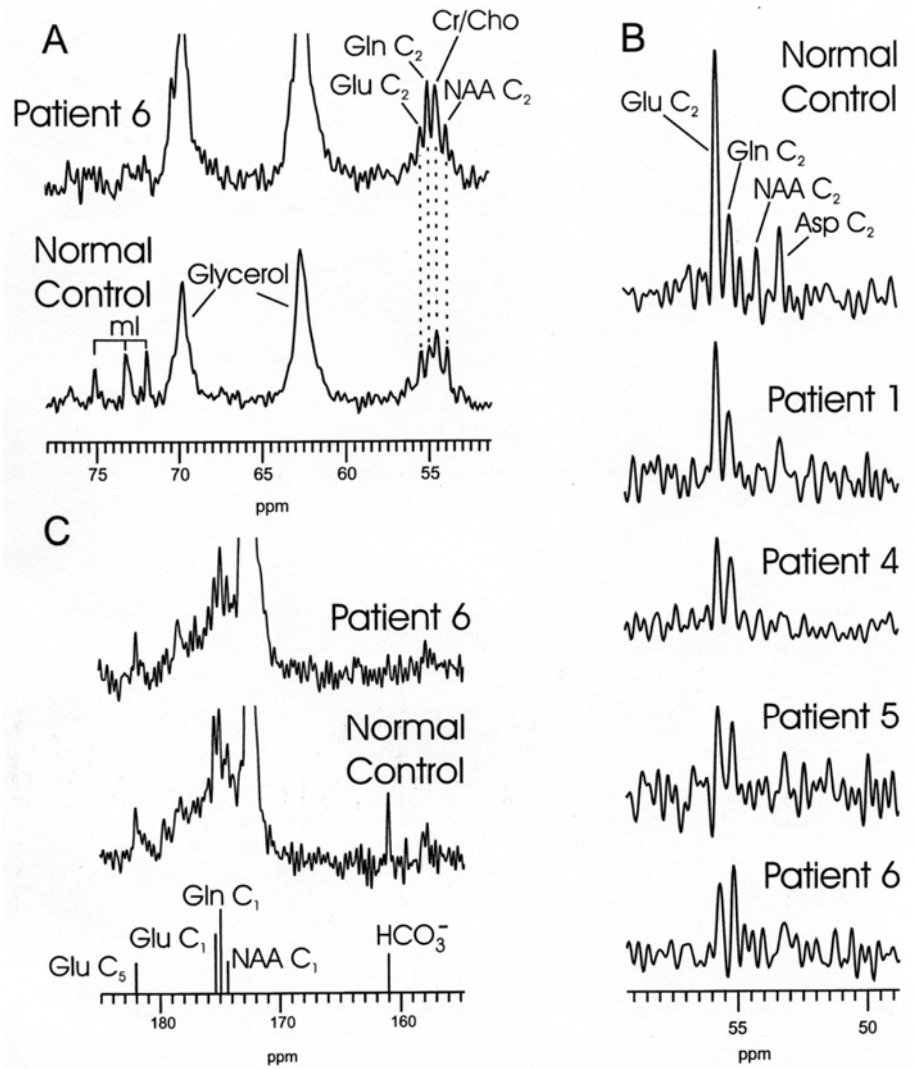
Normal

CHE

Glutamine Enrichment Lags Behind Glutamate



Severity of Clinical HE is reflected by Glutamate Flux *in vivo*



Highly Suggestive of a Glutamate Neurotransmitter Defect

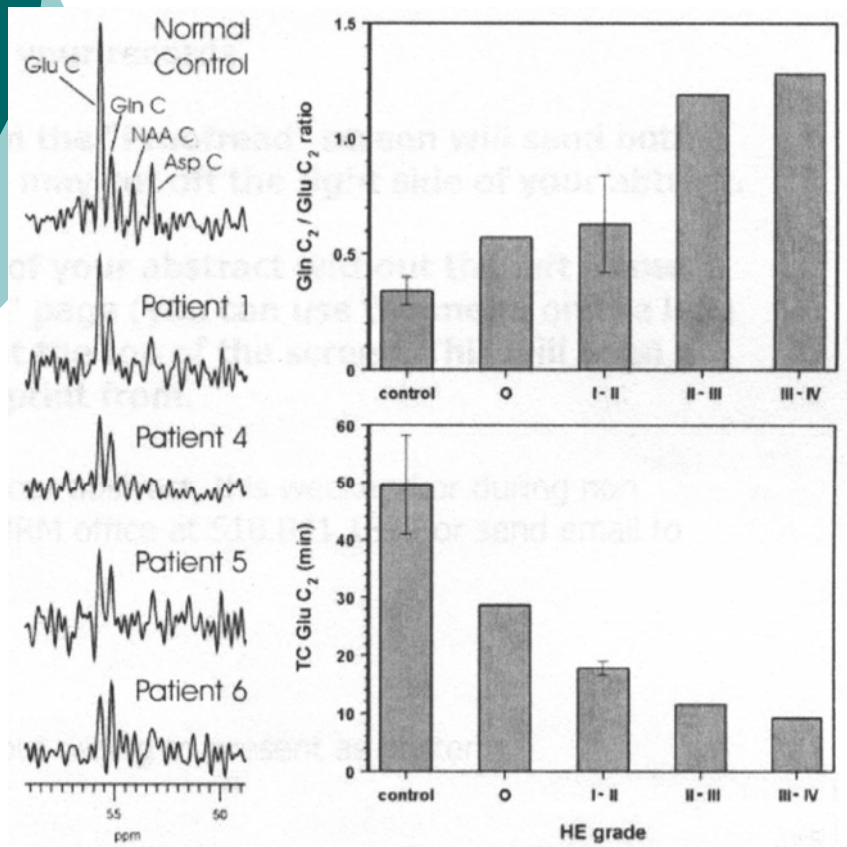
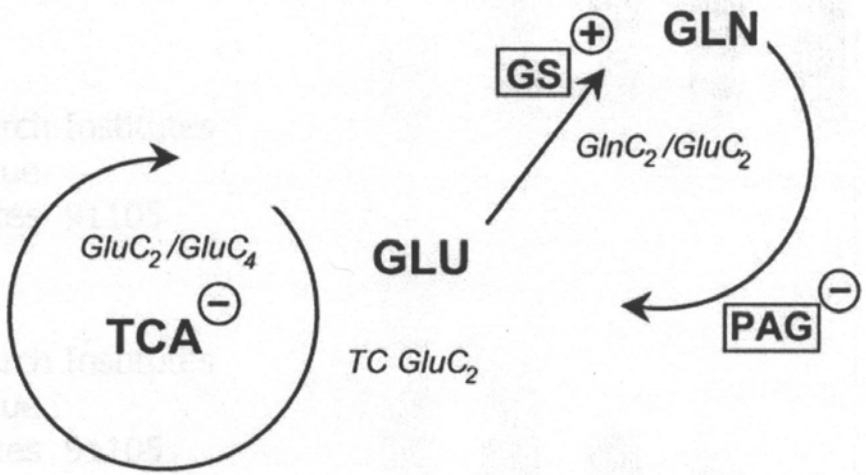
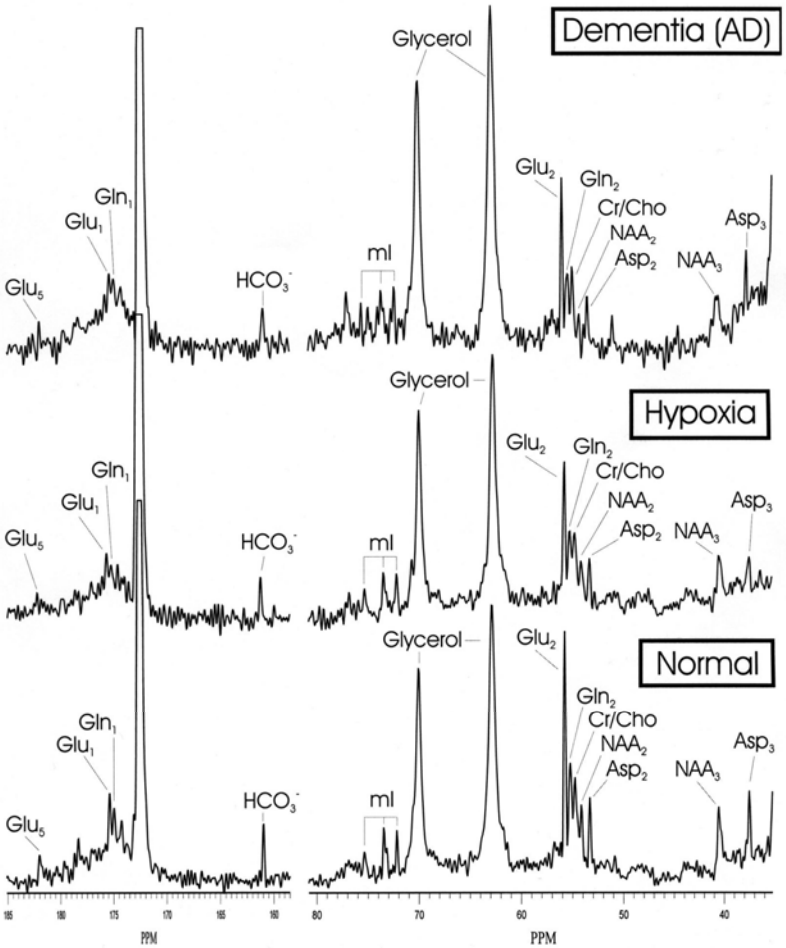


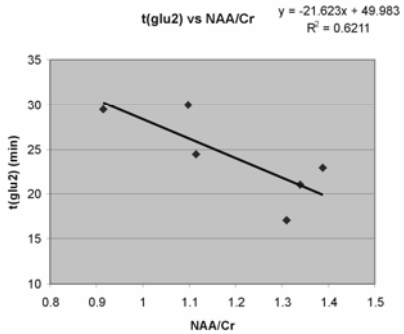
Figure 1: ^{13}C difference spectrum of the brain in normal and four HE patients.

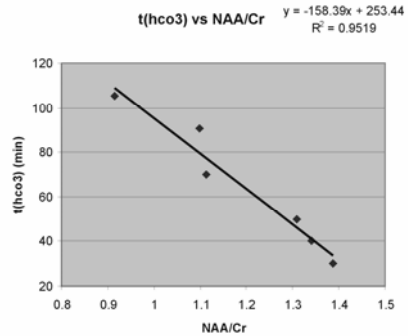
Figure 2: ^{13}C MRS vs. coma grade (HE). Bars indicate SD.

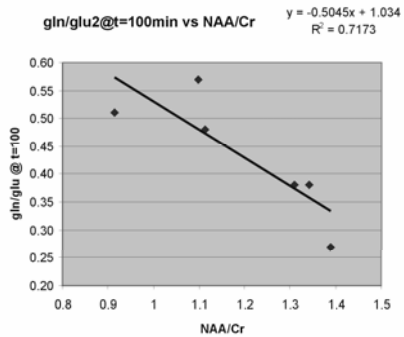


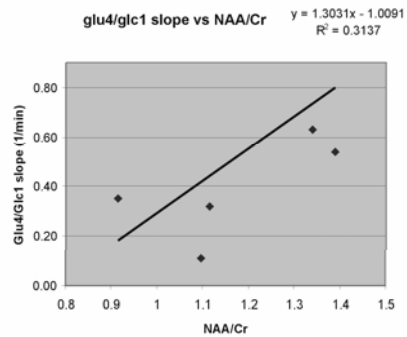
If so, this is a different GNT defect from that recently described in Alzheimer's Disease



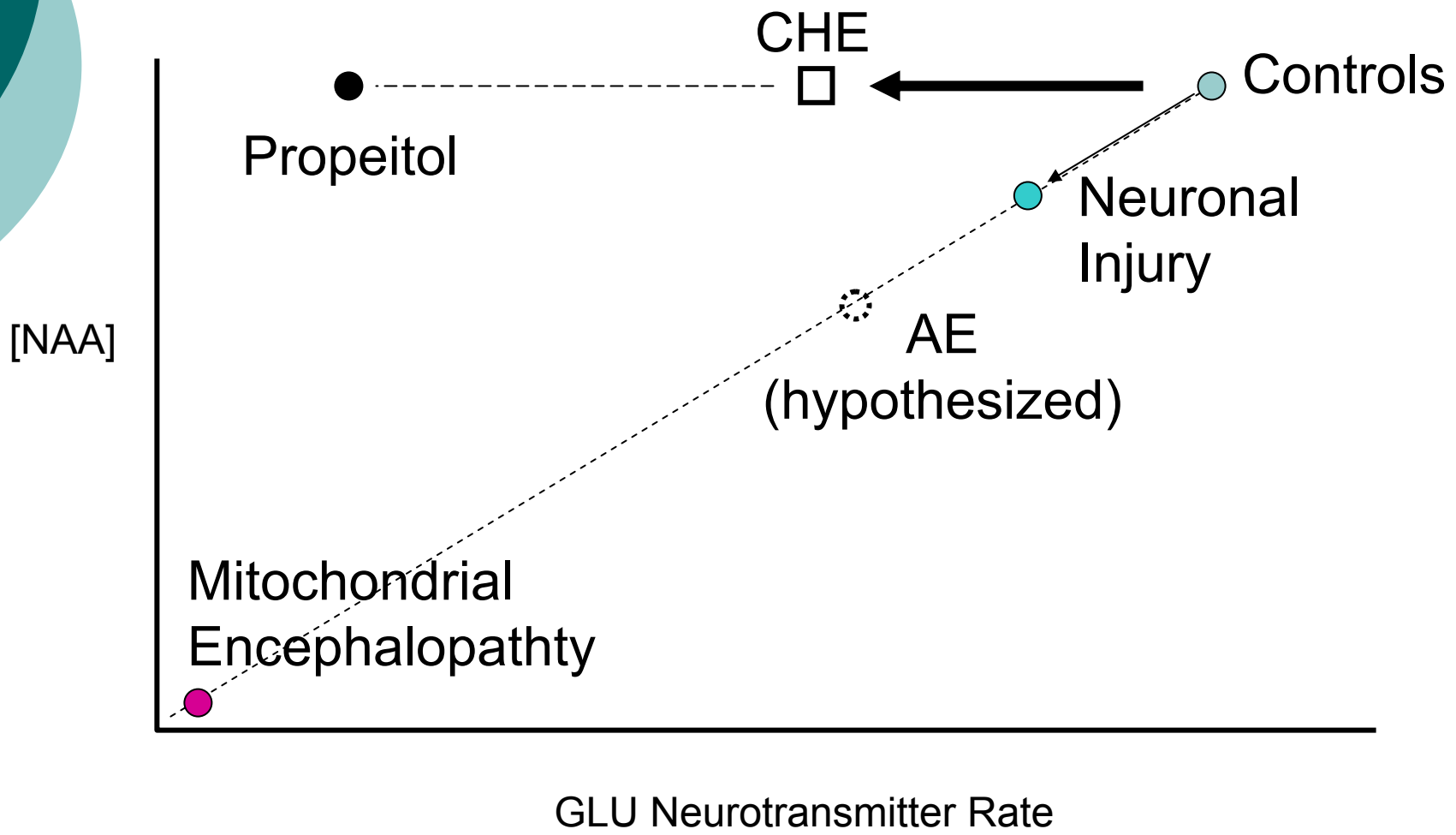








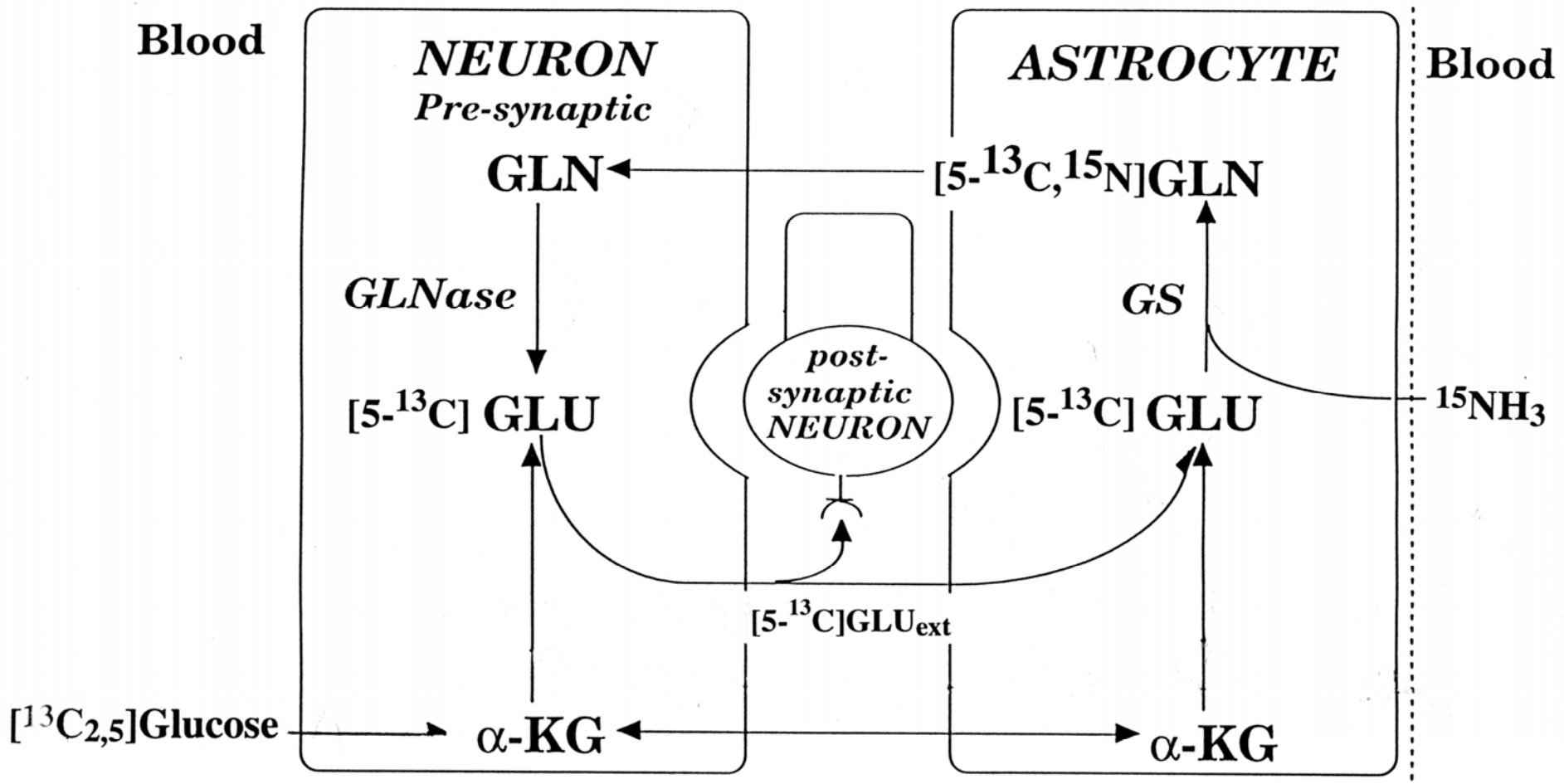
13C MRS might therefore be a powerful tool for distinguishing AE from HE



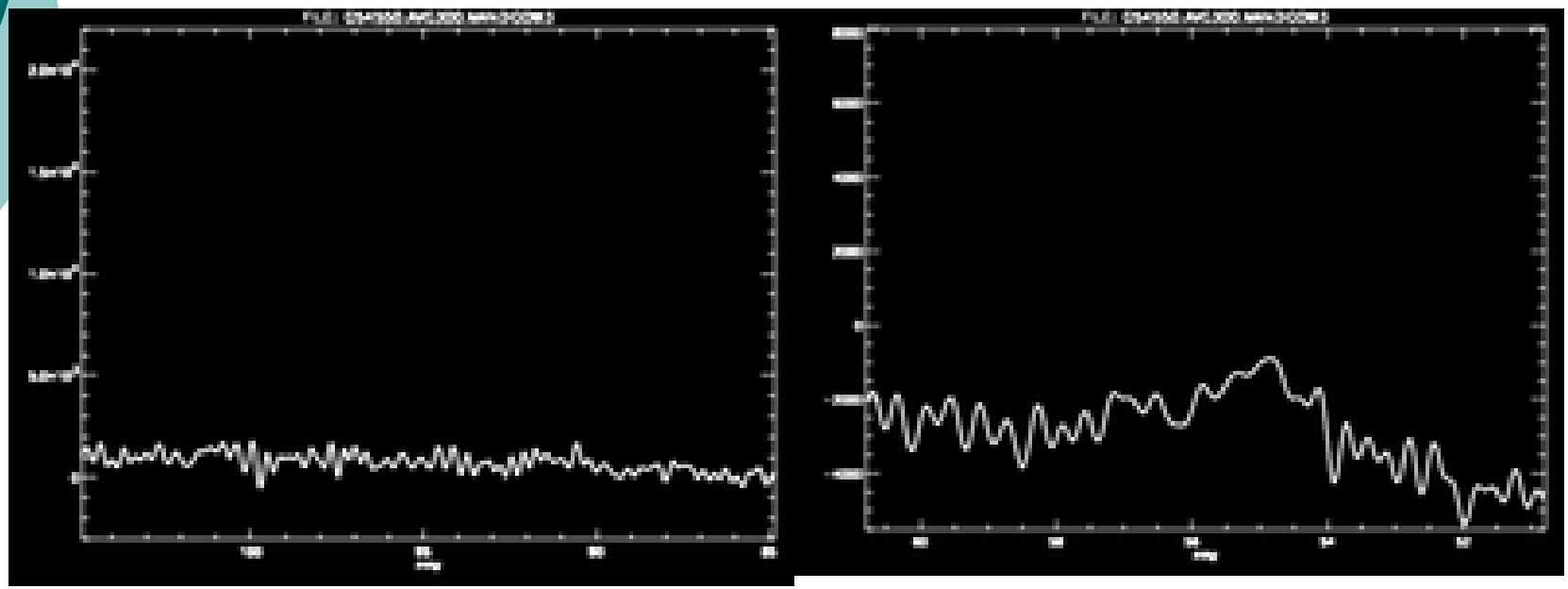
Conclusions I

1. AE is multiple syndromes
2. Some or ALL may involve glutamate axis
3. Based on human 13C MRS, two or more distinct GNT defects

   (Type I = N, Type II = A)
4. Based on more detailed animals studies with ^{13}C, ^{15}N, and bran dialysis plus special inhibitors, at least EIGHT types of GNT defect can be envisioned


Blood
NEURON
Pre-synaptic
ASTROCYTE
Blood
GLN
[5-13C,15N]GLN
GLNase
GS
post-
synaptic
NEURON
[5-13C] GLU
[5-13C] GLU
15NH3
[5-13C]GLUext
[13C2,5]Glucose
α-KG
α-KG

Glucose



Conclusions II

- Hepatic encephalopathy is a distinct form of alcoholic encephalopathy that definitely involves glutamate

Acknowledgements

Thank you!