SUPPLEMENTARY INFORMATION

Brain Energy Metabolism in Intracerebroventricularly Administered Streptozotocin Mouse Model of Alzheimer's disease: A ^{1}H-[^{13}C]-NMR Study

Narayan Datt Soni[1], Akila Ramesh[1], Dipak Roy[1] and Anant Bahadur Patel[1,2]*

[1]NMR Microimaging and Spectroscopy, CSIR-Centre for Cellular and Molecular Biology, Habsiguda, Uppal Road, Hyderabad 500007, India; [2]Academy of Scientific and Innovative Research, Ghaziabad 201 002, India

*Address for Correspondence:

Dr. Anant B. Patel, NMR Microimaging and Spectroscopy, Centre for Cellular and Molecular Biology, Uppal Road, Habsiguda, Hyderabad 500 007, India.

Telephone: +91-40-27192838

Email: abpatel@ccmb.res.in

Running Headline: Neuroenergetics in ICV-STZ mouse model of AD

Assumption Involved in the Estimation of Metabolic Rates

There are certain limitations that need to be considered while interpreting the findings of the study. Firstly, levels of precursors should be stable during the entire period of measurement. We have adopted an established infusion protocol, which raises the blood substrate level within a couple of minutes. A similar protocol has been used in several studies in rats and mice.[1-5] These studies demonstrated that the levels and ^{13}C enrichments of precursors in the blood reach to maximum in 1 min (glucose) / 3 min (acetate), and maintained to the same level throughout the study. Our previous studies involving ^{13}C acetate in mice and rats have indicated an increase in plasma acetate enrichment to ~90% in less than 3 min after the start of infusion.[4,6,7]

Secondly, the behavior of labeling kinetics of different amino acids was considered linear during the study period (7-10 min). In vivo measurements of brain amino acids labeling from glucose / acetate have demonstrated that glutamate-C4 labeling with time could be approximated linear till 20 min.[4,8-12] The labeling of other amino acids including GABA at various carbon positions follows a linear relation with time for a longer period (30 min). Therefore, the linear behavior of ^{13}C labeling kinetics of amino acids till 7-10 min is a valid assumption. In fact, this approach has been used in different studies for the estimation of neurometabolic fluxes in rats and mice brains.[7,11,13,14]

Another assumption involved is the equal distribution of ^{13}C label in glutamate-C2 and glutamate-C3 possibly due to complete scrambling of ^{13}C label at the malate complete thereby equal labeling of oxaloacetate-C2 (OAA-C2) and OAA-C3. The metabolism of [1,6-$^{13}C_2$]glucose via glycolysis provides two molecules of pyruvate-C3, which is metabolized mostly by pyruvate dehydrogenase (PDH) and TCA cycle to incorporate the label into glutamate-C4 and glutamate-C2/C3. We could not measure the labeling of glutamate/glutamine-C2 and aspartate-C2 due to overlap with other metabolites in the NMR spectrum. However, metabolic modeling of the ^{13}C turnover of amino acids from glucose considers complete scrambling of the label at malate.[5,9,11,15] It is due to the symmetric *nature* of succinate (fumarate), which is a precursor for malate (oxaloacetate). Additionally, a smaller fraction of pyruvate-C3 is metabolized by the pyruvate carboxylase (PC) pathway to label oxaloacetate-C3 in the brain, thereby

increase in the labeling of glutamate/glutamine-C2 over glutamate/glutamine-C3. However, PC flux is reported to be very small in the rat cerebrum (~10% of the PDH flux).[9,16] Therefore, the contribution of PC for glutamate/glutamine-C2 labeling is expected to be small in 7 minute of the study. Additionally, the labeling of glutamate-C3 and glutamate-C2 from [1,6-$^{13}C_2$]glucose was reported to be similar till 100 min in the rat cerebrum.[9,17] Hence, we have considered the labeling of glutamate-C2 to be the same as glutamate-C3. It should be noted that glutamate-C3 labeling in our study is almost one-fourth of glutamate-C4 (Table 3S). Therefore, slightly higher labeling of glutamate-C2 over glutamate-C3 will have a minimal impact on the metabolic rates, and will not affect the conclusion of the study.

Lastly, a small amount of ^{13}C label may be lost due to glutamine efflux, and CO_2 by oxidation in the subsequent turns of the TCA cycle. Any loss in TCA cycle intermediates due to oxidation and glutamine efflux is compensated with PC by de novo synthesis of oxaloacetate in astrocytes.[18] As described in the previous section, PC flux being very small relative to PDH, any loss of label due to glutamine efflux will be small. Moreover, the loss will occur in control and ICV-STZ treated mice. Hence, any underestimation in metabolic rates will happen both in the control and STZ-treated mice, therefore will not affect the conclusion of the study.

References:

1. Duarte JM, Lanz B and Gruetter R. Compartmentalized Cerebral Metabolism of [1,6-(13)C]Glucose Determined by in vivo (13)C NMR Spectroscopy at 14.1 T. *Front Neuroenergetics* 2011; 3: 3. DOI: 10.3389/fnene.2011.00003.

2. Fitzpatrick SM, Hetherington HP, Behar KL, et al. The flux from glucose to glutamate in the rat brain in vivo as determined by 1H-observed, 13C-edited NMR spectroscopy. *J Cereb Blood Flow Metab* 1990; 10: 170-179. DOI: 10.1038/jcbfm.1990.32.

3. Patel AB, de Graaf RA, Mason GF, et al. Glutamatergic neurotransmission and neuronal glucose oxidation are coupled during intense neuronal activation. *J Cereb Blood Flow Metab* 2004; 24: 972-985. DOI: 10.1097/01.WCB.0000126234.16188.71.

4. Patel AB, de Graaf RA, Rothman DL, et al. Evaluation of cerebral acetate transport and metabolic rates in the rat brain *in vivo* using ^{1}H-[^{13}C]-NMR. *J Cereb Blood Flow Metab* 2010; 30: 1200-1213. DOI: 10.1038/jcbfm.2010.2.

5. Tiwari V, Ambadipudi S and Patel AB. Glutamatergic and GABAergic TCA cycle and neurotransmitter cycling fluxes in different regions of mouse brain. *J Cereb Blood Flow Metab* 2013; 33: 1523-1531. DOI: 10.1038/jcbfm.2013.114.

6. Mishra PK, Adusumilli M, Deolal P, et al. Impaired neuronal and astroglial metabolic activity in chronic unpredictable mild stress model of depression: Reversal of behavioral and metabolic deficit with lanicemine. *Neurochem Int* 2020; 137: 104750. DOI: 10.1016/j.neuint.2020.104750.

7. Mishra PK, Kumar A, Behar KL, et al. Subanesthetic ketamine reverses neuronal and astroglial metabolic activity deficits in a social defeat model of depression. *J Neurochem* 2018; 146: 722-734. DOI: 10.1111/jnc.14544.

8. de Graaf RA, Mason GF, Patel AB, et al. Regional glucose metabolism and glutamatergic neurotransmission in rat brain in vivo. *Proc Natl Acad Sci U S A* 2004; 101: 12700-12705. DOI: 10.1073/pnas.0405065101.

9. Duarte JM and Gruetter R. Glutamatergic and GABAergic energy metabolism measured in the rat brain by (13) C NMR spectroscopy at 14.1 T. *J Neurochem* 2013; 126: 579-590. DOI: 10.1111/jnc.12333.

10. Gruetter R, Seaquist ER and Ugurbil K. A mathematical model of compartmentalized neurotransmitter metabolism in the human brain. *Am J Physiol Endocrinol Metab* 2001; 281: E100-112. DOI: 10.1152/ajpendo.2001.281.1.E100.

11. Patel AB, de Graaf RA, Mason GF, et al. The contribution of GABA to glutamate/glutamine cycling and energy metabolism in the rat cortex *in vivo*. *Proc Natl Acad Sci U S A* 2005; 102: 5588-5593. DOI: 10.1073/pnas.0501703102.

12. Shen J, Petersen KF, Behar KL, et al. Determination of the rate of the glutamate/glutamine cycle in the human brain by in vivo 13C NMR. *Proc Natl Acad Sci U S A* 1999; 96: 8235-8240. DOI: 10.1073/pnas.96.14.8235.

13. Pati S, Saba K, Salvi SS, et al. Chronic postnatal chemogenetic activation of forebrain excitatory neurons evokes persistent changes in mood behavior. *Elife* 2020; 9. DOI: 10.7554/eLife.56171.

14. Veeraiah P, Noronha JM, Maitra S, et al. Dysfunctional glutamatergic and gamma-aminobutyric acidergic activities in prefrontal cortex of mice in social defeat model of depression. *Biol Psychiatry* 2014; 76: 231-238. DOI: 10.1016/j.biopsych.2013.09.024.

15. Wang J, Jiang L, Jiang Y, et al. Regional metabolite levels and turnover in the awake rat brain under the influence of nicotine. *J Neurochem* 2010; 113: 1447-1458. DOI: 10.1111/j.1471-4159.2010.06684.x.

16. Sibson NR, Mason GF, Shen J, et al. In vivo (13)C NMR measurement of neurotransmitter glutamate cycling, anaplerosis and TCA cycle flux in rat brain during. *J Neurochem* 2001; 76: 975-989. DOI: 10.1046/j.1471-4159.2001.00074.x.

17. Sonnay S, Duarte JM, Just N, et al. Compartmentalised energy metabolism supporting glutamatergic neurotransmission in response to increased activity in the rat cerebral cortex: A 13C MRS study in vivo at 14.1 T. *J Cereb Blood Flow Metab* 2016; 36: 928-940. DOI: 10.1177/0271678X16629482.

18. Shank RP, Bennett GS, Freytag SO, et al. Pyruvate carboxylase: an astrocyte-specific enzyme implicated in the replenishment of amino acid neurotransmitter pools. *Brain Res* 1985; 329: 364-367. DOI: 10.1016/0006-8993(85)90552-9.

Table 1S Concentration (µmol/g) of brain metabolites in STZ-treated (3 mg/kg) mice

Brain Regions	Treatment Groups	Glu	GABA	Gln	Asp	NAA	m-Ino	Tau	Cho	p-Cho	GPC	Cre
Cerebral Cortex	Control	13.0±0.4	2.2±0.1	4.1±0.1	2.9±0.1	6.7±0.3	6.2±0.2	9.2±0.4	0.04±0.01	0.71±0.04	1.4±0.1	12.3±0.5
	ICV-STZ	12.5±0.4*	2.1±0.1	3.9±0.2	2.9±0.1	6.3±0.2*	6.3±0.4	8.2±0.3**	0.05±0.01	0.67±0.04	1.3±0.1	11.4±0.3*
Hippo-campus	Control	12.5±0.5	2.8±0.1	4.6±0.2	2.4±0.1	6.4±0.2	7.0±0.4	7.7±0.3	0.04±0.01	0.79±0.04	1.3±0.1	13.0±0.6
	ICV-STZ	12.0±0.6	2.6±0.2	4.4±0.2	2.4±0.1	5.9±0.2**	7.4±0.6	7.2±0.1*	0.04±0.01	0.77±0.03	1.2±0.0	12.3±0.4*

The concentrations of metabolites were measured in the cortical and hippocampal tissue extracts from unedited ^{1}H-[^{13}C]-NMR spectrum using [2-^{13}C]glycine as reference. Values are presented as mean±SD. *p<0.05 and **p<0.01 when ICV-STZ-treated mice were compared with controls. Abbreviations used are: Asp, aspartate; Cho, Choline; p-Cho, phosphocholine; GPC, glycerophosphocholine; Cre, creatine; GABA, γ-aminobutyric acid; Glu, glutamate; Gln, glutamine; m-Ino, myo-inositol; NAA, N-acetyl aspartate; Tau, Taurine

Table 2S Concentration of ^{13}C labeled amino acids from [2-^{13}C]acetate and $CMR_{Ace(Ox)}$ in ICV-STZ (5 mg/kg) treated mice

Brain Regions	**Treatment Groups**	**Amino Acids** (µmol/g)						**$CMR_{Ace(Ox)}$** (µmol/g/min)
		Glu_{C4}	**$GABA_{C2}$**	**Gln_{C4}**	**Asp_{C3}**	**Glu_{C3}**	**$GABA_{C4}$**	
Cerebral Cortex	Control	0.44±0.03	0.06±0.01	0.46±0.03	0.05±0.01	0.15±0.02	0.02±0.01	0.14±0.01
	ICV-STZ	0.44±0.03	0.06±0.01	0.46±0.03	0.05±0.01	0.15±0.02	0.02±0.01	0.14±0.01
Hippocampus	Control	0.46±0.02	0.07±0.01	0.52±0.03	0.07±0.01	0.14±0.02	0.03±0.01	0.15±0.01
	ICV-STZ	0.45±0.04	0.07±0.01	0.53±0.05	0.07±0.01	0.14±0.02	0.02±0.01	0.15±0.01

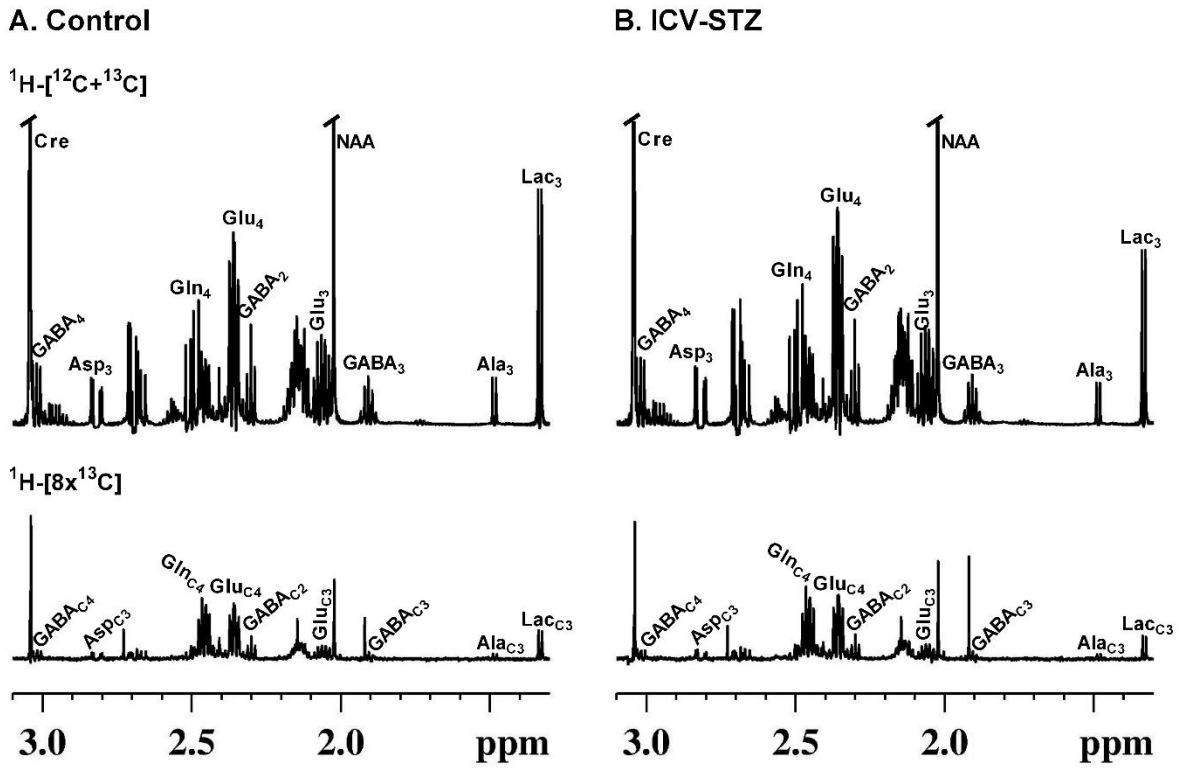
Mice were infused with [2-^{13}C]acetate for 10 min followed by microwave fixation. The concentrations of ^{13}C labeled amino acids were measured in brain tissue extracts from ^{13}C edited spectra using [2-^{13}C]glycine as internal reference. $CMR_{Ace(Ox)}$ was calculated from ^{13}C label trapped into different amino acids using Eqn. 3. Values are presented as mean±SD.

Table 3S Concentration (μmol/g) of ^{13}C labeled amino acids from [1,6-$^{13}C_2$]glucose in ICV-STZ-treated (3 mg/kg) mice

Brain Regions	Treatment Groups	Glu_{C4}	$GABA_{C2}$	Gln_{C4}	Asp_{C3}	Glu_{C3}	$GABA_{C4}$
Cerebral Cortex	Control	2.66±0.17	0.32±0.02	0.44±0.04	0.29±0.02	0.52±0.06	0.08±0.02
	ICV-STZ	2.49±0.09*	0.29±0.01**	0.39±0.04*	0.28±0.02	0.45±0.05*	0.07±0.02
Hippo-campus	Control	2.16±0.15	0.35±0.02	0.37±0.04	0.26±0.02	0.45±0.05	0.10±0.01
	ICV-STZ	1.97±0.09*	0.31±0.02**	0.32±0.04	0.24±0.01	0.41±0.04	0.08±0.01

Mice were infused with [1,6-$^{13}C_2$]glucose for 2 min, and brain metabolism was arrested at 7 min using focussed beam microwave irradiation. The concentrations of ^{13}C labeled amino acids were measured in tissue extracts in ^{13}C edited spectra using [2-^{13}C]glycine as reference. Values are presented as mean±SD. **p<0.01 when ICV-STZ mice compared with control.

Figure 1S



Representative ^{1}H-[^{13}C]-NMR spectra of cortical extracts depicting total and ^{13}C labeled metabolites from [2-^{13}C]acetate. Mice were infused with sodium [2-^{13}C]acetate, and brain metabolism was arrested at 10 min using focussed beam microwave irradiation. Metabolites were extracted from brain tissue, and ^{1}H-[^{13}C]-NMR spectra were recorded at 600 MHz NMR spectrometer. Abbreviation used are: Ala_{C3}, alanine-C3; Asp_{C3}, aspartate-C3; Cre, creatine; $GABA_{C2}$, γ-aminobutyric acid-C2; $GABA_{C3}$, γ-aminobutyric acid-C3; $GABA_{C4}$, γ-aminobutyric acid-C4; Glu_{C3}, glutamate-C3; Glu_{C4}, glutamate-C4; Gln_{C4}, glutamine-C4; Lac_{C3}, Lactate-C3; NAA, N-acetyl aspartate.