In vivo ^{1}H MRS metabolic profiles in *Gad1* haploinsufficient mouse brain

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Introduction

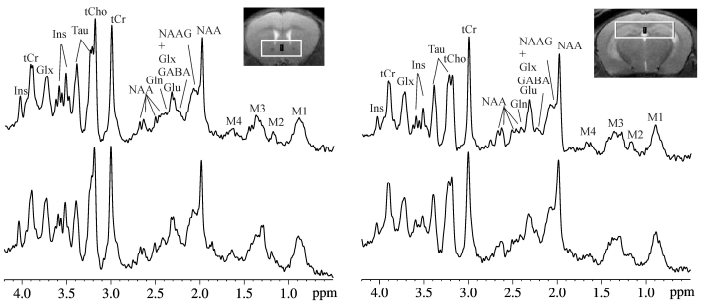

Fig. 1. *In vivo* high resolution ^{1}H MR spectra taken from a B6-*Gad1*$^{tm11.Bgc}$ (top raw) and a B6 mouse (bottom raw) in hippocampus (right) and nucleus accumbens (left) regions, respectively.

GABAergic neurons produce *gamma*-aminobutyric acid (GABA), an inhibitory neurotransmitter in the mammalian central nervous system. GABAergic neurons are differentially distributed throughout the brain and play fundamental role in human disease and function. Abnormal regulation of GABA levels is found in epilepsy, schizophrenia and bipolar disorders. Therapies aim to increase GABA levels in affected brain regions. However, the efficacy of potential therapeutic agents on their role in effecting GABA in specific regions of the brain *in vivo* has been difficult. In this study, we compared *in vivo* neurometabolism levels in wildtype B6 strain mice with their siblings B6-*Gad1*$^{tm11.Bgc}$ mice that lack a single copy of the gene (Gad1) which is the main converting enzyme, glutamatic acid decarboxlase 67 (Gad67), necessary for GABA generation.. Loss of both alleles is lethal, and therefore only haploinsufficiency was evaluated. Successful quantification of GABA, along with other neurotransmitters, will provide a novel preclinical tool to screen anti-epileptic and anti-psychotic drugs that are potential candidates for human patients. We demonstrated the feasibility of using *in vivo* high resolution localized ^{1}H MRS in mouse brain at 7 Tesla.

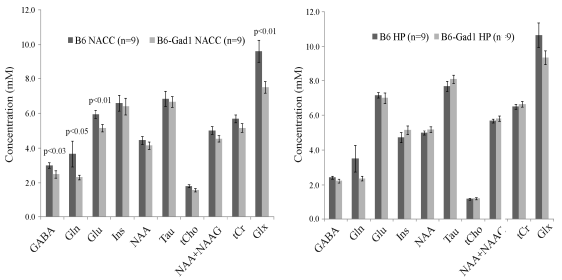

Fig. 2. Comparison of the concentrations of the metabolites between B6-*Gad1*$^{tm11.Bgc}$ and I 6 mice. Data are expressed as mean ± standard error.

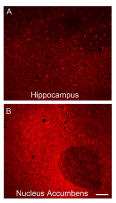

Fig. 3. Immunohistochemistry for GABA. A. Section from dorsalhippocampus shows individual GABA-expressing interneurons. Only a few of the total cells are GABAAergic. B. Section from nucleus accumbens with the majority of cells expressing GABA. Bar = mm in (A) and 500 mm in (B).

Methods

Littermate age-matched male mice (3 - 6 months) were obtained from heterozygous breeding and genotyped using PCR. *In vivo* high resolution ^{1}H MRS experiments were performed on a Bruker BioSpec 70/30USR Avance III 7T scanner. A Bruker four-element ^{1}H surface coil array was used as the receiver and a Bruker 72 mm linear-volume coil as the transmitter. Each mouse was anesthetized in an animal chamber using a gas mixture of O_2 (1 L/min) and isoflurane (3 %) then later maintained at 1-1.5% isoflurane during scanning. A MR compatible small-animal monitoring and gating system was used to monitor the animal respiration rate and body temperature. The animal body temperature was maintained at 36-37^{o}C using a warm water circulation.

Both Proton-density- and T_2- weighted images were obtained using a 2D rapid acquisition with relaxation enhancement (RARE) sequence in the axial plane ($TR/TE_{eff1}/TE_{eff2}$ = 5500/19/57 msec, RARE factor = 4, field of view = 20 x 20 mm^2, slice thickness = 1 mm, in-plane resolution = 114 x 114 μm^2, number of averages (na) = 1) for anatomic references. A customized short-TE PRESS pulse sequence (TR/TE = 2500/10 ms, na = 356)[1] was used for MRS data acquisition with voxel centered on the hippocampus (HP, 1.5 x 6.0 x 0.9 mm^3, n=9) and nucleus accumbens (NACC, 1.5 x 4.0 x 2 mm^3, n=9). LCModel package was used for quantification of the MRS data. The reliability of the major metabolites was estimated in the Cramér-Rao lower bounds (CRLB) from the LCModel analysis. All experimental procedures were approved by the University of Maryland School of Medicine Institutional Animal Care and Use Committee. *In vivo* MRS results from B6 and B6-*Gad1*$^{tm11.Bgc}$ mice were compared using t-tests. Post-hoc immunohistochemistry was performed for GABA using standard laboratory protocols[2].

Results

Axial anatomic images, with the spectroscopic voxel locations in the NACC and HP regions denoted, along with the corresponding spectra from a B6 and a B6-*Gad1*$^{tm1.1Bgc}$ mouse are shown in Fig 1. The *in vivo* ^{1}H spectra demonstrate excellent spectral resolution and sensitivity in both regions. A number of metabolites including GABA, glutamate (Glu), glutamine (Gln), glutamate + glutamine (Glx), myo-inositol (Ins), *N*-acetylaspartate (NAA), taurine (Tau), total Choline (tCho), NAA+ *N*-acetylaspartateglutamate (NAAG), and total Creatine (tCr) were reliability detected. In general, the CRLB values were not more than 15%. Among the metabolites, GABA ($p < 0.03$), Gln ($p < 0.05$), Glu ($P < 0.01$) and Gln+Glu (Glx, $P < 0.01$) concentrations in B6-*Gad1*$^{tm1.1Bgc}$ mice are significantly lower compared to B6 mice in NACC (Fig 2). Interestingly, in HP, GABA ($p < 0.08$), Gln ($P < 0.08$) and Glx ($p < 0.06$) concentrations only marginal lower among B6-*Gad1*$^{tm1.1Bgc}$ mice compared to B6 with Glu concentration showing no differences between the two groups(Fig 2). No statistically significant differences were found in other metabolites including Ins, tCr, tCho, NAA, NAA + NAAG, and Tau concentrations between B6 and B6-Gad1$^{tm1.1Bgc}$ mice in either NACC or HP areas (Fig 2). Cellular localization of GABA in B6 mice by immunohistochemistry demonstrates isolated interneurons in the dorsal hippocampus (Fig. 3A) and most cells in the nucleus accumbens (Fig. 3B). The relative expression of GABA in the nucleus accumbens cells appears to be greater than in the hippocampus confirming the in vivo findings.

Discussion

We demonstrated low GABA, Gln, and Glu levels in NACC in B6-*Gad1*$^{tm1.1Bgc}$ mice using *in vivo* high resolution ^{1}H MRS at 7 T. Because 95% of the neurons in NACC are GABAergic neurons and these neurons are missing a copy of the Gad1 gene, in B6-*Gad1*$^{tm1.1Bgc}$ mice. The low levels of Glu and Gln detected in B6-*Gad1*$^{tm1.1Bgc}$ mice suggest that the GABA-gln cycle at GABAergic synapses in NACC may be suppressed. . Hippocampus is involved in memory formation and storage, and is a common epileptic locus. The excitatory neurons in the hippocampus are under control of local GABAergic interneurons[3,4]. Surprisingly, we did not detected significant low GABA or Gln or Glu levels in this region. GABA biosynthesis is catalyzed by glutamate decarboxylase, which exists in two isoforms, Gad65 and Gad67. While many GABAergic interneurons contain both isoforms, isoform expression levels are heterogeneous, B6-*Gad1*$^{tm1.1Bgc}$ mouse only be suppressed Gad67 gene, not Gad65, and the distributions of Gad67 and Gad65 may be different in NACC and HP. The heterogeneity of isoform expression in brain regions may be used to explain the differences of GABA, Gln, and Glu between NACC and HP.

References

1. Xu et al. *Magn Reson Med* 2012 [Epub ahead of print]. **2.** Bae et al. *Exp Neurol* 221:129-135, 2010. **3.** Klausberger et al. Science 321, 53, 2008. **4.** Traub et al. Annu. Rev. Neurosci. 27, 247-278, 2004.