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Supplementary Data

Impaired Glutamatergic and GABAergic Function at Early Age in AβPPswe-PS1dE9 Mice: Implications for Alzheimer's Disease

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Supplementary Table 1
Concentration (μmol/g) of neurometabolites in wild-type and AβPP-PS1 mice

Brain region	Animal	Glu	GABA	Gln	Asp	NAA	Ino	Tau	Cho
Cortex	Wild-type	13.4 ± 1.2	2.0 ± 0.3	5.6 ± 0.6	2.3 ± 0.4	7.2 ± 0.7	7.2 ± 0.8	12.1 ± 0.9	1.7 ± 0.2
	AβPP-PS1	13.4 ± 1.0	2.2 ± 0.4	5.7 ± 0.4	2.2 ± 0.5	6.8 ± 0.7	7.7 ± 1.0	12.5 ± 0.8	1.8 ± 0.2
Hippocampus	Wild-type	11.8 ± 1.0	3.1 ± 0.6	5.0 ± 0.8	1.7 ± 0.5	6.8 ± 0.8	7.9 ± 1.2	10.7 ± 1.0	1.8 ± 0.2
	AβPP-PS1	10.9 ± 0.7	2.9 ± 0.4	4.8 ± 0.7	2.1 ± 1.2	6.2 ± 0.5	7.8 ± 2.0	11.0 ± 1.7	2.0 ± 0.3
Striatum	Wild-type	11.5 ± 1.6	3.9 ± 0.6	5.3 ± 0.9	1.8 ± 0.6	6.4 ± 1.2	7.4 ± 1.2	10.8 ± 1.6	2.1 ± 0.3
	AβPP-PS1	11.0 ± 1.2	3.7 ± 0.8	5.1 ± 1.0	1.7 ± 0.5	5.9 ± 0.7	7.5 ± 1.3	10.2 ± 2.1	2.1 ± 0.3

Concentration of metabolites were measured relative to [2-^{13}C]glycine added during extraction of metabolites. Abbreviations: Asp, Aspartate; Cho, Choline, GABA, γ-aminobutyric acid; Gln, Glutamine; Glu, Glutamate; Ino, myo-Inositol; NAA, N-acetylaspartate; Tau, Taurine. Two factor ANOVA analysis was carried out to determine the differences in the concentration of neurometabolites in given brain region. The level of neurometabolites in AβPP-PS1 mice was not significantly different (Cortex: $F[1,7] = 0.58$, $p = 0.443$; Hippocampus: $F[1,7] = 0.81$, $p = 0.367$; Striatum: $F[1,7] = 0.2.15$; $p = 0.144$) than wild-type. Values represent mean ± SD.

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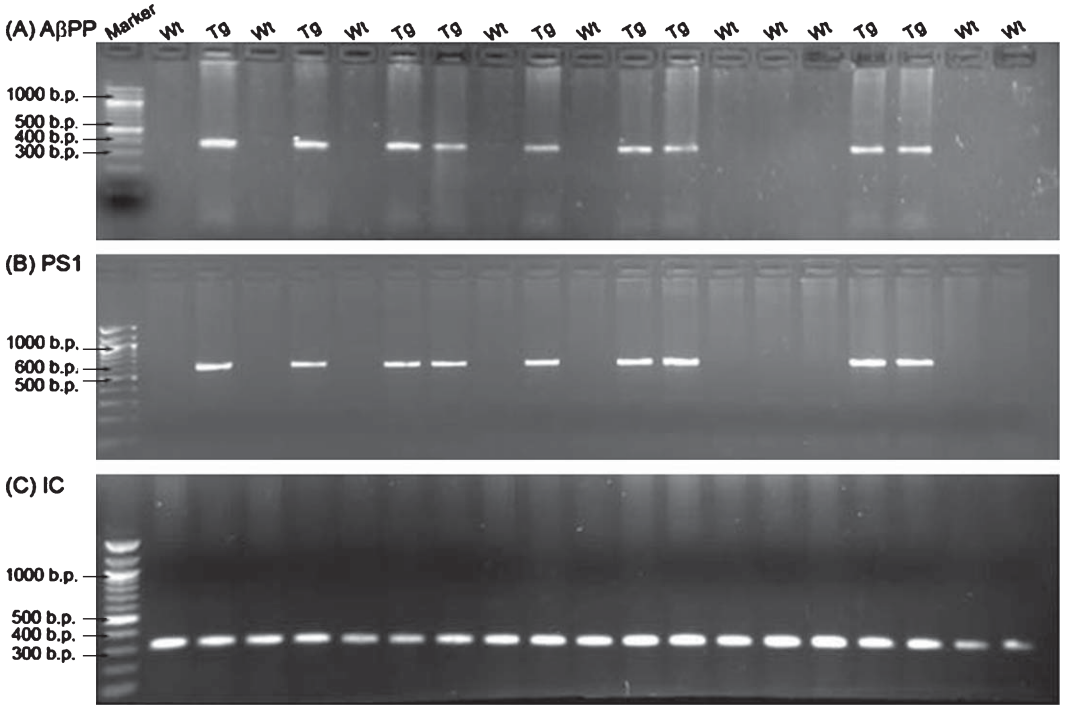
Supplementary Table 2
Concentration (mmol/L) of urethane in plasma of wild-type and AβPP-PS1 mice

Mice	Urethane	
	Time (10 min)	Time (90 min)
Wild-type	24.8 ± 3.2	25.8 ± 5.0
AβPP-PS1	26.5 ± 4.5	24.7 ± 4.2

The concentration of urethane was determined from the ^{1}H NMR spectrum of the filtered plasma (10 kD cut off) and calculated by dividing the intensity of triplet resonance of urethane at 1.24 p.p.m. by the formate intensity. Values represent mean ± SD.

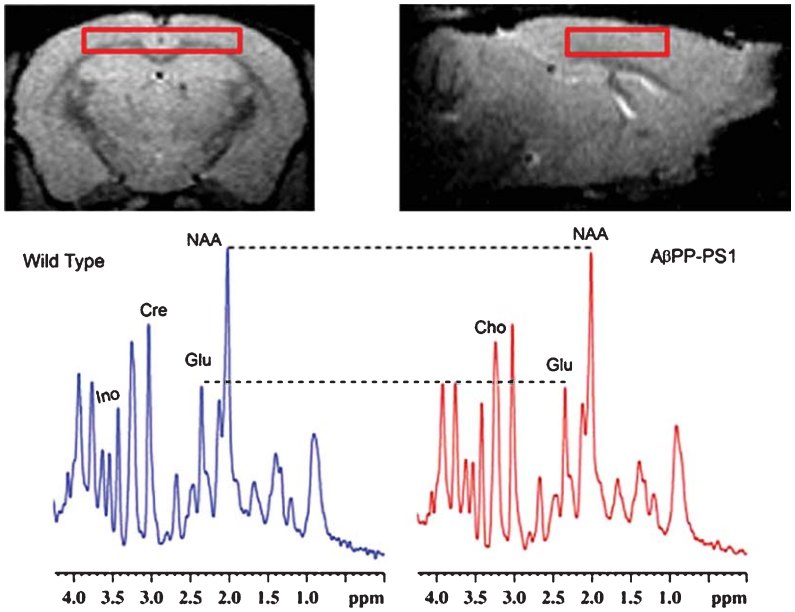


Supplementary Figure 1. Agarose gel showing amplification of genes (A) AβPP, (B) PS1, and (C) Internal control (IC). DNA was isolated from mice tail using Phenol-Chloroform isolation protocol. Isolated DNA was amplified by PCR reaction using specific primers for AβPP, PS1, and IC. The amplified PCR product is separated on 1.5% agarose gel. DNA molecular size markers (b.p.) are indicated on the left. Mice showing amplification for both genes, AβPP (∼377 b.p.) and PS1 (∼608 b.p.), were designated as AβPP-PS1 mice whereas the mice that do not show amplification for AβPP and PS1 genes were used for control. The primers used for amplification of the genes are as follows.

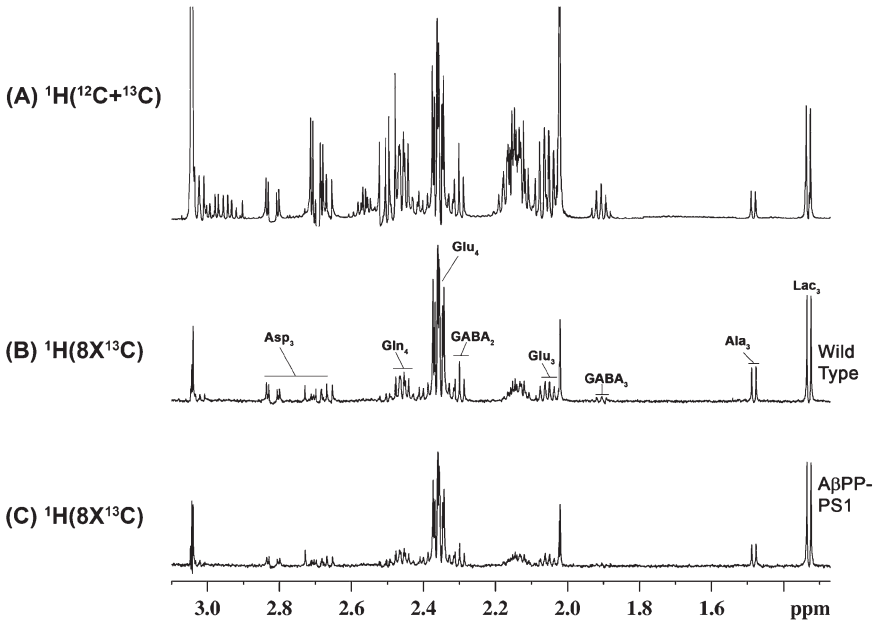
AβPP
Forward Primer: 5′ AGG ACT GAC CAC TCG ACC AG 3′
Reverse Primer: 5′ CGG GGG TCT AGT TCT GCA T 3′

PS1
Forward Primer: 5′ AAT AGA GAA CGG CAG GAG CA 3′
Reverse Primer: 5′ GCC ATG AGG GCA CTA ATC AT 3′

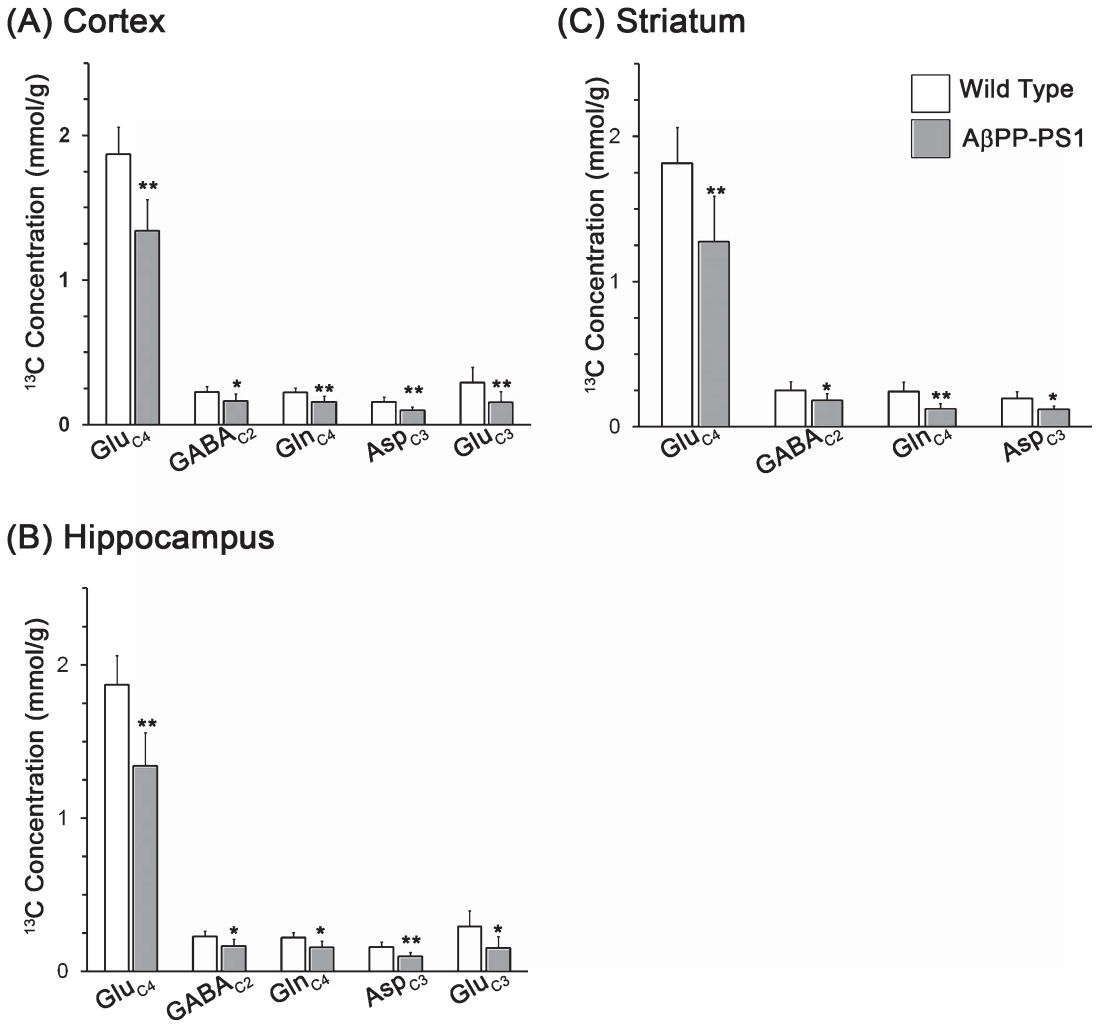
IC
Forward Primer: 5′ CTA GGC CAC AGA ATT GAA AGA TCT 3′
Reverse Primer: 5′ GTA GGT GGA AAT TCT AGC ATC ATC C3′



Supplementary Figure 2. *In vivo* ^{1}H NMR spectra recorded from cerebral cortex of wild-type and AβPP-PS1 mice. Mice were anesthetized with isoflurane (1.5-2.5%) mixed in air (70%):O_2(30%). *In vivo* NMR experiments were performed using a 14.1 T vertical wide bore magnet interfaced with Avance II console (Bruker Biospin, Etlingen Germany). ^{1}H NMR spectra were acquired using a 15 mm diameter transceiver surface coil operating at 600.13 MHz. T1 weighted axial images of brain were acquired using FLASH protocol. Field homogeneity in the NMR voxel was adjusted using FASTMAP method. Localized *in vivo* ^{1}H NMR spectroscopy was carried out using STEAM in conjunction with outer volume suppression, TE/TR = 3.5/3000 ms, from a voxel positioned in cortex ($4\times1.2\times3$ mm^3) and striatum ($2\times2\times2$ mm^3). Water resonance was suppressed using VAPOR method. Rectangular box depicts the size and position of NMR voxel. Abbreviations: Cho, choline; Cre, creatine; Glu, glutamate; Ino, inositol; NAA, N-acetylaspartate.



Supplementary Figure 3. Typical ^{1}H-[^{13}C]-NMR spectra obtained from cerebral cortical tissue extract prepared after 10 min of [1,6-$^{13}C_2$]glucose infusion. (A) ^{13}C Decoupled ^{1}H NMR spectrum. ^{1}H-[^{13}C]-NMR spectra depicting labeling from [1,6-$^{13}C_2$]glucose in (B) wild-type and (C) AβPP-PS1 mice. Abbreviations: Ala_3, alanine-C3; Asp_3, aspartate-C3; $GABA_2$, γ-aminobutyric acid-C2; $GABA_3$, γ-aminobutyric acid-C3; Gln_4, glutamine-C4; Glu_3, glutamate-C3; Glu_4, glutamate-C4; Lac_3, lactate-C3.



Supplementary Figure 4. Concentration of ^{13}C labeled amino acids during 10 min [1,6-$^{13}C_2$]glucose infusion. ^{13}C Enrichment of amino acids was measured in tissue extracts using ^{1}H-[^{13}C]-NMR spectroscopy. ^{13}C Concentration was obtained by multiplying the total concentration with corresponding normalized ^{13}C enrichment. Abbreviations: Asp$_{C3}$, aspartate-C3; GABA$_{C2}$, γ-aminobutyric acid-C2; Gln$_{C4}$, glutamine-C4; Glu$_{C3}$, glutamate-C3; Glu$_{C4}$, glutamate-C4. Values represent mean ± SD. *$p < 0.05$, **$p < 0.01$.