Neurochemical Research (2019) 44:133–146
https://doi.org/10.1007/s11064-018-2658-9

ORIGINAL PAPER

# Effects of Ethanol Exposure on the Neurochemical Profile of a Transgenic Mouse Model with Enhanced Glutamate Release Using In Vivo $^{1}$H MRS

**Wen-Tung Wang[1] · Phil Lee[1,2] · Dongwei Hui[4] · Elias K. Michaelis[4] · In-Young Choi[1,2,3]**

Received: 30 June 2018 / Revised: 5 October 2018 / Accepted: 8 October 2018 / Published online: 17 October 2018


## Abstract

Ethanol (EtOH) intake leads to modulation of glutamatergic transmission, which may contribute to ethanol intoxication, tolerance and dependence. To study metabolic responses to the hyper glutamatergic status at synapses during ethanol exposure, we used Glud1 transgenic (tg) mice that over-express the enzyme glutamate dehydrogenase in brain neurons and release excess glutamate (Glu) in synapses. We measured neurochemical changes in the hippocampus and striatum of tg and wild-type (wt) mice using proton magnetic resonance spectroscopy before and after the animals were fed with diets within which EtOH constituting up to 6.4% of total calories for 24 weeks. In the hippocampus, the EtOH diet led to significant increases in concentrations of EtOH, glutamine (Gln), Glu, phosphocholine (PCho), taurine, and Gln + Glu, when compared with their baseline concentrations. In the striatum, the EtOH diet led to significant increases in concentrations of GABA, Gln, Gln + Glu, and PCho. In general, neurochemical changes were more pronounced in the striatum than the hippocampus in both tg and wt mice. Overall neurochemical changes due to EtOH exposure were very similar in tg and wt mice. This study describes time courses of neurochemical profiles before and during chronic EtOH exposure, which can serve as a reference for future studies investigating ethanol-induced neurochemical changes.

**Keywords** Glud1 transgenic mice · Enhanced glutamate release · Ethanol exposure · Magnetic resonance spectroscopy

## Introduction

A large body of experimental evidence demonstrates that acute and chronic alcohol intake affects the function of the two major neurotransmitters in the brain, γ-aminobutyric acid (GABA), the inhibitory, and glutamate (Glu), the excitatory transmitter [1–4]. Regarding ethanol (EtOH) effects on Glu neurotransmission, acute exposure of neurons to EtOH leads to inhibition of Glu/*N*-methyl-D-aspartate (NMDA) receptors [5–8], In addition, inhibition of NMDA receptors with chemical antagonists produces the same discriminant properties as EtOH administration in experimental animals [9]. It has been reported that acute EtOH exposure brings about increases in the re-uptake of Glu into nerve endings [10–13], decreases in Glu release into the extracellular space in the hippocampus measured by micro-dialysis methods [14], and decreases in Glu levels in the cortex measured by $^{1}$H–[$^{13}$C] magnetic resonance spectroscopy (MRS) [15]. On the other hand, MRS measurements of Glu and glutamine (Gln) levels following repeated episodes of binge drinking, or micro-dialysis measurements following chronic EtOH administration revealed increases rather than decreases in Glu levels in the cortico-limbic and hippocampus regions, respectively [16, 17]. Studies of chronic exposure to EtOH represent the neuro-adaptive changes that counteract the acute effects of neuronal exposure to EtOH, as, for example, the decreases in Glu transport into glia under chronic EtOH intake [18], resulting in elevations in brain Glu levels. It

Special issue: In honor of Elias K. Michaelis.

✉ In-Young Choi
ichoi@kumc.edu

1 Hoglund Brain Imaging Center, University of Kansas Medical Center, Kansas City, KS, USA

2 Department of Molecular & Integrative Physiology, University of Kansas Medical Center, Kansas City, KS, USA

3 Department of Neurology, University of Kansas Medical Center, Kansas City, KS, USA

4 Department of Pharmacology and Toxicology, University of Kansas, Lawrence, KS, USA

should be noted that both acute and chronic effects of EtOH on Glu levels may be brain region-dependent [19].

We speculate that individuals or experimental animals that are deficient in enzymes involved in Glu metabolism might be differentially sensitive to the neurobiological and behavioral effects of EtOH. For example, *Drosophila* mutants for the genes *Glu pyruvate transaminase* [20] and *Glu oxaloacetate transaminase* [21], as well as hypomorphs for the gene of the enzyme *Glu dehydrogenase* (*Glud*) [20] are more sensitive to ethanol-induced inebriation than isogenic controls [20]. We also hypothesize that an adaptive response to the neuro-suppressant effects of chronic EtOH administration might involve increases in Glu transmitter stores and synaptic release in order to overcome the acute effects of EtOH on Glu neurotransmission.

The present study was designed to assess the possibility of adaptive metabolic responses in experimental animals exposed over a prolonged period of time to dietary intake of EtOH. We measured, repeatedly, in two brain regions of the same animals the changes in the concentration of Glu, Glu-related metabolites such as glutamine (Gln) and GABA, and of several other neurochemicals during chronic EtOH administration. We employed non-invasive, in vivo high resolution $^{1}$H MRS at 9.4 T to determine the effects of chronic EtOH administration on the levels of twenty neurochemicals, plus EtOH, in the striatum and hippocampus of wt mice and of mice that overexpress the enzyme Glu dehydrogenase, the *Glud1* transgenic (tg) mice. These tg mice overexpress *Glud1* only in neurons of the central nervous system, release increased levels of Glu at synapses, and are a model of a moderate hyper-glutamatergic state that is not associated with extensive brain damage or substantially reduced lifespan [22, 23]. In neurons, Glu dehydrogenase operates primarily in the direction of the conversion of Glu to 2-oxoglutarate [24], which is then released from mitochondria to the cytosol through the activity of the mitochondrial ketodicarboxylic acid carrier [25, 26]. Cytosolic 2-oxoglutarate is converted to Glu primarily by aspartate aminotransferase [25, 26] and the Glu formed contributes to the neurotransmitter releasable pool of Glu at nerve terminals [25, 27]. Glutamate dehydrogenase may be directly involved in the synthesis of Glu from 2-oxoglutarate by adding $NH_3$ to 2-oxoglutarate, although this reaction is thermodynamically not favored in brain [24].

In this study, we investigated whether chronic EtOH administration to adult wt mice led to adaptive or compensatory changes in Glu, Gln, and GABA, as well as in metabolites associated with Glu interconversions in brains, such as aspartate (Asp) and alanine (Ala), and whether such changes in concentrations of metabolites were the same or different in the hyper-glutamatergic *Glud1* tg as in wt mice. Also, as mitochondrial dysfunction, reactive oxygen species (ROS) formation, cellular oxidative stress, and glutathione (GSH) depletion due to EtOH exposure have been observed in brain [28], changes in the concentrations of the cellular reducing agent glutathione (GSH) and other neurochemical indexes of cell injury, such as *N*-acetyl aspartate (NAA), were also measured during the chronic exposure of the mice to EtOH.

## Methods

### Animals and EtOH Exposure

All animal care and other procedures described in this study were performed according to the guidelines for the care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Kansas Medical Center. The *Glud1* tg mice were generated by inserting the transgene for *Glud1* under the control of the neuron-specific enolase promoter that allowed expression of *Glud1* in mitochondria of only neurons of the CNS [22]. Mice bearing the *Glud1* transgene were genotyped and back-crossed to C57BL/6 for five generations.

The EtOH diet was given to both *Glud1* tg mice and their wt littermates starting at the age of 41 weeks using the commonly used Lieber-DeCarli liquid diet [29], in which the mice were fed a nutritionally balanced liquid diet containing EtOH. Water was provided *ad libitum*. During the first week on the diet, the EtOH content increased from 2.1% of calories ingested for days 1–3 to 4.3% for days 4–7. From day 8 and thereafter, EtOH constituted 6.4% of total calories. MRS data were acquired from both groups of mice 2 weeks before (E0), and 2, 12, and 24 weeks (E2, E12, and E24, respectively) after initiation of the EtOH-containing diet.

### MRS Experiments

The MRS data were measured on wt (n = 15 at E0, 10 males) and *Glud1* tg (n = 28 at E0, 23 males) mice using a Agilent (Varian) 9.4 T INOVA MR system (Agilent Technologies, CA) equipped with 12-cm gradient coils (0.4 T/m, 250 µs) and shim coils (maximum second-order shim strength of 0.4 T/m$^2$, Magnex Sceintific, Abingdon, UK). Among 28 *Glud1* tg mice, 16 mice (10 males) were fed with the EtOH diet and included in the longitudinal study. The scans were scheduled based on the availability of the scanner on the day, instead of the timing relative to the EtOH ingestion. During each experiment, each animal was anesthetized with a gas mixture of air and oxygen (air:oxygen = 2:1) with 1–2% isoflurane and its core temperature was maintained at 37 °C using a circulating water blanket with an automatic temperature controller. After the initial induction of anesthesia using 2% isoflurane, the anesthesia level was adjusted to maintain the animal's physiological conditions. Animals' physiological parameters were respiration rate of 91 ± 17

breaths per minute, heart rate of 516 ± 74 beats per minute, and core temperature of 37 ± 2 °C. A quadrature RF surface coil consisting of two geometrically decoupled loops was placed on top of the animal head to transmit and receive at 400 MHz frequency, and FASTMAP [30] was employed to adjust field homogeneity. The SPin-ECho full Intensity Acquired Localized (SPECIAL) spectroscopy (TE = 3 ms, TR = 4 s) [31] was used to acquire spectral data from a voxel localized either in the hippocampus or in the striatum. The two brain regions were selected because it has been shown that EtOH affects the central nervous system by altering neurotransmitter system in striatum [32, 33], and EtOH is linked to the impairment of hippocampal functions, including memory and learning, which are related to the development and maintenance of alcohol addiction [34, 35]. The voxel size was $1.8 \times 1.2 \times 1.8$ mm$^3$ to $2 \times 1.2 \times 2.2$ mm$^3$ for hippocampus, $1.6 \times 1.7 \times 1.8$ mm$^3$ to $1.7 \times 1.8 \times 2$ mm$^3$ for striatum. The location of the voxels was determined using $T_2$-weighted MR images acquired using a fast spin-echo multi-slice sequence (echo train length = 16, echo spacing = 11 ms, TE/TR = 11/4000 ms, matrix = $256 \times 256$, FOV = $25.6 \times 25.6$ mm, slice thickness = 0.5 mm, and NT = 2). The spectral data were acquired as a series of blocks, each block was averaged from 16 free induction decays (FIDs). Each block was corrected for frequency drift based on the total creatine signal (Cr + PCr) at 3.03 ppm. The blocks were averaged and corrected for residual eddy current effects using the unsuppressed water signal, which was acquired from the same voxel.

Neurochemicals were quantified using LCModel (linear combination of model spectra of metabolite solutions in vitro, version 5.1-7W) [36] and unsuppressed water signals as an internal concentration reference. Neurochemicals that were quantified from each spectrum included Ala, Asp, ascorbate (Asc), beta-hydroxybutyrate (bHB), creatine (Cr), GABA, glucose (Glc), Glu, Gln, GSH, glycerophosphocholine (GPC), phosphocholine (PCho), myo-inositol (mI), lactate (Lac), NAA, *N*-acetyl aspartylglutamate (NAAG), phosphocreatine (PCr), phosphorylethanolamine (PE), serine (Ser), taurine, Glu + Gln, and total creatine (Cr + PCr). The ratio of PCr/Cr was also determined and its error bounds were calculated using formulas of error propagation via standard deviations for each measured variable [37]. Measured macromolecules and simulated EtOH basis sets were also included in the LCModel to estimate their concentrations.

### Statistical Analysis

Mean neurochemical concentrations were calculated using a weighted averaging approach that utilizes spectral fitting error estimates, Cramer-Rao lower bounds (CRLB) in LCModel (https://s-provencher.com/pages/lcmodel.shtml), where weighted average of the concentration $\bar{C}$ was computed by using the following formula:

$$\bar{C} = \sum w_j C_j \sum w_j, \quad (1)$$

where $w_j = 1/\sigma_j^2$ and $\sigma_j = (SD)_j \times C_j/100$. $C_j$ is the concentration of the j$^{th}$ measurement of the neurochemical, *SD* is the estimated standard deviations (i.e., CRLB). All results were presented as mean ± standard deviation of the mean. Statistical analysis was performed using multivariate generalized linear mixed model in R software [38]. Kenward-Rodger approximation was used to estimate degree of freedom, which was used to calculate p values. Correction of multiple comparisons was performed using Dunnett's procedure for comparisons between neurochemical concentrations at each time point and those at the baseline (E0). The limit of significance level was set at $p < 0.05$.

## Results

In this study, shimming using FASTMAP resulted in highly resolved spectra with the water linewidth in the range of 11–16 Hz. Hippocampal and striatal spectra acquired from wt mice before and after the EtOH diet was initiated are shown in Fig. 1 a–f, respectively, while those acquired from *Glud1* tg mice are shown in Fig. 2 a–f, respectively. The difference spectra in C and F of both figures show that concentrations of ethanol were increased at E2, i.e., 2 weeks after the EtOH diet was initiated. Signal-to-noise ratio (SNR) of spectra in each region was similar between the two groups of animals at each of the four time points, e.g., 29.9 ± 5.7 and 28.8 ± 4.2 (mean ± standard deviation) for the hippocampus at E0 in *Glud1* tg and wt mice, respectively.

The concentrations of hippocampal neurochemicals measured in wt and *Glud1* tg mice are shown in the left and right columns, respectively, in Fig. 3; those of striatal neurochemicals in Fig. 4. Compared with the baseline measurements at E0 (wt, n = 15; tg, n = 28), the concentrations of a number of neurochemicals showed significant changes from baseline at E2 (wt and tg, n = 14 per group), E12 (wt and tg, n = 8 per group), and E24 (wt and tg, n = 7 per group). What is apparent from the results shown in Figs. 3 and 4 was the similarity in the patterns of concentration changes for several neurochemicals in the wt and *Glud1* tg mice. This was corroborated by the results of the statistical analysis, indicating no significant difference was detected between the two groups of animals. Statistical analyses indicated the following changes from baseline in the concentrations of neurochemicals during the period of exposure to EtOH. In the hippocampus of wt mice, the EtOH diet led to statistically significant increases in the concentrations of Gln (at

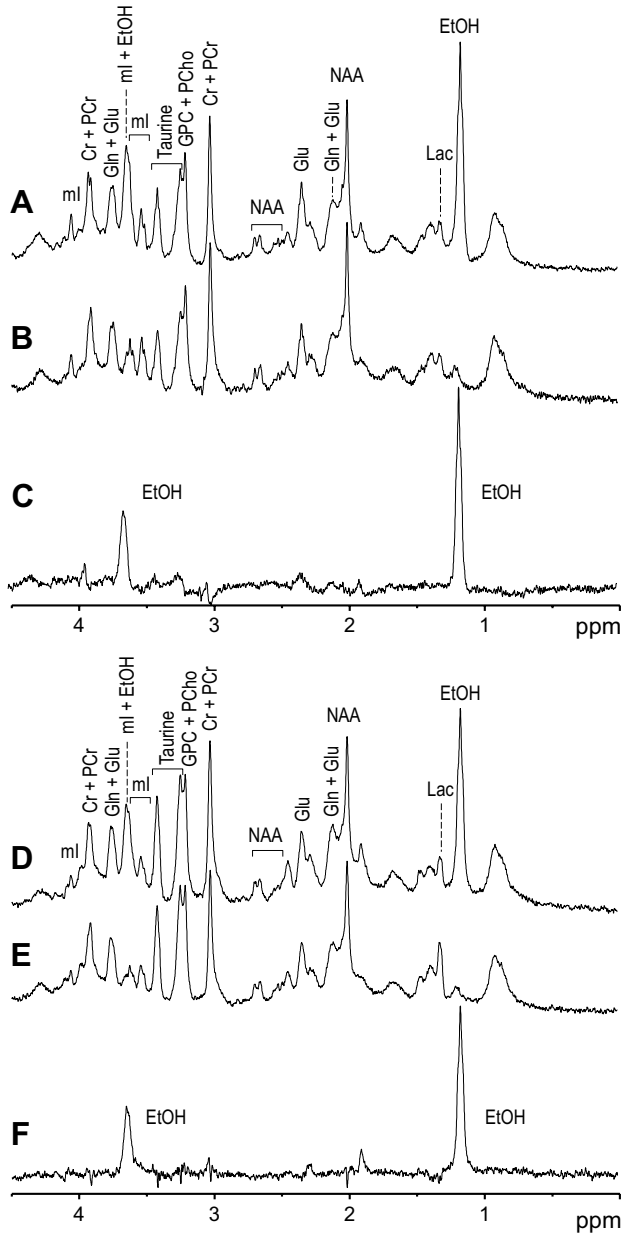


**Fig. 1** In vivo $^{1}$H MR spectra at 9.4 T in the hippocampus (A, B) and the striatum (D, E) of wt mice before (B, E) and after (A, D) introduction of the EtOH diet. Spectra were acquired from volumes of 3.9–6.1 μL localized in the hippocampus and striatum using SPECIAL with TE = 3 ms, TR = 4 s, and 320–480 and 480–640 transients, respectively. Difference between spectra before and after initiation of the EtOH diet are shown in c and f, respectively. The spectra were scaled based on the weighted average of NAA concentrations on E0 and E2 to visually show differences of the corresponding spectra. The difference spectra are not quantitatively accurate because of the linewidth differences between the spectra, and residual spectral distortion due to phase and frequency differences

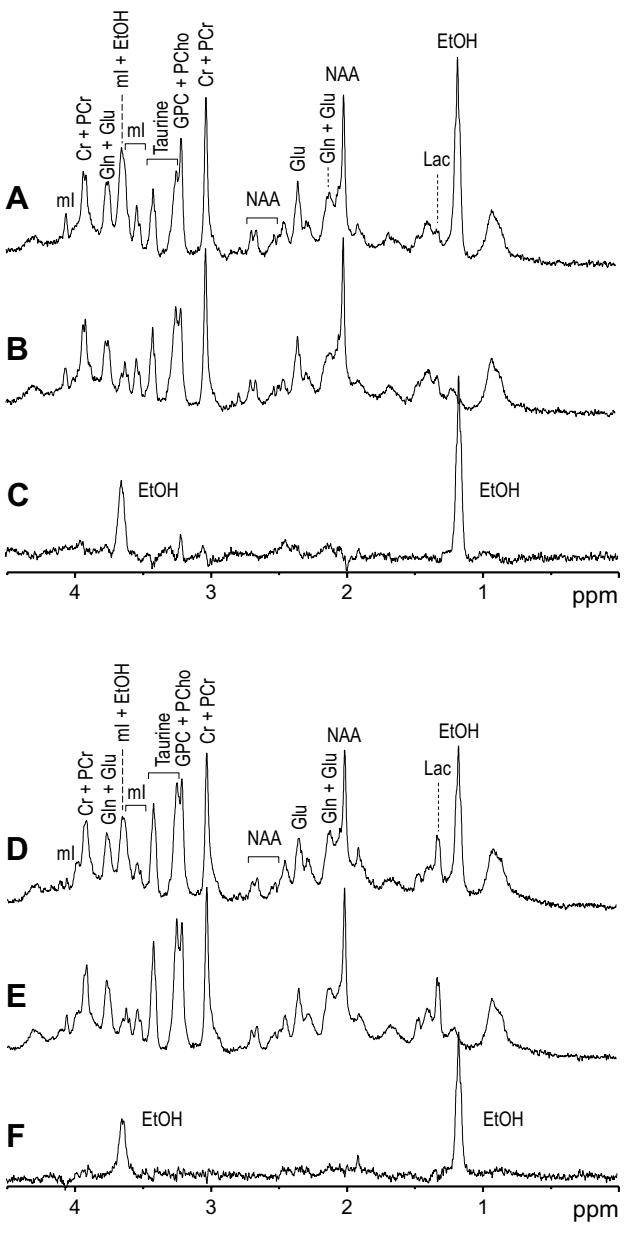


**Fig. 2** In vivo $^{1}$H MR spectra at 9.4 T in the hippocampus (A, B) and the striatum (D, E) of *Glud1* tg mice before (B, E) and after (A, D) introduction of the EtOH diet. Other details of the figure are the same as Fig. 1

E2–E24), Glu (at E12 and E24), Gln + Glu (at E2–E24), PCho (at E2 and E12), taurine (at E12 and E24), and PCr/Cr (at E24). On the other hand, EtOH intake resulted in significant decreases in the concentrations of Cr (at E12 and E24), Glc (at E24), GPC (at E2), Lac (at E2), and Ser (at E12 and E24).

In the hippocampus of tg mice, the EtOH diet led to significant increases in the concentrations of Ala (at E24), Asc (at E24), bHB (at E12), Gln (at E2–E24), Glu (at E12), Gln + Glu (at E2–E24), mI (at E24), PCho (at E2 and E12), and PCr/Cr (at E12 and E24), and significant decreases in the concentrations of Cr (at E2–E24), Cr + PCr (at E24), GPC (at E2 and E12), and GSH (at E12 and E24).

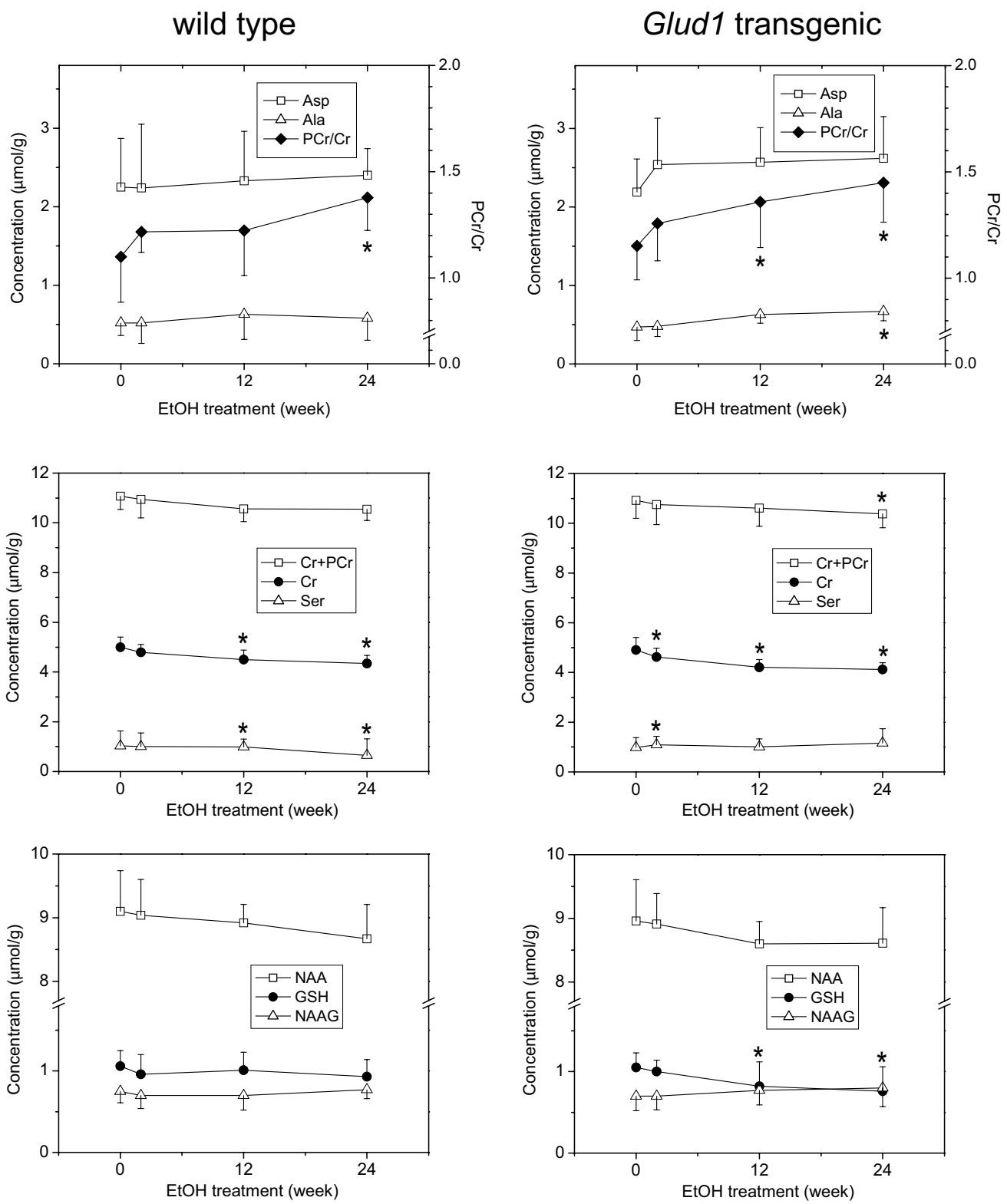


**Fig. 3** Effects of EtOH on the concentration of neurochemicals in the hippocampus of wt and *Glud1* tg mice, shown in the left and right columns, respectively, measured at the indicated intervals of EtOH diet. An asterisk (*) denotes significant differences, $p < 0.05$, between the concentration of a neurochemical at E0 (n = 28 for tg; n = 15 for wt) and other time points of EtOH diet (n = 14, 8, and 7 at E2, E12, and E24 respectively for both tg and wt groups)

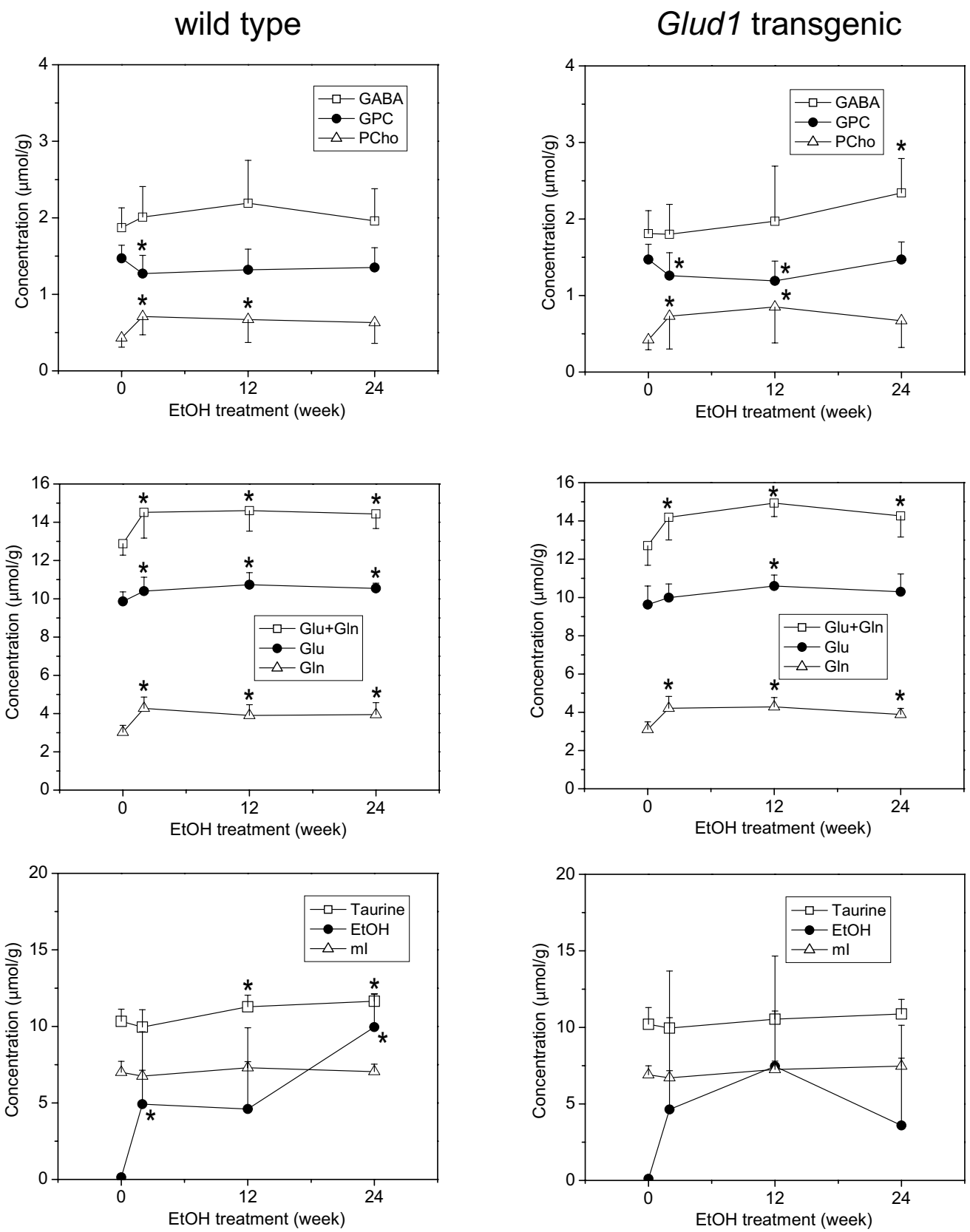


**Fig. 3** (continued)

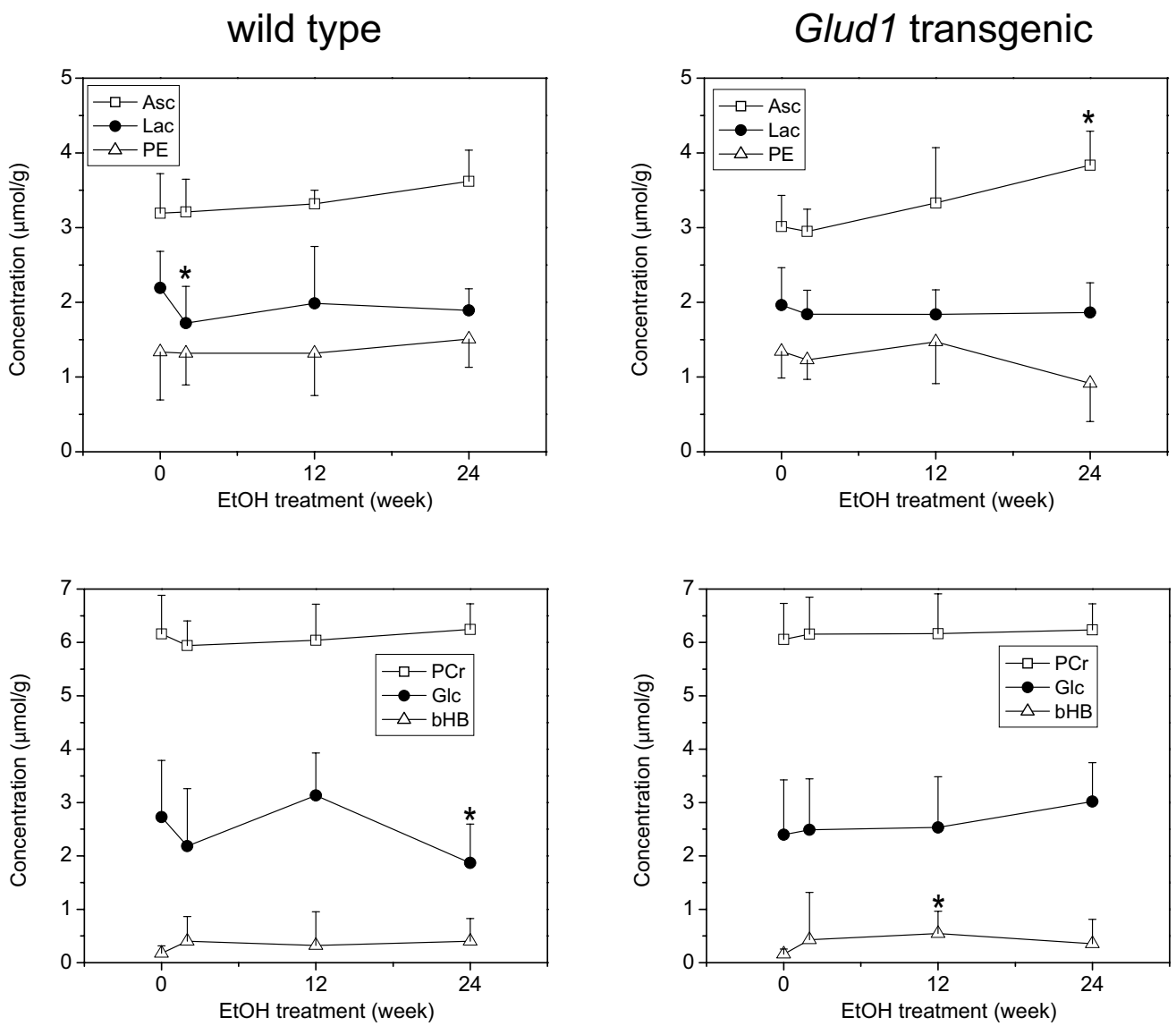


**Fig. 3** (continued)

In the striatum of wt mice, the EtOH diet led to significant increases in the concentrations of bHB (at E12), GABA (at E2 and E12), Gln (at E2–E24), Gln + Glu (at E2 and E24), PCho (at E2 and E24), and PCr/Cr (at E12 and E24), as well as significant decreases in the concentrations of Ala (at E24), Cr (at E2–E24), Cr + PCr (at E2–E24), Glc (at E2), GSH (at E12 and E24), mI (at E2), NAA (at E2), and NAAG (at E24). In tg mice exposed to the EtOH diet, there were significant increases in the concentrations of Asp (at E12 and E24), bHB (at E2 and E24), GABA (at E2–E24), Gln (at E2–E24), NAAG (at E2), PCho (E2 and E24), PE (at E12), and Ser (at E2 and E12), as well as significant decreases in the concentrations of Ala (at E24), Cr (at E12 and E24), Cr + PCr (at E2–E24), GPC (at E2 and E24), GSH (at E24), mI (at E2), and Taurine (at E2 and E12).

## Discussion

The methods used in the present study allowed longitudinal measurements of 20 neurochemicals, as well as macromolecules and EtOH, in two brain regions of wt and tg mice during a period from 41 to 65 weeks of age. The aim of this study was to explore the metabolic response to chronic EtOH intake of cells in two brain regions. In particular, we focused on the metabolic changes in Glu- and GABA-related metabolites and in neurochemical indices of possible oxidative stress and cell injury in hippocampus and striatum. The studies were performed on both wt mice and *Glud1* tg mice so that we could assess whether enhanced Glu metabolism and synaptic Glu release in the tg mice altered the metabolic response of brain cells to chronic EtOH exposure. Our

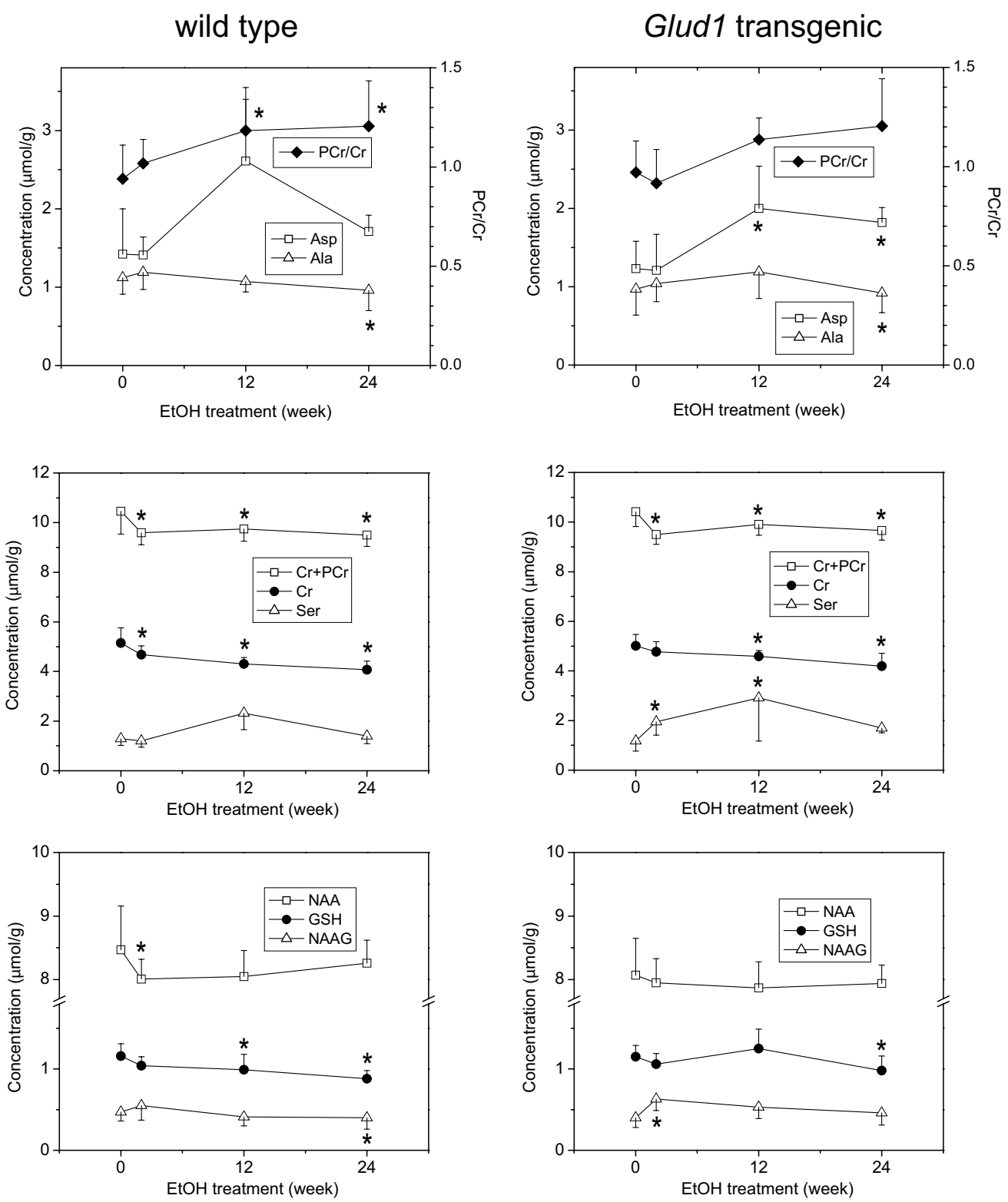


**Fig. 4** Effects of EtOH on the concentration of neurochemicals in the striatum of wt and *Glud1* tg mice, shown in the left and right columns, respectively, measured at the indicated intervals of EtOH diet. An asterisk (*) denotes significant differences, $p < 0.05$, between the concentration of a neurochemical at E0 (n = 28 for tg; n = 15 for wt) and other time points of EtOH diet (n = 14, 8, and 7 at E2, E12, and E24 respectively for both tg and wt groups)

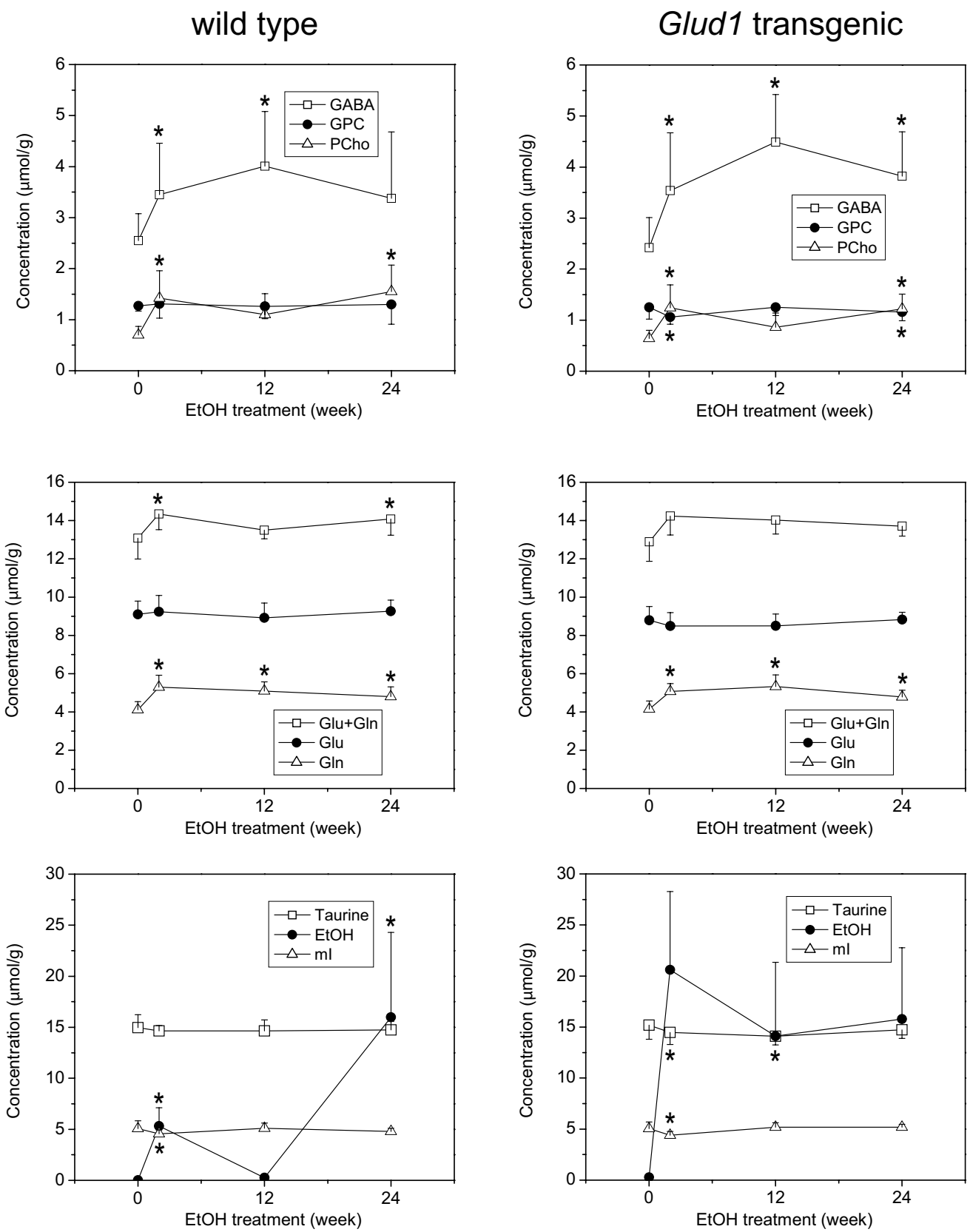


**Fig. 4** (continued)

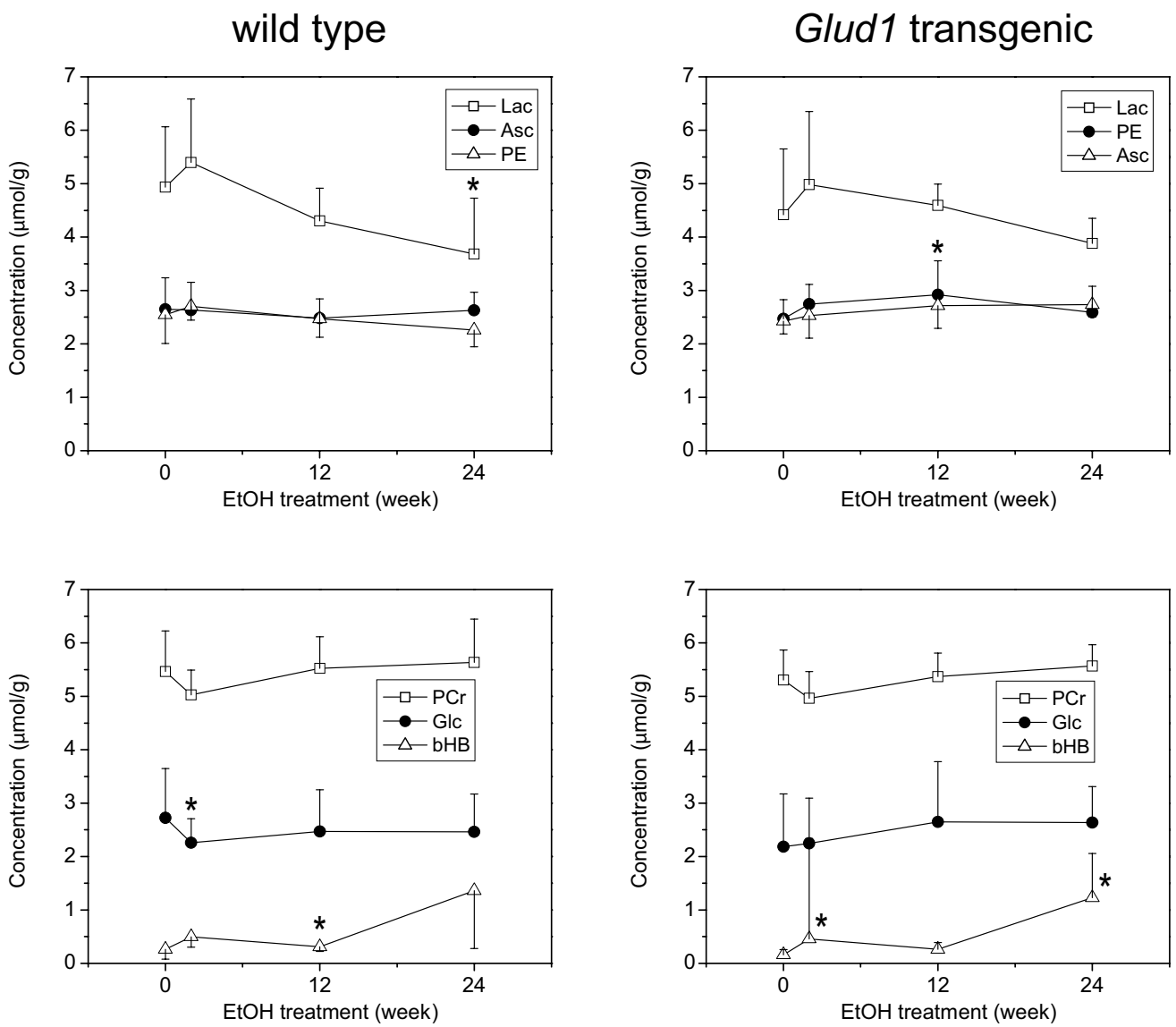


**Fig. 4** (continued)

results show that, except for a few differences at specific time points during chronic EtOH administration, the concentrations of neurochemicals that we monitored changed in very similar patterns in wt and *Glud1* Tg. These results suggest that whatever adaptive or stress-related responses were triggered in brain cells following chronic EtOH exposure, they were consistent regardless of the genetic differences between wt and *Glud1* tg mice.

Some key features of neurochemical changes that resulted from chronic exposure of the mice to EtOH were the increased levels of Glu, Gln, and Glu + Gln in hippocampus and the increased levels of Gln and Glu + Gln in striatum, changes that were in line with the results from some previous studies regarding the effects of chronic EtOH intake on Glu and Gln levels [14–17, 19]. The concentrations of Glu increased significantly in hippocampus, an observation that fits with the known enrichment of Glu neurotransmitter activity in large neurons in hippocampus and with the high sensitivity of this region to EtOH-induced stress [28]. Increases in the levels of the inhibitory transmitter GABA were only observed consistently in the striatum, a region that is fairly enriched in inhibitory interneurons. Overall, these results were suggestive of potential adaptive responses of brain cells to the chronic exposure to EtOH and of the differential effects that EtOH had on glutamatergic and GABAergic neurotransmission and related metabolites in these two regions.

In hepatic encephalopathy, ammonia accumulation in the blood is converted into Gln. Disturbance of Glu-Gln metabolism and accumulation of Gln in astrocytes leads to

decreases in mI in order to regulate the volume of astrocytes [39–43]. Similar to the hepatic encephalopathy, mI levels significantly decreased in striatum at E2 in response to the increases of Glu and Gln. A previous study [44] reported that, in addition to hippocampal Glu and Ala, concentrations of striatal Ala, GABA, Glu, GSH, and Taurine showed significant decreases in aged mouse brain of up to 24 months old when compared to their 6-month-old baseline. Aging effects on these metabolites may not be significant in the present study, because the duration of 5.5 months is much shorter than the 18 months in the previous study [44]. More importantly, effects of EtOH exposure seem to be prominent right after the introduction of the Ethanol diet, as many neurochemicals show obvious alterations at E2.

Acute and chronic effects of EtOH exposure have been studied using rodent models [14, 16, 17, 45–48]. When compared with the control group that was not exposed to EtOH, acute effects of EtOH exposure resulted in significantly reduced concentrations of Glu and Asp, as well as significantly increased concentrations of PCho and Taurine. Chronic EtOH exposure for up to 24 weeks led to significant increases in the concentrations of choline-containing compounds, Glu, and Glu + Gln. While there was no control group in the present study, levels of these metabolites showed similar trends. That is, concentrations of hippocampal PCho, Glu, and Glu + Gln, as well as striatal PCho and Glu + Gln, were significantly increased when compared to their baseline concentrations on E0.

Among the metabolites that decreased following chronic EtOH intake was GSH in wt striatum (at E12 and E24) and in both *Glud1* tg hippocampus (at E12 and E24) and striatum (at E24). The reduction in GSH following EtOH exposure of wt and tg mice was not unexpected as other studies have reported decreases in GSH levels, especially in EtOH-sensitive areas such as the hippocampus. Mitochondrial dysfunction, ROS formation, cellular oxidative stress, and GSH decreases have been observed in brain regions sensitive to EtOH-induced injury [28, 49]. GSH decreases might indicate EtOH-induced neuronal stress which was more pronounced in the tg mice, indicating signs of neuronal injury during the 24-week period on the EtOH diet. This conclusion was based on the observation of moderate, not statistically significant decreases in the NAA levels in both regions and in both wt and tg mice, except for the significant decrease in wt striatal NAA levels at E2. Chronic EtOH exposure also brought about decreases in Cr, a key cellular chemical that provides high energy stores in excitable tissues such as brain and muscle [50]. The Cr decrease in hippocampus, as well as the decreases in Cr and Cr + PCr in striatum, for both wt and tg mice, might be the result of either EtOH suppression of Cr synthesis in liver, kidney or brain [51, 52], or the result of partial inhibition of Cr transport into brain by EtOH. Although the decreases in Cr and PCr in brain were relatively small they may lead to disruption of normal synaptic and excitatory activity. Further, chronic EtOH exposure led to increased PCr/Cr ratio, which could be an indicator of lower demands for ATP and PCr due to decreased neuronal activity in rodent models of EtOH intoxication [53].

In previous studies, we observed small increases in Glu levels in the brains of the *Glud1* tg as compared with wt mice [22, 54]. However, statistical analyses of the data in our current study did not reveal statistically significant differences in the baseline measurements of Glu between wt and tg mice. As indicated above, the differences in Glu levels in the neuron and glial compartments of wt versus tg mice tended to be small, thus suggestive of a differentiation between Glu released at synapses, which was consistently elevated in tg mice [22] and overall levels of Glu in brain tissue.

A limitation of the present study is that metabolite data were acquired from wt and tg mice without fasting. For some of the mice, the spectral data were obtained at a time when the mice were still imbibing their daily EtOH diet while for others the MRS study was performed at some time after they had ingested their EtOH diet. Using this experimental design, variations in the acquisition time of MRS data relative to the dietary intake of EtOH may result in differential weighting of the acute effects of EtOH exposure, possibly masking the differences in wt and tg mice. The acute effects of EtOH might be eliminated or better controlled, if all measurements are obtained under fasting conditions.

It was reported that after 12 days of EtOH feeding using the Lieber-DeCarli liquid diet, the EtOH concentrations in blood were 156.15 ± 43.97 mg/dl in mice of age 14 months [55]. Further, it was found that ethanol elimination rate in mice ranged from 83.2 ± 8.2 to 98.8 ± 9.6 mg/dl/hr (mean ± SEM) [56]. Multiple studies have shown that regardless of the mode of EtOH administration in rodents, EtOH levels in the brain are almost identical to the blood levels and follow very closely the rise and fall of blood levels of EtOH over time post administration [57–59]. A quick calculation would suggest that based on the measurements in previous studies [55], 1 h after EtOH ingestion the concentration of EtOH in blood would be about 20 µmol/g tissue, which is in line with the average brain EtOH concentrations we estimated in the present study that varied between 5 and 20 µmol/g.

The strength of the present study is related to the successful, repeated, in vivo measurements of neurochemicals in two brain regions in a set of wt and tg mice treated chronically with EtOH. A weakness in the present study is the fact that nearly half the mice in either the wt or tg population used for the initial measurements of neurochemicals, died before the chronic EtOH exposure schedule could be completed. It appears that the combination of chronic EtOH exposure and repeated general anesthesia

(isoflurane) exposure was not well tolerated by a substantial number of mice. Previous studies showed that isoflurane affects glutamatergic synapses by reducing presynaptic Glu release and increasing its uptake from the synaptic cleft [60]. It is also shown that repeated exposures to isoflurane may lead to neuronal loss and reduced neurogenesis [61] and concentration increases of taurine in the anterior and posterior cortex as well as Glu in the posterior cortex [62]. Therefore, we cannot rule out potential influence of repeated isoflurane exposures on neurochemical changes observed in the current study, e.g., increased taurine and Glu. However, considering much longer intervals between exposures (i.e., 14, 74 and 84 days in comparison to previous studies with 1 and 10 days) and lower anesthesia levels than previous studies, the potential influence is expected to be much smaller.

The repeated exposure to EtOH and the general anesthetic agent in combination with the possible effects of the timing of the EtOH administration on neurochemical profiles, point to the difficulties in conducting a chronic exposure study using the same subjects repeatedly for the MRS measurement of chemical changes in the brain. Nevertheless, this study does offer time courses of neurochemical profiles before and during chronic EtOH administration which can serve as a reference for future studies investigating ethanol-induced neurochemical changes. In addition, the present study established quite clearly that brain cells upregulate Glu and Gln levels as a likely adaptive response to the well-known suppression of Glu release and Glu receptor function in the central nervous system following the acute administration or intake of EtOH.

**Funding** This study was funded by National Institute on Aging (AG 12993, AG 035982), Kansas City Area Life Sciences Institute (KCALSI-07-13).

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