Oxidation of ethanol in the rat brain and effects associated with chronic ethanol exposure

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It has been reported that chronic and acute alcohol exposure decreases cerebral glucose metabolism and increases acetate oxidation. However, it remains unknown how much ethanol the living brain can oxidize directly and whether such a process would be affected by alcohol exposure. The questions have implications for reward, oxidative damage, and long-term adaptation to drinking. One group of adult male Sprague–Dawley rats was treated with ethanol vapor and the other given room air. After 3 wk the rats – has been reported not to penetrate the blood the development of alcohol dependence (6, 16, 26). and toxic effects of EtOH in the brain, possibly playing a role in then intracerebral AA may mediate behavioral, neurochemical, penetration very slowly (23, 25). Thus, if the brain can oxidize EtOH, does not penetrate the blood (9). These studies provide powerful evidence that EtOH oxidation and AA production occur in the living brain. However, cerebral generation of AA is believed to be extremely slow, and EtOH oxidation has not been quantified directly in the awake brain. Neither have the consequences of chronic alcohol exposure on intracerebral oxidation been reported.

It has proven difficult to discriminate by isotopic means the intracerebral oxidation of EtOH from cerebral consumption of EtOH-derived Ac produced in the liver. When hamsters or rats are infused with 14C/13C-EtOH (32), the liver generates 14C/13C-Ac, whose consumption by the brain yields isotopic labeling that is indistinguishable from that of the EtOH. In this study, a combination of [1, 2-13C]acetate (Ac12) and [2-13C]EtOH (Etoh2) provided positional information that differentiated the metabolism of Ac from that of EtOH (Fig. 1). This strategy was used in EtOH-naive rats and rats exposed to vapor to quantify intracerebral EtOH oxidation and assess the impact of chronic EtOH exposure on intracerebral oxidation of EtOH.

Results

Plasma. Throughout the 2-h infusion, Ac and EtOH were the only substrates whose labeling was detected above natural abundance in the plasma; no labeling appeared for glucose, lactate, and β-hydroxybutyrate. By 30 min after the start of the infusion, blood EtOH levels rose to 25.5 ± 0.9 mM and 23.2 ± 1.0 mM (117 and 107 mg/dL) and rose slightly by 2 hr to 28.5 ± 0.9 and 28.1 ± 1.5 mM (131 and 129 mg/dL), respectively, with no differences between the groups (Fig. 2B).

The concentration of Ac increased within 5 min of the start of the infusion of Ac12 and Etoh2, with the treated animals having more Ac (4.75 ± 0.17 mM) than the naive group (4.09 ± 0.24 mM) (P = 0.04). The treated group kept a constant level, whereas Ac in the naïve rats decreased to 3.51 ± 0.20 mM at 15 min and stayed constant thereafter (3.66 ± 0.18 mM) (Fig. 2B). The difference in plasma Ac may be associated with metabolic tolerance, an ability of exposed rats to metabolize ethanol more quickly (33, 34), if the rate of acetate clearance is elevated in levels of EtOH and Ac, and AA rapidly disappears after sample collection due to its volatility. EtOH oxidation and the formation of AA have been measured in brain homogenates (11, 27–30) and cell cultures (31). In a novel approach, Zimatkín and Buben made the measurement in living brain by intraventricular perfusion (9) of anesthetized rats with 180 mM EtOH and measured AA concentrations rising to ~50 μM in the perfusate (23). When the authors perfused the brain with 85–90 mM EtOH, they again observed AA rise in the perfusate with no measurable AA in the blood (9). These studies provide powerful evidence that EtOH oxidation and AA production occur in the living brain. However, cerebral generation of AA is believed to be extremely slow, and EtOH oxidation has not been quantified directly in the awake brain. Neither have the consequences of chronic alcohol exposure on intracerebral oxidation been reported.

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13C-NMR catalase | alcohol dehydrogenase | cytochrome P450 | acetaldehyde dehydrogenase

The liver is the major organ for the oxidation of ethanol (EtOH) (1, 2), followed by the stomach and other organs. Acetate (Ac) generated from EtOH by the liver is consumed by the brain (3, 4), where it replaces a significant portion of cerebral glucose metabolism in humans and animals (5–8), either decreasing glucose consumption directly or compensating for glucose consumption decreased by some other effect. However, the brain may also oxidize EtOH (9–14), and that capacity is important with respect to several perspectives. The first step of EtOH oxidation generates acetaldehyde (AA), which is toxic, reactive, and potentially carcinogenic. AA is aversive systemically, as has been observed from the unpleasant effects of disulfiram, an inhibitor of AA dehydrogenase, but it has been shown to be rewarding in parts of the brain (12, 15, 16) especially in the posterior ventral tegmental area (17–19) by activating dopamine neurons (20–22). The liver maintains circulating levels of AA at levels of 20–155 μM (23, 24), and AA has been reported not to penetrate the blood–brain barrier or to penetrate very slowly (23, 25). Thus, if the brain can oxidize EtOH, then intracerebral AA may mediate behavioral, neurochemical, and toxic effects of EtOH in the brain, possibly playing a role in the development of alcohol dependence (6, 16, 26).

AA is difficult to measure in living systems. The concentrations of AA in liver, blood, and brain that occur with drinking lie in the micromolar range (23, 24), in contrast to millimolar
exposed rats, although this study was not designed to address that question.

Fig. 34 shows the $^{13}$C spectra from the plasma, with the Ac$_{12}$ doublet around the Ac$_{2}$ singlet. The total $^{13}$C enrichment of Ac (Ac$_{2}$ + Ac$_{12}$) increased to $\sim$85% after 5 min for the EtOH-treated and naive groups and stayed constant during the infusion (Fig. 2C). To use the steady-state Eq. 1 from Materials and Methods, the concentration of Ac and the ratio Ac$_{2}$/Ac$_{12}$ had to be maintained constant for the final hour of the procedure, which was achieved here (Fig. 2D). The combination of rapid metabolism of EtOH and the Ac arriving via infusion led to the appearance of $^{13}$C by the first measured plasma time point, 5 min (Fig. 2C). Then the ratio Ac$_{3}$/Ac$_{12}$ rose to a stable value after 30 min of infusion (Fig. 2D).

**Total Enrichments of Brain Glu and Gln**. The $^{13}$C enrichments of Glu$_{4}$ and Gln$_{4}$ for cortex and subcortex obtained after 2 hr of infusion are shown in Fig. 4A and B. Total enrichments of Gln$_{4}$ in the EtOH-treated group was significantly greater ($P = 0.003$) after EtOH chronic treated in cortex, and although Gln$_{Ac2}$ was also higher, the treatment effect did not reach significance ($P = 0.8$). The results indicate significant intracerebral oxidation of EtOH and that the oxidation is increased by chronic exposure to EtOH.

The observed enrichments were used in Eqs. 5 and 6 to estimate the rates of the cortical astroglial and neuronal tricarboxylic acid (TCA) cycles and Glu–Gln cycling in the cortex of awake rats (35) (Fig. 5). In astroglia of EtOH-naïve rats, EtOH was oxidized at a rate of 0.017 $\pm$ 0.002 $\mu$mol/min/g and in neurons 0.014 $\pm$ 0.003 $\mu$mol/min/g, corresponding to 12.3 $\pm$ 1.4% of astroglial oxidation and 1.7 $\pm$ 0.4% of neuronal oxidation, respectively (Table 1). The cortical EtOH oxidation rate for neurons and astroglia combined, 0.031 $\pm$ 0.003 $\mu$mol/min/g, corresponds to 3.2 $\pm$ 0.5% of the global brain oxidation of all substrates. Chronic alcohol exposure increased the astroglial EtOH oxidation to 0.028 $\pm$ 0.002 $\mu$mol/min/g ($P = 0.001$), corresponding to 20.2 $\pm$ 1.5% of astroglial oxidation, with a significant effect on neuronal EtOH oxidation ($P = 0.1$). TCA cycle and Glu–Gln cycling rates from three other publications (36–38) were also used with Eqs. 5 and 6 to estimate the rates of cortical EtOH oxidation; in all cases astroglial oxidation of EtOH was found to be elevated significantly (Table S1). In the subcortex, $^{13}$C labeling from EtOH oxidation was detected, with no significant impact of EtOH exposure (Fig. 4D). Due to the heterogeneity of the subcortex and its metabolic rates (39), the absolute rates of EtOH oxidation were not estimated. However, the percentages of $^{13}$C labeling in the subcortical brain are similar to those in the cortex (Fig. 4 C and D), which suggests that the average EtOH oxidation of EtOH. Gln also has a singlet and Gln$_{34}$ doublet resonances that are disproportionately large compared with the Gln$_{45}$ doublet, also consistent with EtOH oxidation.

Fig. 4A and B shows that Gln$_{4}$ and Gln$_{14}$ were significantly more labeled after chronic EtOH treatment for cortex ($P = 0.02$ and 0.001, respectively) and subcortex ($P = 0.04$ and 0.04, respectively), and the isotopomer analysis allows the source to be ascribed to Ac$_{2}$ or Etoh$_{2}$, as follows.

**Quantification of the Intracerebral Etoh Oxidation**. Fig. 4C and D shows the $^{13}$C enrichments from Ac$_{12}$, Ac$_{2}$, and Etoh$_{2}$ in the cortex and subcortex regions, based on the isotopomers observed for Glu$_{4}$ and Gln$_{4}$. Gln$_{Etoh2}$ was significantly greater ($P = 0.003$) after EtOH chronic treated in cortex, and although Gln$_{Ac2}$ was also higher, the treatment effect did not reach significance ($P = 0.8$). The results indicate significant intracerebral oxidation of EtOH and that the oxidation is increased by chronic exposure to EtOH.

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Ac is considered to be an astroglia-specific substrate, due to a specific Ac-uptake mechanism in astrocytes (51). However, the activity of acetyl-CoA synthetase is greater in neurons than in astrocytes (51). Therefore, a lack of Ac transport into neurons prevents them from oxidizing Ac from the blood, but one would predict that neurons can oxidize Ac that is derived from Etoh intraneurally. $^{13}$C- and $^{11}$C-$^{13}$C-labeled Etoh have been used to study the fate of Etoh in the brain (10, 52–54). One difficulty has been that isotopically labeled Etoh quickly becomes labeled Ac, whose intracerebral oxidation could not be distinguished from that of Etoh. Xiang and Shen (52) avoided this limitation by using saturation transfer. They detected no exchange between Etoh and AA in the brain, with a detection threshold of 0.001 $\mu$mol/min/g for the rate constant from Etoh to AA. Given our Etoh levels of ~28 mg/kg, the saturation transfer detection threshold corresponds to a rate of 1.7 $\mu$mol/min/g, which is far greater than our finding of 0.037–0.087 $\mu$mol/min/g.

Intracerebral Etoh Oxidation After Chronic Etoh Exposure. It has been shown that the brain consumes Ac and, when Etoh is given to rodents (4–6) and humans (3, 7, 8, 55), consumes its own Ac. Acetyl-CoA synthetase is greater in neurons than in astrocytes (51). Therefore, a lack of Ac transport into neurons prevents them from oxidizing Ac from the blood, but one would predict that neurons can oxidize Ac that is derived from Etoh intraneurally.

**Discussion**

**Metabolic Fate of Etoh in the Brain.** The first step of Etoh oxidation is its conversion to AA by mitochondrial alcohol dehydrogenase, catalase, or cytochrome P450. Alcohol dehydrogenase has been reported to have very low activity in the brain but appears to be heterogeneously distributed, occurring in some types of neurons (40, 41). Cytochrome P450 also has been shown to metabolize Etoh in the brain in neurons and astroglia, depending on the brain regions (42), but at a rate of 0.00051 $\mu$mol/min/g (43), significantly more slowly than the rate of 0.012 $\mu$mol/min/g reported for catalase in brain homogenates (calculated from the 3.5–7.1 $\mu$mol/g protein/h (11), using 0.1 g protein/g brain tissue (44)). Furthermore, inhibition of brain catalase activity in mice inhibits many of the pharmacologic effects of ethanol (45), and inhibition or stimulation of catalase activity in brain homogenates, respectively, reduces or increases the formation of AA (14, 15). Thus, evidence suggests that catalase is likely to be a more major contributor to Etoh oxidation in the brain. Inhibition of brain catalase activity in mice inhibits many of the pharmacologic effects of Etoh (45), and inhibition or stimulation of catalase activity, respectively, reduces or increases the formation of AA in brain homogenates (15). Although catalase is found in neurons and astroglia (46, 47), it occurs to a greater extent in neurons (48). From those previous reports of activity in vitro, one can predict a total rate of AA production of ~0.01 $\mu$mol/min/g from alcohol dehydrogenase, catalase, and cytochrome P450 combined, much slower than indicated by the present data in vivo. A possible explanation is the presence of potential cofactors and other environmental conditions that lead to greater activity in vivo than is measured in other preparations. For example, in the presence of glucose oxidase, the activity of catalase for Etoh significantly increases to ~10-fold (15). In this context, the faster oxidation rates observed in the living rat brain (0.031 and 0.036 $\mu$mol/min/g for naive and treated groups, respectively) are not unreasonable.

The second stage of Etoh metabolism is the conversion of AA to Ac via AA dehydrogenase, which is located in astroglia and neurons in many brain regions (41, 49). The activity of the brain to metabolize aldehydes has long been known (22). It has been shown that the aldehyde-oxidizing capacity of the brain fraction of capillaries and astroglia cells is 5–6 times higher than that of the neurons (49, 50), providing ample capacity to carry out the second step of Etoh oxidation. The rates of oxidation in neurons and glia result from the balance of the latter’s higher catalase activity and the slower neuronal aldolase conversion to Ac.

Significance of Intracerebral Etoh Oxidation. The current study shows that Etoh can provide energy for rat brain, mainly as Ac, which was generated in the liver, but also from the directly oxidation in the brain. The small but significant contribution of Etoh to brain oxidation might provide some energetic reward. When Etoh is oxidized directly by the brain, AA is produced within the brain, where it may serve as a pharmacologic reward (62), despite the fact that AA is aversive in the rest of the body. Another likely impact of intracerebral metabolism of Etoh is cumulative damage due to AA toxicity and oxidative damage from free radical generation (63).

Limitations. This study shows that intracerebral oxidation of Etoh occurs and that its rate is increased in astroglia by chronic Etoh treatment. However, the sensitivity of the technique was insufficient to measure oxidation in particular regions of interest, such as the posterior ventral tegmental area (19, 62). A further limitation pertains to the kinetic modeling. The measurements were conducted at an isotopic steady state, which means that the data contain only relative rates: that is, rates of ethanol oxidation relative to the rates of other pathways. Therefore, the absolute rates of ethanol oxidation in glia and neurons, in $\mu$mol/min/g, were estimated assuming values for the ratios $V_{Cyc}/V_{Ac}$ and $V_{Cyc}/V_{Ac}$. A factor to note is that one purpose of this study was to test whether chronic exposure to Etoh could affect intracerebral metabolism of Etoh; it did, but whether such a mechanism is associated with eventual dependence or not is an important question that remains to be investigated.

Fig. 3. 

**in vivo** oxidation over the subcortical brain comprises a similar fraction of total oxidation.
Materials and Methods

12% to 20% of total astroglial oxidation. associated with an increase in astroglial Etoh consumption from ronal oxidation, and that 3 wk of exposure to Etoh vapor was energy, supplying results showed that Etoh can be oxidized by the brain to provide the discrimination of the oxidation of Ac from that of Etoh. The analogues for Gln follow the same pattern. (Analogous to sively by astroglia (Fig. 1) (51) and labels Glu and Gln (36 Ac12 is infused, it enters the brain and yields [4, 5-13C2]Glu and Gln in the come from Etoh2. The13C-labeling of Gln from Etoh2 was according to:

\[ \text{dGlu}_N = f_{\text{Glu}_N} V_{\text{cycle}} - f_{\text{Etoh}_2} \text{CMRet}_N \]

\[ \text{dGlu}_A = f_{\text{Glu}_A} V_{\text{cycle}} - f_{\text{Etoh}_2} \text{CMRet}_A \]

\[ f_{\text{Glu}_4s} V_{\text{cycle}} - f_{\text{Glu}_4s} V_{\text{tcaN}} = 0. \text{ and} \]

\[ f_{\text{Etoh}_2} \text{CMRet}_N - f_{\text{Glu}_A} (V_{\text{cycle}} + V_{\text{tcaN}}) = 0. \]

\[ f_{\text{Glu}_4s} V_{\text{cycle}} - f_{\text{Glu}_4s} V_{\text{tcaA}} = 0.\]

\[ f_{\text{Etoh}_2} \text{CMRet}_A - f_{\text{Glu}_A} (V_{\text{cycle}} + V_{\text{tcaA}}) = 0. \]

The abbreviations for Gln follow the same pattern. (Analogous to...)}

Conclusions

The liver oxidizes most Etoh in the body (1, 2), but there remains some question of how much, if any, Etoh the brain can oxidize directly (9–13). However, the quantification of direct intracerebral Etoh oxidation has been encumbered by hepatic conversion of isotopically labeled Etoh to abundant Ac that circulates in the blood and is readily consumed by the brain. In this experiment, the combination of i.v. infusion of both Ac12 and Etoh2 allowed the discrimination of the oxidation of Ac from that of Etoh. The results showed that Etoh can be oxidized by the brain to provide energy, supplying ~12% of astroglial oxidation and ~2% of neuronal oxidation, and that 3 wk of exposure to Etoh vapor was associated with an increase in astroglial Etoh consumption from 12% to 20% of total astroglial oxidation.

Materials and Methods

Strategy for Isotopic Labeling and Estimation of Etoh Oxidation Rates. After the 13C-labeled Ac crosses the blood–brain barrier, it is metabolized exclusively by astroglia (Fig. 1) (51) and labels Glu and Gln (36–38, 51, 64). When Ac12 is infused, it enters the brain and yields [4, 5-13C2]Glu and Gln in the first turn of the TCA cycle and [3, 4, 5-13C3]Glu and Gln in the second and subsequent turns (Fig. 1). The total C4 and C345 labeling is proportional to the percent enrichment (PE) of Ac12 in the blood, with a proportionality constant kAc = ([4, 5-13C2]Glu + [3, 4, 5-13C3]Glu)/Ac12. When [2-13C]Etoh is infused simultaneously, the liver quickly converts some of it to [2-13C]acetate (Ac2). In the brain, Ac2 generates [4-13C] and [3, 4-13C2]Glu and Gln, following the same proportion kAc observed from the C45 glutamate labeled by Ac12.

The present study was designed with the intention that Etoh2 would be the only singly labeled substrate in the blood besides Ac2, so any C4 and C345 labeling of glutamate beyond that expected from the proportion kAc must come from Etoh2. The 13C-labeling of Glu from Etoh2 was according to:

\[ \text{where PE is the } 13C \text{ enrichment of Glu at the numbered positions. An equivalent expression exists for Glu.} \]

Etoh oxidation rate was evaluated using steady-state isotopic balance equations:

\[ \frac{\text{dGlu}_N}{\text{dt}} = f_{\text{Glu}_N} V_{\text{cycle}} - f_{\text{Glu}_N} V_{\text{tcaN}} = 0. \]

**Fig. 4.** The 13C labeling in Glu and Gln in the cortex and subcortex for naïve (10 rats) and ethanol-treated (nine rats) groups. (A) The percentage of carbon in cortical Glu and Gln labeled in different patterns: total C4 labeling (Glu and Gln), C4 only (Glu4s, Gln4s), C3,4 (Glu34, Gln34), and C4,5 (Glu45, Gln45). (B) Analogous to A, but for subcortex. (C) The percentage of carbon in Glu and Gln that came from Etoh and Ac. Glu-Ac2, Glu13C content from [1,2-13C]Ac; Glu-Eth, Glu13C content from Eth. The abbreviations for Gln follow the same pattern. (D) Analogous to C but for subcortex. *P < 0.05, **P < 0.01.

**Fig. 5.** The cortical oxidation rates of ethanol in astroglia, neurons, and whole brain, calculated using published rates of VtcaN, VtcaA, and Vcycle, for naïve (10 rats) and ethanol-treated (nine rats) groups. **P < 0.01. **
Table 1. The contribution of ethanol to oxidation in neurons, astroglia, and their sum relative to the TCA cycle in awake rats (35)

<table>
<thead>
<tr>
<th>Cell type</th>
<th>TCA cycle rate, μmol/min/g</th>
<th>EtOH contribution to oxidation</th>
<th>Naive</th>
<th>Treated</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuron</td>
<td>0.81</td>
<td>1.7 ± 0.4%</td>
<td>1.0 ± 0.1%</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Astroglia</td>
<td>0.14</td>
<td>12.3 ± 1.4%</td>
<td>20.2 ± 1.5%</td>
<td>0.001**</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.95</td>
<td>3.2 ± 0.3%</td>
<td>3.8 ± 0.2%</td>
<td>0.1</td>
<td></td>
</tr>
</tbody>
</table>

**P < 0.01.

rates for $V_{cycle}$, $V_{tcaN}$, and $V_{tcaA}$, and the total oxidation rate of EtOH (CMRetox) is the sum of CMRetox and CMRetA.

Animal Procedures. All experiments were carried out in accordance with protocols approved by the Yale Animal Care and Use Committee. Nineteen adult male Sprague–Dawley rats (Charles River Labs, Inc.) were maintained on a 12 h light–dark cycle, and food (Teklad Global 18% Protein Rodent Diet 2018, Harlan Laboratories) and water were available ad libitum. They were allowed a minimum of 2 d to acclimate before the start of the experiments. To familiarize the rats with human interaction and minimize stress on the day of the infusion, they were handled daily until the final day. Room air or EtOH was administered to rats in vapor chambers constructed in-house (65), allowing the EtOH vapor 8 h/d for 3 wk. At the start of the exposure period, the naive rat (n = 10) weighed 156.1 ± 1.8 g, and the treated rats (n = 9) weighed 155.5 ± 1.5 g (P = 0.572). The initial vapor level was set at 18–20 mg/L and gradually increased to 23–25 mg/L over the first week. This inhalation model is associated with minimal hepatic dysfunction (66). To monitor the blood EtOH concentration, 200 μL of blood was drawn from the saphenous vein of one rat in each cage each day after 7 h of the day’s exposure. With three rats housed per cage, each rat was tested once every 3 d. The blood samples were centrifuged to obtain plasma, and the EtOH concentration measured (Analox GM7, Analox Instruments, Inc.). During the exposure periods, the blood EtOH concentrations ranged 150–200 mg/dL, levels that have been shown to induce depressive- and anxiety-like behaviors in rats (67) and slurred speech, double vision, and difficulty walking in humans (68). After 3 wk of EtOH exposure, the weight of the treated rats (301.0 ± 2.8 g) was 3% higher than that of the naive rats (310.7 ± 2.7 g) (P = 0.033).

On the day of infusion, anesthesia was induced with 3% (vol/vol) isoflurane and lowered to 1.5–2.5% for 1–2 min to catheterize one lateral tail vein with a 23-G needle connected with PE-50 tubing (Instech Laboratories, Inc.) (35). The rats then recovered for 30 min, moving and walking freely in the cage before the infusions. EtOH2 (up to 1.373 mg/kg over 2 h) was infused through the tail catheter using a pharmacologically modeled infusion protocol (69). AC2 (up to 322 mg/kg over 2 h) was coadministered by a variable rate infusion over 2 h (2 M dissolved in water, pH = 7.0) according to the following rate schedule: 0–15 s, 1.875 mmol/min/kg; 15 s–45 min, 0.265 mmol/min/kg; 4–14.5 min, 0.15 mmol/min/kg; and 14.5–120 min, 0.075 mmol/min/kg. During the administration, 100 μL blood samples were collected from the saphenous vein at the time points 0, 5, 15, 30, 60, and 120 min; centrifuged at 18,000 × g for 10 min at 2–4 °C; and frozen for later measurement of plasma EtOH2, Ac2, and AC2. After the infusion, the rats were euthanized with focused microwave irradiation (35, 70). The fixed brain was removed and dissected into cortex and subcortex and frozen for later NMR analysis.

Preparation of Plasma Samples for NMR Study. The frozen samples were thawed and centrifuged at 18,000 × g for 5 min. The supernatant was withdrawn and mixed with water to total volume of 150 μL, and an additional 60 μL D2O and 240 μL buffer with 12.5 mM Pi (90 mM KH2PO4 and 35 mM K2HPO4) were added, with 5 mM sodium formate as a concentration and chemical shift reference for 1H-NMR and 150 μL 10 mM [2-13C]glucose as a chemical shift reference for 13C-NMR. The mixture was transferred into a 5 mL NMR tube for NMR analysis.

Preparation of Brain Samples for NMR Study. The frozen brain samples were weighed and placed into a 7 mL homogenizer with 300 μL 0.1 M HCl in EtOH in a wet ice bath and ground. We added 3 mL ice-cold 60% (vol/vol) EtOH with 150 μL 10 mM [2-13C]glucose as an internal standard, and the mixture was homogenized. The homogenizer was washed with 2 mL ice-cold 60% EtOH, and the total mixture was centrifuged at 18,000 × g for 30 min at 2–4 °C. The supernatant was withdrawn, frozen with liquid nitrogen, and lyophilized for NMR analysis.

The lyophilized extracts were dissolved with 60 μL D2O and 540 μL phosphate buffer. The solution was centrifuged at 18,000 × g for 30 min, and the supernatant was collected for [1H-13C]NMR and 13C-NMR analysis.

NMR Analysis for the Brain Tissue Extracts and Plasma Samples. The measurements were performed at 11.7ST on a Bruker vertical bore NMR spectrometer operating at 500.13 MHz for 1H and 125.76 MHz for 13C. 1H-NMR was used to measure the concentrations and total 13C-enrichments of energy substrates in the plasma. 1H-Observed/13C-Edited (POCE) NMR was used to measure the brain concentrations and total 13C-enrichments of Glu and Gln C4 (71). Polarization transfer was used to observe the 13C spectrum to measure plasma AC2 distinctly from AC1 and to distinguish brain Glu and Gln C4 and C5 from Glu and Gln C4 and C5.

For 1H- and POCE-NMR, the magnetic field homogeneity on each sample was optimized with an automated 3D filed mapping algorithm capable of adjusting up to fifth-order spherical harmonics.

1H-NMR. Spectra were obtained with 12 µs hard-pulse nonselective excitations, with free induction decays acquired at 16,384 complex points. Total concentrations were determined relative to the added formate, and the 13C enrichments of Ac were calculated from the ratio of the areas of the satellite peaks relative to the entire integral of the signal for Ac2.

POCE Spectra were obtained with the POCE (71), using an 8-ms echo time, 20-s repetition time, 12-ppm sweep width, and 32,768 complex data points. Briefly, the heteronuclear editing method consists of the acquisition of two spin-echo measurements, one with a broadband inversion pulse applied at the 13C frequency and the other without the inversion pulse. The difference between the spectra represents protons bound to 13C (at twice the true intensity), whereas the subspectrum without the inversion pulse represents the protons for 13C-labeled plus the unlabeled compounds (i.e., the total concentrations). The total 13C enrichments of Glu4 and Glu4 were half of the respective ratios between the Glu4 and Glu4 signals in the difference spectrum and subspectrum without the inversion pulse.

13C-NMR. The pulse sequence Sensitive Nuclei Enhanced by Polarization Transfer (INEPT) (72–74) was involved, and 32,768 complex peaks were acquired after manual adjustment of the magnetic field homogeneity. The enrichments of C4, C5, C6, C7, and C8 of Glu and Gln were calculated according to:

$$\text{f}_{\text{NMR}} = \frac{\text{f}_{\text{C4 Total}}}{\left(\frac{\text{A}_{\text{Glu}_4}}{\text{A}_{\text{Gln}_4} + \text{A}_{\text{Glu}_4}}\right) / \left(\frac{\text{A}_{\text{Gln}_4}}{\text{A}_{\text{Gln}_4} + \text{A}_{\text{Glu}_4}}\right)}$$

[7]

where $\text{A}_{\text{Glu}_4}$ is the area of the Glu C4 singlet resonance, $\text{A}_{\text{Gln}_4}$ is the area of the doublet resonance that represents [3, 4-13C]Gln, and so on for the relevant combinations of labeling. The analogous calculations were performed for C5, C6, and C7.

Statistical Analysis. The significance of differences between means of measured parameters in different treatment groups was assessed using two-tailed t tests, assuming unequal variances. All means are presented ± SEM.

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Supporting Information

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Table S1. Cortical oxidation rates of ethanol in astroglia (CMRet<sub>A</sub>), neurons (CMRet<sub>N</sub>), and the total (CMRet<sub>tot</sub>) based on the published rates of V<sub>tcAN</sub>, V<sub>tcNA</sub>, and V<sub>cycle</sub>

<table>
<thead>
<tr>
<th>Values calculated</th>
<th>Naive</th>
<th>Treated</th>
<th>Naive</th>
<th>Treated</th>
<th>Naive</th>
<th>Treated</th>
<th>Rate, μmol/min/g</th>
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<tr>
<td>Rate</td>
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