

Ethanol Induces Expression of the Glutamate Transporters EAAT1 and EAAT2 in Organotypic Cortical Slice Cultures

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Background: Exposure of the developing brain to ethanol disposes the fetus to fetal alcohol syndrome and causes a number of changes in several neurochemical systems. In particular, the glutamatergic system is affected by ethanol. Thus, increased sensitivity of glutamate receptors and enhanced transmembrane transport of glutamate were found in primary astrocyte cultures. However, in these in vitro studies, changes in the expression of glutamate transporters were not detected. To further characterize the influence of chronic ethanol exposure on the developing brain, we assessed the transcriptional and translational regulation of glutamate transporters in a less artificial in vitro system.

Methods: We exposed postnatal rat cortical organotypic slice cultures to ethanol (100 mM) for 4 and 8 days. Expression of the excitatory amino acid transporters EAAT1 and EAAT2 was analyzed in comparison to untreated controls with semiquantitative reverse transcriptase-polymerase chain reaction. In addition, changes in protein expression were detected by Western blotting.

Results: In ethanol-exposed cortical slices, we observed more prominent fiber outgrowth and significantly increased gene expression (EAAT1: +35%, $p = 0.029$; EAAT2: +251%, $p = 0.015$). These findings could be verified on the protein level, because Western blot analysis revealed significantly higher levels of EAAT1 (+76%; $p = 0.008$) and EAAT2 (+104%; $p = 0.018$) in ethanol-treated slices compared with controls.

Conclusions: Our results are in concert with earlier studies describing the induction of glutamate transport by ethanol. Enhanced expression of EAAT1 and EAAT2 after ethanol exposure can be viewed as a maladaptive process that disposes the developing brain to fetal alcohol syndrome.

Key Words: Glutamate, EAAT, Development, Ethanol, Fetal Alcohol Syndrome.

THE DEVELOPING BRAIN is highly sensitive to ethanol. A severe consequence for the developing brain after ethanol intoxication is fetal alcohol syndrome (FAS), which is characterized by craniofacial malformations, neuropathologic symptoms, and increased mortality (Burd et al., 2004; Olney et al., 2002). In addition, reduced brain volume and various neurobehavioral disturbances, such as learning deficits, have been reported. Alterations in several neurochemical systems may underlie these neurobehavioral disturbances. Thus, functional changes have been found in the serotonergic (Zafar et al., 2000), dopaminergic (Szot et al., 1999), γ -aminobutyric acid (GABAergic), and glutamatergic systems (Butters et al., 2003; Olney et al., 2001; Othman et al., 2002).

One of the major hypotheses, which involves alterations in the glutamatergic system, suggests that ethanol, by blocking NMDA receptors, disrupts synaptogenesis in the developing brain (Olney, 2004). Thus, the administration of both competitive and noncompetitive NMDA receptor antagonists triggers a massive wave of apoptotic neurodegeneration that affects many neurons in the developing brain (Ikonomidou et al., 1999). Ethanol, which also possesses NMDA receptor antagonistic properties (Hundt et al., 1998; Lovinger et al., 1989), triggers a neurodegenerative response that is even more robust than the response to the noncompetitive NMDA receptor antagonist MK-801 (Ikonomidou et al., 2000).

Another change within the glutamatergic system of the developing brain involves the clearance of glutamate after ethanol exposure. Thus, in cortical astroglial cell cultures, uptake of radioactively labeled glutamate was repeatedly found to be increased (Othman et al., 2002; Smith, 1997; Smith and Navratilova, 1999; Smith and Zsigo, 1996). Membrane-bound transporter molecules that form the gene family of excitatory amino acid transporters (EAAT; Danbolt, 2001) accomplish the clearance of glutamate from the synaptic cleft. EAAT1 (formerly named GLAST) and the quantitatively dominating transporter EAAT2 (GLT1)

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are expressed on astroglial cells (Perego et al., 2000; Torp et al., 1994), but gene expression on neuronal cells during development has also been reported (Furuta et al., 1997; Sutherland et al., 1996). So far, no evidence for increased protein expression of EAAT1 or EAAT2 has been found after ethanol exposure *in vitro*.

Compared with dissociated glial or neuronal cell cultures, organotypic tissue cultures more closely model the *in vivo* processes, as monitored by functional and molecular genetic markers (Haydar et al., 1999; Molnar and Blake-more, 1999). Furthermore, organotypic slice cultures of newborn rat cerebral cortex exhibit a period of cortical development that corresponds to the last trimester of human pregnancy (Dobbing and Sands, 1979) and, therefore, likely approximates the situation of ethanol intoxication during pregnancy and the subsequent development of FAS. Effects of ethanol on other than glutamatergic marker molecules have previously been investigated with hippocampal organotypic slice cultures (Belmadani et al., 2003; Diao and Dunwiddie, 1996). Electrophysiological studies on hippocampal slices have shown an inhibition of NMDA receptor-mediated excitatory postsynaptic currents by acute exposure to ethanol and an increase of synaptic transmission after withdrawal as a model of ethanol withdrawal hyperexcitability (Thomas et al., 1998; Thomas and Morrisett, 2000), a process that also involves the metabotropic glutamate type 5 receptor (Harris et al., 2003) and polyamines (Gibson et al., 2003).

We chose cortical organotypic slice cultures to further characterize the influence of chronic ethanol exposure on the developing brain and assessed the transcriptional and translational regulation of glutamate transporters EAAT1 and EAAT2 to test whether enhanced expression of EAAT1 and EAAT2 underlies the observed augmentation of the glutamate uptake system after ethanol exposure.

MATERIALS AND METHODS

Organotypic Cortical Slice Cultures

Wistar rats from our own breeding colony (Central Institute of Mental Health, Mannheim, Germany) were housed and bred in standard hanging rodent cages (Ehret, Emmendingen, Germany) on a 12-hr light/dark cycle with lights on at 7:00 AM. Animals were provided with food (Sniff, Soest, Germany) and water *ad libitum*. All experimental procedures were approved by the respective Committee on Animal Care and Use and performed by following the local Animal Welfare Acts and in accordance with the European Communities Council Directive of November 24, 1986 (86/609/EEC). On postnatal day 1, newborn rats were killed by decapitation. The brain was quickly placed into ice-cold Hanks' buffered salt solution supplemented with glucose (6.5 mg/ml), and a triangular portion of the cerebral cortex containing frontal and parietal subregions was prepared. A tissue chopper (McIlwain, GaLa Instrumente, Bad Schwalbach, Germany) dissected this part into coronal slices as thick as 350 μm . After an incubation of 1 hr at room temperature in glucose-supplemented Hanks' buffered salt solution, the slices were transferred with the inverted part of a Pasteur's pipette on membrane inserts (Costar transwell polyester, 24 mm, 0.4 μm pore size, Corning, Palo Alto, CA) in a six-well culture dish and cultured on the interface between air and medium [Dulbecco's essential Eagle's medium/F12 50:50 supplemented with 5% fetal bovine

serum, N2-supplement (Gibco Life Sciences, Karlsruhe, Germany), streptomycin (50 $\mu\text{g}/\text{ml}$), penicillin (50 IU/ml), and L-glutamine (2 mM)]. Tissue cultures were observed during the period of culture with a Leica microscope (DM-IRB; Wetzlar, Germany), and phase contrast images were acquired by a digital camera (Photometrix Quantix, London, UK) and image-analysis software (IP Lab, Guernsey, UK).

Ethanol Treatment of Organotypic Slice Cultures

Different ethanol concentrations (10, 100, and 250 mM) in the culture medium were tested, including even potentially neurotoxic levels (250 mM). To estimate the evaporation of ethanol, we analyzed representative sets of medium with a commercial kit (Sigma Aldrich, Taufkirchen, Germany). This photometrical assay (340 nm) is based on the oxidation of ethanol to acetaldehyde catalyzed by the enzyme alcohol dehydrogenase. Evaporation of ethanol resulted in a reduced concentration to approximately 57% over 2 days; therefore, complete medium was changed every second day. Treatment periods were either 4 or 8 days. In addition, the multiwell dishes were protected by wrapping with Parafilm (American Can Company, Greenwich, CT).

RNA Isolation and Semiquantitative Reverse Transcriptase-Polymerase Chain Reaction

Total RNA was extracted with a commercial procedure (Quiagen RNeasy Kit, Hilden, Germany) and transcribed into complementary DNA (cDNA) with oligo-deoxythymidine primers (Promega, Mannheim, Germany) and Superscript (Gibco). Semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR; *Taq* polymerase, Promega) amplified specific cDNA fragments: EAAT1 (GenBank accession number S59158, bases 2834 to 3248) with 30 cycles and a 58°C annealing temperature; EAAT2 (GenBank accession number X67857, bases 1190 to 1626) with 31 cycles and a 61°C annealing temperature; and glyceraldehyde phosphate dehydrogenase (GAPDH; GenBank accession number M17701, bases 550 to 1001) with 22 cycles and a 62°C annealing temperature. PCR products were analyzed on ethidium bromide-stained agarose gels; band intensity was determined with the image-analysis software GelDoc 1000 (Bio-Rad, München, Germany) and normalized to the housekeeping enzyme GAPDH.

Real-Time RT-PCR

The expression of pituitary adenylate cyclase-activating polypeptide (PACAP) was evaluated by help of a real-time RT-PCR approach (iCycler, Bio-Rad). The transcripts of the housekeeping gene GAPDH (described previously) and of PACAP (GenBank accession number M63006, bases 531 to 981) were amplified in 1 to 100 diluted cDNAs and negative controls without reverse transcription. We used a commercial master mix (Absolute SYBR Green Fluorescein, ABgene, Epsom, UK) and primer concentrations of 400 nM (PACAP) or 800 nM (GAPDH). The cycle of amplification threshold (CT) and a melting curve to identify the amplification product were analyzed. We assessed the difference of CT numbers between the housekeeping gene and the gene of interest for every sample (ΔCT) and compared the means of the treatment group and controls by using Student's *t* test ($n = 7$) to determine the significance of the $\Delta\Delta\text{CT}$ value.

Western Blotting

Homogenization of cultured tissue was performed in a 2% sodium dodecyl sulfate buffer at 85°C, followed by denaturation at 95°C. After several passages through a 26-gauge needle and centrifugation (10 min; 14,000 rpm), the supernatant was stored at -20°C. Protein content was determined by the photometrical method of Markwell, and equal amounts (100 μg for EAAT1 and 10 μg for EAAT2) were run on 9% polyacrylamide gels (Roth, Karlsruhe, Germany). After transfer to nitrocellulose membranes (Amersham, Little Chalfont, UK) and blocking of nonspecific binding with nonfat milk powder, goat antisera against EAAT1 (Santa

Cruz Biotechnology 7758, 1:2000, Santa Cruz, CA) and EAAT2 (Santa Cruz Biotechnology 7760, 1:4000) were applied. We used a horseradish-coupled secondary antibody (Santa Cruz Biotechnology 2020, 1:6000) and visualized the bands with a chemiluminescence reagent (Perkin Elmer, Wellesley, MA). EAAT1 and EAAT2 were detected in the expected size of monomers between the protein marker bands of alcohol dehydrogenase at 50 kDa and glutamic acid dehydrogenase at 67 kDa. Total protein staining with MemCode (Pierce, Köln, Germany) allowed normalization to the amount of proteins run in the specific lanes of interest. For image analysis, the chemiluminescence films and MemCode-stained membranes were evaluated with a digital camera (Nikon, Tokyo, Japan) and an image-analysis system (Applied Information Systems, Chapel Hill, CA) to determine the protein expression semiquantitatively.

Statistics

Messenger RNA (mRNA) and protein expression were evaluated in independent experiments. The difference between ethanol-treated slices and controls was compared by using Student's *t* test. Statistical levels of $p < 0.05$ were considered significant.

RESULTS

We adopted an organotypic tissue culture system (Haydar et al., 1999; Molnar and Blakemore, 1999) and treated newborn rat cortical slice cultures for different periods of time and with different concentrations of ethanol. Evaporation of ethanol caused a reduction of the initial ethanol concentration over 2 days. Thus, in eight representative samples, a reduction from 100 to 57 mM after 2 days was measured. Therefore, the complete medium was changed every second day.

We consistently observed pronounced fiber outgrowth in the ethanol-treated slices over the entire period of culturing in comparison to untreated cultures of the same age. Typical examples as seen at day 4 and day 8 are documented in Fig. 1.

RT-PCR with cDNA of independent RNA preparations allowed the assessment of EAAT1 and EAAT2 expression

in relation to the constitutively expressed housekeeping gene GAPDH. Pilot experiments with various concentrations of ethanol resulted in an approximated dose-response curve of the induction of EAAT1 on mRNA level (10 mM, +71%; 100 mM, +78%; 250 mM, -1%) and of EAAT2 (10 mM, +38%; 100 mM, +61%; 250 mM, +29%). Because these results were in concert with previous findings on the effects of ethanol on transmembrane glutamate transport in astrocyte cultures (Smith, 1997; Smith and Zsigo, 1996), the following experiments were performed at a concentration of 100 mM applied over 4 days (mRNA induction) or 8 days (protein induction).

We observed induced gene expression of EAAT1 (+35%; $p = 0.029$) and of EAAT2 (+251%; $p = 0.015$). Figure 2 shows the difference of means of four independent RNA preparations and a typical example. After 8 days of culture, we performed Western blot analysis. Comparing lanes of treated and untreated cultures, we found induced protein expression of EAAT1 (+76%; $p = 0.008$) and EAAT2 (+104%; $p = 0.018$). These results are depicted in Fig. 3 as differences of means in addition to a typical pair of bands.

Because the neuropeptide PACAP is known to induce the expression of EAAT1 and EAAT2 (Figiel and Engele, 2000), it represents a candidate molecule for the described effect of ethanol. Therefore, we evaluated its expression in sets of cDNAs derived from cultures treated with 100 mM ethanol and untreated controls. The expression did not differ significantly, as revealed by a Δ CT value (\pm SD) in the treated samples of 15.72 (\pm 2.79) and of 14.72 (\pm 0.99) in the controls.

DISCUSSION

Our results show that exposing the developing cerebral cortex in organotypic slice cultures to ethanol leads to an induced neurite sprouting and an induction of glutamate transporters EAAT1 and EAAT2 on the mRNA and protein level. We used organotypic slice cultures of newborn rat cerebral cortex to investigate the effects of ethanol on the developing brain as closely as possible and selected a period of cortical development that corresponds to the last trimester in human pregnancy (Dobbing and Sands, 1979). This approach mimics maternal ethanol intoxication during pregnancy and likely approximates the subsequent development of FAS.

The finding of induced glutamate transporter expression is functionally in concert with previous results obtained with newborn rat astroglial cell cultures (Smith, 1997; Smith and Zsigo, 1996). In these studies, induced transmembrane transport of glutamate has been described, but with Western blotting analysis, no evidence for increased transporter protein expression could be obtained. However, our investigations showed transcriptional and translational induction of EAAT1 and EAAT2.

This finding raises an important question: how does

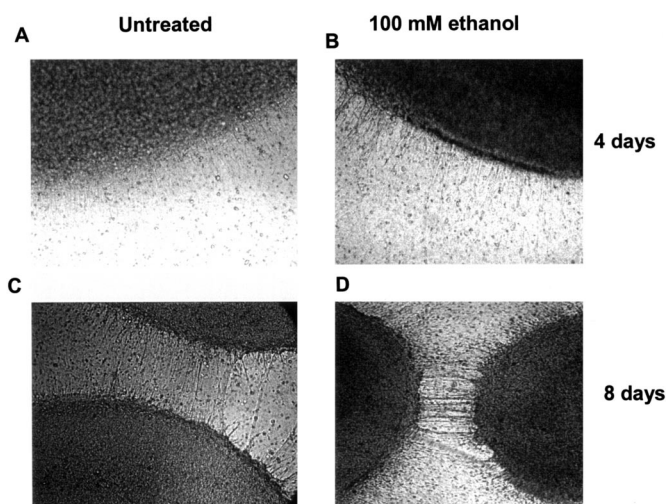


Fig. 1. The exposure of neonatal rat cortex organotypic slice cultures to 100 mM ethanol (B and D) results in pronounced fiber outgrowth in comparison to untreated controls (A and C), as documented after 4 and 8 days of treatment.

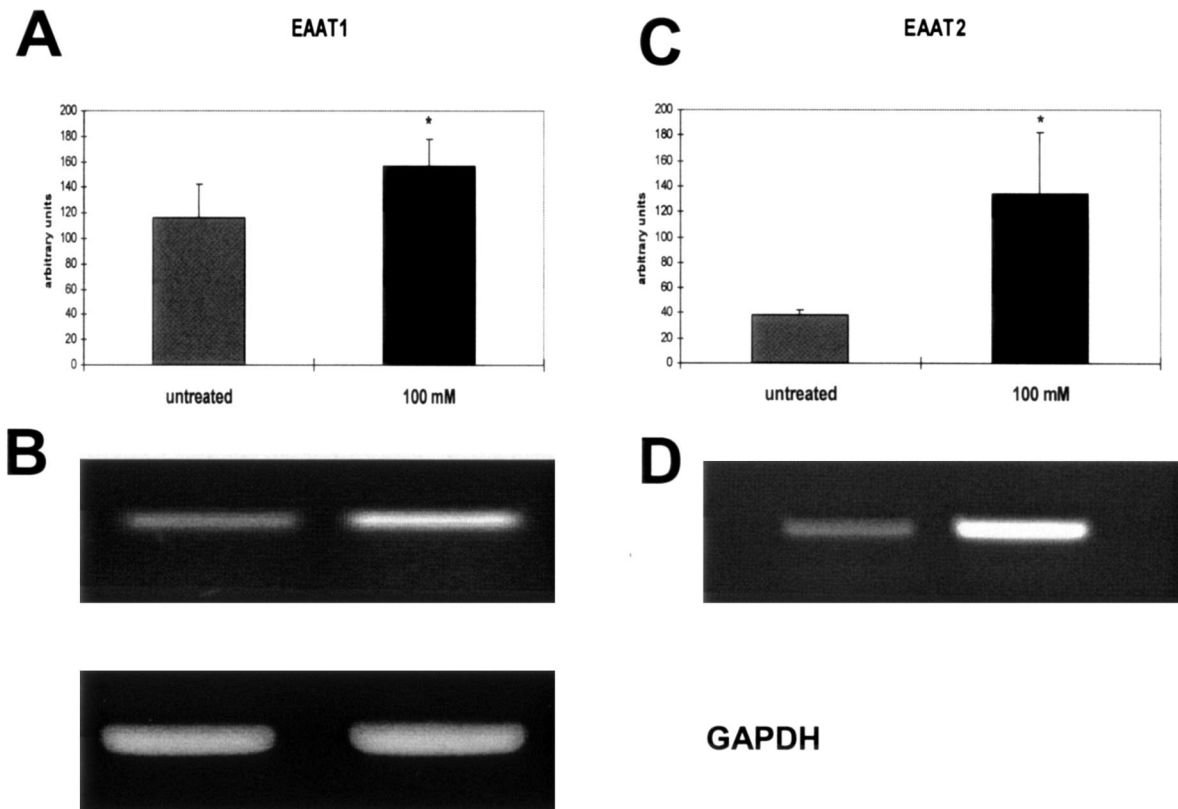


Fig. 2. Semiquantitative RT-PCR of the glutamate transporters EAAT1 (A and B) and EAAT2 (C and D) as evaluated by RT-PCR comparing organotypic cortical slices after ethanol treatment (100 mM for 4 days; $n = 4$) with untreated control slices ($n = 4$). Specific expression was determined in relation to the expression of the housekeeping gene glyceral aldehyde phosphate dehydrogenase (GAPDH). Typical paired results are documented (B and D). Significant induction was observed for both glutamate transporters (EAAT1: +31%, $p = 0.029$; EAAT2: +251%, $p = 0.015$).

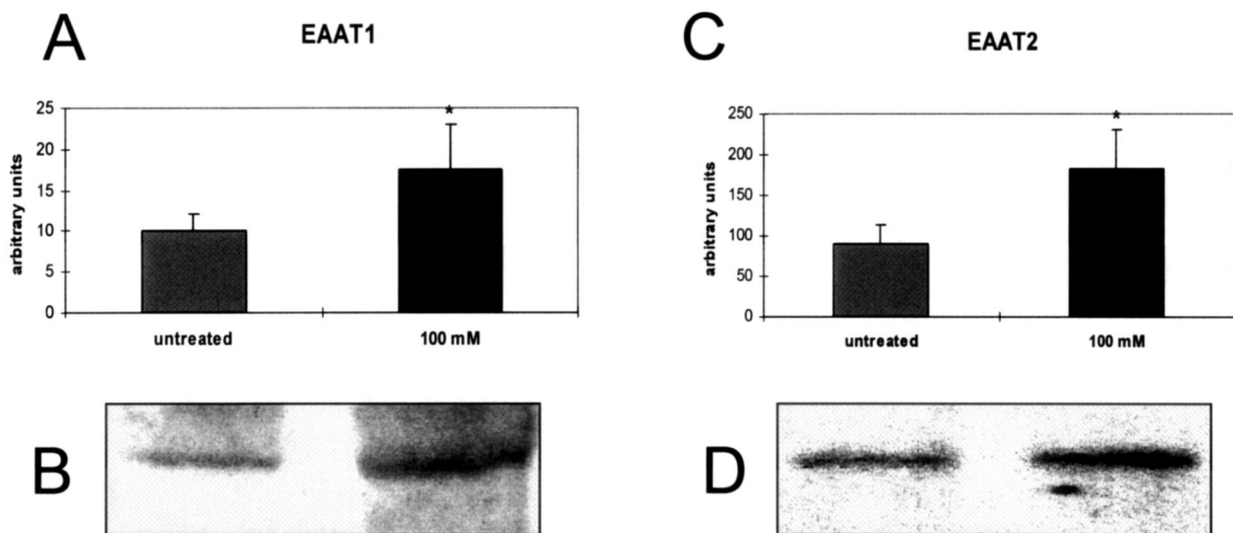


Fig. 3. Western blot analysis of the glutamate transporters EAAT1 (A and B) and EAAT2 (C and D); crude lysates of organotypic cortical slices were treated with 100 mM ethanol for 8 days ($n = 6$) or served as untreated controls ($n = 6$). Typical examples of bands are depicted (B and D). Significant induction of both EAAT1 (+76%; $p = 0.008$) and EAAT2 (+104%; $p = 0.002$) was found.

exposure to ethanol induce the expression of EAAT1 and EAAT2 in organotypic slice cultures, but not in astrocytic cultures? One possible explanation for pronounced expression of glial glutamate transporters could be that ethanol modifies the cellular composition of the cultured slice by

means of induced neuronal cell death, leading to a dominance of glial cells and glial cell markers such as the glutamate transporters EAAT1 and EAAT2. However, this seems to be unlikely because neurite outgrowth as a marker of vital neurons seemed to be stimulated in the ethanol-

treated dishes. With respect to the regulatory mechanism, it is important to note that in organotypic tissue culture systems different cell types and cell/cell contacts between neuronal and glial cells are preserved and allow mutual stimulation with diffusible factors. Potentially neurotoxic levels of ethanol (250 mM) do not exert the described effects. A candidate effector gene of this stimulation could be the neuropeptide PACAP, because it is known to strongly induce EAAT expression (Figiel and Engele, 2000). We therefore assessed the expression of PACAP with a real-time RT-PCR approach and did not find significant differences in Δ CT values. Alternatively, changed expression or sensitivity of the PACAP receptors or other neuronal stimuli may be further candidates for the mechanism of action. Further suggestions about the regulatory mechanisms have to consider the complex effects of ethanol on glutamate receptors with important consequences for the development of hyperexcitability during ethanol withdrawal (Faingold et al., 2000; Harris et al., 2003; Thomas and Morrisett, 2000). In this context, induced glutamate transporter expression leading to an augmented uptake of glutamate from the synaptic cleft and reduced availability of glutamate could conceivably provide a form of neuroprotective adaptation.

In contrast, during critical stages of brain development, enhanced expression of EAAT1 and EAAT2 can be interpreted as a maladaptive process that is involved in the etiology of FAS. Reduced levels of glutamate might not only reduce the synaptic activity of this main excitatory neurotransmitter, but also lead to an imbalanced guidance of neurons, because glutamate serves as a diffusible regulator of cell migration (Behar et al., 1999).

Admittedly, complex human pathology such as FAS cannot be completely mimicked in vitro. Nevertheless, current models of FAS pathogenesis state a blockade of NMDA-mediated neurotransmission and an excessive activation of GABA_A receptors (Olney, 2004). Our results fit the concept of reduced glutamatergic neurotransmission, because induced glutamate transporter expression can result in induced glutamate cleavage. However, these findings cannot easily be generalized to other species, developmental states, or brain regions; they are limited to the period of terminal differentiation of the rat cerebral cortex as monitored by a tissue culture approach. Data of reduced or unchanged glutamate transport, after alcohol exposure in adult animals or in ethanol-preferring animals (Devaud, 2001; Schreiber and Freund, 2000; Spanagel et al., unpublished data, 2004), should not be a surprise, because neuronal plasticity is often reduced in a completely developed animal. We concentrated on glutamate transporter expression and did not assess the glutamate transport functionally, because reports about cell culture approaches consistently describe induced transport as measured by radioactively labeled uptake assays. Other limitations of our study are that changes of mRNA stability and post-

translational modifications may exert additional effects on transcription and function of glutamate transporters.

In conclusion, ethanol induces EAAT1 and EAAT2 expression on the transcriptional and translational level in organotypic cortical slice cultures. This effect is significant for the terminal differentiation of the cerebral cortex with respect to the availability of glutamate and for the molecular correlates of FAS.

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REFERENCES

- Behar TN, Scott CA, Greene CL, Wen X, Smith SV, Maric D, Liu Q-Y, Colton CA, Barker JL (1999) Glutamate acting at NMDA receptors stimulates embryonic cortical neuronal migration. *J Neurosci* 19:4449–4461.
- Belmadani A, Neafsey EJ, Collins MA (2003) Human immunodeficiency virus type 1 gp120 and ethanol coexposure in rat organotypic brain slice cultures: curtailment of gp120-induced neurotoxicity and neurotoxic mediators by moderate but not high ethanol concentrations. *J Neurovirol* 9:45–54.
- Burd L, Klug MG, Martsolf JT (2004) Increased sibling mortality in children with fetal alcohol syndrome. *Addict Biol* 9:179–186.
- Butters NS, Reynolds JN, Brien JF (2003) Effects of chronic prenatal ethanol exposure on cGMP content and glutamate release in the hippocampus of the neonatal guinea pig. *Neurotoxicol Teratol* 25:59–68.
- Danbolt NC (2001) Glutamate uptake. *Progr Neurobiol* 65:1–105.
- Devaud LL (2001) Ethanol dependence has limited effects on GABA or glutamate transporters in rat brain. *Alcohol Clin Exp Res* 25:606–611.
- Diao L, Dunwiddie TV (1996) Interactions between ethanol, endogenous adenosine and adenosine uptake in hippocampal brain slices. *J Pharmacol Exp Ther* 278:542–546.
- Dobbing J, Sands J (1979) Comparative aspects of the brain growth spurt. *Early Hum Dev* 3:79–83.
- Faingold C, Li Y, Evans MS (2000) Decreased GABA and increased glutamate receptor-mediated activity on inferior colliculus neurons in vitro are associated with susceptibility to ethanol withdrawal seizures. *Brain Res* 868:287–295.
- Figiel M, Engele J (2000) Pituitary adenylate cyclase-activating polypeptide (PACAP), a neuron-derived peptide regulating glial glutamate transport and metabolism. *J Neurosci* 20:3596–3605.
- Furuta A, Rothstein JD, Martin LJ (1997) Glutamate transporter protein subtypes are expressed differentially during rat CNS development. *J Neurosci* 17:8363–8375.
- Gibson DA, Harris BR, Prendergast MA, Hart SR, Blanchard JA II, Holley RC, Pedigo NW, Littleton JM (2003) Polyamines contribute to ethanol withdrawal-induced neurotoxicity in rat hippocampal slice cultures through interactions with the NMDA receptor. *Alcohol Clin Exp Res* 27:1099–1106.
- Harris BR, Gibson DA, Prendergast MA, Blanchard JA, Holley RC, Hart SR, et al. (2003) The neurotoxicity induced by ethanol withdrawal in mature organotypic hippocampal slices might involve cross-talk between metabotropic glutamate type 5 receptors and N-methyl-D-aspartate receptors. *Alcohol Clin Exp Res* 27:1724–1735.
- Haydar TF, Bambrick LL, Krueger BK, Rakic P (1999) Organotypic slice cultures for analysis of proliferation, cell death, and migration in the embryonic neocortex. *Brain Res Brain Res Protoc* 4:425–437.
- Hundt W, Danysz W, Hölter SM, Spanagel R (1998) Ethanol and N-methyl-D-aspartate-receptor complex interactions: a detailed drug discrimination study in the rat. *Psychopharmacology* 135:44–51.

- Ikonomidou C, Bittigau P, Ishimaru MJ, Wozniak DF, Koch C, Genz K, et al. (2000) Ethanol-induced apoptotic neurodegeneration and fetal alcohol syndrome. *Science* 287:1056–1060.
- Ikonomidou C, Bosch F, Miksa M, Bittigau P, Vöckler J, Dikranian K, et al. (1999) Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain. *Science* 283:70–74.
- Lovinger DM, White G, Weight FF (1989) Ethanol inhibits NMDA-activated ion current in hippocampal neurons. *Science* 243:1721–1724.
- Molnar Z, Blakemore C (1999) Development of signals influencing the growth and termination of thalamocortical axons in organotypic culture. *Exp Neurol* 156:363–393.
- Olney JW (2004) Fetal alcohol syndrome at the cellular level. *Addict Biol* 9:137–149.
- Olney JW, Wozniak DF, Farber NB, Jevtovic-Todorovic V, Bittigau P, Ikonomidou C (2002) The enigma of fetal alcohol neurotoxicity. *Ann Med* 34:109–119.
- Olney JW, Wozniak DF, Jevtovic-Todorovic V, Ikonomidou C (2001) Glutamate signaling and the fetal alcohol syndrome. *Ment Retard Dev Disabil Res Rev* 7:267–275.
- Othman T, Sinclair CJ, Haughey N, Geiger JD, Parkinson FE (2002) Ethanol alters glutamate but not adenosine uptake in rat astrocytes: evidence for protein kinase C involvement. *Neurochem Res* 27:289–296.
- Perego C, Vanoni C, Bossi M, Massari S, Basudev H, Longhi R, Pietrini G, Pietrini G (2000) The GLT-1 and GLAST glutamate transporters are expressed on morphologically distinct astrocytes and regulated by neuronal activity in primary hippocampal cocultures. *J Neurochem* 75:1076–1084.
- Schreiber R, Freund WD (2000) Glutamate transport is downregulated in the cerebral cortex of alcohol-preferring rats. *Med Sci Monit* 6:649–652.
- Smith TL (1997) Regulation of glutamate uptake in astrocytes continuously exposed to ethanol. *Life Sci* 61:2499–2505.
- Smith TL, Navratilova E (1999) Increased calcium/calmodulin protein kinase activity in astrocytes chronically exposed to ethanol: influences on glutamate transport. *Neurosci Lett* 269:145–148.
- Smith TL, Zsigo A (1996) Increased Na⁺-dependent high affinity uptake of glutamate in astrocytes chronically exposed to ethanol. *Neurosci Lett* 218:142–144.
- Sutherland ML, Delaney TA, Noebels JL (1996) Glutamate transporter mRNA expression in proliferative zones of the developing and adult murine CNS. *J Neurosci* 16:2191–2207.
- Szot P, White SS, Veith RC, Rasmussen DD (1999) Reduced gene expression for dopamine biosynthesis and transport in midbrain neurons of adult male rats exposed prenatally to ethanol. *Alcohol Clin Exp Res* 23:1643–1649.
- Thomas MP, Monaghan DT, Morrisett RA (1998) Evidence for a causative role of N-methyl-D-aspartate receptors in an in vitro model of alcohol withdrawal hyperexcitability. *J Pharmacol Exp Ther* 287:87–97.
- Thomas MP, Morrisett RA (2000) Dynamics of NMDAR-mediated neurotoxicity during chronic ethanol exposure and withdrawal. *Neuropharmacology* 39:218–226.
- Torp R, Danbolt NC, Babaie E, Bjoras M, Seeberg E, Storm-Mathisen J, Ottersen OP (1994) Differential expression of two glial glutamate transporters in the rat brain: an in situ hybridization study. *Eur J Neurosci* 6:936–942.
- Zafar H, Shelat SG, Redei E, Tejani-Butt S (2000) Fetal alcohol exposure alters serotonin transporter sites in rat brain. *Brain Res* 856:184–192.