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Taurine-induced long-lasting potentiation in the rat hippocampus shows a partial dissociation from total hippocampal taurine content and independence from activation of known taurine transporters

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Abstract

Perfusion with high millimolar levels of taurine evoked a long-lasting potentiation (LLP-TAU) of synaptic transmission in the Schaffer-collateral CA1 region of the rat hippocampus. Although LLP-TAU showed some correlations to increases in the total taurine content of hippocampal slices, it could not be blocked by the taurine transport inhibitor guanidinoethanesulfonic acid (GES), which was able to significantly reduce total slice taurine uptake. Inhibition of GABA transport by either nipecotic acid or β-guanidinopropionate failed to abolish LLP-TAU and had no significant effect on taurine uptake. The combination of GES and nipecotic acid also had no significant effect on LLP-TAU. Experiments with transportable structural analogs of taurine (β-aminoisobutyric acid, homotaurine, and isethionic acid) suggest that activation of classical taurine transport pathways does not always yield a robust LLP-TAU. Hippocampal LLP-TAU could be significantly attenuated, however, by pre-incubation with submillimolar levels of taurine. In summary, the development of LLP-TAU in the rat hippocampus appears to be associated with the intracellular accumulation rather than the activation of known transporters of taurine, but the precise means of its accumulation remains to be identified.

Keywords: GABA, hippocampus, potentiation, rat, taurine, transporters.

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Taurine (2-aminoethanesulfonic acid) is accumulated to millimolar levels in the brain (Palkovits *et al*. 1986) and appears to play an important role in a variety of the brain's physiological processes: osmoregulation (Pasantes-Morales *et al*. 2000), membrane stabilization (Huxtable 1989), calcium homeostasis (Foos and Wu 2002), and neuromodulation (Huxtable 1989; Hussy *et al*. 2001). Taurine's neuroactive properties have led to speculation that it may be a neurotransmitter (Kuriyama *et al*. 1983). Although taurine has yet to meet all of the criteria of a classical neurotransmitter, it nonetheless exhibits an unusual, divergent neurochemistry. Acutely, taurine shows neuroinhibitory properties as a partial agonist at $GABA_A$ and glycine receptors (Haas and Hosli 1973; Horikoshi *et al*. 1988; Hussy *et al*. 1997, 2001). Prolonged exposure to millimolar concentrations, however, produces an increase in synaptic function that is not strictly dependent on taurine's actions at inhibitory ionotropic receptors (Galarreta *et al*. 1996; del Olmo *et al*. 2000; Chepkova *et al*. 2002; Sergeeva *et al*. 2003). This long-lasting potentiation of synaptic transmission has been termed LLP-TAU (Galarreta *et al*. 1996).

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Abbreviations used: ACSF, artificial cerebrospinal fluid; βAIB, β-aminoisobutyric acid; βGPA, β-guanidinopropionic acid; EFP, evoked field potentials; fEPSP, excitatory postsynaptic potential; GAT, GABA transporter; GES, guanidinoethanesulfonic acid; HTAU, homotaurine; ISA, isethionic acid; LLP, long-lasting potentiation; NPA, nipecotic acid; OPA, o-pthalaldehyde; PCA, perchloric acid; RP-HPLC, reversed-phase high-performance liquid chromatographic; SR, stratum radiatum; TAU, taurine; TAUT, taurine transporter.

LLP-TAU has been demonstrated in the glutamatergic pathways of both hippocampal (Galarreta *et al*. 1996; del Olmo *et al*. 2000, del Olmo *et al*. 2003; Sergeeva *et al*. 2003) and corticostriatal slices (Chepkova *et al*. 2002; Sergeeva *et al*. 2003). There are two prevailing hypotheses for the proximate mechanism behind LLP-TAU induction. The first, based on data from rat hippocampal slices, centers on the electrogenic nature of sodium-dependent amino acid transport (Holopainen *et al*. 1990; Loo *et al*. 1996; Barakat *et al*. 2002). Accordingly, mass activation of taurine transporters may produce sufficient depolarization to activate low voltage-activated Ca^{2+} channels and initiate a cascade of Ca^{2+}-dependent events that in turn results in enhanced synaptic efficiency (del Olmo *et al*. 2000). Indeed, other taurine transport system substrates, like β-alanine and GABA, induce a long-lasting potentiation while poorly transported analogs do not (Galarreta *et al*. 1996). The second hypothesis posits an intracellular mechanism independent of plasma membrane transporter activation. In murine hippocampal slices LLP-TAU was blocked by nipecotic acid (NPA), a competitive inhibitor and translocatable substrate of GABA transporters (GATs; Sergeeva *et al.* 2003). NPA alone was unable to elicit significant long-lasting potentiation, suggesting that GAT activation is not related to LLP-TAU induction. The apparent discrepancy between these two hypotheses may be partially explained by a poor understanding of the mechanisms of hippocampal taurine uptake in rats and mice.

In brain slices, taurine uptake shows two saturable components – a high-affinity/low-capacity and low-affinity/high-capacity system (Huxtable 1989) – and a non-saturable component (Lahdesmaki and Oja 1973; Kontro and Oja 1983; Oja and Saransaari 1996). The saturable high-affinity component is thought to be mediated by transporters exclusive to taurine and its β-amino acid homologs. Two such transporters have been cloned, TAUT-1 and TAUT-2 (Liu *et al*. 1992; Smith *et al*. 1992). In the rat hippocampus, TAUT-1 protein is not detectable, whereas TAUT-2 protein is expressed in the CA1 region (Pow *et al*. 2002). In the mouse hippocampus, TAUT-1 protein is limited to the CA3 region (Sergeeva *et al*. 2003); whether TAUT-2 protein is expressed in the mouse hippocampus is not known. Equally unclear is the identity of the low-affinity component of taurine transport. Both non-saturable transport (Oja and Saransaari 1996) – possibly diffusion – and GABA transporters (Sivakami *et al*. 1992; Liu *et al*. 1993) have been implicated. Of the four GAT subtypes cloned from the rodent brain, only GAT-3 (rat) and GAT-4 (mouse) have been shown capable of transporting taurine (Liu *et al*. 1993). In the rat hippocampus, GAT-3 protein is either non-detectable or weakly expressed (Ikegaki *et al*. 1994; Ribak *et al*. 1996). Localization of GAT4 protein expression in murine hippocampus has yet to be shown, though its mRNA is diffusely found throughout this structure (Evans *et al*. 1996).

In this study, we have attempted to further elucidate the dependence of LLP-TAU on intracellular taurine content and taurine transport in the Schaffer-collateral CA1 region of the rat hippocampus. We report that LLP-TAU is partially associated with an increase in intracellular taurine content. Gradually raising whole-slice TAU levels with submillimolar concentrations of taurine, in fact, produces an attenuation of the LLP-TAU caused by subsequent exposures to high extracellular taurine. A significant fraction of hippocampal taurine uptake is sensitive to high-affinity taurine transport inhibition, but the development of LLP-TAU is not. In contrast to findings in mice, neither LLP-TAU nor taurine transport is attenuated by GAT inhibitors. Finally, activation of known taurine transport pathways with taurine structural analogs does not always result in a long-lasting enhancement of postsynaptic responses.

Materials and methods

Preparation of hippocampal slices

Experiments were conducted on transverse hippocampal slices (400 μm) obtained from 3- to 5-month-old Sprague-Dawley rats (Harlan, Indianapolis, IN, USA). Rats were anesthetized with 2-bromo-2-chloro-1,1,1-trifluoroethane (Halothane, Halocarbon, River Edge, NJ, USA) and quickly decapitated. Brains were rapidly removed and briefly (< 30 s) immersed in ice-cold artificial cerebrospinal fluid (ACSF) composed of (in mM): 125 NaCl, 3.3 KCl, 1.25 KH_2PO_4, 1.0 $MgSO_4$, 20 $NaHCO_3$, 2.0 $CaCl_2$, and 10 [D]-glucose saturated with a gas mixture of 95% O_2 and 5% CO_2. Hippocampi were subsequently dissected from surrounding tissue and cut into 400-μm thick transverse slices using a McIlwain tissue chopper. Slices were separated in pre-gassed ACSF and then incubated in a glass holding chamber at room temperature (21–24°C). After an equilibration period of at least 1 h (total time spent in the holding chamber ranged from 1 to 7 h with no observable change in response viability), a slice was transferred to a submersion-type recording chamber (Warner Instruments, Hampden, CT, USA), covered with a nylon tissue harp, and continuously perfused at a flow rate of 2.8–3.0 mL/min with ACSF saturated with 95% O_2/5% CO_2. ACSF in the recording chamber was maintained at 28–30°C.

Electrophysiological recordings

Prior to each recording, a concentric bipolar stimulating electrode (stainless steel OP: 200 μm; Pt/Ir IP: 25 μm; Frederick Haer & Co, Bowdoinham, ME, USA) was placed in the stratum radiatum (SR) of CA1 for stimulation of Schaffer collateral-commissural fibers. To record evoked field potentials (EFP), a borosilicate glass micropipette filled with 4 M NaCl was placed in the SR approximately 200–600 μm from the stimulating electrode. Synaptic responses were elicited by the delivery of 50 μs constant current biphasic square at 0.05 Hz. Typical EFPs consisted of two negative deflections: a presynaptic fiber volley followed by a population excitatory postsynaptic potential (fEPSP; see Fig. 1f for an illustration of these components on a representative EFP trace). To control for between-slice variations in excitability, test currents were applied to each slice prior to recording to establish the maximum amplitude

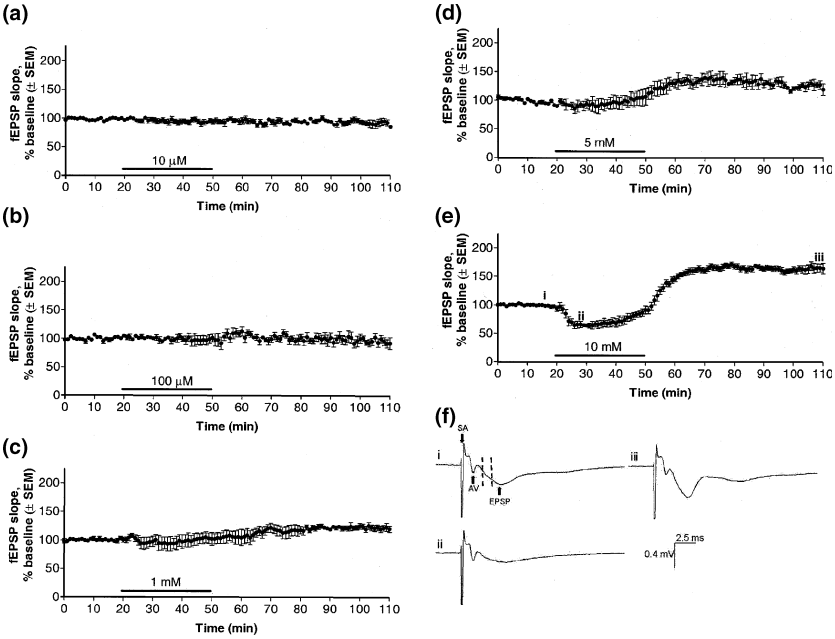

Fig. 1 The effects of a 30-min perfusion with varying doses of taurine (TAU) on hippocampal field fEPSP responses. Tested doses of TAU: (a) 10 μM ($n = 5$), (b) 100 μM ($n = 4$), (c) 1 mM ($n = 4$), (d) 5 mM ($n = 4$), (e) 10 mM ($n = 9$). Black bar indicates the period of TAU application to the recording chamber. (f) Average field potentials (average of five traces) taken from different time points during the course of a representative experiment using a 10 mM TAU perfusion concentration. Time points are identified by lower case Roman numerals and correspondingly indicated in (e). Salient features of the evoked field potential are marked in the top trace: stimulus artifact (SA), afferent volley (AV), population excitatory postsynaptic potential (EPSP), and the time intervals used for slope calculation (dashed lines).

fEPSP at the given electrode positions. Stimulus intensity was adjusted accordingly to yield a postsynaptic field component with a slope 50% that of the maximal response. Stimulus currents ranged 0.06–0.2 mA and average baseline fEPSP slope for control experiments was 0.27 ± 0.006 mV/ ms. Stability of the fEPSP response was evaluated by comparing the first 5 min of baseline recording against the last 5 min of baseline recording. Experiments were conducted using slices that showed a baseline drift of less than 10% as determined by this comparison.

Data acquisition and analysis

Signals were recorded using a Grass P-511 preamplifier, filtered at 10 kHz and digitized at 20 kHz by a Digidata 1322A analog interface (Axon Instruments, Union City, CA, USA) using the program Clampex 9.0 (Axon Instruments) on a Dell Dimension 4500 computer. fEPSP slopes were calculated off-line as the ratio of voltage and time differences between time points 10 and 90% along the rising phase of each individual fEPSP.

All electrophysiological data were normalized with respect to the mean fEPSP slope collected within the first 5 min of baseline recording initialization. For the purposes of presentation clarity, three consecutive, normalized responses (1-min recording) were averaged to yield a single data point. Results are expressed as means ± SEM relative to baseline (100%).

Drugs and solutions

Drugs were applied to the perfusion solution via a syringe pump. Stock solutions were freshly prepared prior to each experiment at a concentration 50× greater than final desired bath concentration. The syringe pump speed (3.3–3.5 mL/h) was adjusted accordingly to achieve the necessary dilution. β-aminoisobutyric acid, β-guanidinopropionic acid, homotaurine, isethionic acid, nipecotic acid, and taurine were obtained from Sigma (St Louis, MO, USA). Guanidinoethanesulfonic acid was a gift from Dr Ryan Huxtable (University of Arizona).

Analysis of slice TAU content using reversed-phase high-performance liquid chromatographic (RP-HPLC)

Following electrophysiological recording, tissue not part of the hippocampus proper was carefully removed from the slice. Slices were then quickly washed with room temperature ACSF, placed in ice-cold 0.2 M perchloric acid (PCA) with 200 mg/L EDTA, and homogenized. An aliquot of the homogenized suspension was removed for protein analysis as outlined by the Bradford method (Bradford 1976) using bovine serum albumin as a protein standard. Homogenates were centrifuged to yield a protein-free PCA supernatant that was separated from the pellet and frozen at −20°C until preparation for amino acid analysis.

Amino acid analysis of the PCA supernatant was conducted by pre-column o-phthalaldehyde (OPA) derivatization and RP-HPLC coupled with electrochemical detection using a previously described method (Dawson and Wallace 1992). Briefly, an aliquot of PCA supernatant was diluted with 0.1 M sodium tetraborate buffer (pH = 10.0) and derivatized with o-phthalaldehyde and mercaptoethanol. Isocratic separation of the derivatized amino acids was accomplished on a C18 analytical column (3 μm particle size, 4.6 mm internal diameter × 10.0 mm length; Varian, Walnut Creek, CA, USA) using a mobile phase composed of 73% 0.1 mM sodium phosphate dibasic buffer with 0.13 mM EDTA (pH = 6.0) and 27% methanol. OPA-derivatized amino acids were detected by electrochemical detection (oxidation potential = +0.750V vs. Ag/AgCl reference electrode) at a sensitivity of 20 nA and quantified against external standards. Amino acid values for each slice were normalized by total protein content and expressed as means ± SEM.

Statistical analysis

Statistical differences were analyzed by ANOVA followed by an appropriate post test (Dunnet's or Newman-Keuls) or by Student's *t*-test (two-tailed) using Prism 3 (GraphPad Software Inc., San Diego, CA, USA).

Results

Relationship of taurine perfusion concentration to evoked field responses and total intracellular taurine content

Effects of taurine concentration on fEPSPs

Taurine's dual ability to depress synaptic field responses and facilitate their long-term enhancement is illustrated Fig. 1. The most robust demonstration of these effects was seen with 10 mM taurine. Bath application of 10 mM taurine initially elicited a marked depression in the fEPSP component of evoked field potentials (fEPSP depression to 65.3 ± 2.0%, $n = 9$, 10–15 min after introduction of 10 mM taurine). This depression was effectively abolished by either 30 μM bicuculline methiodide or 100 μM picrotoxin (data not shown). At concentrations lower than 10 mM, taurine perfusion did not evoke any observable depression of postsynaptic responses.

fEPSP slope increased during the last 5 min of the 10 mM taurine perfusion, resulting in a partial recovery (87.9 ± 5.9%, $n = 9$) towards pre-application levels by the end of the taurine perfusion period. No such trend was observed at lower taurine concentrations. Washout of taurine was associated with a non-linear dose-dependent increase in fEPSP slope that reached a maximum 20 min after cessation of taurine perfusion. The greatest enhancement of fEPSP slope 20–25 min after washout was observed following 10 mM taurine perfusion (165.1 ± 4.5%, $n = 9$). The potentiation achieved by 10 mM taurine persisted with negligible decay over the remainder of the washout period (166.7 ± 6.7%, $n = 9$, 55–60 min after washout). In fact, perfusion with 10 mM taurine yielded a stable potentiation that persisted for at least 2 h (162.4 ± 12.5%, $n = 2$). Unlike the taurine-induced depression, potentiation persisted in the presence of either 30 μM bicuculline methiodide or 100 μM picrotoxin (data not shown). Relative to the 10 mM taurine perfusion, smaller enhancements in fEPSP slope were seen following perfusions with 5 mM (135.4 ± 9.5%, $n = 4$, 20–25 min after washout; 127.1 ± 6.6%, $n = 4$, 55–60 min after washout) and 1 mM (116.2 ± 9.1%, $n = 4$, 20–25 min after washout; 120.9 ± 5.3%, $n = 4$, 55–60 min after washout). No sustainable potentiation was observed following perfusion with either 100 or 10 μM taurine.

Effects of taurine concentration on slice taurine content

Following a 110-min control perfusion, the intracellular taurine content of hippocampal slices was 32.5 ± 6.9 nmol/mg protein ($n = 4$) as measured by HPLC. This value represented a ~50% drop relative to the taurine content of freshly dissected hippocampi (66.1 ± 14.8 nmol/mg protein, $n = 4$). The taurine content of hippocampal slices increased in a non-linear dose-dependent fashion following 30 min of taurine perfusion (Table 1).

Table 1 Effects of the indicated treatment perfusions on the total taurine (TAU) content of hippocampal slices following a 60-min washout

Experiment/treatment	Taurine content 60 min after indicated treatment washout (nmol/mg protein)
Taurine dose response	
Control (0 mM TAU)	32.5 ± 6.9[bd] ($n = 4$)
10 μM TAU	34.2 ± 4.0[bd] ($n = 5$)
100 μM TAU	76.6 ± 17.2[ad] ($n = 4$)
1 mM TAU	87.2 ± 28.1[ad] ($n = 4$)
5 mM TAU	247.6 ± 41.5[c] ($n = 4$)
10 mM TAU	349.3 ± 29.6 ($n = 9$)
TAU uptake inhibition studies	
1 mM GES/10 mM TAU	145.0e ± 8.7 ($n = 5$)
1 mM NPA/10 mM TAU	338.4 ± 46.6 ($n = 5$)
1 mM βGPA/10 mM TAU	419.6 ± 66.6 ($n = 4$)
1 mM NPA/1 mM GES/10 mM TAU	501.0 ± 68.1 ($n = 4$)
TAU structural analogs	
10 mM βAIB	31.4 ± 8.6 ($n = 4$)
10 mM HTAU	35.1 ± 10.3 ($n = 4$) (HTAU content: 368.4 ± 88.5)
10 mM ISA	28.7 ± 9.4 ($n = 4$)
5–7-h pre-incubation with 100 μm TAU	
Control slices (no additional TAU perfusion)	134.6 ± 17.4 ($n = 8$)
10 mm TAU perfused slices	441.8 ± 66.8 ($n = 8$)

Significant differences between the effects of various concentrations of taurine or taurine structural analogs were determined by analysis of variance and Neuman-Keuls post test ([a]$p < 0.01$ vs. 5 mm TAU, [b]$p < 0.001$ vs. 5 mm TAU; [c]$p < 0.05$ vs. 10 mm TAU, [d]$p < 0.001$ vs. 10 mm TAU). Significant differences between taurine uptake in the absence or presence of inhibitors were determined by analysis of variance and Dunnet's post test using 10 mm taurine perfusion in the absence of inhibitors as the control ([e]$p < 0.05$ vs. 10 mm).

Pharmacological inhibition of taurine transporter systems and its effects on fEPSP slope and slice taurine content

Guanidinoethane sulfonate (GES) is a widely used competitive inhibitor of high-affinity taurine transport (Huxtable *et al*. 1979; Liu *et al*. 1992). GES was perfused 10 min prior to and during the standard 30-min 10 mM taurine application (Fig. 2a). At a concentration of 1 mM, GES had no effect on the maintenance of LLP-TAU 55–60 min after washout (156.9 ± 21.3%, $n = 5$, vs. 166.7 ± 6.7% with 10 mM taurine alone, $n = 9$, $p > 0.05$). However, relative to slices perfused with taurine alone, the taurine content of slices exposed to both GES and taurine was significantly ($p < 0.05$) reduced by 58.6% (Table 1). By itself, 10 mM GES produced fEPSP depression that was comparable in magnitude to 10 mM taurine (mean depression to 56.8 ± 6.6%, $n = 3$, 10–15 min after introduction of 10 mM GES; Fig. 3a). In contrast to taurine, 10 mM GES alone produced only a slight, sustainable potentiation of fEPSP (113.0 ± 7.1%, $n = 3$, 55–60 min after washout).

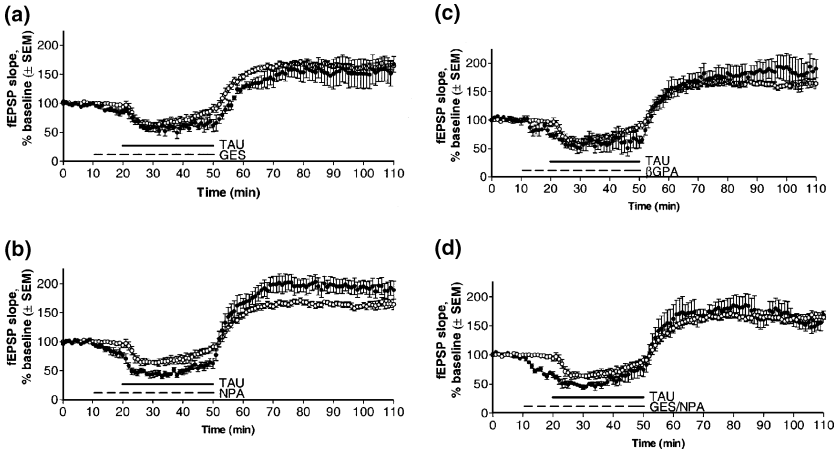

Fig. 2 Pharmacological blockade of putative TAU transporters and its effects on fEPSP slope during the course of a 30-min 10 mM TAU perfusion. (a) Competitive blockade of high-affinity taurine transporters with 1 mM GES ($n = 5$). (b) Competitive blockade of GABA transporters with 1 mM NPA ($n = 5$), a broad spectrum GAT antagonist. (c) Taurine-sensitive GABA transporters were blocked with 1 mM βGPA ($n = 4$). (d) Competitive inhibition of both TAU and GABA transporters with a combination of 1 mM GES and 1 mM NPA ($n = 4$). Open circles, experiments with 10 mM taurine alone. Closed circles, experiments with 10 mM taurine and indicated transport blocker. Perfusion of the transporter antagonist is indicated by a dashed bar while TAU perfusion is represented by a solid bar.

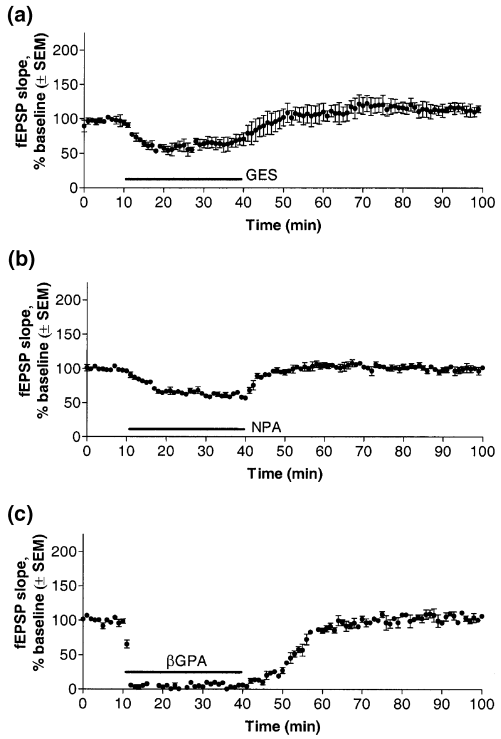

Fig. 3 Evaluation of commonly used competitive inhibitors of GABA and TAU transporters for their effects on evoked fEPSP slope in the absence of exogenously perfused taurine. (a) 10 mM GES ($n = 3$), (b) 1 mM NPA ($n = 3$) and (c) 10 mM βGPA ($n = 3$).

NPA and β-guanidinopropionic (βGPA), two competitive inhibitors of GATs, were examined for their effects on LLP-TAU induction. NPA is a broad spectrum GAT inhibitor, albeit with higher affinity for taurine insensitive GABA transporters, while βGPA shows high specificity for the taurine sensitive GABA transporters GAT-3 and GAT-4 (Liu *et al.* 1993). Both inhibitors were applied 10 min before and during the standard 30-min taurine perfusion (Figs 2b and c). At a concentration of 1 mM, NPA failed to attenuate the magnitude of sustainable LLP-TAU 55–60 min after washout (191.1 ± 13.7%, $n = 5$, vs. 166.7 ± 6.7% with 10 mM TAU alone, $n = 9$, $p > 0.05$). However, it did produce a significant enhancement of synaptic depression (average fEPSPs falling to 41.2 ± 6.5% ($n = 5$) of baseline vs. 61.7 ± 5.3% with 10 mM TAU alone, $n = 9$, $p < 0.05$) during taurine perfusion and a larger initial enhancement of fEPSPs (200.3 ± 14.6%, $n = 5$, vs. 165.1 ± 4.5% with 10 mM TAU alone, $n = 9$, $p < 0.05$) 20 min after washout of taurine and NPA. Perfusion of 1 mM NPA alone for 30 min depressed fEPSPs to 60.7 ± 0.7% ($n = 3$) in the last 5 min of perfusion. Recovery to baseline levels was achieved after 20–25 min of washout (103.1 ± 4.0%, $n = 3$; Fig. 3b) and remained stable for at least another 40 min of washout (99.6 ± 4.3%, $n = 3$). Blockade with 1 mM βGPA did not cause any significant effect on the development of LLP-TAU by 10 mM taurine (186.0 ± 21.6%, $n = 4$, $p > 0.05$; Fig. 2c). Thirty minutes of perfusion with 10 mM βGPA alone produced a complete abrogation of fEPSPs (1.6 ± 8.3%, $n = 3$; Fig. 3c) followed by a return to baseline levels after 20–25 min of perfusion (105.3 ± 7.6%, $n = 3$) that was maintained until the end of a 60-min washout (103.5 ± 7.0%, $n = 3$). Not surprisingly, unlike GES, neither NPA nor βGPA had any significant effect on taurine sequestration in these slices (Table 1).

Bath application of 1 mM GES and 1 mM NPA 10 min prior to and concurrent with a 30-min 10 mM taurine

perfusion had no significant effect on the development of LLP-TAU (152.4 ± 10.8%, $n = 4$, vs. 166.7 ± 6.7% with 10 mM taurine alone, $n = 9$, $p > 0.05$; Fig. 2d). Although GES alone was able to significantly reduce total taurine uptake, the combination of GES and NPA had no effect on the total uptake of taurine (Table 1).

Transportable taurine structural analogs and their ability to induce long-lasting potentiation

We decided to further evaluate the hypothesis that activation of taurine transporters underlies the induction of LLP-TAU by using unconventional taurine structural analogs. These analogs, illustrated in Fig. 4, display divergent affinities for the various components of the taurine transport system.

β-aminoisobutyric acid (3-amino-2-methyl-propanoic acid, AIB) effectively antagonizes high-affinity taurine uptake (Trachtman *et al.* 1992). Like taurine, perfusion with 10 mM βAIB produced a depression in fEPSP slope (79.7 ± 10.8%, $n = 4$; Fig. 5a). Additionally, βAIB produced an enhancement in fEPSP slope (143.9 ± 8.1%, $n = 4$, $p < 0.05$ vs. baseline responses) that was comparable with that seen with 10 mM taurine 55–60 min after washout (143.9 ± 8.1%, $n = 4$ vs. 166.7 ± 6.7% with 10 mM TAU alone, $n = 9$, $p > 0.05$).

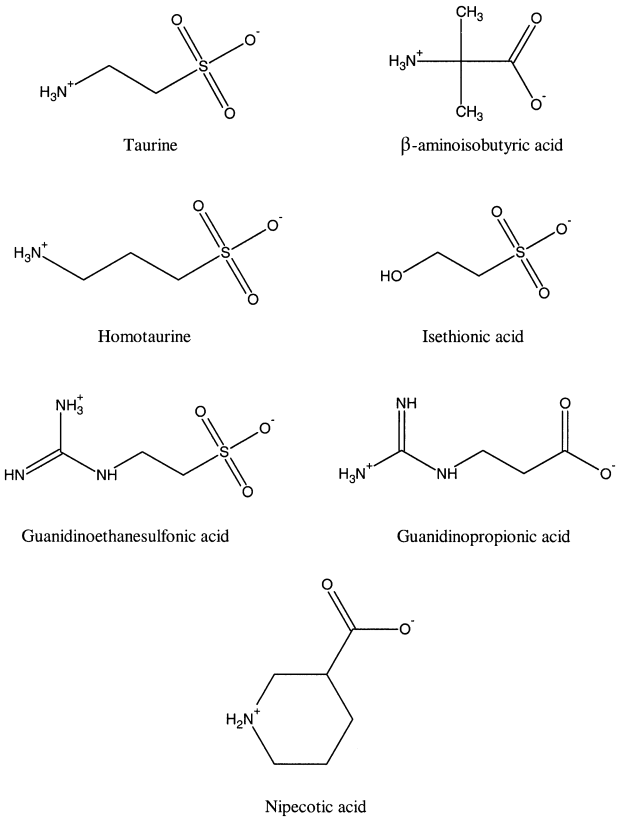

Fig. 4 Structural depictions of the taurine isomers evaluated in this study.

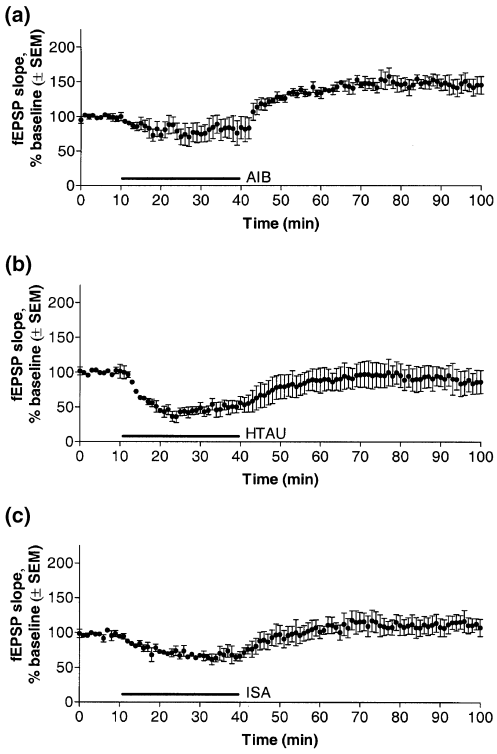

Fig. 5 Testing of additional taurine structural analogs to evaluate their ability to mimic the effects of taurine on fEPSP slope. (a) Thirty minutes of 10 mM βAIB perfusion ($n = 4$). (b) Thirty minutes of 10 mM HTAU perfusion ($n = 4$). (c) Thirty minutes of 10 mM ISE perfusion ($n = 4$).

Homotaurine (3-aminopropanesulfonic acid, HTAU) is also capable of competing for high-affinity taurine binding (Smith *et al.* 1992; Petegnief *et al.* 1995). Bath perfusion with 10 mM HTAU depressed fEPSP slopes to 41.6 ± 6.2% ($n = 4$) 10–15 min after the start of perfusion (Fig. 5b). The magnitude of this depression was significantly greater than that achieved by 10 mM taurine (41.6 ± 6.2%, $n = 4$ vs. 65.3 ± 2.0%, $n = 9$, with 10 mM taurine, $p < 0.001$). Following HTAU washout, fEPSP slope gradually returned towards baseline levels with no evidence of potentiation after 55–60 min into the washout period (86.7 ± 17.3%, $n = 4$, $p > 0.05$ vs. baseline responses). Because HTAU was resolvable by our chromatographic separation, we could quantify the amount present in the slices. Indeed, HPLC analysis revealed that HTAU was taken up during the course of the HTAU perfusion. Absolute levels of this amino acid (368.4 ± 88.5 nmol/mg protein) were similar to those seen with taurine following a perfusion with 10 mM taurine (Table 1). HTAU was not detectable in control slices (< 2.0 nmol/mg protein).

Isethionic acid (2-hydroxyethanesulfonic acid, ISA) is transported into rat brain by a mechanism that is non-saturable and passive (Ikaheimo *et al.* 1982). This process is not mutually exclusive to taurine transport, however, as ISA

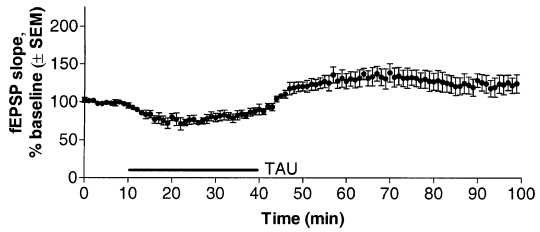

Fig. 6 Effect of 5–7-h pre-incubation with 100 μM taurine (TAU) on fEPSP slopes following subsequent exposure to a 30-min 10 mM TAU perfusion ($n = 6$). Solid bar, 10 mM TAU perfusion.

non-competitively blocks taurine uptake at high taurine concentrations. Analagous to taurine, fEPSP slopes were depressed following bath application of 10 mM ISA (71.2 ± 3.6%, $n = 3$; Fig. 5c). Perfusion with 10 mM ISA for 30 min, however, did not induce significant potentiation 55–60 min after washout (104.3 ± 10.2%, $n = 3$, $p > 0.05$ vs. baseline responses).

Prolonged incubation with 100 μM taurine attenuates LLP-TAU induced by 10 mM taurine

Significant attenuation of LLP-TAU could be attained with 5–7-h pre-incubation with 100 μM taurine (123.0 ± 11.4%, $n = 6$, vs. 166.7 ± 6.7% with 10 mM TAU alone, $n = 9$, $p < 0.01$; Fig. 6). Of the six slices tested, LLP-TAU was completely blocked in two slices (91.4 ± 1.3%) and greatly reduced in the remaining four (138.8 ± 8.7%). The attenuation of LLP-TAU was not due to a change in viability associated with the length of incubation; slices incubated in taurine-free ACSF for 5–7 h displayed robust LLP-TAU (161.2 ± 9.6%, $n = 3$) 60 min after the standard 10 mM taurine perfusion. The magnitude of taurine-induced depression was not significantly affected by pre-incubation with 100 μM TAU (74.8 ± 6.0%, $n = 6$, $p > 0.05$). Other electrophysiological parameters, such as threshold stimulus intensities and maximal fEPSP slope, were not significantly affected by the pre-incubation (data not shown).

Prolonged incubation with 100 μM taurine significantly increased intracellular taurine content relative to slices not exposed to taurine (134.6 ± 17.4 nmol/mg protein, $n = 8$ vs. 32.5 ± 6.9 nmol/mg protein, $n = 4$, $p < 0.05$; Table 1). After adjusting for the increase in taurine associated with pre-incubation, the total amount of taurine uptake by these slices following exposure to 10 mM taurine for 30 min (average = 307.0 nmol/mg protein) was comparable with those not exposed to a prolonged incubation with 100 μM taurine (average = 316.5 nmol/mg protein).

Discussion

Our investigation of LLP-TAU within the rat Schaffer collateral-CA1 pathway has uncovered several unique aspects of both LLP-TAU and taurine transport within the hippocampus. Although the intracellular accumulation of taurine is coincident with the development of LLP-TAU, there is no clear relationship between the induction of LLP-TAU and intracellular taurine concentration or the actual process of taurine uptake. Moreover, we have found that activation of classical transport pathways does not always appear to be sufficient for the induction of LLP-TAU and pharmacological blockade of them does not significantly inhibit it. A diagram illustrating the significance of these findings in relationship to previous proposed mechanisms of LLP-TAU induction is shown in Table 2.

If LLP-TAU is a consequence of intracellular taurine sequestration, then it might be expected that a positive association exists between taurine uptake and degree of LLP-TAU. In this study, there was a positive association between the magnitude of sustainable LLP-TAU and total taurine uptake by the hippocampal slice. However, this relationship was not strictly linear (Fig. 7). Perfusion with high concentrations of taurine was generally associated with an increase in both the degree of attainable potentiation as well as the total taurine content. Deviation from this trend, however, was seen following perfusion with either 100 μM or 1 mM taurine. Both concentrations resulted in equivalent intracellular taurine levels, but significant enhancement of evoked fEPSP slope occurred only with 1 mM taurine. The lack of a completely linear relationship between these two parameters may be due to the fact that our amino acid analysis represents the average intracellular taurine content across many different cellular populations within the hippocampus and is not expressly limited to those of the Schaffer collateral-CA1 region. Consequently, cells within this region could show taurine transport kinetics that differ from surrounding tissues, but the disparity would be lost in the final average of the analysis. Overall, however, the dose–response data may be used to argue for a partial association between intracellular taurine accumulation and LLP-TAU. Additionally, the dose–response data indirectly argue against a role for TAUTs in LLP-TAU. With a K_m ranging between 4 and 40 μM (Liu *et al*. 1992; Smith *et al*. 1992), the activity of TAUTs would be expected to be near saturation at the low millimolar perfusion concentrations.

It is important to note that not all substrates of traditionally defined taurine transport systems were capable of eliciting a clearly defined LLP-TAU, consistent with the idea that the activation of transporters per se does not cause LLP-TAU. The distant β-amino acid structural analog of taurine, βAIB, produced a sustainable enhancement of evoked fEPSP responses. GES, in keeping with the observation of Galarreta *et al*. (1996), produced a much lower degree potentiation. HTAU and ISA, in contrast, displayed no discernable potentiation. The HPLC method used in this study could not detect GES or ISA, but our ability to detect HTAU definitely confirmed that at least this amino acid was being taken up to the same extent as taurine. Presumably, if the

Table 2 A comparison of evidence both for and against the proposed mechanisms of hippocampal LLP-TAU as gleaned from existing studies and this investigation

Proposed mechanism of hippocampal LLP-TAU	Previous evidence	Results of this study
Activation of taurine transport systems	1. Dependence upon extracellular taurine and sodium concentration as well as bath perfusion temperature (Galarreta *et al.* 1996). 2. Magnitude of potentiation caused by taurine analogs (β-alanine, GABA, GES, N-methyl taurine) correlated with affinity for taurine transporters (Galarreta *et al.* 1996). 3. Electrogenic nature of taurine transport (Halopainen *et al.* 1990; Loo *et al.* 1996; Barakat et al. 2002) 4. Blockade of LLP-TAU by 50 μm nickel, inhibitor of low threshold Ca^{2+} channels (del Olmo *et al.* 2000)	Not all transportable taurine analogs were capable of inducing LLP-TAU. In particular, HTAU was accumulated to substantial levels in slices but did not elicit potentiation. Conclusion: analog experiments suggest that activation of taurine transporters is not sufficient for induction of LLP-TAU.
Intracellular taurine concentration	1. Dependence upon extracellular taurine and sodium concentration as well as bath perfusion temperature (Galarreta *et al.* 1996). 2. Blockade of GABA transporters (putative low-affinity taurine transporters) with NPA inhibited LLP-TAU in murine hippocampus. Activation of GABA transporters with NPA alone, however, did not imitate LLP-TAU.	1. At perfusion concentrations > 1 mm, intracellular taurine levels of hippocampus correlated with degree of LLP-TAU. 2. Although GES attenuated taurine uptake, it had no effect on LLP-TAU. GABA transport inhibitors had no effect on taurine uptake nor LLP-TAU. 3. Slowly raising intracellular taurine levels with submillimolar taurine incubation attenuated LLP-TAU. Conclusion: although intracellular taurine concentration is more closely associated with LLP-TAU induction than transporter activation, imperfect reconciliations remain.

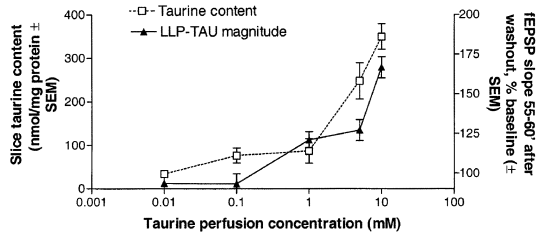

Fig. 7 A comparative summary of the effect of TAU perfusion concentration on total slice taurine content and LLP-TAU.

only requisite for LLP-TAU induction is the activation of TAU transporters, then it should have occurred following HTAU application. The absence of any discernable LLP-TAU accompanying exposure to 10 mM HTAU could mean that, unlike other cell types of the hippocampus, those in the CA1 region contain a transport system that recognizes TAU and its β-amino acid homologs but not HTAU as translocatable substrates. Alternatively, HTAU could be accumulated by the principal cells of the CA1 region but not act with the same potency as TAU at an unidentified intracellular target for LLP-TAU induction.

GES blocked taurine uptake by almost 60%. This suggests that TAUT-2, which shows a high degree of sensitivity to GES (Liu *et al.* 1992) and is the only high-affinity taurine transporter expressed in the rat hippocampus (Pow *et al.* 2002), is a quantitatively important contributor to hippocampal taurine accumulation. Despite its ability to attenuate taurine uptake – to levels only slightly higher than those achieved by 1 mM taurine perfusion – GES did not produce a significant alteration in the development of LLP-TAU. In some elements of the hippocampus, including the CA1 region, Pow *et al.* (2002) have shown a discordant association between intracellular taurine accumulation and TAUT-2 expression, i.e. cells appeared to accumulate taurine in the absence of detectable TAUT-2 expression. Other systems of taurine accumulation therefore might exist in the hippocampus and could conceivably contribute to LLP-TAU induction.

Because experiments with GES eliminated the proximal involvement of known high-affinity taurine transporters in LLP-TAU, we next examined the potential contribution of GABA transporters. Although they are low-affinity taurine transporters, GABA transporters could play an important role in light of the high concentrations of taurine required to elicit a robust potentiation. Indeed, Sergeeva *et al.* (2003) have recently reported a GES-resilient form of LLP-TAU in the Schaffer collateral-CA1 pathway of murine hippocampus that could be completely abolished by the broad-spectrum GABA transporter antagonist NPA at a concentration of 1 mM. In this study, however, 1 mM NPA failed to block the

development of LPP-TAU and showed no significant effect on taurine uptake. Instead, NPA increased the taurine-induced depression of postsynaptic responses and produced a significant initial enhancement of synaptic transmission following washout that later decayed to levels slightly above those seen with taurine alone. The cause for these two effects may be due to the ability of NPA to increase extracellular GABA levels (Blume *et al*. 1981; Solis and Nicoll 1992). GABA released by NPA may activate $GABA_A$ receptors and NPA may also directly activate $GABA_A$ receptors (Barrett-Jolley 2001), explaining the additional depression of postsynaptic activity in the presence of taurine. As for the enhanced potentiation, Galarreta *et al*. 1996) previously demonstrated that 10 mM GABA perfusion could induce a sustainable potentiation (LLP-GABA) with a similar time course to LLP-TAU. Given their similar time courses, the proximate mechanism behind LLP-GABA induction was presumed to be the same as LLP-TAU. Although NPA alone did not produce a sustainable potentiation in this study, it cannot be ruled out that GABA released by NPA would have an additive interaction with the taurine perfusion to produce the observed initial enhancement of LLP-TAU. Moreover, this additive effect could conceivably mask any attenuation of true LLP-TAU. Divorcing LLP-TAU and LLP-GABA pharmacologically is difficult given how little is definitely known about their respective mechanisms of induction. However, the initial enhancement of LLP-TAU caused by NPA disappeared in the presence of GES. Although commonly used as a high-affinity taurine transport antagonist, GES has also been shown to be an inhibitor of GABA uptake in cortical synaptosome preparations (Li and Lombardini 1990). Presuming that intracellular entry of GABA is the cause of the initial NPA-induced enhancement of LLP-TAU and that GES blocks it, simultaneous use of both compounds suggests that NPA does not diminish true LLP-TAU. The possibility of an interaction between NPA and taurine transport remains, however, as NPA was able to interfere with the ability of GES to attenuate taurine uptake. The mechanism for this phenomenon is at present unknown.

Because of the shortcomings of NPA as a non-specific GABA transporter antagonist and its low affinity for GAT3 (Liu *et al*. 1993), βGPA was tested. Despite its ability to specifically inhibit GABA transporters that transport taurine (Liu *et al*. 1993), it had no significant effect on LLP-TAU. Furthermore, it produced no significant change in taurine uptake. Taken together, these results suggest that taurine permeable GABA transporters do not play an important role in either the induction of LLP-TAU or total hippocampal taurine transport in the rat, even under conditions of high extracellular taurine.

The failure of GABA transporter antagonists to block taurine uptake is not entirely unexpected given the paucity of GAT-3 protein expression within the CA1 region of the rat hippocampus (Ikegaki *et al*. 1994) and the generally weak immunoreactivity of GAT-3 across the hippocampus as a whole (Ikegaki *et al*. 1994; Ribak *et al*. 1996). However, it is not clear why GABA transport antagonists block LLP-TAU in the hippocampus of mice but not rats. This difference, combined with the fact that the forebrains of mice contain approximately 1.6 times the amount of taurine as rats (Puka *et al*. 1991), suggests that these species may have disparate mechanisms and pathways of taurine accumulation. There may be, for instance, differences in GAT subtype expression between these two species. Comparative studies, however, suggest similar distributions of taurine sensitive GABA transporters in the hippocampi of adult mice and rats (Jursky *et al*. 1994; Evans *et al*. 1996; Jursky and Nelson 1999). The basis of this interspecies difference in taurine sequestration awaits further exploration.

Chepkova *et al*. (2002) have previously shown that pre-incubation with 1 mM TAU blocks LLP-TAU in murine corticostriatal preparations by saturation of LLP-TAU. We demonstrate that a similar phenomenon, albeit of lower magnitude, occurs in rat hippocampal slices following pre-incubation with a 10-fold lower dose of taurine. More importantly, we show that this is associated with an increase in whole-slice taurine content caused by the pre-incubation. Several possibilities exist to explain this observed attenuation: attainment of near maximal potentiation caused by the pre-incubation, desensitization of taurine uptake, or desensitization of some element in the LLP-TAU pathway that is independent of taurine transport. The maximal fEPSP slope of slices pre-incubation with 100 μM did not differ significantly from control slices, suggesting that pre-potentiation is not a major contributor. Similarly, desensitization of taurine transport does not appear to be an important causal factor as taurine pre-incubation produced no significant change in net uptake following subsequent taurine exposure. Therefore, it appears that the pre-incubation period desensitized an unknown uptake-independent component(s) of the LLP-TAU induction pathway to subsequent rapid increases in intracellular TAU accompanying the 10-mM perfusion. Again, this observation indirectly argues for the intracellular taurine accumulation rather than taurine transport as a being closely linked to LLP-TAU.

The physiological relevance of LLP-TAU is an important question that has been posed several times before. Outside of extreme pathological conditions that liberate intracellular taurine stores, such as ischemia or anisosmotic shock, the brain's interstitial spaces do not achieve the high extracellular taurine concentrations required to rapidly elevate intracellular taurine levels in *in vitro* slices over a 30 min perfusion period and induce LLP-TAU. It is interesting to note, however, that hippocampal slices show a significant depletion of intracellular taurine relative to *in situ* tissues. The traumatizing process of *in vitro* slice preparation creates an environment that favors taurine depletion by combining substantial

mechanical injury with prolonged exposure to nominally taurine-free ACSF (Hamberger *et al*. 1982; Olson *et al*. 2003). Repletion of intracellular taurine stores to near 'normal' levels had an effect on synaptic transmission, as revealed by the attenuation of LLP-TAU by pre-incubation with 100 μM TAU or the modest potentiation induced by a 1-mM taurine perfusion. This could mean that the synaptic strengths of slices is lower than those found in *in vivo* circuits, partly as a consequence of lower taurine content. A similar situation has been documented for choline, another diffusible micronutrient that also undergoes significant depletion as a result of slice preparation. The reintroduction of choline into hippocampal slice preparations at physiologically relevant concentrations has been shown to have subtle albeit widespread effects on neuronal activity (Uteshev *et al*. 2003). Additional work remains, however, to prove that taurine has a role in synaptic transmission at physiologically relevant concentrations. Research needs to be continued at the cellular level to resolve whether LLP-TAU is the product of a novel electrogenic transport process or a specific intracellular target of action.

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