AUTHOR QUERY FORM

ELSEVIER	**Journal: YNBDI** **Article Number: 2113**	**Please e-mail or fax your responses and any corrections to:** **E-mail: corrections.essd@elsevier.spitech.com** **Fax: +1 61 9699 6721**

Dear Author,

Any queries or remarks that have arisen during the processing of your manuscript are listed below and highlighted by flags in the proof. Please check your proof carefully and mark all corrections at the appropriate place in the proof (e.g., by using on-screen annotation in the PDF file) or compile them in a separate list.

For correction or revision of any artwork, please consult http://www.elsevier.com/artworkinstructions.

Articles in Special Issues: Please ensure that the words 'this issue' are added (in the list and text) to any references to other articles in this Special Issue.

Uncited references: References that occur in the reference list but not in the text – please position each reference in the text or delete it from the list.
Missing references: References listed below were noted in the text but are missing from the reference list – please make the list complete or remove the references from the text.

Location in article	Query / remark Please insert your reply or correction at the corresponding line in the proof
Q1	USA was provided as country name. Please check.
Q2	'sISPCs' was changed to 'sIPSCs' for consistency. Please check.
Q3	'23º' was changed to '23 °C'. Please check.

Electronic file usage
Sometimes we are unable to process the electronic file of your article and/or artwork. If this is the case, we have proceeded by:

☐ Scanning (parts of) your article ☐ Rekeying (parts of) your article ☐ Scanning the artwork

Thank you for your assistance.

Neurobiology of Disease xxx (2010) xxx–xxx

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

Increase of $GABA_A$ receptor-mediated tonic inhibition in dentate granule cells after traumatic brain injury

Zakaria Mtchedlishvili [a,b,*], Eka Lepsveridze [d], Hong Xu [a], Elena A. Kharlamov [a,b], Bo Lu [a], Kevin M. Kelly [a,b,c]

[a] Center for Neuroscience Research, Allegheny-Singer Research Institute, Allegheny General Hospital, Pittsburgh, PA, USA
[b] Department of Neurology, Drexel University College of Medicine, Philadelphia, PA, USA
[c] Department of Neurobiology and Anatomy, Drexel University College of Medicine, Philadelphia, PA, USA
[d] Ilia Chavchavadze State University, Faculty of Life Sciences, Tbilisi, Georgia

ARTICLE INFO

Article history:
Received 11 November 2009
Revised 10 March 2010
Accepted 10 March 2010
Available online xxxx

Keywords:
Traumatic brain injury
Dentate granule cells
$GABA_A$ receptor
Diazepam
Furosemide
THIP
Bicuculline
mIPSCs
Synaptic currents
Tonic currents

ABSTRACT

Traumatic brain injury (TBI) can result in altered inhibitory neurotransmission, hippocampal dysfunction, and cognitive impairments. GABAergic spontaneous and miniature inhibitory postsynaptic currents (sIPSCs and mIPSCs) and tonic (extrasynaptic) whole cell currents were recorded in control rat hippocampal dentate granule cells (DGCs) and at 90 days after controlled cortical impact (CCI). At 34 °C, in CCI DGCs, sIPSC frequency and amplitude were unchanged, whereas mIPSC frequency was decreased (3.10 ± 0.84 Hz, $n = 16$, and 2.44 ± 0.67 Hz, $n = 7$, $p < 0.05$). At 23 °C, 300 nM diazepam increased peak amplitude of mIPSCs in control and CCI DGCs, but the increase was 20% higher in control (26.81 ± 2.2 pA and 42.60 ± 1.22 pA, $n = 9$, $p = 0.031$) compared to CCI DGCs (33.46 ± 2.98 pA and 46.13 ± 1.09 pA, $n = 10$, $p = 0.047$). At 34 °C, diazepam did not prolong decay time constants (6.59 ± 0.12 ms and 6.62 ± 0.98 ms, $n = 9$, $p = 0.12$), the latter suggesting that CCI resulted in benzodiazepine-insensitive pharmacology in synaptic $GABA_A$ receptors ($GABA_A$Rs). In CCI DGCs, peak amplitude of mIPSCs was inhibited by 100 μM furosemide (51.30 ± 0.80 pA at baseline and 43.50 ± 5.30 pA after furosemide, $n = 5$, $p < 0.001$), a noncompetitive antagonist of $GABA_A$Rs with an enhanced affinity to α4 subunit-containing receptors. Potentiation of tonic current by the $GABA_A$R δ subunit-preferring competitive agonist THIP (1 and 3 μM) was increased in CCI DGCs (47% and 198%) compared to control DGCs (13% and 162%), suggesting the presence of larger tonic current in CCI DGCs; THIP (1 μM) had no effect on mIPSCs. Taken together, these results demonstrate alterations in synaptic and extrasynaptic $GABA_A$Rs in DGCs following CCI.

Introduction

Cognitive deficits are among the most enduring and frequently reported impairments following traumatic brain injury (TBI) (Levin et al., 1979; Hall and Bornstein, 1991), which can also result in depression, posttraumatic stress disorder, and posttraumatic epilepsy (PTE). TBI results in complex pathophysiology that involves cascades of short- and long-term changes at subcellular and cellular levels. The hippocampus is particularly vulnerable to TBI (DeRidder et al., 2006; Pullela et al., 2006; Saatman et al., 2006; Tran et al., 2006; Bonislawski et al., 2007), and can undergo long-term changes in its physiological function and contribute to alterations in neurotransmission. $GABA_A$ receptor ($GABA_A$R)-mediated inhibition is critical for keeping local circuit excitatory activity under homeostatic control and is intimately involved in the regulation of cognitive processes, especially in learning and memory (Castellano et al., 1993; Collinson et al., 2002; Das et al., 2004). $GABA_A$R-mediated signaling can be either potentiated or decreased in different animal models of TBI. Enhancement of paired-pulse inhibition was described in the dentate gyrus in the fluid percussion injury (FPI) model in rat (Reeves et al., 1997) and in the controlled cortical impact (CCI) model in mouse (Hunt et al., 2009). However, efficacy of GABA was reported to be reduced in dentate granule cells (DGCs) after FPI due to functional alterations in the chloride/potassium pump (Bonislawski et al., 2007).

Little is known about long-term alterations of postsynaptic $GABA_A$Rs in DGCs after head trauma. GABA regulates synchronous neuronal oscillations that are critical for cognitive functions such as object perception, selective attention and working memory, and spatial memory (Engel and Singer, 2001; Buzsaki and Draguhn, 2004), as well as consciousness (Llinas and Ribary, 2001). Thus, disruptions in GABAergic signaling after head trauma are very likely to have an impact on memory and cognitive capacities. Positive allosteric modulators of $GABA_A$Rs impair memory processing (Arolfo and Brioni, 1991; Mayo et al., 1993; Kant et al., 1996; Krazem et al., 2001; Johansson et al., 2002; Silvers et al., 2003; Turkmen et al., 2006), whereas $GABA_A$R blockers or inverse agonists often potentiate cognitive and memory performance (Brioni and McGaugh, 1988; Raffalli-Sebille et al., 1990; Mayo et al.,

* Corresponding author. Allegheny General Hospital, 9J9 ST., 320 East North Avenue, Pittsburgh, PA 15212, USA. Fax: +1 412 359 6847.
E-mail address: zmtchedl@wpahs.org (Z. Mtchedlishvili).
Available online on ScienceDirect (www.sciencedirect.com).

0969-9961/$ – see front matter © 2010 Published by Elsevier Inc.
doi:10.1016/j.nbd.2010.03.012

1992; Dahhaoui et al., 1994; Atack et al., 2006; Collinson et al., 2006; Dawson et al., 2006). Alterations in $GABA_AR$-mediated transmission are also among key factors that result in hyperexcitability in the hippocampus and subsequent epileptogenesis (Otis et al., 1994; Gibbs et al., 1997; Brooks-Kayal et al., 1998; Nusser et al., 1998; Shumate et al., 1998; Pirker et al., 2003).

$GABA_ARs$ are members of a ligand-gated ion channel superfamily assembled from subunits of seven different classes: α(1–6), β(1–3), γ(1–3), δ, ε, θ, and π (Sieghart and Sperk, 2002). They maintain two forms of inhibition: tonic and phasic. Tonic inhibition results from activation of extrasynaptic $GABA_ARs$ by low concentrations of ambient GABA and is strongly represented in DGCs (Rossi and Hamann, 1998; Stell and Mody, 2002; Mody and Pearce, 2004). Conversely, phasic (synaptic) inhibition is mediated by activation of postsynaptic receptors by saturating concentrations of vesicular GABA. Tonic inhibition in DGCs is mediated by α4 and δ subunit-containing, high-affinity, slowly desensitizing, extrasynaptically located $GABA_ARs$ (Peng et al., 2002; Wei et al., 2003), whereas phasic inhibition in DGCs is mediated by α1 and γ2 subunit-containing, rapidly desensitizing, synaptically located receptors with low affinity for GABA (Sun et al., 2004; Mangan et al., 2005). Because TBI is often followed by degenerative changes in the hippocampus, cognitive disturbances, and epileptogenesis, we sought to study potential chronic changes in tonic and phasic $GABA_AR$-mediated inhibition in DGCs after TBI. Rats were subjected to CCI and electrophysiological changes were studied *in vitro* using hippocampal slice preparations from control and brain-injured animals.

Materials and methods

CCI model of TBI and surgery

All procedures involving animals were approved by the Institutional Animal Care and Use Committee of the Allegheny-Singer Research Institute (ASRI) and were carried out according to NIH guidelines and regulations. Animals were housed individually in the ASRI vivarium, maintained in a 12 hour light/12 hour dark cycle environment with controlled temperature (23 ± 2 °C), and food and water were given *ad libitum*. The CCI procedure was performed according to Dixon et al. (1991) with some modifications. Briefly, male Sprague–Dawley rats (2–3-mo old) were anesthetized with an initial dose of 4% isoflurane mixed with oxygen and positioned in a stereotaxic frame (David Kopf Instruments, Tujunga, CA). The concentration of isoflurane was reduced to 2–3% after the animals were deeply anesthetized. Body temperature was monitored throughout the procedure using a rectal probe and maintained at 37 ± 2 °C with a heating pad (Harvard Apparatus). A craniectomy was performed over the right parietal cortex within the boundaries of bregma and lambda while leaving the dura intact. A 1.975-cm-diameter pneumatic impactor, attached to a double-acting, stroke-constrained, pneumatic cylinder with a 5.0 cm stroke (Pittsburgh Precision Instruments, Pittsburgh, PA) was used to deliver CCI. The cylinder was rigidly mounted in a vertical position on a crossbar, which was adjusted in the vertical axis over the animal's head. The animal's head was secured in the stereotaxic frame with ear bars, and the impactor was positioned 4–5 mm laterally to the longitudinal line. The lower rod end had an attached 5.5-mm impactor tip (i.e., that part of the shaft that comes into contact with the exposed dura mater). The upper rod end was attached to the transducer core of a linear variable differential transformer. The impactor velocity was adjusted to 4 m/s, the impact duration was 100 ms, and the depth of tissue depression was 2.8 mm. Following CCI and the cessation of cortical bleeding, the scalp was sutured, and the animal was returned to the vivarium for recovery. Animals were sacrificed 90 days after CCI for electrophysiological and histological studies.

Video monitoring

A subset of CCI-injured animals underwent continuous video monitoring for one week prior to sacrifice to assess for possible behavioral seizure activity. Animals were housed individually in multiple monitoring chambers in satellite vivaria and maintained on the 12 hour light/12 hour dark cycle. Animal behavior was monitored by closed-circuit television cameras, with and without infrared capability, that were connected to video splitter units (Advanced Technology Video, Inc., Redmond, Washington). Digital video files (Diva, Stellate Systems) were recorded directly to high capacity hard disk drives using removable hard drive bays. The recordings were reviewed visually offline and played at 2× speed to detect any behavioral seizure activity according to a standard classification scale (Racine, 1972).

Electrophysiology

Brains were dissected free and immersed in cold (2–4 °C) artificial cerebrospinal fluid (ACSF) saturated with 95% O_2–5% CO_2. The ACSF consisted of the following (in mM): 127.0 NaCl, 2.0 KCl, 1.5 $CaCl_2$, 1.5 $MgSO_4$, 25.7 $NaHCO_3$, 1.1 KH_2PO_4, and 10.0 glucose (osmolarity, 300–305 mOsm). The brains were mounted on a vibratome stage (Camden Instruments, UK) and 350-μm-thick coronal sections were cut. Slices were maintained in continuously oxygenated ACSF at 32 °C in a holding chamber for a minimum of 30–45 min and then at room temperature (23 °C) in a recording chamber mounted on the stage of a Nikon FN1 microscope (Tokyo, Japan) equipped with a 64× water-immersion objective, infrared differential interference contrast optics, and video. For recordings at 34 °C, the bath temperature was maintained by an SH-27B inline solution heater and TC-324B temperature controller (Warner Instruments, Hamden, CT). Patch electrodes (final resistances of 4–6 MΩ) were pulled from borosilicate glass (Garner Glass, Clairmont, CA) on a horizontal Flaming–Brown microelectrode puller (model P-97; Sutter Instruments, Novato, CA) using a two-stage pull protocol. Electrode tips were filled with a filtered internal recording solution consisting of the following (in mM): 153.3 CsCl, 1.0 $MgCl_2$, 10.0 HEPES, 5.0 EGTA, 3.0 ATP Mg^{2+} salt, and 0.1 GTP Na^+ salt, pH 7.2, (with CsOH; osmolarity 285–295 mOsm). The extensive injury to the hippocampus ipsilateral to the CCI-subjected hemisphere made the slices containing the ipsilateral dentate gyrus unsuitable for patch-clamp recordings. For this reason, the recordings were obtained from the lower blade of the dentate gyrus from the contralateral hemisphere. 50 μM DL-2-amino-5-phosphonovaleric acid (AP-5) and 20 μM 6,7-dinitroquinoxaline-2,3-dione (DNQX) were included in ACSF for all recordings to block glutamatergic transmission. The membrane-impermeable Na^+ channel blocker N-(2,6-dimethylphenylcarbamoylmethyl) triethylammonium bromide (QX-314) in the final concentration of 10 μM was included in the internal solution to block Na^+ channels from the inside of the clamped cells thereby preventing the cells from escaping the clamp during the recordings of spontaneous inhibitory postsynaptic currents (sIPSCs) and tonic currents. Tetrodotoxin (TTX; 1 μM) was included in the ACSF to block voltage-gated Na^+ channels in the slice for the recordings of miniature inhibitory postsynaptic currents (mIPSCs). QX-314 was omitted from the internal solution for recordings of mIPSCs. Diazepam was dissolved in DMSO to a 5 mM concentration (first stock solution) and diluted to 50 μM with ACSF (second stock solution) on the day of an experiment. The second stock was than diluted to a 300 nM final concentration in one of the slice perfusion reservoirs. Furosemide was dissolved to a 100 mM stock solution in methanol, and was dissolved to a final 100 μM concentration on the day of an experiment. All salts were obtained from Sigma (St. Louis, MO). All other reagents used in this study were obtained from Tocris Bioscience (Ellisville, MO). Control and CCI DGCs were visually identified and voltage-clamped to −70 mV under

Please cite this article as: Mtchedlishvili, Z., et al., Increase of $GABA_A$ receptor-mediated tonic inhibition in dentate granule cells after traumatic brain injury, Neurobiol. Dis. (2010), doi:10.1016/j.nbd.2010.03.012

isotonic chloride conditions ($E_{Cl} = 0$ mV) with a Multiclamp 700B amplifier (Molecular Devices, Palo Alto, CA). Whole cell capacitance and series resistance were compensated by 80%. Recordings were performed when series resistance after compensation was 20 MΩ or less. Access resistance was monitored with 10 ms, −4 mV test pulses, delivered once every 60 s. If at any time during the experiment the series resistance increased by 25%, the recording was terminated and the data were not used for statistical analysis. Current traces were filtered at 5 kHz, digitized at 10 kHz using a Digidata 1442 digitizer, and acquired on a computer hard drive using pClamp 10.1 software (Molecular Devices).

Measurement of synaptic currents

sIPSCs and mIPSCs were analyzed with Mini Analysis software (Synaptosoft, Leonia, NJ). The detection threshold was set at five times of root mean square. After detection, peak amplitude, frequency, and decay time constants were analyzed. Following the point of peak amplitude, the 0 to 90% region of current decay was assessed by 100 iterations of each event by group analysis for best curve fit. Decay time constants of mIPSCs were fit as a monoexponential function, with Mini Analysis software, using a simplex-based algorithm. Approximately 500 individual events were used from each recording for analysis of peak amplitudes and frequency of sIPSCs and mIPSCs, and decay time constants of mIPSCs.

Measurement of tonic currents

To measure tonic inhibition, the 30-s epoch immediately before application of the drug (baseline) and a 30-s epoch after 3 min of continuous perfusion with a drug were compared. The built-in Gaussian function of ClampFit (Axon Instruments) was used to determine Gaussian fits in the raw traces (entire segments with sIPSCs present). All-point frequency distribution histograms using a 2-pA bin size were derived from these 30-s epochs.

The comparisons of obtained Gaussian means of all-point histogram distributions were performed with GraphPad Prism 4 (Mountain View, CA). Gaussian means (mean baseline current) were used as measures of tonic current. In control DGCs, Gaussian fits of all-point histograms were compared to histograms derived from the same epochs with synaptic currents removed by a custom-designed algorithm in MATLAB (data not shown). No significant difference was found between the Gaussian means of all-point histograms and those derived from the epochs with synaptic events removed. Therefore, we deemed it sufficient to use all-point histograms to study tonic currents. Each DGC was treated as a nested unit for statistical considerations. One DGC was recorded per slice to avoid potential contamination of a drug used in a previous recording in the same slice.

Statistical considerations

To validate the significance of the effects of diazepam and furosemide on peak amplitudes of mIPSCs, cumulative probability (fraction) distributions of peak amplitudes at baseline (prior to drug application) and during continuous application of the drug were compared with the Kolmogorov–Smirnov (KS) test for each DGC using the built-in KS analysis option in Mini analysis software (Synaptosoft, Leonia, NJ). All DGCs tested with the KS test demonstrated non-Gaussian distributions of mIPSC peak amplitudes. Cumulative fraction distributions of mIPSC peak amplitudes also were compared between control and CCI DGCs. Means of median amplitudes, mean frequencies, and mean decay time constants were compared with the two-tailed t test ± standard error of the mean (SEM). Whole cell capacitance comparisons were performed with the Mann–Whitney test ± SEM. Statistical significance was set at $p < 0.05$.

Perfusion and preparation of tissue sections

At 90 days post-CCI, some animals were deeply anesthetized and transcardially perfused with phosphate buffered saline (PBS; pH 7.4), followed by 4% paraformaldehyde solution. The brains were removed, post-fixed in the same solution, and cryoprotected in 30% sucrose. Gross examination of the brains was performed to confirm the presence of TBI. Forty-micron-thick coronal and horizontal sections were cut on a cryostat, collected, and stored in cryoprotectant solution (phosphate buffer/ethylene glycol/glycerol) at −20 °C. Standard histological (Nissl) techniques were used for subsequent identification of brain cytoarchitecture and morphological changes following CCI.

Nissl staining

Sections were washed in PBS, mounted on gelatin-coated slides, and air-dried overnight at room temperature. Slides were dehydrated, stained with 0.5% cresyl violet solution, rinsed in distilled water, dehydrated again through graded alcohols and xylene, and covered with Permount and glass coverslips.

Results

General considerations

Twenty-three CCI-injured and 34 non-injured (control) animals were used in the experiments. CCI performed in our laboratory produces a severe brain injury associated with an ~1% mortality rate during the first 24 h (unpublished data); there was no additional mortality before animal sacrifice at 90 days after CCI. Of the 23 injured animals used in the study, 11 animals were monitored continuously for a one-week period prior to sacrifice and subsequent voltage-clamp recordings; control animals were not monitored. A total of 1918 h of video recordings was obtained during these recording sessions, of which 1078 h (924 h lights on/154 h lights off) were of adequate technical quality for review. No seizure was detected in any animal, similar to historical video-EEG recordings of control Sprague–Dawley rats of similar age (Kharlamov et al., 2003). Fig. 1 shows a representative rat brain (Fig. 1A) and structural and morphological changes in the hippocampus with Nissl (Figs. 1B,C) 90 days after CCI.

Properties of synaptic receptors in DGCs following CCI

DGCs receive powerful inhibitory input from GABAergic interneurons, which form predominantly perisomatic synapses on DGCs (Traub and Miles, 1991; Halasy and Somogyi, 1993; Buhl et al., 1994; Buckmaster and Schwartzkroin, 1995; Soltesz and Mody, 1995; Miles et al., 1996). Decreased feed-forward inhibition in the dentate gyrus has been reported within days after FPI and has been attributed to loss of hilar GABAergic interneurons (Toth et al., 1997). It is important to note that previous studies have focused on relatively short-term effects of TBI on GABAergic transmission in the hippocampus, typically assessing tissue one to a few weeks post-TBI. However, neurological consequences of head trauma, such as cognitive impairments, posttraumatic stress disorder, and PTE often manifest themselves after months or even years following the initial insult. To investigate long-term effects of TBI on GABAergic transmission, whole cell patch-clamp recordings of sIPSCs and mIPSCs were performed in rat control and CCI DGCs 90 days after lesioning. Slices were superfused with oxygenated ACSF that contained 50 μM DL-APV and 20 μM DNQX to block excitatory transmission. Comparison of frequency and peak amplitude of sIPSCs in control and CCI DGCs did not reveal significant changes. The frequency of sIPSCs was 4.95 ± 1.27 Hz in control ($n = 27$ DGCs, 6 animals), and 5.58 ± 1.96 Hz in CCI DGCs ($n = 16$ DGCs, 4 animals, $p = 0.93$, two-tailed t test). Within the same group of cells, the means

Please cite this article as: Mtchedlishvili, Z., et al., Increase of $GABA_A$ receptor-mediated tonic inhibition in dentate granule cells after traumatic brain injury, Neurobiol. Dis. (2010), doi:10.1016/j.nbd.2010.03.012

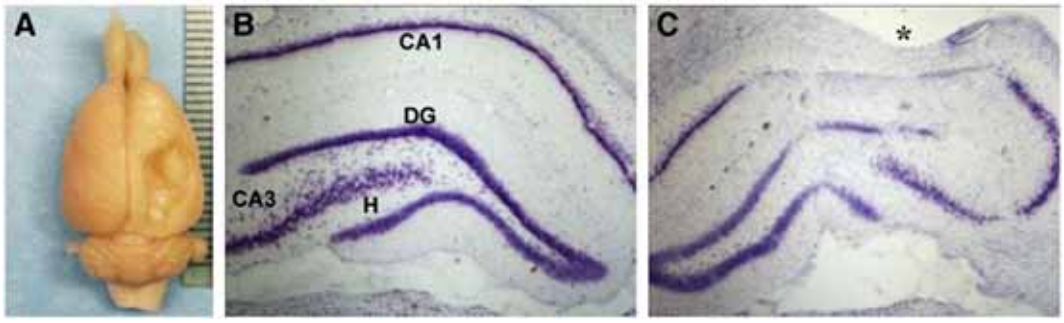

Fig. 1. Brain morphology 90 days following CCI. (A) A perfused brain of an adult rat demonstrates a loss of cortical tissue and a necrotic cavity at the impact site. (B,C) A representative Nissl-stained coronal section shows evidence of cell loss in the ipsilateral hippocampus (C) in CA1, CA3, and dentate gyrus compared to the contralateral side (B). An asterisk (*) marks the necrotic cavity at the cortical impact site (C). Abbreviations: H: hilus; DG: dentate granule cell layer.

of median amplitudes were compared. The mean of median amplitude of sIPSCs was 60.7 ± 2.74 pA in control, and 58.5 ± 5.17 pA in CCI DGCs ($p = 0.91$, two-tailed t test). Because apparent individual sIPSCs can be multiple summated events due to a high probability of synchronous release of transmitter, it is difficult to reliably interpret the absence of amplitude and decay time changes of sIPSCs after CCI as a lack of postsynaptic effect. To further test the possibility that properties of synaptic currents might have been altered by CCI, mIPSCs were recorded in DGCs in the presence of 1 μM TTX in slices containing the dentate gyrus from control and CCI-injured animals. TTX was used to block voltage-gated Na^+ channels and thereby block action potential-evoked release of the transmitter. mIPSCs in DGCs are generated by action potential-independent quantal release of transmitter from presynaptic GABAergic terminals (Edwards et al., 1990). Therefore, alterations of amplitude and decay time constants of mIPSCs can be interpreted as postsynaptic effects.

The timing and strength of synaptic transmission are profoundly dependent on temperature, which affects the rate of exocytosis (Micheva and Smith, 2005), vesicle pool depletion (Kushmerick et al., 2006), and probability of vesicle release (Hardingham and Larkman, 1998; Volgushev et al., 2004). At room, but not at physiological temperature, $GABA_A$R affinity to GABA can increase in many types of hippocampal and cortical neurons by allosteric modulation (Perrais and Ropert, 1999; Hájos et al., 2000). To determine whether the recording temperature could alter possible effects of CCI on mIPSCs, recordings were conducted at 23 °C and near-physiological (34 °C) temperatures and are presented in Table 1.

At 23 °C, the frequency of mIPSCs was significantly decreased in DGCs of CCI animals (1.03 ± 0.18 Hz, $n = 9$, 4 control animals, and 0.67 ± 0.09 Hz, $n = 10$, 4 CCI animals; $p < 0.05$, two-tailed t test), in agreement with a previous finding of decreased frequency of mIPSCs in the FPI model of TBI at room temperature (Toth et al., 1997). No differences were found between means of medians of peak amplitudes (26.81 ± 2.20 pA in control, $n = 9$, and 33.46 ± 2.98 pA in CCI DGCs, $n = 10$, $p = 0.16$, two-tailed t test), as well as in decay time constants in the same neurons (5.12 ± 0.29 ms in control, $n = 9$, and 5.48 ± 0.03 ms in CCI DGCs, $n = 10$, $p = 0.48$, two-tailed t test).

Recordings at 34 °C revealed a decrease of mIPSC frequency in the CCI group (3.10 ± 0.84 Hz in control DGCs, $n = 16$, 3 animals, and 2.44 ± 0.67 Hz in CCI DGCs, $n = 7$, 2 animals, $p < 0.05$, two-tailed t test). Comparison of means of median amplitudes of mIPSCs within the same group of cells did not demonstrate an alteration of peak amplitude in the CCI group (62.10 ± 0.73 pA in control, $n = 16$, and 53.10 ± 0.59 pA in CCI DGCs, $n = 7$, $p = 0.09$, two-tailed t test). An increase of peak amplitudes of mIPSCs in both groups of cells at 34 °C compared to 23 °C was in agreement with a previous finding that increasing temperature from room to near-physiological temperature causes an increase of chord conductance of the $GABA_A$R and increased amplitude of mIPSCs (Perrais and Ropert, 1999). Within the same group of control and CCI DGCs, we did not detect a significant change of decay time constants (5.97 ± 0.07 ms in control, $n = 12$, and 6.59 ± 0.12 ms in CCI DGCs, $n = 9$, $p = 0.19$, two-tailed t test).

Loss of diazepam potentiation of synaptic current decay, and decreased amplitude potentiation after CCI

In DGCs, $GABA_A$ α1 and γ2 subunit-containing receptors are preferentially localized in synapses, whereas α4 and δ subunit-containing $GABA_A$Rs are preferentially localized in the extrasynaptic membrane where they mediate tonic inhibition (Nusser et al., 1995; Sur et al., 1999; Stell and Mody, 2002; Wei et al., 2003; Sun et al., 2004; Mtchedlishvili and Kapur, 2006; Glykys et al., 2008). The γ2 subunit plays a key role in clustering of $GABA_A$Rs (Essrich et al., 1998; Kneussel

Table 1
Summary of parameters of mIPSCs and effects of diazepam in control and CCI DGCs.

mIPSCs	Temperature	Control			CCI		
		Baseline	300 nM diazepam	n	Baseline	300 nM diazepam	n
Frequency (Hz)	23 °C	1.03 ± 0.18[a]	0.96 ± 0.19	9	0.67 ± 0.09[a]	1.21 ± 0.21	10
	34 °C	3.10 ± 0.84[a]	3.59 ± 1.22	16	2.44 ± 0.67[a]	1.42 ± 0.27	7
Peak amplitude (pA)	23 °C	26.81 ± 2.20[b]	42.60 ± 1.22[b]	9	33.46 ± 2.98[b]	46.13 ± 1.09[b]	10
	34 °C	62.10 ± 0.73	59.85 ± 0.67	16	53.10 ± 0.59[b]	36.06 ± 7.09[b]	7
Decay time (ms)	23 °C	5.12 ± 0.29[c]	6.59 ± 0.08[c]	9	5.48 ± 0.03	5.61 ± 0.08	10
	34 °C	5.97 ± 0.07[c]	7.22 ± 0.19[c]	12	6.59 ± 0.12	6.62 ± 0.98	9

The values represent mean ± S.E.M. n indicates number of cells. $p < 0.05$, two-tailed t test.
[a] Indicates significant differences in frequency of mIPSCs at 23 °C and 34 °C under baseline conditions in control and CCI groups.
[b] Indicates significant differences in peak amplitude of mIPSCs at 23 °C in control and CCI DGCs before and after 300 nM diazepam, and at 34 °C in CCI DGCs before and after 300 nM diazepam.
[c] Indicates significant differences in decay time constants of mIPSCs at 23 °C and 34 °C in control DGCs before and after 300 nM diazepam.

Please cite this article as: Mtchedlishvili, Z., et al., Increase of $GABA_A$ receptor-mediated tonic inhibition in dentate granule cells after traumatic brain injury, Neurobiol. Dis. (2010), doi:10.1016/j.nbd.2010.03.012

and Betz, 2000; Kittler and Moss, 2003; Alldred et al., 2005) and in phasic GABAergic inhibition (Nusser and Mody, 2002). However, some γ2 subunit expression has been documented in extrasynaptic locations (Somogyi et al., 1996; Nusser et al., 1998; Sassoè-Pognetto et al., 2000). The inhibitory network of the dentate gyrus undergoes substantial perturbations after TBI, such as loss of GABAergic interneurons and sprouting of excitatory fibers (Santhakumar et al., 2000, 2001; Golarai et al., 2001). A net result of these perturbations is increased neuronal activity in the tissue surrounding the injury site. Because increased neuronal activity in hippocampal neurons causes increased internalization of γ2 subunit-containing, but not δ subunit-containing $GABA_A$Rs (Goodkin et al., 2008; Terunuma et al., 2008; Joshi and Kapur, 2009), we tested the possibility that tonic and phasic currents might be altered differentially after CCI. γ2 subunit-containing receptors are sensitive to the benzodiazepine diazepam, whereas α4/δ subunits render the receptors diazepam-insensitive.

Recordings of mIPSCs from control and CCI DGCs were performed with 300 nM diazepam. The effects of diazepam on mIPSC frequency, peak amplitude, and decay time constants at 23 °C and 34 °C are summarized in Table 1. There was no significant effect on mIPSC frequency by diazepam at 23 °C in control (1.03 ± 0.18 Hz vs. 0.96 ± 0.19 Hz, $n = 9$, 4 animals, $p = 0.43$, two-tailed t test) and CCI DGCs (0.67 ± 0.09 Hz vs. 1.21 ± 0.21 Hz, $n = 10$, 4 animals, $p = 0.11$, two-tailed t test), and at 34 °C in control (3.10 ± 0.84 Hz vs. 3.59 ± 1.22 Hz, $n = 16$, 3 animals, $p = 0.57$, two-tailed t test) and CCI DGCs (2.44 ± 0.67 Hz vs. 1.42 ± 0.27 Hz, $n = 7$, 2 animals, $p = 0.17$, two-tailed t test).

Peak amplitude and decay time constants of mIPSCs were altered differentially by diazepam in control and CCI DGCs at 23 °C and in CCI DGCs at 34 °C (Figs. 2A–H). At 23 °C, diazepam increased the mean of median peak amplitude of mIPSCs in both control and CCI groups, but the increase was 20% higher in control DGCs than in CCI DGCs. The significance of the diazepam effect on peak amplitude was validated in individual DGCs by comparing cumulative fraction distributions by the KS test, before and after diazepam. The KS test suggested a statistically significant effect of diazepam ($p < 0.05$) in all control and CCI DGCs, thus rejecting the null hypothesis that the amplitude populations were equal before and after diazepam. Because mIPSC amplitudes are skewed (demonstrate a non-Gaussian distribution), means of median peak amplitudes were subsequently compared. In control DGCs, diazepam increased the amplitude (26.81 ± 2.20 pA and 42.60 ± 1.22 pA, $n = 9$, 3 animals, $p = 0.031$, two-tailed t test) and in CCI DGCs (33.46 ± 2.98 pA and 46.13 ± 1.09 pA, $n = 10$, 5 animals, $p = 0.047$, two-tailed t test). At 34 °C, diazepam did not increase peak amplitude in control DGCs (62.10 ± 0.73 pA vs. 59.85 ± 0.67 pA, $n = 16$, 3 animals, $p = 0.11$ two-tailed t test). However, diazepam decreased peak amplitude in CCI DGCs (53.10 ± 0.59 pA vs. 36.06 ± 7.09 pA, $n = 7$, 2 animals, $p = 0.03$, two-tailed t test).

In control DGCs, diazepam prolonged decay time constants by 21.5% at 23 °C (5.12 ± 0.29 ms and 6.59 ± 0.08 ms, $n = 9$, 5 animals, $p = 0.002$, two-tailed t test) and by 21% at 34 °C (5.97 ± 0.07 ms and 7.22 ± 0.19 ms, $n = 12$, 6 animals, $p = 0.001$, two-tailed t test). In CCI DGCs, diazepam's prolongation of decay time constants was not significant: 8.9% at 23 °C (5.48 ± 0.03 ms and 5.61 ± 0.08 ms, $n = 10$, 5 animals, $p = 0.093$, two-tailed t test) and by 0.4% at 34 °C (6.59 ± 0.12 ms and 6.62 ± 0.98 ms, $n = 9$, 4 animals, $p = 0.12$, two-tailed t test).

Potentiation of inhibition of synaptic currents by furosemide after CCI

The diuretic furosemide is a noncompetitive antagonist of $GABA_A$Rs that demonstrates an enhanced affinity to α4 subunit-containing recombinant receptors (Wafford et al., 1996) and does not discriminate between γ and δ subunit-containing receptors (Korpi and Luddens, 1997). Recordings were performed with furosemide at 23 °C and 34 °C and were qualitatively similar; data from recordings at 34 °C are presented. The effects of furosemide on typical recordings of mIPSCs are presented in Figs. 3A–D. The significance of the furosemide effect on peak amplitude was validated in individual DGCs by comparing cumulative fraction distributions by the KS test, before and after furosemide. The KS test suggested a statistically significant effect ($p < 0.05$) of furosemide in all CCI, but not in control, DGCs. Furosemide (100 μM) was bath-applied to control DGCs and did not alter the mean of median peak amplitudes (49.80 ± 4.20 pA at baseline, and 45.20 ± 4.40 pA in the presence of furosemide, $n = 6$, 2 animals, $p = 0.32$, two-tailed t test), frequency (0.39 ± 0.05 Hz at baseline, and 0.36 ± 0.06 Hz in the presence of furosemide, $n = 6$, $p = 0.85$, two-tailed t test), or decay time constants (6.42 ± 0.30 ms at baseline, and 7.13 ± 0.44 ms in the presence of furosemide, $n = 6$, $p = 0.13$, two-tailed t test). In CCI DGCs, 100 μM furosemide decreased the mean of median peak amplitudes (51.30 ± 0.80 pA at baseline, and 43.50 ± 5.30 pA in the presence of furosemide, $n = 5$, 2 animals, $p < 0.001$, two-tailed t test). Furosemide did not alter the frequency (0.42 ± 0.09 Hz at baseline, and 0.38 ± 0.11 Hz in the presence of furosemide, $n = 6$, $p = 0.34$, two-tailed t test) or decay time constants (5.26 ± 0.30 ms at baseline and 5.40 ± 0.20 ms in the presence of furosemide $n = 5$, $p = 0.73$, two-tailed t test).

Increase of tonic inhibition in DGCs after CCI

In order to assess potential alterations in tonic currents in CCI DGCs, measurements of whole cell capacitance were obtained to ensure that potential differences in tonic current were not attributable to significant differences in cell membrane areas. Measurements of whole cell capacitance revealed similar values in control and CCI DGCs (46.40 ± 6.50 pF in control, $n = 36$, 9 animals, and 36.30 ± 8.50 pF, $n = 27$, 8 animals, in CCI DGCs, $p = 0.11$, Mann–Whitney test), suggesting relative homogeneity in cell membrane areas in control and CCI DGC groups.

4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol (THIP) is a low affinity agonist of $GABA_A$Rs with preferential affinity for δ subunits (Adkins et al., 2001; Brown et al., 2002). 1 and 3 μM THIP were used for potentiation and 20 μM bicuculline was used for blockade of tonic currents. The recordings were performed at 34 °C. Quantitative measurement of tonic current was performed by comparing Gaussian distributions of all-point histograms of 30-s epochs immediately before the opening of the THIP-containing reservoir (baseline) and 3 min after opening the reservoir. Both concentrations of THIP increased baseline noise and shifted holding current in both control and CCI DGCs. The data of tonic current alteration are presented in Table 2. 1 μM THIP shifted holding current in control DGCs from 98.7 ± 30.4 pA to 114.7 ± 20.4 pA (13% increase, $n = 13$, 6 animals $p = 0.0005$, two-tailed t test) whereas in CCI DGCs, THIP shifted holding current from 119.8 ± 34.6 pA to 176.3 ± 42.2 pA (47% increase, $n = 9$, 4 animals, $p = 0.0003$, two-tailed t test). To confirm that 1 μM THIP caused a greater potentiation of tonic current in CCI DGCs, we repeated this experiment by increasing the concentration of THIP by a half-log. 3 μM THIP shifted holding current in control DGCs from 88.9 ± 20.6 pA to 233.7 ± 50.4 pA (162% increase), $n = 11$, 3 animals, $p = 0.0005$, two-tailed t test, Figs. 4A,B), whereas in CCI DGCs, holding current was shifted from 111.6 ± 30.8 pA to 334.0 ± 69.6 pA) (198% increase, $n = 7$, 2 animals, $p = 0.0001$, two-tailed t test, Figs. 4C,D). A steeper rise time of THIP-induced current in CCI DGCs suggests that, compared to control DGCs, activation of a larger number of receptors by THIP results in a higher rate of current amplitude increase.

In order to corroborate the presence of larger tonic current in CCI DGCs, 20 μM bicuculline was bath-applied to the same DGCs where holding current was potentiated by 1 and 3 μM THIP. We found that substantial amounts of time were required for THIP-induced current to equilibrate to a steady level, likely due to a very slow desensitization rate. In many cases, the quality of the recording during protracted application of THIP would often deteriorate to such an extent that application of bicuculline was not feasible. Our aim was to detect the

Please cite this article as: Mtchedlishvili, Z., et al., Increase of $GABA_A$ receptor-mediated tonic inhibition in dentate granule cells after traumatic brain injury, Neurobiol. Dis. (2010), doi:10.1016/j.nbd.2010.03.012

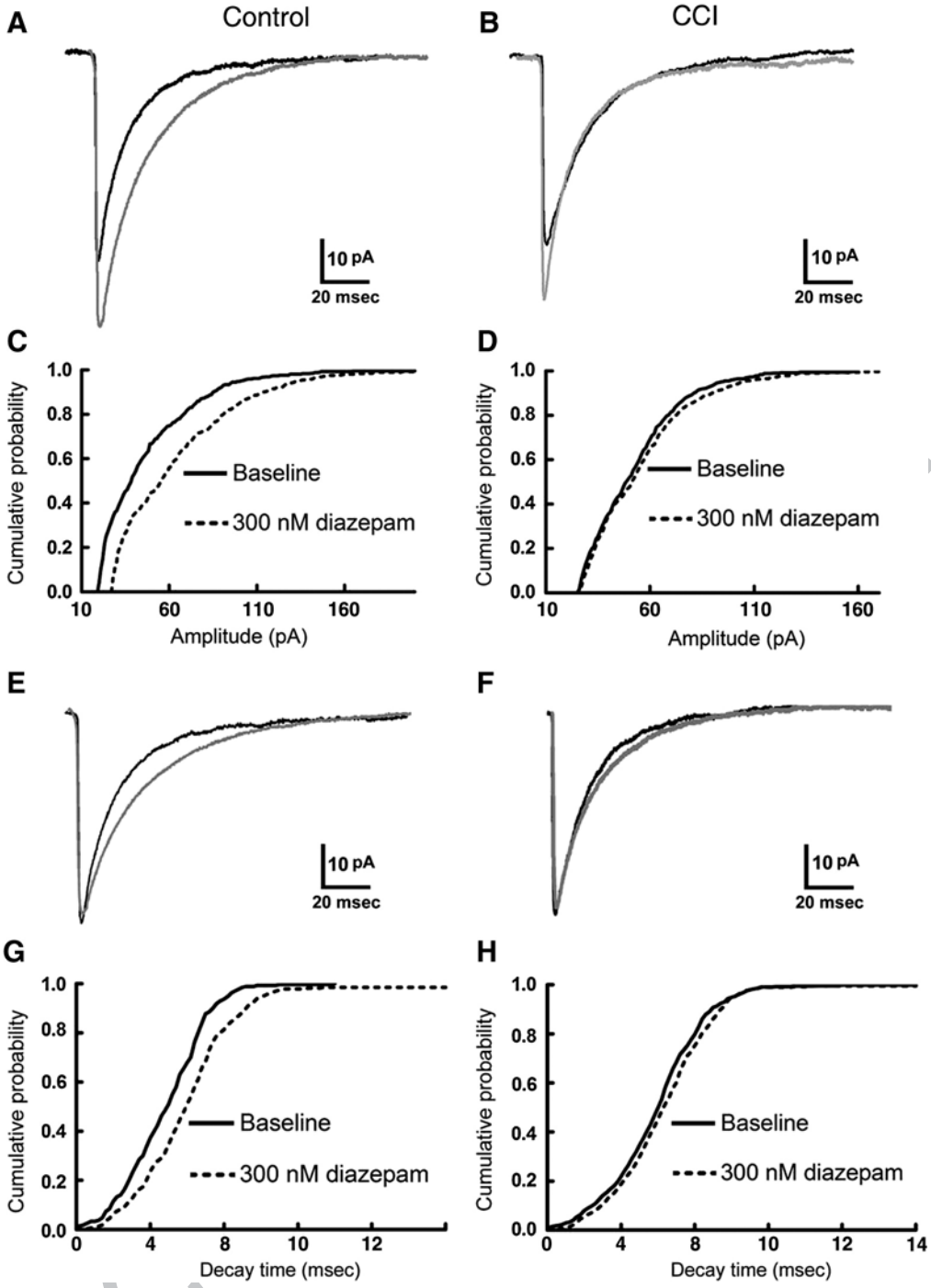

Fig. 2. Loss of diazepam sensitivity in DGCs after CCI. Typical examples of mIPSCs in control and CCI DGCs (averages from single neurons) and cumulative probability plots of peak amplitude and decay time constants at baseline (solid line) and in the continuous presence of 300 nM diazepam (dotted line). DGCs were voltage-clamped to −70 mV and recorded at 23 °C in the presence of 50 μM AP-5, 20 μM DNQX, and 1 μM TTX. (A) An averaged trace from a control DGC at baseline (black) and after application of 300 nM diazepam (grey). Each trace is obtained from ~500 individual mIPSCs in this and subsequent traces. (B) An averaged trace from a CCI DGC at baseline (black) and after application of 300 nM diazepam (grey) Note the stronger potentiation of peak amplitude by 300 nM diazepam in the control DGC compared to the CCI DGC. (C,D) Cumulative probability plots of the peak amplitudes of mIPSCs recorded from the same neurons (A,B), respectively. Note the larger rightward shift of the curve in the presence of diazepam in the control DGC (C) compared to the CCI DGC (D). (E,F) For the same DGCs as in (A) and (B), the peak amplitudes of mIPSCs were normalized to the amplitudes of the traces recorded under continuous application of 300 nM diazepam to demonstrate the effects of diazepam on decay times in control and CCI DGCs. Note the loss of potentiation of decay time by 300 nM diazepam in mIPSCs recorded in the CCI DGC. (G,H) Cumulative probability plots of decay times of mIPSCs recorded from the same control and CCI DGCs (E,F) Note the larger rightward shift of the curve in the presence of diazepam in the control DGC (G) compared to the CCI DGC (H).

relative, rather than the absolute, difference in tonic current. For these reasons, we considered it essential that bicuculline was tested shortly after THIP-induced current reached its peak.

Slow inward current occurs in response to bicuculline application accompanied by loss of synaptic currents and reduction of baseline noise. Reduction of baseline noise occurs because the closure of chloride channels causes an increase of membrane resistance. Several studies have used the shift of holding current by $GABA_AR$ antagonists to measure tonic inhibition (Bai et al., 2001; Hamann et al., 2002; Nusser and Mody, 2002; Mody and Pearce, 2004; Mangan et al., 2005; Mtchedlishvili and Kapur, 2006). Bicuculline blocked most synaptic events, decreased baseline noise, and shifted holding current in both

Please cite this article as: Mtchedlishvili, Z., et al., Increase of $GABA_A$ receptor-mediated tonic inhibition in dentate granule cells after traumatic brain injury, Neurobiol. Dis. (2010), doi:10.1016/j.nbd.2010.03.012

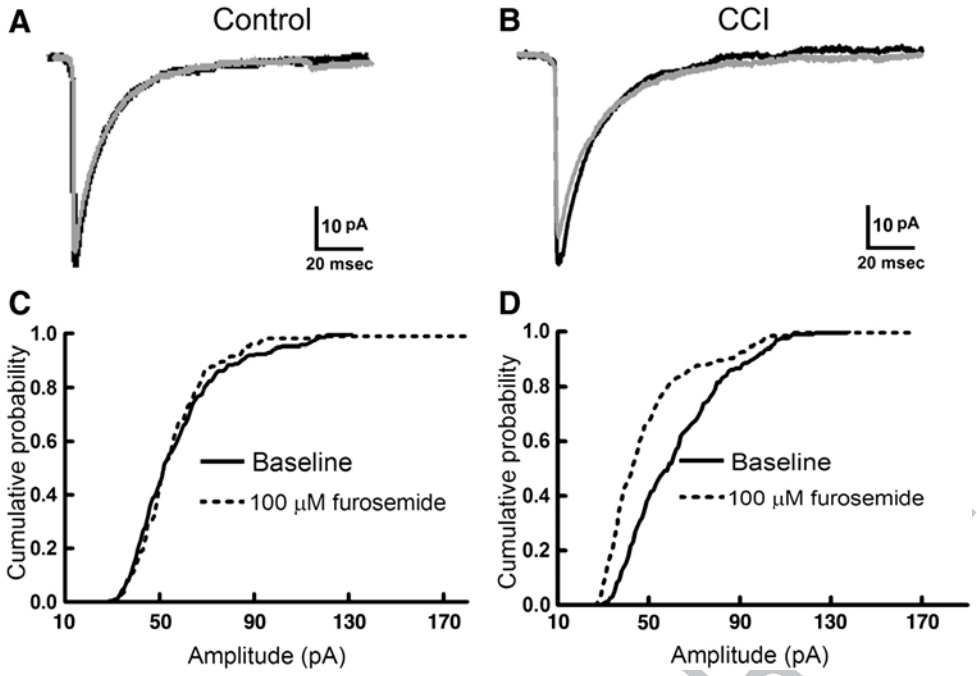

Fig. 3. Potentiation of furosemide inhibition of mIPSCs in DGCs after CCI. Typical examples of mIPSCs in control and CCI DGCs (averages from single neurons) and cumulative probability plots of peak amplitude at baseline (solid line) and in the continuous presence of 100 μM furosemide (dotted line). DGCs were voltage-clamped to −70 mV and recorded at 23 °C in the presence of 50 μM AP-5, 20 μM DNQX, and 1 μM TTX. Averaged traces from a control (A) and CCI DGC (B) before and after application of 100 μM furosemide. Each averaged trace is obtained from ~500 individual mIPSCs. The averaged baseline trace is shown in black, and the averaged trace recorded in the presence of furosemide is shown in grey. Note the decrease of peak amplitude by 100 μM furosemide in the CCI DGC. (C,D) Cumulative probability plots of the peak amplitudes of mIPSCs recorded from the same neurons. Note the larger leftward shift of the curve in the presence of furosemide in the CCI DGC.

control (Fig. 4A) and CCI DGCs (Fig. 4C). However, it caused a greater inhibition of the currents recorded in CCI DGCs than in control DGCs (Table 2). In the DGCs recorded in the presence of 1 μM THIP, bicuculline decreased the holding current compared to the baseline in control DGCs from 98.7 ± 30.4 pA to 54.7 ± 14.5 pA (54% decrease, $n = 13$, $p < 0.0001$, two-tailed t test), whereas in CCI DGCs, bicuculline decreased holding current from 119.8 ± 34.6 pA to 21.2 ± 7.9 pA (83% decrease, $n = 9$, $p < 0.0001$, two-tailed t test). In the current traces acquired from the recordings with 3 μM THIP, the decrease of holding current by bicuculline was similar to that observed in the traces obtained from 1 μM THIP recordings. Bicuculline decreased holding current compared to baseline level in control DGCs from 88.9 ± 20.6 pA to 41.1 ± 6.8 pA (45% decrease, $n = 11$, $p < 0.0001$, two-tailed t test), and in CCI DGCs from 111.6 ± 30.8 pA to 29.2 ± 6.0 pA (74% decrease, $n = 7$, $p = 0.0004$, two-tailed t test).

Properties of synaptic receptors do not contribute to the increase of tonic inhibition

Potentiation of tonic inhibition after TBI, revealed by THIP in DGCs from animals 90 days after CCI, suggested that there was an increased number of high-affinity, and possibly δ subunit-containing, $GABA_A$Rs. We questioned whether the increased number of high-affinity $GABA_A$Rs was restricted to extrasynaptic receptors, or whether the injury might cause abnormal insertion of δ subunit-containing receptors into synapses in DGCs after CCI.

mIPSCs were recorded in control and CCI-injured DGCs voltage-clamped at −70 mV.1 μM THIP was bath-applied to slices after stable recordings were achieved. In control DGCs, 1 μM THIP did not alter the baseline frequency of mIPSCs (0.95 ± 0.21 Hz at baseline, and 0.82 ± 0.14 Hz in the presence of THIP, $n = 4$, 1 animal, $p = 0.54$, two-tailed t test, Fig. 5A). In the same group of control DGCs, comparison of the means of median amplitudes of mIPSCs before and after application of THIP did not demonstrate a statistically significant alteration of peak amplitude by 1 μM THIP (50.78 ± 2.4 pA at baseline, and 53.70 ± 1.93 pA after application of THIP, $n = 4$, $p = 0.37$, two-tailed t test, Fig. 5C). In addition, 1 μM THIP did not alter the decay time constants of mIPSCs (5.24 ± 0.08 ms at baseline, and 5.65 ± 0.09 ms after application of THIP, $n = 4$, $p = 0.54$, two-tailed t test, Fig. 5E).

Next, we studied the effects of 1 μM THIP on synaptic currents in CCI DGCs. 1 μM THIP did not alter the frequency of mIPSCs (1.09 ± 0.12 Hz at baseline, and 1.63 ± 1.13 Hz in the presence of THIP, $n = 7$; 1 animal, $p = 0.54$, two-tailed t test, Fig. 5B). In the same group of CCI DGCs, comparison of the means of median amplitudes of mIPSCs before and after application of 1 μM THIP did not demonstrate a

Table 2
Summary of effects of 1 and 3 μM THIP and 20 μM bicuculline on tonic currents in control and CCI DGCs.

	Control				CCI			
	Baseline	1 μM THIP	Bicuculline	n	Baseline	1 μM THIP	Bicuculline	n
Tonic current (pA)	98.7 ± 30.4	114.7 ± 20.4	54.7 ± 14.5	13	119.8 ± 34.6	176.3 ± 42.2	21.2 ± 7.9	9
	Baseline	3 μM THIP	Bicuculline	n	Baseline	3 μM THIP	Bicuculline	n
Tonic current (pA)	88.9 ± 20.6	233.7 ± 50.4	41.1 ± 6.8	11	111.6 ± 30.8	334 ± 69.6	29.2 ± 6.0	7

The values represent Gaussian mean ± S.E.M. n indicates number of cells.

Please cite this article as: Mtchedlishvili, Z., et al., Increase of $GABA_A$ receptor-mediated tonic inhibition in dentate granule cells after traumatic brain injury, Neurobiol. Dis. (2010), doi:10.1016/j.nbd.2010.03.012

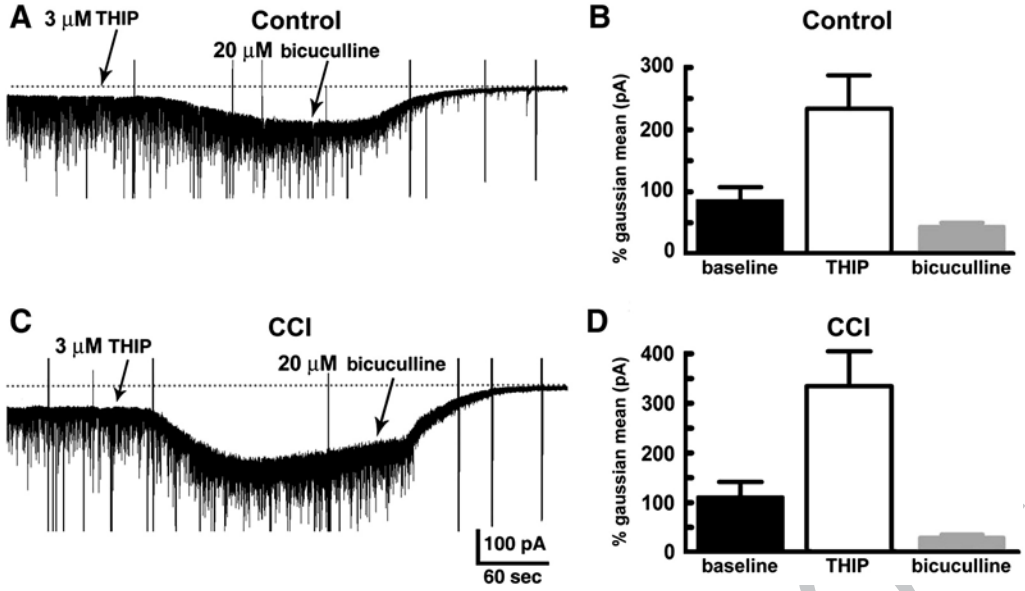

Fig. 4. Increase of tonic inhibition in DGCs after CCI. The low affinity $GABA_A$ receptor agonist 3 μM THIP caused a larger potentiation, and the $GABA_AR$ antagonist 20 μM bicuculline caused a larger inhibition, of the holding current in a CCI DGC compared to a control DGC. Typical recordings at 34 °C from a DGC from a control (A) and a CCI (C) animal 90 days after lesioning. DGCs were voltage-clamped to −70 mV and baseline epochs were selected immediately prior to opening the reservoir containing 3 μM THIP (arrows), and an epoch corresponding to THIP selected 3 min after the valve opening. THIP caused an outward shift of the holding current and potentiation of baseline noise in both control (A) and CCI (C) DGCs. Note the larger shift of the holding current in the DGC from a CCI animal. Bicuculline inhibited tonic current in both cells, but caused a larger shift of holding current in CCI DGCs (shown by dotted line). (B–D) Summary of the effects of 3 μM THIP and 20 μM bicuculline on tonic inhibition in control and CCI DGCs. (B) In control DGCs, 3 μM THIP caused a 162% increase of holding current compared to the baseline. 20 μM bicuculline caused a 54% decrease of holding current compared to the baseline. (D) In CCI DGCs, 3 μM THIP caused a 198% increase of holding current compared to the baseline. 20 μM bicuculline caused an 83% decrease of holding current compared to the baseline. The bars represent Gaussian means of 30-s epochs acquired immediately before THIP application (baseline), at the peak of THIP-evoked current (THIP), and 5 min after application of 20 μM bicuculline (bicuculline). Error bars are mean ± S.E.M. Augmentation of holding current by THIP and inhibition by bicuculline were significant within control ($n = 11$), and CCI DGCs ($n = 7$, $p < 0.05$, paired t test). An enhanced effect of THIP and bicuculline in CCI DGCs compared to control DGCs was significant ($p < 0.05$, two-tailed t test).

statistically significant alteration of peak amplitude by 1 μM THIP (61.9 ± 2.2 pA at baseline, and 82.1 ± 4.4 pA in the presence of 1 μM THIP, $p = 0.08$, two-tailed t test, Fig. 5D). 1 μM THIP did not change the decay time constants of mIPSCs (7.8 ± 1.1 ms in control, and 7.4 ± 0.7 ms in the CCI group, $p = 0.63$, two-tailed t test, Fig. 5F). These results, corroborated by our finding that δ subunit immunoreactivity is not present in synaptic locations in control and CCI DGCs (unpublished data), suggest that synaptic $GABA_ARs$ do not contribute to tonic current mediated by δ subunit-expressing $GABA_ARs$.

Discussion

The principal findings in DGCs 90 days after CCI were: 1) an increase of $GABA_AR$-mediated tonic currents; 2) a decreased frequency of mIPSCs; and 3) a loss of diazepam potentiation and an increase of furosemide inhibition of synaptic $GABA_ARs$.

A number of experimental models have been developed over the last few decades that simulate different aspects of the clinical condition of TBI with varying degrees of accuracy. Two of the most commonly used models are FPI and CCI. The advantage of the FPI model is its relative simplicity and its ability to produce significant injury in the brain, including axonal injury and intraparenchymal hemorrhages (Povlishock et al., 1983). However, the fluid pulse of FPI enters the calvarium and disperses diffusely within the epidural space, as demonstrated with high speed cineradiography (Dixon et al., 1988), making tissue displacement difficult to quantify. An advantage of the CCI model is that it can produce a more precise and reproducible injury as demonstrated by experiments measuring the effect that the velocity and depth of impact have on the severity of injury (Dixon et al., 1991). Recent reports indicate that the CCI model can be successfully applied to induce PTE in mice (Hunt et al., 2009) and in rats (Statler et al., 2009).

Increased tonic inhibition in CCI DGCs

In the present study, we have shown an increase of $GABA_AR$-mediated tonic currents in DGCs 90 days after CCI. Several mechanisms, both pre- and postsynaptic, can account for increased tonic conductance in CCI DGCs. Elevation of ambient levels of GABA can increase tonic current by increased spillover of vesicular GABA caused by increased firing of GABAergic interneurons (Kaneda et al., 1995; Brickley et al., 1996; Wall and Usowicz, 1997; Hamann et al., 2002; Bright et al., 2007), release of GABA from glial sources (Liu et al., 2000; Kozlov et al., 2006), or impairment of GABA transporters (Richerson and Wu, 2003). Larger THIP-evoked tonic current in CCI DGCs suggests an increased density of δ subunit-containing peri- and extrasynaptic $GABA_ARs$. The δ subunit-containing GABARs possess distinct biophysical and pharmacological properties. These receptors have high affinity to GABA (Brown et al., 2002; Sundstrom-Poromaa et al., 2002) and a slow rate of desensitization (Bianchi and Macdonald, 2002; Brown et al., 2002). These properties allow δ subunit-containing GABARs to "sense" ambient levels of GABA in a submicromolar and low micromolar range (Glykys et al., 2008). In DGCs, α5βxγ2 $GABA_ARs$ can contribute to tonic current, which is increased after status epilepticus (Zhan and Nadler, 2009). It is possible that differential patterns of trafficking of receptor subunits play a role in an increased expression of δ-containing $GABA_ARs$, which remain on the membrane surface, whereas γ2-containing $GABA_ARs$ are internalized under conditions of increased neuronal activity (Goodkin et al., 2008) resulting in unchanged (Goodkin et al., 2005) or increased (Naylor et al., 2005) tonic current.

It has been established that TBI induces upregulation of brain-derived neurotrophic factor (BDNF) in the tissue surrounding the injury site (Hicks et al., 1997). BDNF plays a crucial role in neuronal maturation by regulating intracellular Cl^- levels and altering GABAergic signaling from depolarizing to hyperpolarizing (Hübner et al., 2001; Rivera et al., 2002) and improving memory (Falkenberg et al., 1992). In injured

Please cite this article as: Mtchedlishvili, Z., et al., Increase of $GABA_A$ receptor-mediated tonic inhibition in dentate granule cells after traumatic brain injury, Neurobiol. Dis. (2010), doi:10.1016/j.nbd.2010.03.012

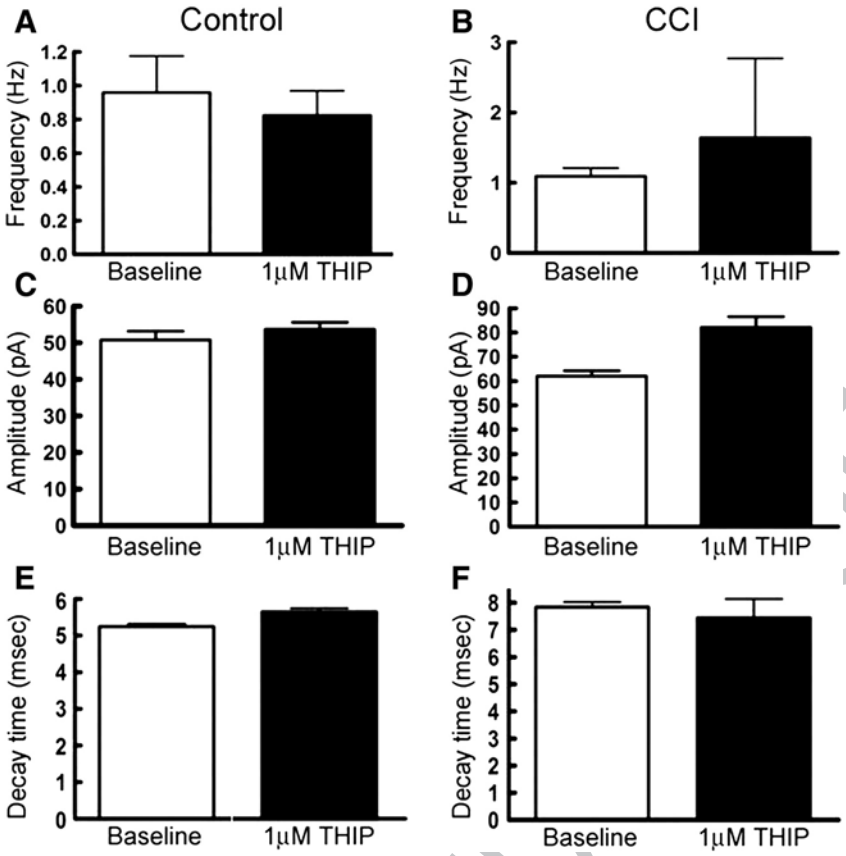

Fig. 5. Low-affinity GABA agonist does not affect synaptic receptors in DGCs from control animals. Recordings in control DGCs were performed at 23 °C. (A) 1 μM THIP did not alter the frequency of mIPSCs (0.95 ± 0.21 Hz at baseline, and 0.68 ± 0.07 Hz in the presence of THIP, $n = 4$, $p = 0.54$, two-tailed t test). (C) Comparison of means of median amplitudes of mIPSCs before and after application of THIP did not demonstrate a statistically significant alteration of peak amplitude by 1 μM THIP (50.78 ± 2.4 pA in the baseline, and 53.70 ± 1.93 pA after application of THIP, $n = 4$, $p = 0.37$, two-tailed t test). (E) 1 μM THIP did not alter the decay time constants of mIPSCs (5.24 ± 0.08 ms and 5.65 ± 0.09 ms, $n = 4$, $p = 0.54$, two-tailed t test). THIP did not alter synaptic currents in DGCs after CCI. (B) THIP did not alter the frequency of mIPSCs (1.09 ± 0.12 Hz in the baseline, and 1.63 ± 1.13 Hz in the presence of THIP, $n = 7$, $p = 0.54$, two-tailed t test). (D) Comparison of means of median amplitudes of mIPSCs before and after application of 1 μM THIP did not demonstrate a statistically significant alteration of peak amplitude by 1 μM THIP (61.9 ± 2.2 pA in the baseline, and 82.1 ± 4.4 pA in the presence of 1 μM THIP, $n = 7$ $p = 0.08$; two-tailed t test). (F) 1 μM THIP did not change the decay time constants of mIPSCs (7.8 ± 1.1 ms and 7.4 ± 0.7 ms; $n = 7$, $p = 0.63$, two-tailed t test).

tissue, increased levels of BDNF (Yang et al., 1996) and its receptor trkB (Binder et al., 1999) are believed to promote excitatory axonal sprouting and therefore are considered epileptogenic after brain injury (Dinocourt et al., 2006) and in kindling (Ernfors et al., 1991; Merlio et al., 1993; Elmer et al., 1998). Exogenous BDNF inhibits internalization and significantly promotes surface expression of δ subunit-containing $GABA_A$Rs in organotypic hippocampal neurons (Joshi and Kapur, 2009). Elevated BDNF in the hippocampus after head trauma, in addition to persistently increased excitatory signaling due to mossy fiber sprouting, may cause a larger surface expression of δ subunit-containing $GABA_A$Rs in DGCs, which results in larger tonic $GABA_A$R-mediated currents.

Altered properties of synaptic receptors

Unchanged amplitudes of synaptic currents after CCI suggest that levels of γ2 subunit-containing $GABA_A$Rs are either unaltered or decreased acutely after injury and are restored to control levels 90 days following injury. In CCI DGCs, benzodiazepine-insensitive pharmacology of synaptic receptors is consistent with co-expression of α4 and γ subunits in the synapse.

Under normal circumstances, α4 is co-expressed with δ subunits in extrasynaptic receptors (Nusser et al., 1998; Sun et al., 2004), but it can be expressed synaptically in DGCs in epilepsy (Payne et al., 2006; Sun et al., 2007). Decrease of allosteric modulation of $GABA_A$Rs of DGCs by diazepam, characterized by decreased prolongation of decay time constants and potentiation of peak amplitude has been characterized under pathological conditions, such as following acute seizures (Kapur and Macdonald 1997; Goodkin et al., 2005; Naylor et al., 2005, Feng et al., 2008), as well as in chronic epilepsy (Cohen et al., 2003; Peng et al., 2004). Loss of potentiation of decay time constants and reduced potentiation of peak amplitudes of mIPSCs in CCI DGCs suggest that the synaptic $GABA_A$Rs undergo changes leading to benzodiazepine insensitivity similar to those found in epilepsy. The possibility of the presence of α4 subunit-containing receptors in GABAergic synapses in DGCs was further suggested by increased furosemide inhibition of $GABA_A$R-mediated mIPSCs in CCI DGCs. Furosemide is an α4-preferring noncompetitive antagonist of $GABA_A$Rs (Wafford et al., 1996) and does not discriminate between and γ and δ subunit-containing receptors (Korpi and Luddens, 1997). Furosemide selectively inhibits the amplitude of fast, but not slow, mIPSCs in CA1 pyramidal neurons (Banks et al., 1998), suggesting a contribution of α4-containing $GABA_A$Rs to these events.

Factors other than subunit variations that could affect diazepam sensitivity might include phosphorylation state and/or mutations of $GABA_A$R subunits. Phosphorylation of $GABA_A$Rs by protein kinase C has been shown to reduce diazepam potency (Gao and Greenfield, 2005; Qi et al., 2007), and mutations of the γ2 subunit can eliminate (Wallace et al., 2001) or reduce (Bowser et al., 2002; Eugène et al., 2007) benzodiazepine sensitivity. A recent report suggests that γ2 subunit mutations at the α1γ2 interface (Arg43 and Glu117), where the benzodiazepine binding site is located, result in a loss of benzodiazepine sensitivity (Goldschen-Ohm et al., 2010). Mutations of histidine residues in the α1 subunit have been linked to diazepam insensitivity

Please cite this article as: Mtchedlishvili, Z., et al., Increase of $GABA_A$ receptor-mediated tonic inhibition in dentate granule cells after traumatic brain injury, Neurobiol. Dis. (2010), doi:10.1016/j.nbd.2010.03.012

(Rudolph and Möhler, 2006). In addition, our finding of decreased frequency of mIPSCs, but not sIPSCs, might suggest decreased probability of release. Similar observations were reported in DGCs in the FPI model of TBI (Toth et al., 1997).

Potential pathophysiological consequences of increased tonic inhibition after CCI

An increase of GABAergic tonic inhibition in DGCs 90 days after CCI may suggest the existence of a novel mechanism by which TBI contributes to the impairment of cognitive and memory functions. It has been recognized that increased $GABA_AR$-mediated tonic inhibition is negatively correlated with cognitive and memory functions (Caraiscos et al., 2004), whereas positive modulators of $GABA_ARs$, such as benzodiazepines, have amnesic effects and adversely affect memory (Maubach, 2003). The role of GABAergic tonic current mediated by δ subunit-containing receptors in memory and cognition is beginning to emerge. A recent finding suggests that potentiation of tonic inhibition in area CA3 interneurons mediated by δ subunit-containing $GABA_ARs$ can suppress CA3 gamma-frequency oscillations, which are critical for memory encoding and retrieval (Mann and Mody, 2009). Increased levels of the GABA synthesizing enzyme GAD67 in the prefrontal cortex has been correlated with decline of working memory (Kobori et al., 2006). In the pendulum model of TBI, impaired spatial memory was improved after blockade of $GABA_ARs$ and was attributed to disinhibition of presynaptic cholinergic transmission (O'Dell and Hamm, 1995). Taken together, these results suggest that concomitant alterations in tonic and phasic inhibition in DGCs may occur after TBI.

TBI is a major risk factor for the development of PTE. It has been suggested that the dentate gyrus functions as a "gate" in preventing the reverberation of excessive excitatory drive through the trisynaptic circuit formed by the mossy fiber to CA3 to CA1 pyramidal neuron. Breakdown of this gate could be a critical event in the development of seizure activity within the temporal lobe (Collins et al., 1983; Stringer and Lothman, 1989; Heinemann et al., 1992). Alterations of GABAergic signaling in DGCs after an epileptogenic insult have been long recognized as an important part of a wide array of molecular, cellular, and circuit level changes in the dentate gyrus. Although we did not document seizures in the 11 CCI-injured animals that underwent limited video monitoring before sacrifice and electrophysiological studies, it is possible that these animals demonstrated convulsive activity before they were monitored or had non-convulsive seizures, which would have been undetectable by video recordings without concomitant EEG. In addition, it is possible that the 12 CCI-injured animals that were not monitored might have had undetected convulsive or non-convulsive seizures at any point in time following cortical impact. If animals had become epileptic during the 90-day period following lesioning, the results of the present study would need to be interpreted in a potentially different context than that of TBI-induced changes alone.

In summary, the present findings support differential alterations of tonic and phasic $GABA_AR$-mediated inhibition in DGCs after a single episode of CCI. The net result of these differential alterations is that the amplitude of tonic current was increased and benzodiazepine sensitivity of synaptic receptors was lost. These findings demonstrate for the first time that tonic inhibition can be altered after CCI in DGCs. Additional studies are required to elucidate the relationship between TBI, increased tonic inhibition in the dentate gyrus, cognitive performance, and the development of PTE.

Acknowledgments

The study was supported by Health Research Formula Fund RFA 01-07-26, Pennsylvania Department of Health (ZM), a Research Grant from the Epilepsy Foundation (ZM), and a grant from the Georgian National Science Scholars Program (EL). We thank Teresa M. Hentosz for assistance in the preparation of the manuscript.

References

Adkins, C.E., Pillai, G.V., Kerby, J., Bonnert, T.P., Haldon, C., McKernan, R.M., Gonzalez, J.E., Oades, K., Whiting, P.J., Simpson, P.B., 2001. alpha4beta3delta GABA(A) receptors characterized by fluorescence resonance energy transfer-derived measurements of membrane potential. J. Biol. Chem. 276, 38934–38939.

Alldred, M.J., Mulder-Rosi, J., Lingenfelter, S.E., Chen, G., Lüscher, B., 2005. Distinct gamma2 subunit domains mediate clustering and synaptic function of postsynaptic GABAA receptors and gephyrin. J. Neurosci. 25, 594–603.

Arolfo, M.P., Brioni, J.D., 1991. Diazepam impairs place learning in the Morris water maze. Behav. Neural. Biol. 55, 131–136.

Atack, J.R., Bayley, P.J., Seabrook, G.R., Wafford, K.A., McKernan, R.M., Dawson, G.R., 2006. L-655, 708 enhances cognition in rats but is not proconvulsant at a dose selective for alpha5-containing GABAA receptors. Neuropharmacology 51, 1023–1029.

Bai, D., Zhu, G., Pennefather, P., Jackson, M.F., MacDonald, J.F., Orser, B.A., 2001. Distinct functional and pharmacological properties of tonic and quantal inhibitory postsynaptic currents mediated by gamma-aminobutyric acid (A) receptors in hippocampal neurons. Mol. Pharmacol. 59, 814–824.

Banks, M.I., Li, T.B., Pearce, R.A., 1998. The synaptic basis of GABAA, slow. J. Neurosci. 18, 1305–1317.

Bianchi, M.T., Macdonald, R.L., 2002. Slow phases of GABA(A) receptor desensitization: structural determinants and possible relevance for synaptic function. J. Physiol. 544, 3–18.

Binder, D.K., Routbort, M.J., McNamara, J.O., 1999. Immunohistochemical evidence of seizure-induced activation of trk receptors in the mossy fiber pathway of adult rat hippocampus. J. Neurosci. 19, 4616–4626.

Bonislawski, D.P., Schwarzbach, E.P., Cohen, A.S., 2007. Brain injury impairs dentate gyrus inhibitory efficacy. Neurobiol. Dis. 25, 163–169.

Bowser, D.N., Wagner, D.A., Czajkowski, C., Cromer, B.A., Parker, M.W., Wallace, R.H., Harkin, L.A., Mulley, J.C., Marini, C., Berkovic, S.F., Williams, D.A., Jones, M.V., Petrou, S., 2002. Altered kinetics and benzodiazepine sensitivity of a GABAA receptor subunit mutation [gamma 2(R43Q)] found in human epilepsy. Proc. Natl. Acad. Sci. U.S.A. 99, 15170–15175.

Brickley, S.G., Cull-Candy, S.G., Farrant, M., 1996. Development of a tonic form of synaptic inhibition in rat cerebellar granule cells resulting from persistent activation of GABAA receptors. J. Physiol. 497, 753–759.

Bright, D.P., Aller, M.I., Brickley, S.G., 2007. Synaptic release generates a tonic GABA(A) receptor-mediated conductance that modulates burst precision in thalamic relay neurons. J. Neurosci. 27, 2560–2569.

Brioni, J.D., McGaugh, J.L., 1988. Post-training administration of GABAergic antagonists enhances retention of aversively motivated tasks. Psychopharmacology (Berl.) 96, 505–510.

Brooks-Kayal, A.R., Shumate, M.D., Jin, H., Rikhter, T.Y., Coulter, D.A., 1998. Selective changes in single cell GABA(A) receptor subunit expression and function in temporal lobe epilepsy. Nat. Med. 4, 1166–1172.

Brown, N., Kerby, J., Bonnert, T.P., Whiting, P.J., Wafford, K.A., 2002. Pharmacological characterization of a novel cell line expressing human alpha(4)beta(3)delta GABA (A) receptors. Br. J. Pharmacol. 136, 965–974.

Buckmaster, P.S., Schwartzkroin, P.A., 1995. Physiological and morphological heterogeneity of dentate gyrus-hilus interneurons in the gerbil hippocampus in vivo. Eur. J. Neurosci. 7, 1393–1402.

Buhl, E.H., Halasy, K., Somogyi, P., 1994. Diverse sources of hippocampal unitary inhibitory postsynaptic potentials and the number of synaptic release sites. Nature 368, 823–828.

Buzsaki, G., Draguhn, A., 2004. Neuronal oscillations in cortical networks. Science 304, 1926–1929.

Caraiscos, V.B., Elliott, E.M., You-Ten, K.E., Cheng, V.Y., Belelli, D., Newell, J.G., Jackson, M.F., Lambert, J.J., Rosahl, T.W., Wafford, K.A., MacDonald, J.F., Orser, B.A., 2004. Tonic inhibition in mouse hippocampal CA1 pyramidal neurons is mediated by alpha5 subunit-containing gamma-aminobutyric acid type A receptors. Proc. Natl. Acad. Sci. U.S.A. 101, 3662–3667.

Castellano, C., Cestari, V., Cabib, S., Puglisi-Allegra, S., 1993. Strain-dependent effects of post-training GABA receptor agonists and antagonists on memory storage in mice. Psychopharmacology (Berl.) 111, 134–138.

Cohen, A.S., Lin, D.D., Quirk, G.L., Coulter, D.A., 2003. Dentate granule cell $GABA_A$ receptors in epileptic hippocampus: enhanced synaptic efficacy and altered pharmacology. Eur. J. Neurosci. 17, 1607–1616.

Collins, R.C., Tearse, R.G., Lothman, E.W., 1983. Functional anatomy of limbic seizures: focal discharges from medial entorhinal cortex in rat. Brain Res. 280, 25–40.

Collinson, N., Kuenzi, F.M., Jarolimek, W., Maubach, K.A., Cothliff, R., Sur, C., Smith, A., Out, F.M., Howell, O., Atack, J.R., McKernan, R.M., Seabrook, G.R., Dawson, G.R., Whiting, P.J., Rosahl, T.W., 2002. Enhanced learning and memory and altered GABAergic synaptic transmission in mice lacking the alpha 5 subunit of the GABAA receptor. J. Neurosci. 22, 5572–5580.

Collinson, N., Atack, J.R., Laughton, P., Dawson, G.R., Stephens, D.N., 2006. An inverse agonist selective for alpha5 subunit-containing GABAA receptors improves encoding and recall but not consolidation in the Morris water maze. Psychopharmacology (Berl.) 188, 619–628.

Dahhaoui, M., Caston, J., Stelz, T., 1994. Effects of inhibition of the GABAergic systems by picrotoxin on retention of a nociceptive experience in the rat, with special reference to the influence of cerebellar cortex output. Physiol. Behav. 56, 257–264.

Please cite this article as: Mtchedlishvili, Z., et al., Increase of $GABA_A$ receptor-mediated tonic inhibition in dentate granule cells after traumatic brain injury, Neurobiol. Dis. (2010), doi:10.1016/j.nbd.2010.03.012

Das, P., Bell-Horner, C.L., Huang, R.Q., Raut, A., Gonzales, E.B., Chen, Z.L., Covey, D.F., Dillon, G.H., 2004. Inhibition of type A GABA receptors by L-type calcium channel blockers. Neuroscience 124, 195–206.

Dawson, G.R., Maubach, K.A., Collinson, N., Cobain, M., Everitt, B.J., MacLeod, A.M., Choudhury, H.I., McDonald, L.M., Pillai, G., Rycroft, W., Smith, A.J., Sternfeld, F., Tattersall, F.D., Wafford, K.A., Reynolds, D.S., Seabrook, G.R., Atack, J.R., 2006. An inverse agonist selective for alpha5 subunit-containing GABAA receptors enhances cognition. J. Pharmacol. Exp. Ther. 316, 1335–1345.

DeRidder, M.N., Simon, M.J., Siman, R., Auberson, Y.P., Raghupathi, R., Meaney, D.F., 2006. Traumatic mechanical injury to the hippocampus in vitro causes regional caspase-3 and calpain activation that is influenced by NMDA receptor subunit composition. Neurobiol. Dis. 22, 165–176.

Dinocourt, C., Gallagher, S.E., Thompson, S.M., 2006. Injury-induced axonal sprouting in the hippocampus is initiated by activation of trkB receptors. Eur. J. Neurosci. 24, 1857–1866.

Dixon, C.E., Lighthall, J.W., Anderson, T.E., 1988. Physiologic, histopathologic, and cineradiographic characterization of a new fluid-percussion model of experimental brain injury in the rat. J. Neurotrauma 5, 91–104.

Dixon, C.E., Clifton, G.L., Lighthall, J.W., Yaghmai, A.A., Hayes, R.L., 1991. A controlled cortical impact model of traumatic brain injury in the rat. J. Neurosci. Methods 39, 253–262.

Edwards, F.A., Konnerth, A., Sakmann, B., 1990. Quantal analysis of inhibitory synaptic transmission in the dentate gyrus of rat hippocampal slices: a patch-clamp study. J. Physiol. 430, 213–249.

Elmer, E., Kokaia, Z., Kokaia, M., Carnahan, J., Nawa, H., Lindvall, O., 1998. Dynamic changes of brain-derived neurotrophic factor protein levels in the rat forebrain after single and recurring kindling-induced seizures. Neuroscience 83, 351–362.

Engel, A.K., Singer, W., 2001. Temporal binding and the neural correlates of sensory awareness. Trends Cogn. Sci. 5, 16–25.

Ernfors, P., Bengzon, J., Kokaia, Z., Persson, H., Lindvall, O., 1991. Increased levels of messenger RNAs for neurotrophic factors in the brain during kindling epileptogenesis. Neuron 7, 165–176.

Essrich, C., Lorez, M., Benson, J., Fritschy, J.M., Luscher, B., 1998. Postsynaptic clustering of major $GABA_A$ receptor subtypes requires the γ2 subunit and gephyrin. Nat. Neurosci. 1, 563–571.

Eugène, E., Depienne, C., Baulac, S., Baulac, M., Fritschy, J.M., Le Guern, E., Miles, R., Poncer, J.C., 2007. GABA(A) receptor gamma 2 subunit mutations linked to human epileptic syndromes differentially affect phasic and tonic inhibition. J. Neurosci. 27, 14108–14116.

Falkenberg, T., Mohammed, A.K., Henriksson, B., Persson, H., Winblad, B., Lindefors, N., 1992. Increased expression of brain-derived neurotrophic factor mRNA in rat hippocampus is associated with improved spatial memory and enriched environment. Neurosci. Lett. 138, 153–156.

Feng, H.J., Mathews, G.C., Kao, C., Macdonald, R.L., 2008. Alterations of GABAA-receptor function and allosteric modulation during development of status epilepticus. J. Neurophysiol. 99, 1285–1293.

Gao, L., Greenfield, L.J., 2005. Activation of protein kinase C reduces benzodiazepine potency at GABAA receptors in NT2-N neurons. Neuropharmacology 48, 333–342.

Gibbs III, J.W., Sombati, S., DeLorenzo, R.J., Coulter, D.A., 1997. Physiological and pharmacological alterations in postsynaptic GABA(A) receptor function in a hippocampal culture model of chronic spontaneous seizures. J. Neurophysiol. 77, 2139–2152.

Glykys, J., Mann, E.O., Mody, I., 2008. Which GABA(A) receptor subunits are necessary for tonic inhibition in the hippocampus? J. Neurosci. 28, 1421–1426.

Golarai, G., Greenwood, A.C., Feeney, D.M., Connor, J.A., 2001. Physiological and structural evidence for hippocampal involvement in persistent seizure susceptibility after traumatic brain injury. J. Neurosci. 21, 8523–8537.

Goldschen-Ohm, M.P., Wagner, D.A., Petrou, S., Jones, M.V., 2010. An epilepsy-related region in the GABA(A) receptor mediates long-distance effects on GABA and benzodiazepine binding sites. Mol. Pharmacol. 77, 35–45.

Goodkin, H.P., Yeh, J.L., Kapur, J., 2005. Status epilepticus increases the intracellular accumulation of GABAA receptors. J. Neurosci. 25, 5511–5520.

Goodkin, H.P., Joshi, S., Mtchedlishvili, Z., Brar, J., Kapur, J., 2008. Subunit-specific trafficking of GABA(A) receptors during status epilepticus. J. Neurosci. 28, 2527–2538.

Hájos, N., Nusser, Z., Rancz, E.A., Freund, T.F., Mody, I., 2000. Cell type- and synapse-specific variability in synaptic GABAA receptor occupancy. Eur. J. Neurosci. 12, 810–818.

Halasy, K., Somogyi, P., 1993. Subdivisions in the multiple GABAergic innervation of granule cells in the dentate gyrus of the rat hippocampus. Eur. J. Neurosci. 5, 411–429.

Hall, S., Bornstein, R.A., 1991. The relationship between intelligence and memory following minor or mild closed head injury: greater impairment in memory than intelligence. J. Neurosurg. 75, 378–381.

Hamann, M., Rossi, D.J., Attwell, D., 2002. Tonic and spillover inhibition of granule cells control information flow through cerebellar cortex. Neuron 33, 625–633.

Hardingham, N.R., Larkman, A.U., 1998. Rapid report: the reliability of excitatory synaptic transmission in slices of rat visual cortex in vitro is temperature dependent. J. Physiol. 507, 249–256.

Heinemann, U., Beck, H., Dreier, J.P., Ficker, E., Stabel, J., Zhang, C.L., 1992. The dentate gyrus as a regulated gate for the propagation of epileptiform activity. Epilepsy Res. 7, 273–280.

Hicks, R.R., Numan, S., Dhillon, H.S., Prasad, M.R., Seroogy, K.B., 1997. Alterations in BDNF and NT-3 mRNAs in rat hippocampus after experimental brain trauma. Brain Res. 48, 401–406.

Hübner, C.A., Stein, V., Hermans-Borgmeyer, I., Meyer, T., Ballanyi, K., Jentsch, T.J., 2001. Disruption of KCC2 reveals an essential role of K–Cl cotransport already in early synaptic inhibition. Neuron 30, 515–524.

Hunt, R.F., Scheff, S.W., Smith, B.N., 2009. Posttraumatic epilepsy after controlled cortical impact injury in mice. Exp. Neurol. 215, 243–252.

Johansson, I.M., Birzniece, V., Lindblad, C., Olsson, T., Backstrom, T., 2002. Allopregnanolone inhibits learning in the Morris water maze. Brain Res. 934, 125–131.

Joshi, S., Kapur, J., 2009. Slow intracellular accumulation of GABA(A) receptor delta subunit is modulated by brain-derived neurotrophic factor. Neuroscience 164, 507–519.

Kaneda, M., Farrant, M., Cull-Candy, S.G., 1995. Whole-cell and single-channel currents activated by GABA and glycine in granule cells of the rat cerebellum. J. Physiol. 485, 419–435.

Kant, G.J., Wylie, R.M., Vasilakis, A.A., Ghosh, S., 1996. Effects of triazolam and diazepam on learning and memory as assessed using a water maze. Pharmacol. Biochem. Behav. 53, 317–322.

Kapur, J., Macdonald, R.L., 1997. Rapid seizure-induced reduction of benzodiazepine and Zn2+ sensitivity of hippocampal dentate granule cell GABAA receptors. J. Neurosci. 17, 7532–7540.

Kharlamov, E.A., Jukkola, P.I., Schmitt, K.L., Kelly, K.M., 2003. Electrobehavioral characteristics of epileptic rats following photothrombotic brain infarction. Epilepsy Res. 56, 185–203.

Kittler, J.T., Moss, S.J., 2003. Modulation of GABAA receptor activity by phosphorylation and receptor trafficking: implications for the efficacy of synaptic inhibition. Curr. Opin. Neurobiol. 13, 341–347.

Kneussel, M., Betz, H., 2000. Receptors, gephyrin and gephyrin-associated proteins: novel insights into the assembly of inhibitory postsynaptic membrane specializations. J. Physiol. 525, 1–9.

Kobori, N., Clifton, G.L., Dash, P.K., 2006. Enhanced catecholamine synthesis in the prefrontal cortex after traumatic brain injury: implications for prefrontal dysfunction. J. Neurotrauma 23, 1094–1102.

Korpi, E.R., Luddens, H., 1997. Furosemide interactions with brain GABA(A) receptors. Br. J. Pharmacol. 120, 741–748.

Kozlov, A.S., Angulo, M.C., Audinat, E., Charpak, S., 2006. Target cell-specific modulation of neuronal activity by astrocytes. Proc. Natl. Acad. Sci. U.S.A. 27, 10058–10063.

Krazem, A., Borde, N., Beracochea, D., 2001. Effects of diazepam and beta-CCM on working memory in mice: relationships with emotional reactivity. Pharmacol. Biochem. Behav. 68, 235–244.

Kushmerick, C., Renden, R., von Gersdorff, H., 2006. Physiological temperatures reduce the rate of vesicle pool depletion and short-term depression via an acceleration of vesicle recruitment. J. Neurosci. 26, 1366–1377.

Levin, H.S., Grossman, R.G., Rose, J.E., Teasdale, G., 1979. Long-term neuropsychological outcome of closed head injury. J. Neurosurg. 50, 412–422.

Liu, Q.Y., Schaffner, A.E., Chang, Y.H., Maric, D., Barker, J.L., 2000. Persistent activation of GABA(A) receptor/Cl(−) channels by astrocyte-derived GABA in cultured embryonic rat hippocampal neurons. J. Neurophysiol. 84, 1392–1403.

Llinas, R., Ribary, U., 2001. Consciousness and the brain. The thalamocortical dialogue in health and disease. Ann. N.Y. Acad. Sci. 929, 166–175.

Mangan, P.S., Sun, C., Carpenter, M., Goodkin, H.P., Sieghart, W., Kapur, J., 2005. Cultured hippocampal pyramidal neurons express two kinds of GABAA receptors. Mol. Pharmacol. 67, 775–788.

Mann, E.O., Mody, I., 2009. Control of hippocampal gamma oscillation frequency by tonic inhibition and excitation of interneurons. Nat. Neurosci. 13, 205–212.

Maubach, K., 2003. GABA(A) receptor subtype selective cognition enhancers. Curr. Drug Targets CNS Neurol. Disord. 2, 233–239.

Mayo, W., Dellu, F., Cherkaoui, J., Chapouthier, G., Dodd, R.H., Le, M.M., Simon, H., 1992. Cognitive enhancing properties of beta-CCM infused into the nucleus basalis magnocellularis of the rat. Brain Res. 589, 109–114.

Mayo, W., Dellu, F., Robel, P., Cherkaoui, J., Le, M.M., Baulieu, E.E., Simon, H., 1993. Infusion of neurosteroids into the nucleus basalis magnocellularis affects cognitive processes in the rat. Brain Res. 607, 324–328.

Merlio, J.P., Ernfors, P., Kokaia, Z., Middlemas, D.S., Bengzon, J., Kokaia, M., Smith, M.L., Siesjo, B.K., Hunter, T., Lindvall, O., Persson, H., 1993. Increased production of the TrkB protein tyrosine kinase receptor after brain insults. Neuron 10, 151–164.

Micheva, K.D., Smith, S.J., 2005. Strong effects of subphysiological temperature on the function and plasticity of mammalian presynaptic terminals. J. Neurosci. 25, 7481–7488.

Miles, R., Tóth, K., Gulyás, A.I., Hájos, N., Freund, T.F., 1996. Differences between somatic and dendritic inhibition in the hippocampus. Neuron 16, 815–823.

Mody, I., Pearce, R.A., 2004. Diversity of inhibitory neurotransmission through GABA(A) receptors. Trends Neurosci. 27, 569–575.

Mtchedlishvili, Z., Kapur, J., 2006. High-affinity, slowly desensitizing GABAA receptors mediate tonic inhibition in hippocampal dentate granule cells. Mol. Pharmacol. 69, 564–575.

Naylor, D.E., Liu, H., Wasterlain, C.G., 2005. Trafficking of GABA(A) receptors, loss of inhibition, and a mechanism for pharmacoresistance in status epilepticus. J. Neurosci. 25, 7724–7733.

Nusser, Z., Mody, I., 2002. Selective modulation of tonic and phasic inhibitions in dentate gyrus granule cells. J. Neurophysiol. 87, 2624–2628.

Nusser, Z., Roberts, J.D., Baude, A., Richards, J.G., Somogyi, P., 1995. Relative densities of synaptic and extrasynaptic GABAA receptors on cerebellar granule cells as determined by a quantitative immunogold method. J. Neurosci. 15, 2948–2960.

Nusser, Z., Hajos, N., Somogyi, P., Mody, I., 1998. Increased number of synaptic GABA(A) receptors underlies potentiation at hippocampal inhibitory synapses. Nature 395, 172–177.

O'Dell, D.M., Hamm, R.J., 1995. Chronic postinjury administration of MDL 26, 479 (Suritozole), a negative modulator at the GABAA receptor, and cognitive impairment in rats following traumatic brain injury. J. Neurosurg. 83, 878–883.

Otis, T.S., De, K.Y., Mody, I., 1994. Lasting potentiation of inhibition is associated with an increased number of gamma-aminobutyric acid type A receptors activated during miniature inhibitory postsynaptic currents. Proc. Natl. Acad. Sci. U.S.A. 91, 7698–7702.

Payne, H.L., Donoghue, P.S., Connelly, W.M., Hinterreiter, S., Tiwari, P., Ives, J.H., Hann, V., Sieghart, W., Lees, G., Thompson, C.L., 2006. Aberrant GABA(A) receptor expression in the dentate gyrus of the epileptic mutant mouse stargazer. J. Neurosci. 26, 8600–8608.

Please cite this article as: Mtchedlishvili, Z., et al., Increase of $GABA_A$ receptor-mediated tonic inhibition in dentate granule cells after traumatic brain injury, Neurobiol. Dis. (2010), doi:10.1016/j.nbd.2010.03.012

Peng, Z., Hauer, B., Mihalek, R.M., Homanics, G.E., Sieghart, W., Olsen, R.W., Houser, C.R., 2002. GABA(A) receptor changes in delta subunit-deficient mice: altered expression of alpha4 and gamma2 subunits in the forebrain. J. Comp. Neurol. 446, 179–197.

Peng, Z., Huang, C.S., Stell, B.M., Mody, I., Houser, C.R., 2004. Altered expression of the δ subunit of the $GABA_A$ receptor in a mouse model of temporal lobe epilepsy. J. Neurosci. 24, 8629–8639.

Perrais, D., Ropert, N., 1999. Effect of zolpidem on miniature IPSCs and occupancy of postsynaptic $GABA_A$ receptors in central synapses. J. Neurosci. 19, 578–588.

Pirker, S., Schwarzer, C., Czech, T., Baumgartner, C., Pockberger, H., Maier, H., Hauer, B., Sieghart, W., Furtinger, S., Sperk, G., 2003. Increased expression of GABA(A) receptor beta-subunits in the hippocampus of patients with temporal lobe epilepsy. J. Neuropathol. Exp. Neurol. 62, 820–834.

Povlishock, J.T., Becker, D.P., Cheng, C.L., Vaughan, G.W., 1983. Axonal change in minor head injury. J. Neuropathol. Exp. Neurol. 42, 225–242.

Pullela, R., Raber, J., Pfankuch, T., Ferriero, D.M., Claus, C.P., Koh, S.E., Yamauchi, T., Rola, R., Fike, J.R., Noble-Haeusslein, L.J., 2006. Traumatic injury to the immature brain results in progressive neuronal loss, hyperactivity and delayed cognitive impairments. Dev. Neurosci. 28, 396–409.

Qi, Z.H., Song, M., Wallace, M.J., Wang, D., Newton, P.M., McMahon, T., Chou, W.H., Zhang, C., Shokat, K.M., Messing, R.O., 2007. Protein kinase C epsilon regulates gamma-aminobutyrate type A receptor sensitivity to ethanol and benzodiazepines through phosphorylation of gamma2 subunits. J. Biol. Chem. 282, 33052–33063.

Racine, R.J., 1972. Modification of seizure activity by electrical stimulation. II. Motor seizure. Electroencephalogr. Clin. Neurophysiol. 32, 281–294.

Raffalli-Sebille, M.J., Chapouthier, G., Venault, P., Dodd, R.H., 1990. Methyl beta-carboline-3-carboxylate enhances performance in a multiple-trial learning task in mice. Pharmacol. Biochem. Behav. 35, 281–284.

Reeves, T.M., Lyeth, B.G., Phillips, L.L., Hamm, R.J., Povlishock, J.T., 1997. The effects of traumatic brain injury on inhibition in the hippocampus and dentate gyrus. Brain Res. 757, 119–132.

Richerson, G.B., Wu, Y., 2003. Dynamic equilibrium of neurotransmitter transporters: not just for reuptake anymore. J. Neurophysiol. 90, 1363–1374.

Rivera, C., Li, H., Thomas-Crusells, J., Lahtinen, H., Viitanen, T., Nanobashvili, A., Kokaia, Z., Airaksinen, M.S., Voipio, J., Kaila, K., Saarma, M., 2002. BDNF-induced TrkB activation down-regulates the K^+–Cl^- cotransporter KCC2 and impairs neuronal Cl^- extrusion. J. Cell Biol. 159, 747–752.

Rossi, D.J., Hamann, M., 1998. Spillover-mediated transmission at inhibitory synapses promoted by high affinity alpha6 subunit GABA(A) receptors and glomerular geometry. Neuron 20, 783–795.

Rudolph, U., Möhler, H., 2006. GABA-based therapeutic approaches: GABAA receptor subtype functions. Curr. Opin. Pharmacol. 6, 18–23.

Saatman, K.E., Feeko, K.J., Pape, R.L., Raghupathi, R., 2006. Differential behavioral and histopathological responses to graded cortical impact injury in mice. J. Neurotrauma 23, 1241–1253.

Santhakumar, V., Bender, R., Frotscher, M., Ross, S.T., Hollrigel, G.S., Toth, Z., Soltesz, I., 2000. Granule cell hyperexcitability in the early post-traumatic rat dentate gyrus: the 'irritable mossy cell' hypothesis. J. Physiol. 524, 117–134.

Santhakumar, V., Ratzliff, A.D., Jeng, J., Toth, Z., Soltesz, I., 2001. Long-term hyperexcitability in the hippocampus after experimental head trauma. Ann. Neurol. 50, 708–717.

Sassoè-Pognetto, M., Panzanelli, P., Sieghart, W., Fritschy, J.M., 2000. Colocalization of multiple $GABA_A$ receptor subtypes with gephyrin at postsynaptic sites. J. Comp. Neurol. 420, 481–498.

Shumate, M.D., Lin, D.D., Gibbs III, J.W., Holloway, K.L., Coulter, D.A., 1998. GABA(A) receptor function in epileptic human dentate granule cells: comparison to epileptic and control rat. Epilepsy Res. 32, 114–128.

Sieghart, W., Sperk, G., 2002. Subunit composition, distribution and function of GABA(A) receptor subtypes. Curr. Top. Med. Chem. 2, 795–816.

Silvers, J.M., Tokunaga, S., Berry, R.B., White, A.M., Matthews, D.B., 2003. Impairments in spatial learning and memory: ethanol, allopregnanolone, and the hippocampus. Brain Res. Brain Res. Rev. 43, 275–284.

Soltesz, I., Mody, I., 1995. Ca (2+)-dependent plasticity of miniature inhibitory postsynaptic currents after amputation of dendrites in central neurons. J. Neurophysiol. 73, 1763–1773.

Somogyi, P., Fritschy, J.M., Benke, D., Roberts, J.D., Sieghart, W., 1996. The gamma 2 subunit of the GABAA receptor is concentrated in synaptic junctions containing the alpha 1 and beta 2/3 subunits in hippocampus, cerebellum and globus pallidus. Neuropharmacology 35, 1425–1444.

Statler, K.D., Scheerlinck, P., Pouliot, W., Hamilton, M., White, H.S., Dudek, F.E., 2009. A potential model of pediatric posttraumatic epilepsy. Epilepsy Res. 86, 221–223.

Stell, B.M., Mody, I., 2002. Receptors with different affinities mediate phasic and tonic GABA(A) conductances in hippocampal neurons. J. Neurosci. 22, RC223.

Stringer, J.L., Lothman, E.W., 1989. Model of spontaneous hippocampal epilepsy in the anesthetized rat: electrographic, [K+]0, and [Ca2+]0 response patterns. Epilepsy Res. 4, 177–186.

Sun, C., Sieghart, W., Kapur, J., 2004. Distribution of alpha1, alpha4, gamma2, and delta subunits of GABAA receptors in hippocampal granule cells. Brain Res. 1029, 207–216.

Sun, C., Mtchedlishvili, Z., Erisir, A., Kapur, J., 2007. Diminished neurosteroid sensitivity of synaptic inhibition and altered location of the alpha4 subunit of GABA(A) receptors in an animal model of epilepsy. J. Neurosci. 27, 12641–12650.

Sundstrom-Poromaa, I., Smith, D.H., Gong, Q.H., Sabado, T.N., Li, X., Light, A., Wiedmann, M., Williams, K., Smith, S.S., 2002. Hormonally regulated alpha(4)beta(2)delta GABA(A) receptors are a target for alcohol. Nat. Neurosci. 8, 721–722.

Sur, C., Farrar, S.J., Kerby, J., Whiting, P.J., Atack, J.R., McKernan, R.M., 1999. Preferential coassembly of alpha4 and delta subunits of the gamma-aminobutyric acidA receptor in rat thalamus. Mol. Pharmacol. 56, 110–115.

Terunuma, M., Xu, J., Vithlani, M., Sieghart, W., Kittler, J., Pangalos, M., Haydon, P.G., Coulter, D.A., Moss, S.J., 2008. Deficits in phosphorylation of $GABA_A$ receptors by intimately associated protein kinase C activity underlie compromised synaptic inhibition during status epilepticus. J. Neurosci. 28, 376–384.

Toth, Z., Hollrigel, G.S., Gorcs, T., Soltesz, I., 1997. Instantaneous perturbation of dentate interneuronal networks by a pressure wave-transient delivered to the neocortex. J. Neurosci. 17, 8106–8117.

Tran, L.D., Lifshitz, J., Witgen, B.M., Schwarzbach, E., Cohen, A.S., Grady, M.S., 2006. Response of the contralateral hippocampus to lateral fluid percussion brain injury. J. Neurotrauma 23, 1330–1342.

Traub, R.D., Miles, R., 1991. Multiple modes of neuronal population activity emerge after modifying specific synapses in a model of the CA3 region of the hippocampus. Ann. N.Y. Acad. Sci. 627, 277–290.

Turkmen, S., Lofgren, M., Birzniece, V., Backstrom, T., Johansson, I.M., 2006. Tolerance development to Morris water maze test impairments induced by acute allopregnanolone. Neuroscience 139, 651–659.

Volgushev, M., Kudryashov, I., Chistiakova, M., Mukovski, M., Niesmann, J., Eysel, U.T., 2004. Probability of transmitter release at neocortical synapses at different temperatures. J. Neurophysiol. 92, 212–220.

Wafford, K.A., Thompson, S.A., Thomas, D., Sikela, J., Wilcox, A.S., Whiting, P.J., 1996. Functional characterization of human γ-aminobutyric $acid_A$ receptors containing the α4 subunit. Mol. Pharmacol. 50, 670–678.

Wall, M.J., Usowicz, M.M., 1997. Development of action potential-dependent and independent spontaneous GABAA receptor-mediated currents in granule cells of postnatal rat cerebellum. Eur. J. Neurosci. 9, 533–548.

Wallace, R.H., Marini, C., Petrou, S., Harkin, L.A., Bowser, D.N., Panchal, R.G., Williams, D.A., Sutherland, G.R., Mulley, J.C., Scheffer, I.E., Berkovic, S.F., 2001. Mutant GABA (A) receptor gamma2-subunit in childhood absence epilepsy and febrile seizures. Nat. Genet. 28, 49–52.

Wei, W., Zhang, N., Peng, Z., Houser, C.R., Mody, I., 2003. Perisynaptic localization of delta subunit-containing GABA(A) receptors and their activation by GABA spillover in the mouse dentate gyrus. J. Neurosci. 23, 10650–10661.

Yang, K., Perez-Polo, J.R., Mu, X.S., Yan, H.Q., Xue, J.J., Iwamoto, Y., Liu, S.J., Dixon, C.E., Hayes, R.L., 1996. Increased expression of brain-derived neurotrophic factor but not neurotrophin-3 mRNA in rat brain after cortical impact injury. J. Neurosci. Res. 44, 157–164.

Zhan, R.Z., Nadler, J.V., 2009. Enhanced tonic GABA current in normotopic and hilar ectopic dentate granule cells after pilocarpine-induced status epilepticus. J. Neurophysiol. 102, 670–681.

Please cite this article as: Mtchedlishvili, Z., et al., Increase of $GABA_A$ receptor-mediated tonic inhibition in dentate granule cells after traumatic brain injury, Neurobiol. Dis. (2010), doi:10.1016/j.nbd.2010.03.012