Enduring Changes in Tonic GABA_A Receptor Signaling in Dentate Granule Cells After Controlled Cortical Impact Brain Injury in Mice

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ABBREVIATIONS

Gamma-aminobutyric acid (GABA), tonic GABA current ($I_{\text{TonicGABA}}$), type A GABA receptor (GABA_AR), controlled cortical impact (CCI), dentate gyrus (DG), traumatic brain injury (TBI), dentate granule cell (DGC), 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridine-3-ol hydrochloride (THIP), 11,12,13,13a-Tetrahydro-7-methoxy-9-oxo-9H-imidazo[1,5-a]pyrrolo[2,1-c][1,4]benzodiazepine-1-carboxylic acid, ethyl ester (L655,708), $3\alpha,21$ -Dihydroxy- 5α -pregnan-20-one (THDOC), quantitative real-time polymerase chain reaction (qRT-PCR), artificial cerebrospinal fluid (ACSF), spontaneous inhibitory postsynaptic currents (sIPSCs), fluid percussion injury (FPI), type B GABA receptor (GABA_BR), protein kinase A (PKA), protein kinase C (PKC), calmodullin kinase II (CamKII), rous sarcoma oncogene cellular homolog (Src)

ABSTRACT

Changes in functional GABA_AR signaling in hippocampus have previously been evaluated using pre-clinical animal models of either diffuse brain injury or extreme focal brain injury that precludes measurement of cells located ipsilateral to injury. As a result, there is little information about the status of functional GABA_AR signaling in dentate granule cells (DGCs) located ipsilateral to focal brain injury, where significant cellular changes have been documented. We used whole-cell patch-clamp recordings from hippocampal slices to measure changes in GABA_ARs in dentate granule cells (DGCs) at 1-2 weeks and 8-13 weeks after controlled cortical impact (CCI) brain injury. Synaptic

and tonic GABA_AR currents ($I_{\text{TonicGABA}}$) were measured in DGCs at baseline conditions and during application of the GABA_AR agonist 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridine-3-ol hydrochloride (THIP) to assess in the function of δ subunit-containing GABA_ARs. DGCs ipsilateral to CCI exhibited no changes in the amplitude of resting tonic GABA_AR current ($I_{\text{TonicGABA}}$) relative to DGCs after sham-injury or contralateral to CCI. In contrast, there was a significant reduction in the THIP-evoked $I_{\text{TonicGABA}}$ in DGCs ipsilateral to CCI at both time-points. Tonic GABAergic inhibition of DGCs ipsilateral to injury also exhibited reduced responsiveness to the neurosteroid THDOC. $I_{\text{TonicGABA}}$ in DGCs ipsilateral to CCI did not exhibit a change in sensitivity to L655,708, an inverse agonist with selectivity for α_5 subunit-containing GABA_ARs, suggesting a lack of functional change in GABA_ARs containing this subunit. At the 8-13 week time-point, gene expression of GABA_AR subunits expected to contribute to $I_{\text{TonicGABA}}$ (i.e., α_4 , α_5 and δ) was not significantly altered by CCI injury in isolated dentate gyrus. Collectively, these results demonstrate enduring functional changes in $I_{\text{TonicGABA}}$ in DGCs ipsilateral to focal brain injury that occur independent of altered gene expression.

KEYWORDS

GABA_A receptors, tonic GABA current, controlled cortical impact, traumatic brain injury, post-traumatic epilepsy

INTRODUCTION

Type A GABA receptors (GABA_AR's) are prominent inhibitory signaling components of the central nervous system and are formed as heteropentamers of α_{1-6} , β_{1-4} , γ_{1-3} , δ , ϵ , θ and π subunits (summarized in Barnard et al., 1998; Kapur and Macdonald, 1999; Laurie et al., 1992; Macdonald and Olsen, 1994; Whiting et al., 1999). The subunit composition of these pentamers varies by cell type and brain location and confers receptor kinetics and pharmacological response profiles (summarized in Davies et al., 1997; Draguhn et al., 1990; Farrant and Nusser, 2005; Glykys and Mody, 2007; Lüddens and Wisden, 1991; Verdoorn et al., 1990). In adult rodents, dentate granule cells (DGCs) express functional subunit compositions that are either predominantly $\alpha_1\beta\gamma_2$ for synaptic signaling or predominantly $\alpha_4\beta\delta$ for tonic current signaling, although other subunit combinations can occur (summarized in Brown et al., 2002; Chang et al., 1996; Farrant and Nusser, 2005; Glykys et al., 2008; Mody, 2001; Mtchedlishvili and Kapur, 2006; Semyanov et al., 2004; Stell et al., 2003; Wei et al., 2003).

Pathology-induced changes in the activity or pharmacological responsiveness of GABA_ARs may contribute to cellular and network hyperexcitability and disrupted information encoding that accompanies cognitive impairment, behavioral and motor dysfunction, and epileptogenesis after brain injury. Further, changes in functional GABA_AR signaling have been reported to reflect rearrangements in GABA_AR subunit composition (Brooks-Kayal et al., 1998; Cossart et al., 2001; Gibbs et al., 1997; Leroy et al., 2004). Aberrant GABA signaling and pharmacological responsiveness of GABA_ARs have been reported after a variety of brain insults in animal models, including cerebral

ischemia(Kharlamov et al., 2008; Redecker et al., 2002), fluid percussion injury (Drexel et al., 2015; Gupta et al., 2012; Pavlov et al., 2011; Raible et al., 2012), kindling-induced status epilepticus (Sun et al., 2007) and chemoconvulsant-induced status epilepticus (Peng et al., 2004; Zhan and Nadler, 2009; Zhang et al., 2007; Zhang et al., 2012). Acute seizures alone may also drive GABA_AR subunit reorganization (Kapur and Macdonald, 1997).

Traumatic brain injury (TBI) leads to reorganization of brain networks, which can include selective cell loss, aberrant axonal arrangements, and synaptic reorganization(Hunt et al., 2009, 2010, 2011; Lowenstein et al., 1992; Santhakumar et al., 2000; Santhakumar et al., 2001; Scheff et al., 2005; Swartz et al., 2006). Controlled cortical impact (CCI) is a model of focal TBI used to investigate mechanisms of brain repair (Dixon et al., 1991; Hall et al., 2005; Lighthall, 1988; Scheff et al., 1997) and posttraumatic epilepsy (Hunt et al., 2013; Hunt et al., 2012; Hunt et al., 2009, 2010, 2011). At present, functional changes in GABA_AR subunits after focal brain injury have been characterized after extreme CCI that results in a loss of ipsilateral hippocampus, rendering it impractical to perform electrophysiological recordings or molecular analysis near the injury epicenter(Kharlamov et al., 2011; Mtchedlishvili et al., 2010), where many cellular changes in DG circuitry occur (Hunt et al., 2012; Hunt et al., 2009, 2010, 2011). As a result, understanding GABA_AR subunit composition and signaling in the context of posttraumatic epileptogenesis and brain reorganization, requires assessment of changes in the injured hemisphere.

Here, functional changes in GABA_AR populations in DGCs after focal brain trauma were assessed using whole-cell patch-clamp recordings of DGCs 1-2 or 8-12

weeks after CCI injury. Effects of pharmacological agents having relative selectivity for GABA_ARs containing α_5 and δ subunits were assessed, since these subunits contribute predominantly to tonic GABA_AR-mediated currents in DGCs (summarized in Brown et al., 2002; Chang et al., 1996; Farrant and Nusser, 2005; Glykys et al., 2008; Mody, 2001; Mtchedlishvili and Kapur, 2006; Semyanov et al., 2004; Stell et al., 2003; Wei et al., 2003). Brain injury-induced changes in gene expression of GABA_AR subunits in the dentate gyrus were also examined using quantitative real-time polymerase chain reaction (qRT-PCR). We tested the hypotheses that functional GABA_AR signaling and GABA_AR subunit composition are altered in DGCs located ipsilateral to CCI and maintained for months after injury.

METHODS

Animals. Six-to-eight-week-old adult male CD-1 mice were housed under a normal 14/10 hour light/dark cycle and given food and water *ad libitum*. All animals were acclimated to the University of Kentucky vivarium for at least one week prior to experimentation. All procedures were approved by the University of Kentucky Animal Care and Use Committee and adhered to NIH guidelines for the care and use of laboratory animals.

Brain Injury. Mice were administered unilateral, focal contusion injury using controlled cortical impact (CCI) as previously reported (Hunt et al., 2009, 2010; Scheff et al., 1997). Briefly, animals were anesthetized using 2% isoflurane and placed in a

stereotaxic frame. A midline incision was made to reveal the skull and a 4 mm craniotomy was performed lateral to the sagittal suture and centered between bregma and lambda. The skull cap was removed without damage to the underlying dura. A computer controlled, pneumatically driven impactor fitted with a 3 mm stainless-steel beveled tip was used to compress the cortex (TBI-0310; Precision Systems and Instrumentation; Fairfax Station; VA). Impact parameters were set to 1.0 mm depth (hard stop), 3.5 m/sec velocity, and 500 ms duration. For sham-injured controls, craniotomy was performed but no impact was administered to the brain. The incision was sutured and the animals were allowed to recover. All mice given CCI survived and remained otherwise healthy until experimentation. These parameters result in a well-characterized injury (Guo et al., 2013; Hunt et al., 2012; Hunt et al., 2009, 2010, 2011). The animals in this study were not monitored for seizure activity. Previous studies showed that 30-50% of similarly-injured mice exhibit spontaneous seizures by about eight weeks post-injury (Guo et al., 2013; Hunt et al., 2009, 2010). In this study, we did not observe a sorting of the inhibitory changes into this proportion of animals (i.e., the changes were observed consistently across mice), so we did not seek to further test a relationship between these changes and seizure expression.

Hippocampal Slice Preparation. Mice were anesthetized by isoflurane inhalation to effect (lack of tail pinch response) and decapitated while anesthetized. The brain was then rapidly removed and immediately immersed in ice-cold (0–4°C), oxygenated (95% O₂-5% CO₂) artificial cerebrospinal fluid (ACSF) containing (in mM) 124 NaCl, 3 KCl, 26 NaHCO₃, 1.4 NaH₂PO₄, 11 glucose, 1.3 CaCl₂, 1.3 MgCl₂; pH = 7.2–7.4, with an

osmolality of 290–305 mosmol/kg H₂O. ACSF always contained the glutamate receptor antagonist kynurenic acid (1 mM; Sigma-Aldrich; St. Louis, MO). The brain was blocked, mounted on a sectioning stage, and 350 μ M slices were cut in the coronal plane with a vibrating microtome (Vibratome Series 1000; Technical Products International; St. Louis, MO). Slices were cut in the coronal plane to ensure consistency of tranverse slices from the dorsal one-third of the hippocampus. Each hippocampus was isolated from surrounding brain areas, making sure to completely remove entorhinal cortex, and their order maintained so that the location relative to the injury within each hippocampus was known. The slices were then transferred to a holding chamber where they were superfused with warmed (32–34°C) ACSF. The ACSF used for recordings was identical to that used in the dissection, except when drugs were added as described.

Electrophysiology. After an equilibration period of at least 1 hour, slices were transferred to a recording chamber on an upright, fixed-stage microscope equipped with infrared, differential interference contrast (IR-DIC; Olympus BX51WI; Melville, NY), where they were continually superfused with warmed (32–34°C) ACSF. Whole-cell patch-clamp recordings were performed from visualized hippocampal DGCs. Recording pipettes were pulled (P-87, Sutter Instruments; Novato, CA) from borosilicate glass capillaries with 1.65 mm outer diameter and 0.45 mm wall thickness (King Precision Glass; Claremont, CA). Open tip resistance was 2–5 M Ω , seal resistance was 1–5 G Ω , series resistance was <25 M Ω (mean=12.02 $\pm$ 0.68 M Ω , n=85), uncompensated. Recordings in which series resistance varied by more than 20% were discarded. Recording pipettes were filled with (in mM) 140 Cs-gluconate, 1 NaCl, 5

EGTA, 10 HEPES, 1 MgCl₂, 1 CaCl₂, 3 KOH, 2 ATP; pH =7.15–7.30. Using Cs⁺ as the primary cation carrier blocks K⁺ conductances, including GABA_B receptor-mediated responses, providing for improved voltage-clamp at 0 mV while measuring outward Cl⁻ currents. Neural activity was recorded using Axon Instruments Axopatch 200B and Multiclamp 700B patch-clamp amplifiers (Molecular Devices; Sunnyvale, CA), acquired at 10-20 kHz and low-pass filtered at 5 kHz using Digidata 1320A and 1322A digitizers and pClamp 10.3 software (Molecular Devices). Synaptic currents were analyzed off-line on a PC-style computer with pCLAMP programs (Molecular Devices) or Minianalysis 6.0.3 (Synaptosoft; Decatur, GA). Inhibitory postsynaptic currents were blocked by the GABA_A receptor antagonist bicuculline (30 μM). A value of 3x the root mean squared noise level for a given recording was used as the detection limit for synaptic currents. A single-exponential IPSC decay time constant was measured in Minianalysis using a Fraction of Peak to Find a Decay Time setting of 0.37 with no weighted adjustments. Spontaneous inhibitory postsynaptic currents (sIPSCs) and tonic GABA_A currents (I_{TonicGABA}) were recorded in voltage-clamp mode with a voltage command of 0 mV. GABA_AR-mediated responses, including tonic GABA_AR inhibition, can exhibit voltage-dependent outward rectification (Pavlov et al., 2009; Weiss, 1988). Under conditions of low extracellular GABA concentration in slices, more positive membrane potentials result in increased open GABA_AR probability (Weiss, 1988; Weiss et al., 1988), increased activation and decay kinetics (Akaike et al., 1986; Collingridge et al., 1984; Segal and Barker, 1984) and reduced receptor desensitization (Yoon, 1994). Thus, at depolarized membrane potentials, such as the 0 mV voltage-clamp recordings used here, the currents generated during GABA binding may be anticipated to be larger,

slower and less desensitizing relative to those at more hyperpolarized voltage commands.

A minimum of 5 minutes following establishment of whole-cell configuration was used in order to allow equilibration of the intracellular and recording pipette contents. Added to the ACSF for specific experiments were the following: bicucullinemethiodide (30 μ M; Tocris Bioscience; Minneapolis, MN), 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridine-3-ol hydrochloride (THIP; gaboxadol; 3 μ M; Sigma-Aldrich), 3 α ,21-Dihydroxy-5 α -pregnan-20-one (THDOC; 1 μ M; Sigma-Aldrich) (Belelli and Lambert, 2005; Maguire and Mody, 2007), nipecotic acid (0.5 mM; Sigma-Aldrich) and 11,12,13,13a-Tetrahydro-7-methoxy-9-oxo-9H-imidazo[1,5-a]pyrrolo[2,1-c][1,4]benzodiazepine-1-carboxylic acid, ethyl ester (L655,708; 5 μ M; Tocris Bioscience). Unless otherwise stated, neurons were recorded for 5 minutes under baseline conditions, 10 minutes during THIP or L655,708 application, and then for 5 minutes during application of bicuculline-methiodide.

RNA Isolation & cDNA. Isolated dentate gyrus was harvested from each hemisphere and processed according to previously described methodology (Boychuk et al., 2015; Hideo et al., 2009). The tissue was visualized with a dissecting microscope (SMZ800; Nikon; Melville, NY) and was submerged in ice-cold (0–4°C) ACSF for all steps of dissection. The dorsal half of each isolated dentate gyrus was selected for transcript analysis because the greatest proportion of cell signaling changes in the ipsilateral dentate gyrus (eg., mossy fiber sprouting and interneuron loss) are observed in this region after CCI (Hunt et al., 2012; Hunt et al., 2009, 2010, 2011). Resulting isolated,

dorsal halves of dentate gyrus ipsilateral and contralateral to CCI or sham-injury were treated as individual samples. Each sample was immediately homogenized in ice-cold 500 μ L of Trizol (Sigma-Aldrich). Chloroform (100-250 μ l) was added and tubes were vortexed for 15 s and then maintained at 4°C for 20 min and subsequently centrifuged at 12,000 rpm for 15 min at 4°C. The pellet was discarded and the RNA supernatant was transferred into fresh 1.5 ml centrifuge tubes, mixed with 500 μ L of ice-cold propanol, incubated at room temperature for 10 min, and centrifuged at 12,000 rpm for 10 min at 4°C. Propanol was decanted and RNA was washed by re-suspension in 500 μ L 75% ethanol followed by centrifugation at 7500-12,000 rpm for 10 min at 4°C. The wash step was repeated, the ethanol decanted, and RNA samples were air-dried for 10-20 min. RNA was then re-dissolved in 20 μ L ddH₂O. Spectrophotometry (Nanodrop, Fisher Scientific, Wilmington, DE) was used to determine mRNA concentration and purity.

TaqMan Quantitative Real-time Polymerase Chain Reaction (qRT-PCR). qRT-PCR was performed as previously described (Boyчук et al., 2015). Samples of mRNA (2 μ g) were used to create cDNA using SuperScript II reverse transcriptase kit (Invitrogen, Carlsbad, CA) using a Mastercycler (Eppendorf, Hauppauge, NY). All qRT-PCR reactions were run in triplicate in 96-well optical grade plates using a 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA). Each run consisted of 1 cycle of 50°C for 2 min, then 1 cycle of 95°C for 10 min, and 40 cycles of 95°C for 15 sec, and 60°C for 1 min. Total volume for each run was 20 μ L containing 10 μ L of TaqMan 2x PCR Master Mix (Applied Biosystems), 1 μ L of primer (Applied Biosystems) and a

combination of cDNA and ddH₂O that provided a cDNA concentration of 50 ng. All reactions used forward and reverse primer concentrations of 100 nM. Probe concentrations for all reactions were 50 nM. Primer and Taqman probe sets were purchased from Applied Biosystems. The sequences for each were generated from the listed references within GenBank: α 4: Mm00802631_m1; α 5: Mm00621092_m1; δ : Mm01266203_g1; β -actin: Mm00607939_s1. No-template and no-RT controls were run for each plate and results discarded if false positives were present.

Data Analysis and Statistics. For electrophysiology data, individual 3 minute samples of recording were used to measure neuronal responses (sIPSCs or $I_{\text{TonicGABA}}$) under baseline conditions or in the presence of pharmacological compounds. Resting $I_{\text{TonicGABA}}$ was measured by subtracting mean steady-state holding current values at baseline from mean steady-state holding current values in the presence of bicuculline. Drug-modified $I_{\text{TonicGABA}}$ was measured by subtracting mean steady-state holding current values during drug application from mean steady-state holding current values in the presence of bicuculline. $I_{\text{TonicGABA}}$ values were normalized to cell capacitance (Glykys and Mody, 2007). Current variance analysis of $I_{\text{TonicGABA}}$ was not performed in this study, since macroscopic current is considered effective for measuring activity of receptors mediating $I_{\text{TonicGABA}}$ when intracellular chloride concentration is low (Bright and Smart, 2013). For sIPSCs, event frequency, amplitude and decay time constant were measured using MiniAnalysis (Synaptosoft, Decatur, GA). Groups were compared by one-way ANOVA followed by Tukey post hoc comparison or two-tailed student's t test. All electrophysiological parameters were averaged across neurons (i.e., n = number of

neurons). For gene expression data, fold change in receptor subunit expression was calculated by the formula $2^{-\Delta\Delta C_t}$, using β -actin as an internal reference (Livak and Schmittgen, 2001; Schmittgen and Livak, 2008). Relative mRNA abundance was presented as fold change; the ΔC_t values were used to determine statistical significance using two-tailed, unpaired, t-test (Wood and Giroux, 2003). Data are expressed as mean $\pm$ SEM and significance was set at $p < 0.05$.

RESULTS

Synaptic and tonic GABA_AR-mediated currents were assessed at both 1-2 weeks and 8-13 following CCI or sham injury. Comparisons were made between DGCs from sham animals (i.e., sham), DGCs contralateral to CCI injury (i.e., contralateral DGCs) and DGCs ipsilateral to CCI injury (i.e., ipsilateral DGCs).

Effect of THIP on tonic GABA_AR current 1-2 weeks post-injury. A previous study using a more severe CCI injury reported changes in resting $I_{\text{TonicGABA}}$ in DGCs contralateral to CCI (Mtchedlishvili et al., 2010) but this conductance has not been studied in DGCs located ipsilateral to CCI. Here, the current amplitude of $I_{\text{TonicGABA}}$ was assessed in DGCs from uninjured and injured hemispheres using voltage-clamp recordings prior to drug application, during application of THIP, and during co-application of THIP and the GABA_AR antagonist bicuculline (Fig. 1). Current amplitudes were normalized to cell capacitance in order to account for differences in cell size. THIP activates non- δ subunit-containing GABA_ARs only at high concentrations, whereas THIP

concentrations of $\leq 3 \mu\text{M}$ primarily activate $\alpha_4\beta_x\delta$ subunit-containing GABA_ARs (Adkins et al., 2001; Brown et al., 2002; Ebert et al., 1994; Glykys and Mody, 2007; í Stórustovu and Ebert, 2006; Stell et al., 2003).

Resting $I_{\text{TonicGABA}}$ was calculated as the difference between normalized current amplitude at baseline and normalized current amplitude during GABA_AR block using THIP+bicuculline. Resting $I_{\text{TonicGABA}}$ in DGCs ipsilateral to CCI injury (mean= 0.69 ± 0.16 pA/pF; n=9) was not significantly different than in DGCs from sham operated mice (mean= 0.73 ± 0.11 pA/pF; n=9; Tukey, $p=0.98$) or DGCs contralateral to CCI (mean= 0.87 ± 0.12 pA/pF; n=9; Tukey, $p=0.62$) (1-Way ANOVA; $F(2,24)=0.50$, $p=0.61$; Fig. 1A-D). Thus, resting $I_{\text{TonicGABA}}$ was similar across all groups 2 weeks after CCI.

THIP application resulted in a significant outward shift in holding current (2-Way ANOVA; $F(1,24)=110.10$, $p<0.00010$; Fig. 1A-D). Holding current was significantly changed in sham DGCs (Bonferroni, $p<0.00010$), contralateral DGCs (Bonferroni, $p<0.00010$) and ipsilateral DGCs (Bonferroni, $p=0.0063$). The responsiveness of $I_{\text{TonicGABA}}$ to the agonist THIP was calculated as the difference between current amplitude during THIP and THIP+bicuculline application. There was a significant reduction in THIP responsiveness of $I_{\text{TonicGABA}}$ in ipsilateral DGCs (mean= 1.84 ± 0.32 pA/pF) relative to sham DGCs (mean= 3.31 ± 0.34 pA/pF; Tukey, $p=0.032$) and contralateral DGCs (mean= 3.21 ± 0.47 pA/pF; Tukey, $p=0.047$; 1-Way ANOVA; $F(2,24)=4.59$, $p=0.021$; Fig. 1D). Sham DGCs and contralateral DGCs did not differ in resting $I_{\text{TonicGABA}}$ (Tukey, $p=0.73$) or in $I_{\text{TonicGABA}}$ THIP responsiveness (Tukey, $p>0.99$; Fig. 1A,B). Thus, whereas resting (i.e., bicuculline-sensitive) $I_{\text{TonicGABA}}$ in DGCs is not altered after focal brain injury, the $I_{\text{TonicGABA}}$ in these cells exhibited reduced

responsiveness to THIP 1-2 weeks after CCI.

Effect of THIP on sIPSC frequency 1-2 weeks post-injury. Brain injury results in a loss of GABAergic hilar interneurons (Lowenstein et al., 1992) and this cell loss may contribute to the reduced GABAergic synaptic drive to DGCs after CCI (Hunt et al., 2011). After seizures, GABAergic neurons located presynaptically to DGCs may undergo GABA_AR subunit modification in a manner that is predicted to alter their responsiveness to THIP (Peng et al., 2004; Zhang et al., 2007). Here, we determined whether sIPSC frequency in DGCs was affected by THIP application and whether this property was altered with focal brain injury (Fig. 2A-D). Under baseline conditions, sIPSC frequency in ipsilateral DGCs (mean = 1.16 ± 0.06 Hz; n=9) was significantly lower than in sham DGCs (mean = 2.43 ± 0.22 Hz; n=9; Tukey, p=0.0001) and contralateral DGCs (mean = 2.39 ± 0.22 Hz; n=9; Tukey, p=0.0002; 1-Way ANOVA; $F(2,24)=15.72$, p<0.0001; Fig. 2A-D), similar to a previous report (Hunt et al., 2011).

Application of THIP significantly reduced sIPSC frequency in sham DGCs (mean = 1.86 ± 0.10 Hz; Bonferroni, p=0.0006) and contralateral DGCs (mean = 1.91 ± 0.17 Hz; Bonferroni, p=0.0037), whereas sIPSC frequency in ipsilateral DGCs failed to show this reduction (mean = 1.03 ± 0.07 Hz; Bonferroni, p>0.99; 2-Way ANOVA; $F(1,24)=26.98$, p<0.0001; Fig. 2A-D). sIPSC frequency in sham DGCs and contralateral DGCs did not significantly differ at baseline (Tukey, p=0.99) and were reduced to similar levels by THIP application (Tukey, p=0.95; Fig. 2A,B,D). As a result, while CCI injury results in reduced spontaneous inhibitory synaptic drive to DGCs ipsilateral to injury, the reduction in sIPSC frequency that was observed in control DGCs during THIP

application was not observed in DGCs ipsilateral to CCI.

Effect of THIP on sIPSC amplitude 1-2 weeks post-injury. The effect of THIP application on sIPSC amplitude was assessed in DGCs from sham-injured control and CCI-injured mice (Fig. 2A-C,E). At baseline, sIPSC amplitude was not different in DGCs from injured versus uninjured mice (1-Way ANOVA; $F(2,24)=0.045$, $p=0.96$). Application of THIP failed to significantly alter sIPSC amplitude in sham DGCs (22.39 versus 24.65 pA; $n=9$; Bonferroni, $p>0.99$), contralateral DGCs (22.46 versus 23.98 pA; $n=9$; Bonferroni, $p>0.99$) or ipsilateral DGCs (21.21 versus 23.51 pA; $n=9$; Bonferroni, $p>0.99$) (2-Way ANOVA; $F(1,24)=1.32$, $p=0.26$; Fig. 2A-C,E).

Effect of THIP on sIPSC decay time constant 1-2 weeks post-injury. We then determined whether sIPSC decay time constant in DGCs was altered by THIP application or after CCI injury (Fig. 2A-C,F). At baseline, there was a non-significant trend toward increased sIPSC decay time constant 1-2 weeks after CCI in DGCs ipsilateral to the injury (mean: 19.18 ± 2.27 ms; $n=9$) versus DGCs from sham-injured mice (mean: 14.45 ± 1.16 ms; $n=9$; Tukey, $p=0.12$) or DGCs contralateral to the injury (mean: 14.77 ± 1.17 ms; $n=9$; Tukey, $p=0.15$; 1-Way ANOVA; $F(2,24)=2.67$, $p=0.089$). THIP did not significantly affect sIPSC decay time constant in ipsilateral DGCs (mean: 18.85 ± 2.23 ms), sham DGCs (mean: 15.23 ± 1.20 ms) or contralateral DGCs (mean: 15.15 ± 1.02 ms; 2-Way ANOVA; $F(1,24)=0.24$, $p=0.63$; Fig. 2A-C,F). Collectively, these data suggest changes in THIP sensitivity of $I_{\text{TonicGABA}}$ and sIPSC frequency by 1-2 weeks in DGCs ipsilateral to CCI injury, whereas postsynaptic changes in the sensitivity of

sIPSCs to THIP were not apparent.

Effect of THIP on tonic GABA_AR current 8-13 weeks post-injury. Studies have reported changes in GABA_AR subunit organization shortly after brain injury that resolve over time (Drexel et al., 2015; Pavlov et al., 2011). Since a significant reduction in THIP-induced $I_{\text{TonicGABA}}$ was detected 1-2 weeks after CCI, we determined whether the reduction in THIP-induced $I_{\text{TonicGABA}}$ persisted 8-13 weeks after CCI (Fig. 3A-D). Similar to the earlier time-point, the amplitude of resting $I_{\text{TonicGABA}}$ (baseline – bicuculline) was unaffected 8-13 weeks after CCI: ipsilateral DGCs (mean = 0.72 ± 0.15 pA/pF; n=7) versus sham DGCs (mean = 0.78 ± 0.34 pA/pF; n=7; Tukey, p=0.98) or versus contralateral DGCs (mean = 0.88 ± 0.17 pA/pF; n=8; Tukey, p=0.88; 1-Way ANOVA; $F(2,19)=0.12$, p=0.88; Fig. 3A-D).

THIP application resulted in a significant increase in normalized tonic GABA_AR current in sham DGCs (Bonferroni, p<0.00010) and contralateral DGCs (Bonferroni, p<0.00010; Fig. 3A,B). At this time-point however, THIP application alone failed to significantly affect $I_{\text{TonicGABA}}$ in ipsilateral DGCs (Bonferroni, p=0.11) (2-Way ANOVA; $F(1,19)=87.31$, p<0.00010; Fig. 3C). Ipsilateral DGCs (mean = 1.67 ± 0.24 pA/pF) exhibited a significantly lower THIP-induced tonic current amplitude compared to sham DGCs (mean = 3.63 ± 0.37 pA/pF; Tukey, p=0.0020) and contralateral DGCs (mean = 3.81 ± 0.37 pA/pF; Tukey, p=0.0006) 8-13 weeks after CCI (1-Way ANOVA; $F(2,19)=12.17$, p=0.0004; Fig. 3A-D). Thus, focal brain injury significantly reduced THIP responsiveness in DGC $I_{\text{TonicGABA}}$ within two weeks of CCI and this effect persisted 8-13 weeks post-injury. Sham DGCs and contralateral DGCs did not significantly differ from

each other in the amplitude of normalized tonic GABA_AR current at baseline (Tukey, $p=0.95$) or in the presence of THIP (Tukey, $p=0.92$).

Effect of the neurosteroid THDOC on tonic GABA_AR currents 3-5 weeks post-injury. Tonic inhibition of DGCs is enhanced by neurosteroids acting at δ subunit-containing GABA_ARs (Stell et al., 2003). In this present study, the reduced response to THIP in DGCs located ipsilateral to CCI suggests a reduction in δ subunit-containing GABA_AR signaling, and propounds a potential reduction in neurosteroid responses (Farrant and Nusser, 2005; Mtchedlishvili and Kapur, 2006; Semyanov et al., 2004; Stell et al., 2003; Wei et al., 2003). Accordingly, an additional experiment was undertaken to test whether neurosteroid regulation of GABAergic signaling in DGCs was affected by focal brain injury. The effect of THDOC on $I_{\text{TonicGABA}}$ was measured in DGCs from uninjured and injured hemispheres. Effects of THDOC (1 μM) were calculated as the difference between normalized current amplitude during THDOC application and THDOC+bicuculline. In order to evoke measurable THDOC responses, recordings were performed in the presence of the GABA reuptake inhibitor nipecotic acid (0.5 mM) (Stell et al., 2003; Zhang et al., 2007).

There was no difference in resting $I_{\text{TonicGABA}}$ (baseline – bicuculline) between DGCs from sham animals (mean = 1.47 ± 0.57 pA/pF; $n=7$) and DGCs from the contralateral hemisphere of CCI-injured animals (mean = 1.13 ± 0.30 pA/pF; $n=7$) ($T(12)=0.54$, $p=0.60$). Similarly, DGCs from sham animals (mean = 11.61 ± 0.86 pA/pF) and DGCs from the contralateral hemisphere of CCI-injured animals (mean = 12.29 ± 2.21 pA/pF) did not significantly differ in $I_{\text{TonicGABA}}$ in the presence of THDOC (THDOC –

bicuculline) ($T(12) = 0.28$, $p = 0.78$). DGCs from sham animals and from the contralateral hemisphere of CCI-injured animals were combined into a single control group (Butler et al., 2015). As observed throughout this study, resting $I_{\text{TonicGABA}}$ in DGCs located ipsilateral to CCI (mean = 1.49 ± 0.32 pA/pF; $n = 7$) did not significantly differ from control DGCs (mean = 1.30 ± 0.31 pA/pF; $n = 14$; $T(19) = 0.38$, $p = 0.71$). In contrast, DGCs located ipsilateral to CCI (mean = 8.55 ± 1.51 pA/pF; $n = 7$) exhibited a significantly smaller $I_{\text{TonicGABA}}$ in the presence of THDOC compared to control DGCs (mean = 11.95 ± 1.14 pA/pF; $n = 14$; $T(19) = 1.75$, $p = 0.048$; Fig. 3E). These findings indicate that DGCs ipsilateral to focal brain injury exhibit a reduction in the $I_{\text{TonicGABA}}$ response to the neurosteroid, THDOC.

Effect of THIP on sIPSC frequency 8-13 weeks post-injury. Since we observed a robust reduction in THIP's ability to reduce sIPSC frequency in DGCs 1-2 weeks after brain injury, we determined whether this loss of responsiveness persisted 8-13 weeks post-injury (Fig. 4A-D). Under baseline conditions, sIPSC frequency was significantly lower at this time-point in ipsilateral DGCs (mean = 1.33 ± 0.10 Hz; $n = 7$) compared to contralateral DGCs (mean = 2.15 ± 0.25 Hz; $n = 8$; Tukey, $p = 0.0084$) and sham DGCs (mean = 2.18 ± 0.10 Hz; $n = 7$; Tukey, $p = 0.0090$; 1-Way ANOVA; $F(2,19) = 7.47$, $p = 0.0040$; Fig. 4A-D). There was no difference in sIPSC frequency between contralateral DGCs and sham DGCs at baseline (Tukey, $p = 0.99$).

DGCs from sham-injured mice exhibited a significant reduction in sIPSC frequency in response to THIP application (mean = 1.54 ± 0.16 Hz; Bonferroni, $p = 0.012$), whereas contralateral DGCs exhibited a non-significant trend for a THIP-induced

reduction in synaptic frequency (mean=1.72± 0.16 Hz; Bonferroni, $p=0.090$; 2-Way ANOVA; $F(1,19)=14.46$, $p=0.0012$; Fig. 4A,B,D). There was no difference in sIPSC frequency during THIP application between contralateral DGCs and Sham DGCs (Tukey, $p=0.66$). In contrast, THIP application did not change sIPSC frequency in DGCs ipsilateral to injury (mean=1.14± 0.11 Hz; Bonferroni, $p>0.99$; Fig. 4C). Thus, in addition to displaying reduced inhibitory synaptic input overall, DGCs ipsilateral to injury displayed little effect of THIP on sIPSC frequency relative to control cells, suggesting a presynaptic decrease in THIP responsiveness of local inhibitory neurons that persists for months after CCI-injury.

Effect of THIP on sIPSC amplitude 8-13 weeks post-injury. Despite a lack of change in sIPSC amplitude in DGCs at the 1-2 week time-point, we determined whether THIP altered sIPSC amplitude 8-13 weeks post-injury (Fig. 4A-C,E). There was no significant difference in sIPSC amplitude between injured and control DGCs at baseline (1-Way ANOVA; $F(2,19)=0.085$, $p=0.92$) or in the presence of THIP (1-Way ANOVA; $F(2,19)=0.0022$, $p>0.99$; Fig. 4A-C,E). sIPSC amplitude was not altered by THIP in sham DGCs (20.36 versus 21.02 pA; $n=7$; Bonferroni, $p>0.99$), contralateral DGCs (20.59 versus 20.81 pA; $n=8$; Bonferroni, $p>0.99$) or ipsilateral DGCs (21.42 versus 20.93 pA; $n=7$; Bonferroni, $p>0.99$; 2-Way ANOVA; $F(1,19)=0.011$, $p=0.92$).

Effect of THIP on sIPSC decay time constant 8-13 weeks post-Injury. We also assessed the effects of THIP on sIPSC decay time constant in DGCs 8-13 weeks CCI (Fig. 4A-C,F). Prior to THIP application, sIPSC decay time constant was significantly

greater in ipsilateral DGCs (mean=19.04± 1.29; n=7) versus sham DGCs (mean=14.10± 1.34; n=7; Tukey, p=0.031) or contralateral DGCs at baseline (mean=13.31± 1.09; n=8; Tukey, p=0.0096; 1-Way ANOVA; F(2,19)=6.27, p=0.0081) and in the presence of THIP (1-Way ANOVA; F(2,19)=5.93, p=0.010) (Fig. 4A-C,F). THIP application failed to significantly change sIPSC decay time constant in sham DGCs (mean= 13.28± 1.33 ms; Bonferroni, p>0.99), contralateral DGCs (mean= 13.27± 1.26 ms; Bonferroni, p>0.99) or ipsilateral DGCs (mean= 18.77± 1.27 ms; Bonferroni, p>0.99; 2-Way ANOVA; F(1,19)=0.23, p=0.63; Fig. 4A-C,F). sIPSC decay time constant was not significantly different between sham DGCs and contralateral DGCs at baseline (p=0.96) or in the presence of THIP (p>0.99). Thus, THIP did not affect sIPSC decay time constant in uninjured or injured DGCs.

Effect of L655,708 on tonic GABA_AR currents 8-13 weeks post-injury. THIP exhibits selectivity for δ subunit-containing GABA_ARs and the δ subunit typically pairs with the α_4 subunit to form $\alpha_4\beta_x\delta$ subunit-containing GABA_ARs in DGCs (summarized in Brown et al., 2002; Chang et al., 1996; Farrant and Nusser, 2005; Glykys et al., 2008; Hoestgaard-Jensen et al., 2014; Mody, 2001; Mtchedlishvili and Kapur, 2006; Semyanov et al., 2004; Stell et al., 2003; Wei et al., 2003). At the concentration used here, however, THIP could conceivably activate $\alpha_5\beta_3\gamma_x$ GABA_AR subunit combinations and the emergence of functional α_5 -containing GABA_ARs has been reported in a chemoconvulsant model of epilepsy (Ebert et al., 1994; Kusama et al., 1993; Zhan and Nadler, 2009). To address these possibilities, the effect of the inverse agonist L655,708 on $I_{\text{TonicGABA}}$ in DGCs was tested 8-13 weeks after CCI due to its relative selectivity for α_5

subunit-containing GABA_ARs (Fig. 5) (Bonin et al., 2007; Caraiscos et al., 2004; Quirk et al., 1996).

The current amplitude of $I_{\text{TonicGABA}}$ was measured in DGCs from uninjured and injured hemispheres using voltage-clamp recordings during baseline and application of L655,708. Effects of L655,708 on $I_{\text{TonicGABA}}$ were calculated as the difference between current amplitude during baseline and current amplitude during L655,708. As observed in earlier experiments, resting $I_{\text{TonicGABA}}$ (baseline – bicuculline) was similar in ipsilateral DGCs (mean= 0.62 ± 0.19 pA/pF) compared to contralateral DGCs (mean= 0.67 ± 0.26 pA/pF) ($T(13) = 0.17$, $p=0.87$; Fig. 5). No significant difference in responses of $I_{\text{TonicGABA}}$ to L655,708 was detected in ipsilateral DGCs (mean= 0.43 ± 0.16 pA/pF; $n=8$) compared to contralateral DGCs (mean= 0.35 ± 0.11 pA/pF; $n=7$; $T(13) = 0.41$, $p=0.69$; Fig. 5). Since responses to L655,708 were not different between the groups, it seemed unlikely that CCI injury induced the emergence of α_5 subunit-containing GABA_ARs in DGCs ipsilateral to injury.

Gene expression of α_4 , α_5 and δ GABA_AR subunits indentate gyrus 8-13 weeks post-injury. The dorsal half of isolated dentate gyrus was analyzed for α_4 , α_5 and δ GABA_AR subunit mRNA expression using samples from sham-injured DG and DG contralateral and ipsilateral to CCI at the 8-13 week time-point (Fig. 6). Contralateral ($n=4$) and sham ($n=7$) isolated dorsal dentate gyrus did not significantly differ in α_4 subunit expression ($T(9) = 0.066$, $p=0.95$). There was no significant difference in the expression of the GABA_AR α_4 subunit in ipsilateral ($n=10$) versus control ($n=11$) isolated dorsal dentate gyrus ($T(19) = 0.50$, $p=0.62$; two-tailed, unpaired, t-test; Fig. 6A).

Contralateral (n=3) and sham (n=5) isolated dorsal dentate gyrus did not significantly differ in α_5 subunit expression ($T(7)= 0.15$, $p=0.88$). There was no significant difference in the expression of the GABA_AR α_5 subunit in ipsilateral (n=10) versus control (n=8) isolated dorsal dentate gyrus ($T(16)= 0.29$, $p=0.78$; two-tailed, unpaired, t-test; Fig. 6B). Similarly, contralateral (n=6) and sham (n=11) isolated dorsal dentate gyrus did not significantly differ in δ subunit expression ($T(15)= 0.11$, $p=0.92$). There was no significant difference in the expression of the GABA_AR δ subunit in ipsilateral (n=19) versus control (n=17) isolated dorsal dentate gyrus ($T(34)= 0.70$, $p=0.49$; two-tailed, unpaired, t-test; Fig. 6C). These results were not consistent with changes in mRNA expression of GABA_AR α_4 , α_5 and δ subunit expression 8-13 weeks after CCI injury.

DISCUSSION

Although altered expression and distribution of GABA_AR subunits has been reported in animal models of epilepsy, little is known about GABA_AR function in DGCs ipsilateral to CCI brain injury, which also results in post-traumatic epileptogenesis (Brooks-Kayal et al., 1998; Cossart et al., 2001; Gibbs et al., 1997; Hunt et al., 2009; Leroy et al., 2004). Similar to results after focal brain injury reported here, $I_{\text{TonicGABA}}$ in DGCs from mice that survive pilocarpine-induced status epilepticus exhibits no change in resting amplitude, but its sensitivity to neurosteroids is reduced in a manner consistent with a functional reduction of δ subunit-containing GABA_ARs (Mihalek et al., 1999; Zhang et al., 2007). Changes in $I_{\text{TonicGABA}}$ function are paralleled by a reported decrease in δ subunit immunoreactivity and increase in α_4 subunit and γ_2 subunit

immunoreactivity in the dentate gyrus of pilocarpine-treated mice (Peng et al., 2004). Ultrastructurally, there is both a shift in γ subunit immunogold-labeling from synaptic to perisynaptic sites and a reduction in δ subunit immunogold-labeling within the dendritic fields of DGCs in the molecular layer (Peng et al., 2004). Analogous changes in the distribution and protein expression of GABA_AR subunits may account for the reduced $I_{\text{TonicGABA}}$ THIP and THDOC responses that were observed here after CCI, since gene expression was not altered. The reduced DGC responsiveness to THDOC suggests a reduction in neurosteroid-dependent regulation of GABAergic signaling that may disrupt their ability to maintain appropriate levels of excitability (Bianchi and Macdonald, 2003; Mtchedlishvili et al., 2001; Stell et al., 2003; Wohlfarth et al., 2002; Zhang et al., 2007).

Brain injuries differ in the resulting patterns of cell loss, reactive plasticity, and circuit reorganization (reviewed in Hunt et al., 2013). Controlled cortical impact and fluid percussion injury (FPI) are common brain injury models used to study mechanisms of brain repair and posttraumatic epilepsy. Changes in tonic GABA_A currents have not previously been reported for DGCs located ipsilateral to focal brain injury using CCI. After severe CCI, however, which prohibited recordings in ipsilateral DGCs due to extensive damage to the dorsal dentate gyrus, resting and THIP-sensitive $I_{\text{TonicGABA}}$ in DGCs contralateral to injury were enhanced 90 days after injury (Mtchedlishvili et al., 2010). Using a somewhat less severe CCI injury, which spares the ipsilateral hippocampus and results in posttraumatic epilepsy in ~40% of mice (Hunt et al., 2009), we detected no differences in resting or THIP-induced $I_{\text{TonicGABA}}$ between DGCs from the contralateral hemisphere of CCI versus sham-treatment. Additionally, the present study found no difference in resting $I_{\text{TonicGABA}}$ in DGCs ipsilateral to CCI, similar to findings

using a pilocarpine-treated mouse model of acquired TLE (Zhang et al., 2007), whereas resting $I_{\text{TonicGABA}}$ was increased in the pilocarpine-treated rat model of TLE (Zhan and Nadler, 2009). One week following FPI, the amplitude of resting $I_{\text{TonicGABA}}$ was increased in DGCs ipsilateral to FPI and this was accompanied by an increase in the effect of THIP on $I_{\text{TonicGABA}}$ (Gupta et al., 2012), suggesting an increased contribution of δ subunit-containing GABA_A Rs. Others have reported that the resting $I_{\text{TonicGABA}}$ amplitude and responsiveness to THIP are unaltered in DGCs at 1 and 6 months post-FPI (Pavlov et al., 2011). Conversely, we consistently observed reduced THIP responsiveness in DGCs ipsilateral to CCI injury 1-2 weeks post-injury, which persisted for months after the injury. $I_{\text{TonicGABA}}$ in DGCs represents an important form of GABAergic signaling in DGCs due to its continuous charge transfer over time (Mody and Pearce, 2004). Small changes in $I_{\text{TonicGABA}}$ would be expected to impact the ability of dentate gyrus to filter incoming perforant path input and may compromise its ability to regulate excitability (reviewed in Coulter and Carlson, 2007). The reduced capacity of $I_{\text{TonicGABA}}$ in DGCs ipsilateral to CCI injury may limit the ability of GABA to inhibit neuronal activity when GABA release is increased in the dentate gyrus (e.g., during a seizure).

In the present study, DGCs ipsilateral to CCI injury also exhibited reduced responses to the neurosteroid THDOC. In DGCs, neurosteroids regulate tonic inhibitory signaling by enhancing the efficacy of GABA_A Rs that contain the δ subunit (Bianchi and Macdonald, 2003; Mtchedlishvili et al., 2001; Stell et al., 2003; Wohlfarth et al., 2002; Zhang et al., 2007). In DGCs ipsilateral to CCI, the reduced responsiveness to both THIP and THDOC provides convergent evidence of a loss of functional δ subunit-containing GABA_A Rs with this injury. The reduced THDOC response also suggests

a possible loss of neurosteroid regulation of tonic GABAergic inhibition in DGCs after focal brain injury. A reduction in neurosteroid-dependent $I_{\text{TonicGABA}}$ modulation in DGCs has also been observed following chemoconvulsant-induced status epilepticus (Mtchedlishvili et al., 2001; Zhang et al., 2007). The reduced regulation of tonic GABAergic inhibition may disrupt information encoding in the dentate gyrus, and this effect could be exaggerated by physiological processes that affect neurosteroids, including stress (Belelli and Lambert, 2005; Maguire and Mody, 2007; Maguire et al., 2005; Majewska et al., 1986).

Reduced inhibitory synaptic drive to DGCs has been reported in models of TBI, although the sites of this disruption vary by injury severity. With FPI, decreased sIPSC frequency in ipsilateral DGCs has been observed within one week (Gupta et al., 2012; Santhakumar et al., 2001) or one month (Pavlov et al., 2011). This reduced GABA release progresses over time, and by six months post-injury reduced IPSC frequency is observed in DGCs from both hemispheres (Pavlov et al., 2011; Santhakumar et al., 2001), consistent with the diffuse tissue damage caused by FPI. A reduction in the frequency of synaptic inhibition in DGCs located contralateral to a severe CCI injury has also been observed (Mtchedlishvili et al., 2010). At the post-injury time-points in the present study, we observed reduced sIPSC frequency in DGCs ipsilateral to CCI, but no change in sIPSC frequency was detected in DGCs contralateral to CCI. The decreased sIPSC frequency after CCI observed here is consistent with a previous report using a similar injury model (Hunt et al., 2011) and suggests that changes after focal brain injury may be a function of severity and spread of direct injury.

Subunit composition of GABA_ARs dictates their kinetics and responsiveness

to endogenous agonists and pharmacological compounds (summarized in Davies et al., 1997; Draguhn et al., 1990; Farrant and Nusser, 2005; Glykys and Mody, 2007; Lüddens and Wisden, 1991; Verdoorn et al., 1990). In DGCs, synaptic inhibition is predominantly mediated by $\alpha_1\beta\gamma_2$ subunit assemblies that impart a low affinity for GABA and fast kinetics to the resulting GABA_ARs (summarized in Brown et al., 2002; Chang et al., 1996; Farrant and Nusser, 2005; Glykys et al., 2008; Mody, 2001; Mtchedlishvili and Kapur, 2006; Semyanov et al., 2004; Stell et al., 2003; Wei et al., 2003). Extrasynaptic GABA_ARs may also modulate synaptic inhibition, as mice lacking the δ GABA_AR subunit exhibit altered properties of IPSCs (Nusser et al., 1998; Spigelman et al., 2002; Wei et al., 2003). However, neither sIPSC amplitude nor decay time constant was significantly altered after CCI, implying that the reduced δ subunit-mediated responses did not affect phasic inhibition directly. DGCs also express functional $\alpha_4\beta\delta$ subunit assemblies that are considered the major source of $I_{\text{TonicGABA}}$; these receptors have a high affinity for GABA, desensitize slowly, and are activated by THIP and neurosteroids (Brown et al., 2002; Chang et al., 1996; Farrant and Nusser, 2005; Glykys et al., 2008; Mody, 2001; Mtchedlishvili and Kapur, 2006; Semyanov et al., 2004; Stell et al., 2003; Wei et al., 2003). DGCs in mice lacking the δ GABA_AR subunit exhibit only a small residual $I_{\text{TonicGABA}}$, suggesting that the δ subunit prominently contributes to $I_{\text{TonicGABA}}$ in these cells (Glykys et al., 2008; Glykys and Mody, 2007; Stell et al., 2003). In bi-transgenic mice lacking both δ and α_5 GABA_AR subunits, $I_{\text{TonicGABA}}$ in DGCs is reduced by >90% (Glykys et al., 2008), suggesting that α_5 subunit-containing GABA_AR(s) may also contribute to this conductance in DGCs (Glykys et al., 2008).

Although THIP may activate non- δ subunit-containing GABA_ARs at high concentrations, THIP concentrations of $\leq 3 \mu\text{M}$ primarily activate $\alpha_4\beta\delta$ subunit-containing GABA_ARs (Adkins et al., 2001; Brown et al., 2002; Ebert et al., 1994; Glykys and Mody, 2007; í Stórustovu and Ebert, 2006; Stell et al., 2003). Responses to concentrations of $\leq 10 \mu\text{M}$ THIP have been observed in cell culture expression systems expressing one of the following GABA_AR combinations: $\alpha_4\beta_3\delta$, $\alpha_6\beta_3\delta$ or $\alpha_5\beta_3$ (Adkins et al., 2001; Brown et al., 2002; Ebert et al., 1994; Glykys and Mody, 2007; í Stórustovu and Ebert, 2006; Kusama et al., 1993; Stell et al., 2003). It is unclear how lack of the γ or δ subunit for the $\alpha_5\beta_3$ combination affects activity of the resulting GABA_ARs, and the $\alpha_6\beta_3\delta$ subunit combination is unlikely to contribute significantly to GABAergic signaling in DGCs (Glykys et al., 2008; Glykys and Mody, 2007; Stell et al., 2003). GABA_ARs containing α_5 subunits exhibit 50% maximal normalized responses to $\leq 40 \mu\text{M}$ THIP, suggesting that they could respond to lower THIP concentrations (Ebert et al., 1994). GABA_ARs containing α_5 subunits contribute to $I_{\text{TonicGABA}}$ in DGCs (Ebert et al., 1994; Glykys et al., 2008) and an increase in α_5 GABA_AR subunit-mediated $I_{\text{TonicGABA}}$ has been observed in DGCs from pilocarpine-treated rats (Zhan and Nadler, 2009). THIP responses of $I_{\text{TonicGABA}}$ were reduced in DGCs ipsilateral to CCI to an estimated $< 60\%$ of control levels despite resting $I_{\text{TonicGABA}}$ being unaffected by CCI. Conversely, we failed to detect any injury-induced change of $I_{\text{TonicGABA}}$ in DGCs in response to the inverse agonist L655,708, which possesses partial selectivity for the α_5 GABA_AR subunit (Quirk et al., 1996). If an increase in α_5 subunit-containing GABA_ARs was responsible for maintaining resting $I_{\text{TonicGABA}}$, then DGCs located ipsilateral to CCI should have exhibited an increased sensitivity to L655,708. We conclude, therefore, that the reduced response to THIP in

DGCs ipsilateral to injury represented a functional reduction in the contribution of δ subunit-containing GABARs to $I_{\text{TonicGABA}}$.

We also detected injury-dependent differences in how THIP affects sIPSC frequency in DGCs. Control DGCs exhibited a reduction in sIPSC frequency with THIP application, whereas sIPSC frequency in DGCs ipsilateral to CCI was unaltered by THIP. The effect of THIP on sIPSC frequency is consistent with a pre-synaptic reduction in the THIP responsiveness of local inhibitory neurons after focal brain injury. In pilocarpine-treated mice, interneurons within the dentate molecular and granular cell layers exhibit increased δ subunit immunoreactivity (Peng et al., 2004). Increased δ subunit-containing GABA_ARs in interneurons is not consistent with the reduced effect of THIP on sIPSC frequency observed here. Differing effects of pilocarpine-induced status epilepticus and CCI on hippocampal GABAergic interneurons, including changes associated with class-specific GABA cell loss, reorganization of GABA synapses along the somato-dendritic axis, or modifications to GABAergic terminals may underlie these differences in inhibitory network responses to THIP (Hunt et al., 2011; Peng et al., 2004; Zhang et al., 2007).

The present study did not detect changes in GABA_AR subunit gene expression, despite changes in the functional GABA_A currents in DGCs ipsilateral to CCI. This dissociation suggests that, at least in the CCI model used here, other mechanisms subserve these functional alterations in GABAergic signaling. Protein expression of GABA_AR subunits, post-transcriptional or post-translational receptor modifications, GABA transporters, and chloride transporters contribute to $I_{\text{TonicGABA}}$ function (Clarkson et al., 2010; Farrant and Nusser, 2005; Glykys and Mody, 2007; Mody, 2001; Mody and

Pearce, 2004; Rivera et al., 2004; Stell et al., 2003; Van Den Pol et al., 1996) and changes in any of these mechanisms may underlie the CCI-altered GABA_AR currents observed in this study. In a model of CCI that results in similar damage to the present one, no change in hippocampal protein expression of α_1 , β_2 , β_3 , γ_2 or δ GABA_AR subunits was detected at either 1 or 16 weeks post-injury (Raible et al., 2015). Severe CCI that ablates ipsilateral hippocampus results in increased δ GABA_AR subunit in contralateral hippocampus (Kharlamov et al., 2011). Thus, whereas contracoup effects of severe injury may include genomic changes in GABA_AR subunits, injury-induced changes in GABA_AR currents of DGCs ipsilateral to focal injury most likely occur subsequent to post-translational modification of the receptor,

GABA_AR trafficking and membrane distribution are reportedly altered in association with epilepsy development after pilocarpine-induced status epilepticus (Peng et al., 2004; Wei et al., 2003). Intracellular signals including protein kinase A (PKA), protein kinase C (PKC), calmodulin kinase II (CamKII), the proto-oncogene tyrosine protein kinase Src and a variety of phosphatases can all potentially modulate GABA_AR function by regulating GABA_AR trafficking and surface expression (summarized in Nakamura et al., 2015). However, it remains unknown how these GABA_AR signaling modifiers specifically affect synaptic versus tonic conductances, interact with different subunit compositions in different neuron types, or operate in combination to affect inhibitory signaling after CCI injury. In the context of brain injury, it may be important to relate changes in functional GABA_AR signaling to this broader class of GABA_AR modifiers. In addition, $I_{\text{TonicGABA}}$ is sensitive to GABA_BR signaling in DGCs (Tao et al., 2013) and this may also affect tonic GABAergic signaling after brain-

injury.

Correlations between gene expression of GABA_AR subunits and their corresponding functional signaling have been inconsistent across models of brain injury (Drexel et al., 2015; Kharlamov et al., 2011; Mtchedlishvili et al., 2010; Pavlov et al., 2011; Raible et al., 2012; Raible et al., 2015). Comparisons between studies are made more difficult because many molecular studies have sampled from whole hippocampus rather than with individual subfields or cell-types and there are disparate time-points of sampling. There are also differences in how each model is performed, including injury type and severity, across laboratories. Here, the dorsal DG was sampled ipsilateral to injury, corresponding to the region where responses to THIP were altered by injury. In the FPI model, most changes in GABA_AR subunit expression appear to be reversed within months of injury and do not consistently parallel changes in tonic GABA_AR currents (Drexel et al., 2015; Pavlov et al., 2011).

CONCLUSIONS

Results here demonstrate that long-lasting functional reductions in $I_{\text{TonicGABA}}$ responsiveness to THIP and THDOC occur in DGCs located ipsilateral to focal brain injury. These changes in pharmacological responsiveness may represent functional changes in GABA_AR subunit composition in DGCs and interneurons after focal brain injury, but do not appear to involve changes in gene transcription for the α_4 , α_5 , or δ subunits. Many of the changes in GABA_AR signaling ipsilateral to CCI share similarity with those observed in chemo-convulsant models of acquired epilepsy, whereas they

are different from changes in GABA_AR signaling in DGCs after FPI or in contralateral DGCs after more severe CCI. These changes may contribute to chronic brain disruption and seizure phenotype after focal TBI and highlight that individuals with brain injury may exhibit variable responses to pharmacotherapies targeting GABA_ARs, depending on injury type and severity. Reduced neurosteroid-dependent regulation of I_{TonicGABA} in DGCs after focal brain injury may have detrimental consequences to excitability of this brain region and may be exacerbated during periods of stress or altered hormonal signaling.

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JAB, CRB, KCH and BNS contributed to the conception and design of the work; acquisition and analysis were performed by JAB, CRB and KCH; interpretation of the data was performed by JAB, CRB, KCH and BNS. JAB, CRB and KCH and BNS drafted

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REFERENCES CITED

- 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. $\alpha 4\beta 3\delta$ GABA_A Receptors Characterized by Fluorescence Resonance Energy Transfer-derived Measurements of Membrane Potential. *J. Biol. Chem.* 276, 38934-38939.
- Akaike, N., Inoue, M., Krishtal, O., 1986. 'Concentration-clamp' study of gamma-aminobutyric acid induced chloride current kinetics in frog sensory neurones. *J. Physiol. (Lond)*. 379, 171-185.
- Barnard, E., Skolnick, P., Olsen, R., Mohler, H., Sieghart, W., Biggio, G., Braestrup, C., Bateson, A., Langer, S., 1998. International Union of Pharmacology. XV. Subtypes of γ -aminobutyric acid_A receptors: classification on the basis of subunit structure and receptor function. *Pharmacol. Rev.* 50, 291-314.
- Belelli, D., Lambert, J.J., 2005. Neurosteroids: endogenous regulators of the GABA_A receptor. *Nat. Rev. Neurosci.* 6, 565-575.
- Bianchi, M.T., Macdonald, R.L., 2003. Neurosteroids shift partial agonist activation of GABA_A receptor channels from low-to high-efficacy gating patterns. *J. Neurosci.* 23, 10934-10943.
- Bonin, R.P., Martin, L.J., MacDonald, J.F., Orser, B.A., 2007. $\alpha 5$ GABA_A receptors regulate the intrinsic excitability of mouse hippocampal pyramidal neurons. *J. Neurophysiol.* 98, 2244-2254.
- Boychuk, C.R., Halmos, K., Smith, B.N., 2015. Diabetes induces GABA receptor plasticity in murine vagal motor neurons. *J. Neurophysiol.* 4, 698-706.
- Bright, D.P., Smart, T.G., 2013. Methods for recording and measuring tonic GABA_A receptor-mediated inhibition. *Front. Neural Circuits* 7, 1-13.
- 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., Whiting, P., Wafford, K., 2002. Pharmacological characterization of a novel cell line expressing human $\alpha 4\beta 3\delta$ GABA_A receptors. *Br. J. Pharmacol.* 136, 965-974.
- Butler, C.R., Boychuk, J.A., Smith, B.N., 2015. Effects of Rapamycin Treatment on Neurogenesis and Synaptic Reorganization in the Dentate Gyrus after Controlled Cortical Impact Injury in Mice. *Front. Syst. Neurosci.* 9, 163.
- 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., 2004. Tonic inhibition in

mouse hippocampal CA1 pyramidal neurons is mediated by $\alpha 5$ subunit-containing γ -aminobutyric acid type A receptors. *Proc. Natl. Acad. Sci. U. S. A.* 101, 3662-3667.

Chang, Y., Wang, R., Barot, S., Weiss, D.S., 1996. Stoichiometry of a recombinant GABAA receptor. *J. Neurosci.* 16, 5415-5424.

Clarkson, A.N., Huang, B.S., MacIsaac, S.E., Mody, I., Carmichael, S.T., 2010. Reducing excessive GABA-mediated tonic inhibition promotes functional recovery after stroke. *Nature* 468, 305-309.

Collingridge, G.L., Gage, P.W., Robertson, B., 1984. Inhibitory post-synaptic currents in rat hippocampal CA1 neurones. *J. Physiol. (Lond.)* 356, 551.

Cossart, R., Dinocourt, C., Hirsch, J.C., Merchán-Pérez, A., De Felipe, J., Ben-Ari, Y., Esclapez, M., Bernard, C., 2001. Dendritic but not somatic GABAergic inhibition is decreased in experimental epilepsy. *Nat. Neurosci.* 4, 52-62.

Coulter, D.A., Carlson, G.C., 2007. Functional regulation of the dentate gyrus by GABA-mediated inhibition. *Prog. Brain Res.* 163, 235-812.

Davies, P.A., Hanna, M.C., Hales, T.G., Kirkness, E.F., 1997. Insensitivity to anaesthetic agents conferred by a class of GABAA receptor subunit. *Nature* 385, 820-823.

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

Draguhn, A., Verdorn, T.A., Ewert, M., Seeburg, P.H., Sakmann, B., 1990. Functional and molecular distinction between recombinant rat GABA A receptor subtypes by Zn^{2+} . *Neuron* 5, 781-788.

Drexel, M., Puhakka, N., Kirchmair, E., Hörtnagl, H., Pitkänen, A., Sperk, G., 2015. Expression of GABA receptor subunits in the hippocampus and thalamus after experimental traumatic brain injury. *Neuropharmacol.* 88, 122-133.

Ebert, B., Wafford, K.A., Whiting, P.J., Krogsgaard-Larsen, P., Kemp, J.A., 1994. Molecular pharmacology of gamma-aminobutyric acid type A receptor agonists and partial agonists in oocytes injected with different alpha, beta, and gamma receptor subunit combinations. *Mol. Pharmacol.* 46, 957-963.

Farrant, M., Nusser, Z., 2005. Variations on an inhibitory theme: phasic and tonic activation of GABAA receptors. *Nat. Rev. Neurosci.* 6, 215-229.

Gibbs, J.W., Shumate, M.D., Coulter, D.A., 1997. Differential epilepsy-associated alterations in postsynaptic GABAA receptor function in dentate granule and CA1 neurons. *J. Neurophysiol.* 77, 1924-1938.

Glykys, J., Mann, E.O., Mody, I., 2008. Which GABAA receptor subunits are necessary for tonic inhibition in the hippocampus? *J. Neurosci.* 28, 1421-1426.

Glykys, J., Mody, I., 2007. Activation of GABA A receptors: views from outside the synaptic cleft. *Neuron* 56, 763-770.

Guo, D., Zeng, L., Brody, D.L., Wong, M., 2013. Rapamycin attenuates the development of posttraumatic epilepsy in a mouse model of traumatic brain injury. *PLoS One* 8, e64078.

Gupta, A., Elgammal, F.S., Proddutur, A., Shah, S., Santhakumar, V., 2012. Decrease in tonic inhibition contributes to increase in dentate semilunar granule cell excitability after brain injury. *J. Neurosci.* 32, 2523-2537.

Hall, E.D., Sullivan, P.G., Gibson, T.R., Pavel, K.M., Thompson, B.M., Scheff, S.W., 2005. Spatial and temporal characteristics of neurodegeneration after controlled cortical impact in mice: more than a focal brain injury. *J. Neurotrauma* 22, 252-265.

Hideo, H., Keiko, T., Nobuyuki, Y., Tsuyoshi, M., 2009. Dissection of hippocampal dentate gyrus from adult mouse. *J. Vis. Exp.* 33, e1543.

Hoestgaard-Jensen, K., Dalby, N.O., Krall, J., Hammer, H., Krogsgaard-Larsen, P., Frølund, B., Jensen, A.A., 2014. Probing $\alpha 4\beta\delta$ GABAA receptor heterogeneity: differential regional effects of a functionally selective $\alpha 4\beta 1\delta/\alpha 4\beta 3\delta$ receptor agonist on tonic and phasic inhibition in rat brain. *J. Neurosci.* 34, 16256-16272.

Hunt, R.F., Boychuk, J.A., Smith, B.N., 2013. Neural circuit mechanisms of post-traumatic epilepsy. *Front. Cell Neurosci.* 7, 89.

Hunt, R.F., Haselhorst, L.A., Schoch, K.M., Bach, E.C., Rios-Pilier, J., Scheff, S.W., Saatman, K.E., Smith, B.N., 2012. Posttraumatic mossy fiber sprouting is related to the degree of cortical damage in three mouse strains. *Epilepsy Res.* 99, 167-170.

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

Hunt, R.F., Scheff, S.W., Smith, B.N., 2010. Regionally localized recurrent excitation in the dentate gyrus of a cortical contusion model of posttraumatic epilepsy. *J. Neurophysiol.* 103, 1490-1500.

Hunt, R.F., Scheff, S.W., Smith, B.N., 2011. Synaptic reorganization of inhibitory hilar interneuron circuitry after traumatic brain injury in mice. *J. Neurosci.* 31, 6880-6890.

Í Stórustovu, S., Ebert, B., 2006. Pharmacological characterization of agonists at δ -containing GABAA receptors: functional selectivity for extrasynaptic receptors is dependent on the absence of $\gamma 2$. *J. Pharmacol. Exp. Ther.* 316, 1351-1359.

Kapur, J., Macdonald, R.L., 1997. Rapid seizure-induced reduction of benzodiazepine and Zn^{2+} sensitivity of hippocampal dentate granule cell GABAA receptors. *J. Neurosci.* 17, 7532-7540.

Kapur, J., Macdonald, R.L., 1999. Postnatal development of hippocampal dentate granule cell Gamma-aminobutyric acidA receptor pharmacological properties. *Mol. Pharmacol.* 55, 444-452.

Kharlamov, E.A., Downey, K.L., Jukkola, P.I., Grayson, D.R., Kelly, K.M., 2008. Expression of GABA A receptor $\alpha 1$ subunit mRNA and protein in rat neocortex following photothrombotic infarction. *Brain Res.* 1210, 29-38.

Kharlamov, E.A., Lepsveridze, E., Meparishvili, M., Solomonina, R.O., Lu, B., Miller, E.R., Kelly, K.M., Mtchedlishvili, Z., 2011. Alterations of GABA A and glutamate receptor subunits and heat shock protein in rat hippocampus following traumatic brain injury and in posttraumatic epilepsy. *Epilepsy Res.* 95, 20-34.

Kusama, T., Spivak, C., Whiting, P., Dawson, V., Schaeffer, J., Uhl, G., 1993. Pharmacology of GABA A $\rho 1$ and GABA A α/β receptors expressed in *Xenopus* oocytes and COS cells. *Br. J. Pharmacol.* 109, 200-206.

Laurie, D., Wisden, W., Seeburg, P., 1992. The distribution of thirteen GABAA receptor subunit mRNAs in the rat brain. III. Embryonic and postnatal development. *J. Neurosci.* 12, 4151-4172.

Leroy, C., Poisbeau, P., Keller, A., Nehlig, A., 2004. Pharmacological plasticity of GABAA receptors at dentate gyrus synapses in a rat model of temporal lobe epilepsy. *J. Physiol. (Lond).* 557, 473-487.

Lighthall, J.W., 1988. Controlled cortical impact: a new experimental brain injury model. *J. Neurotrauma* 5, 1-15.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2- $\Delta\Delta CT$ method. *Methods* 25, 402-408.

Lowenstein, D.H., Thomas, M.J., Smith, D.H., McIntosh, T.K., 1992. Selective vulnerability of dentate hilar neurons following traumatic brain injury: a potential mechanistic link between head trauma and disorders of the hippocampus. *J. Neurosci.* 12, 4846-4853.

Lüddens, H., Wisden, W., 1991. Function and pharmacology of multiple GABA A receptor subunits. *Trends Pharmacol. Sci.* 12, 49-51.

Macdonald, R.L., Olsen, R.W., 1994. GABAA receptor channels. *Annu. Rev. Neurosci.* 17, 569-602.

Maguire, J., Mody, I., 2007. Neurosteroid synthesis-mediated regulation of GABA(A) receptors: relevance to the ovarian cycle and stress. *J. Neurosci.* 27, 2155-2162.

Maguire, J.L., Stell, B.M., Rafizadeh, M., Mody, I., 2005. Ovarian cycle-linked changes in GABAA receptors mediating tonic inhibition alter seizure susceptibility and anxiety. *Nat. Neurosci.* 8, 797-804.

Majewska, M.D., Harrison, N.L., Schwartz, R.D., Barker, J.L., Paul, S.M., 1986. Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. *Science* 232, 1004-1007.

Mihalek, R.M., Banerjee, P.K., Korpi, E.R., Quinlan, J.J., Firestone, L.L., Mi, Z.-P., Lagenaur, C., Tretter, V., Sieghart, W., Anagnostaras, S.G., 1999. Attenuated sensitivity to neuroactive steroids in γ -aminobutyrate type A receptor delta subunit knockout mice. *Proc. Natl. Acad. Sci. U. S. A.* 96, 12905-12910.

Mody, I., 2001. Distinguishing between GABAA receptors responsible for tonic and phasic conductances. *Neurochem. Res.* 26, 907-913.

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

Mtchedlishvili, Z., Bertram, E.H., Kapur, J., 2001. Diminished allopregnanolone enhancement of GABAA receptor currents in a rat model of chronic temporal lobe epilepsy. *J. Physiol. (Lond).* 537, 453-465.

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

Mtchedlishvili, Z., Lepsveridze, E., Xu, H., Kharlamov, E.A., Lu, B., Kelly, K.M., 2010. Increase of GABA A receptor-mediated tonic inhibition in dentate granule cells after traumatic brain injury. *Neurobiol. Dis.* 38, 464-475.

Nakamura, Y., Darnieder, L.M., Deeb, T.Z., Moss, S.J., 2015. Chapter Four-Regulation of GABA A Rs by Phosphorylation. *Adv. Pharmacol.* 72, 97-146.

Nusser, Z., Sieghart, W., Somogyi, P., 1998. Segregation of different GABAA receptors to synaptic and extrasynaptic membranes of cerebellar granule cells. *J. Neurosci.* 18, 1693-1703.

Pavlov, I., Huusko, N., Drexel, M., Kirchmair, E., Sperk, G., Pitkänen, A., Walker, M., 2011. Progressive loss of phasic, but not tonic, GABA A receptor-mediated inhibition in dentate granule cells in a model of post-traumatic epilepsy in rats. *Neuroscience* 194, 208-219.

Pavlov, I., Savtchenko, L.P., Kullmann, D.M., Semyanov, A., Walker, M.C., 2009. Outwardly rectifying tonically active GABAA receptors in pyramidal cells modulate neuronal offset, not gain. *J. Neurosci.* 29, 15341-15350.

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

Quirk, K., Blurton, P., Fletcher, S., Leeson, P., Tang, F., Mellilo, D., Ragan, C., McKernan, R., 1996. [3 H] L-655,708, a Novel Ligand Selective for the Benzodiazepine Site of GABA A Receptors which Contain the $\alpha 5$ Subunit. *Neuropharmacol.* 35, 1331-1335.

Raible, D.J., Frey, L.C., Cruz Del Angel, Y., Russek, S.J., Brooks-Kayal, A.R., 2012. GABAa receptor regulation after experimental traumatic brain injury. *J. Neurotrauma* 29, 2548-2554.

Raible, D.J., Frey, L.C., Del Angel, Y.C., Carlsen, J., Hund, D., Russek, S.J., Smith, B., Brooks-Kayal, A.R., 2015. JAK/STAT pathway regulation of GABA A receptor expression after differing severities of experimental TBI. *Exp. Neurol.* 271, 445-456.

Redecker, C., Wang, W., Fritschy, J.-M., Witte, O.W., 2002. Widespread and Long-Lasting Alterations in GABAA-Receptor Subtypes After Focal Cortical Infarcts in Rats: Mediation by NMDA-Dependent Processes. *J. Cereb. Blood Flow Metab.* 22, 1463-1475.

Rivera, C., Voipio, J., Thomas-Crusells, J., Li, H., Emri, Z., Sipilä, S., Payne, J.A., Minichiello, L., Saarma, M., Kaila, K., 2004. Mechanism of activity-dependent downregulation of the neuron-specific K-Cl cotransporter KCC2. *J. Neurosci.* 24, 4683-4691.

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. (Lond).* 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.

Scheff, S., Price, D., Hicks, R., Baldwin, S., Robinson, S., Brackney, C., 2005. Synaptogenesis in the hippocampal CA1 field following traumatic brain injury. *J. Neurotrauma* 22, 719-732.

Scheff, S.W., Baldwin, S.A., Brown, R.W., Kraemer, P.J., 1997. Morris water maze deficits in rats following traumatic brain injury: lateral controlled cortical impact. *J. Neurotrauma* 14, 615-627.

Schmittgen, T.D., Livak, K.J., 2008. Analyzing real-time PCR data by the comparative CT method. *Nat. Protoc.* 3, 1101-1108.

Segal, M., Barker, J.L., 1984. Rat hippocampal neurons in culture: voltage-clamp analysis of inhibitory synaptic connections. *J. Neurophysiol.* 52, 469-487.

Semyanov, A., Walker, M.C., Kullmann, D.M., Silver, R.A., 2004. Tonically active GABA A receptors: modulating gain and maintaining the tone. *Trends Neurosci.* 27, 262-269.

Spigelman, I., Li, Z., Banerjee, P.K., Mihalek, R.M., Homanics, G.E., Olsen, R.W., 2002. Behavior and Physiology of Mice Lacking the GABAA Receptor δ Subunit. *Epilepsia* 43, 3-8.

Stell, B.M., Brickley, S.G., Tang, C., Farrant, M., Mody, I., 2003. Neuroactive steroids reduce neuronal excitability by selectively enhancing tonic inhibition mediated by δ subunit-containing GABAA receptors. *Proc. Natl. Acad. Sci. U. S. A.* 100, 14439-14444.

Sun, C., Mtchedlishvili, Z., Erisir, A., Kapur, J., 2007. Diminished neurosteroid sensitivity of synaptic inhibition and altered location of the $\alpha 4$ subunit of GABAA receptors in an animal model of epilepsy. *J. Neurosci.* 27, 12641-12650.

Swartz, B.E., Houser, C.R., Tomiyasu, U., Walsh, G.O., DeSalles, A., Rich, J.R., Delgado-Escueta, A., 2006. Hippocampal cell loss in posttraumatic human epilepsy. *Epilepsia* 47, 1373-1382.

Tao, W., Higgs, M.H., Spain, W.J., Ransom, C.B., 2013. Postsynaptic GABAB receptors enhance extrasynaptic GABAA receptor function in dentate gyrus granule cells. *J. Neurosci.* 33, 3738-3743.

Van Den Pol, A.N., Obrietan, K., Chen, G., 1996. Excitatory actions of GABA after neuronal trauma. *J. Neurosci.* 16, 4283-4292.

Verdoorn, T.A., Draguhn, A., Ymer, S., Seeburg, P.H., Sakmann, B., 1990. Functional properties of recombinant rat GABA A receptors depend upon subunit composition. *Neuron* 4, 919-928.

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.

Weiss, D.S., 1988. Membrane potential modulates the activation of GABA-gated channels. *J. Neurophysiol.* 59, 514-527.

Weiss, D.S., Barnes, E., Hablitz, J.J., 1988. Whole-cell and single-channel recordings of GABA-gated currents in cultured chick cerebral neurons. *J. Neurophysiol.* 59, 495-513.

Whiting, P.J., Bonnert, T.P., McKernan, R.M., Farrar, S., Bourdelles, B.L., Heavens, R.P., Smith, D.W., Hewson, L., Rigby, M.R., Sirinathsinghji, D.J., 1999. Molecular and Functional Diversity of the Expanding GABA-A Receptor Gene Family. *Ann. N. Y. Acad. Sci.* 868, 645-653.

Wohlfarth, K.M., Bianchi, M.T., Macdonald, R.L., 2002. Enhanced Neurosteroid Potentiation of Ternary GABAARs Containing the δ Subunit. *J. Neurosci.* 22, 1541-1549.

Wood, C.E., Giroux, D., 2003. Central Nervous System Prostaglandin Endoperoxide Synthase-1 and- 2 Responses to Oestradiol and Cerebral Hypoperfusion in Late-Gestation Fetal Sheep. *J. Physiol. (Lond).* 549, 573-581.

Yoon, K., 1994. Voltage-dependent modulation of GABAA receptor channel desensitization in rat hippocampal neurons. *J. Neurophysiol.* 71, 2151-2160.

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.

Zhang, N., Wei, W., Mody, I., Houser, C.R., 2007. Altered localization of GABA(A) receptor subunits on dentate granule cell dendrites influences tonic and phasic inhibition in a mouse model of epilepsy. *J. Neurosci.* 27, 7520-7531.

Zhang, W., Huguenard, J.R., Buckmaster, P.S., 2012. Increased excitatory synaptic input to granule cells from hilar and CA3 regions in a rat model of temporal lobe epilepsy. *J. Neurosci.* 32, 1183-1196.

FIGURE CAPTIONS

Figure 1. Effect of THIP on tonic GABAergic currents ($I_{\text{TonicGABA}}$) in dentate granule cells (DGCs) 1-2 weeks following controlled cortical impact (CCI)-induced brain injury. A-C. Representative voltage-clamp recordings of DGCs during baseline, application of THIP (3 μ M), a partial selective agonist for GABA_AR δ subunits, and THIP+bicuculline application demonstrating $I_{\text{TonicGABA}}$ in DGCs. Membrane is voltage-clamped at 0 mV for all traces. A. Sham-injured. B. Contralateral to CCI-injury. C. Ipsilateral to CCI-injury. D. Normalized current amplitude of resting $I_{\text{TonicGABA}}$ was similar across control and injured conditions. THIP significantly increased $I_{\text{TonicGABA}}$ in DGCs from control and injured conditions (# indicates $p < 0.05$). DGCs ipsilateral to CCI ($n=9$) displayed an $I_{\text{TonicGABA}}$ with significantly reduced responses to THIP relative to DGCs after sham-injury ($n=9$) and contralateral to the injury ($n=9$). * indicates $p < 0.05$ versus controls.

Figure 2. Effect of THIP on synaptic GABAergic signaling in dentate granule cells (DGCs) 1-2 weeks following CCI-induced brain injury. A-C. Representative voltage-clamp recordings of spontaneous inhibitory post-synaptic currents (sIPSCs) in DGCs during baseline and THIP application (3 μ M). Membrane is voltage-clamped at 0 mV for all traces. The expanded traces are selected from regions indicated by small dashed lines below each representative image in Figure 1A-C. A. Sham-injured. B. Contralateral to CCI-injury. C. Ipsilateral to CCI-injury. D. DGCs ipsilateral to CCI injury ($n=9$)

displayed lower sIPSC frequency relative to DGCs from sham-injured mice (n=9) and DGCs contralateral to CCI (n=9) (* indicates $p < 0.05$ versus controls). DGCs from sham-injured mice and DGCs contralateral to CCI exhibited a reduction in sIPSC frequency with THIP application (# indicates $p < 0.05$). E. sIPSC amplitude in DGCs was similar between injured and control conditions. F. sIPSC decay time constant in DGCs was similar between injured and control conditions.

Figure 3. Effect of THIP and THDOC on $I_{\text{TonicGABA}}$ in DGCs >3 weeks following CCI-induced brain injury. A-C. Representative voltage-clamp recordings of DGCs during baseline, THIP application ($3\mu\text{M}$) and THIP+bicuculline application demonstrating $I_{\text{TonicGABA}}$ in DGCs 8-13 weeks post-injury. Membrane is voltage-clamped at 0 mV for all traces. A. Sham-injured. B. Contralateral to CCI-injury. C. Ipsilateral to CCI-injury. D. Normalized current amplitude of resting $I_{\text{TonicGABA}}$ was similar across all groups. THIP significantly increased $I_{\text{TonicGABA}}$ in DGCs from sham-injured mice and DGCs contralateral to CCI (# indicates $p < 0.05$). At 8-13 weeks post-injury, DGCs ipsilateral to CCI (n=7) exhibited an $I_{\text{TonicGABA}}$ with significantly reduced response to THIP relative to DGCs from sham-injured mice (n=7) and DGCs contralateral to CCI (n=8). E. At 3-5 weeks post-injury, $I_{\text{TonicGABA}}$ in the presence of THDOC ($1\mu\text{M}$) was reduced in DGCs located ipsilateral to CCI (n=7) relative to control DGCs (sham and contralateral cells; n=14). * indicates $p < 0.05$ versus controls.

Figure 4. Effect of THIP on synaptic GABAergic signaling in DGCs 8-13 weeks following CCI-induced brain injury. A-C. Representative voltage-clamp recordings of

sIPSCs in DGCs during baseline and THIP application (3 μ M). Membrane is voltage-clamped at 0 mV for all traces. The expanded traces are selected from regions indicated by small dashed lines below each representative image in Figure 3A-C. A. Sham-injured. B. Contralateral to CCI-injury. C. Ipsilateral to CCI-injury. D. DGCs ipsilateral to CCI (n=7) displayed reduced sIPSC frequency relative to DGCs from sham-injured mice (n=7) and DGCs contralateral to CCI (n=8); * indicates $p < 0.05$ versus controls. In the presence of THIP, DGCs ipsilateral to CCI exhibited reduced sIPSC frequency relative to DGCs contralateral to CCI (^ indicates $p < 0.05$). DGCs from sham-injured mice exhibited a reduction in sIPSC frequency with THIP application (# indicates $p < 0.05$). E. sIPSC amplitude in DGCs was similar between injured and control conditions. F. DGCs ipsilateral to CCI displayed significantly increased sIPSC decay time constant relative to sham and contralateral DGCs. * indicates $p < 0.05$ versus controls.

Figure 5. Effect of L655,708 on $I_{\text{TonicGABA}}$ in DGCs 8-13 weeks following CCI-induced brain injury. Analysis of resting and L655,708-treated (5 μ M) normalized current amplitudes from voltage clamp recordings of DGCs located contralateral and ipsilateral to CCI. Normalized current amplitude of resting $I_{\text{TonicGABA}}$ was similar between contralateral (n=7) and ipsilateral (n=8) DGCs ($p > 0.05$). The effect of L655,708 on $I_{\text{TonicGABA}}$ was similar between contralateral and ipsilateral DGCs ($p > 0.05$).

Figure 6. mRNA expression of α_4 , α_5 and δ GABA_AR subunits in dorsal dentate gyrus (DG) 8-13 weeks post-injury. Isolated dorsal DG from sham-injured mice and from the contralateral hemisphere in CCI-injured mice (control) are compared

graphically to the dorsal DG ipsilateral to CCI injury. A. No change in mRNA transcription was detected for the GABA_AR α_4 subunit (α_4 control DG: n=11, ipsilateral DG: n=10; p>0.05). B. No transcriptional change was detected for the GABA_AR α_5 subunit (α_5 control DG: n=8, ipsilateral DG: n=10; p>0.05). C. No change in transcription was detected for GABA_AR δ subunit (control DG: n=17, ipsilateral DG: n=19; p>0.05). Cycles thresholds were used for statistical analysis and data are presented as relative change using the $2^{-\Delta\Delta CT}$ method.

Figure 1
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A Sham

B Contralateral To CCI

C Ipsilateral To CCI

D Tonic GABA_AR Current

■ Sham ■ Contralateral To CCI □ Ipsilateral To CCI

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