GABA$_A$ Receptor Regulation after Experimental Traumatic Brain Injury

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Abstract

The gamma-aminobutyric acid (GABA) type A receptor (GABA$_A$R) is responsible for most fast synaptic inhibition in the adult brain. The GABA$_A$R protein is composed of multiple subunits that determine the distribution, properties, and dynamics of the receptor. Several studies have shown that the Janus kinase/signal transducer and activator of transcription (JaK/STAT) and early growth response 3 (Egr3) signaling pathways can alter GABA$_A$R subunit expression after status epilepticus (SE). In this study we investigated changes in these pathways after experimental TBI in the rat using a lateral fluid percussion injury (FPI) model. Our results demonstrated changes in the expression of several GABA$_A$R subunit levels after injury, including GABA$_A$R α1 and α4 subunits. This change appears to be transcriptional, and there is an associated increase in the phosphorylation of STAT3, and an increase in the expression of Egr3 and inducible cAMP element repressor (ICER) after FPI. These findings suggest that the activation of the JaK/STAT and Egr3 pathways after TBI may regulate injury-related changes in GABA$_A$R subunit expression.

Key words: Egr3 pathway; GABA$_A$ receptor; JaK/STAT pathway; traumatic brain injury

Introduction

TRAUMATIC BRAIN INJURY (TBI) is a major public health problem worldwide, and has been called the signature injury of the U.S. conflicts in Iraq and Afghanistan. Each year, TBI is a leading cause of mortality and neurological morbidity.[1,2] In addition, up to 50% of TBI survivors can go on to develop epilepsy,[3,4] and some develop temporal lobe epilepsy (TLE).[5,6] Preventive and therapeutic treatments for many of these patients have been unsuccessful due to a lack of knowledge of the cellular and molecular mechanisms by which brain trauma causes TLE. This lack of understanding limits how current medications are being implemented and designed.

Trauma to the brain initiates a complex sequence of molecular responses involving a number of signaling pathways. One such pathway is the Janus kinase/signal transducer and activator of transcription (JaK/STAT) pathway, which has been shown to be activated after TBI,[7–9] and following other brain insults such as stroke[10,11] and status epilepticus (SE).[12,13] Following SE, the JaK/STAT pathway has been shown to regulate gamma-aminobutyric acid (GABA) type A receptor (GABA$_A$R) subunit α1 expression in the hippocampus by increasing the phosphorylation of STAT3.[13] Phosphorylated STAT3 (pSTAT3) subsequently decreases tran-

scription of the GABA$_A$R α1 gene (*Gabra1*), by increasing the expression of the cyclic AMP response element-binding protein (CREB) family transcriptional inhibitor inducible cAMP element repressor (ICER).[13] ICER binds to the *Gabra1* promoter with phosphorylated CREB (pCREB) to inhibit the expression of *Gabra1* (Fig. 1).[13] The decreased levels of α1 subunit-containing GABA$_A$R are thought to contribute to the increased hyperexcitability seen in the hippocampus of animals following SE, and to contribute to the subsequent development of TLE.[14–17] Decreases in the GABA$_A$R α1 subunit have been reported in injured hippocampus after TBI,[18] but whether the JaK/STAT pathway regulates this decrease is not known. Other GABA$_A$R subunits are also modulated after cerebral insults such as SE,[14,15,19,20] and may also contribute to the alterations of neural excitability.

Lateral fluid percussion injury (FPI) in the rat was used for our studies to determine if there were acute alterations in GABA$_A$R subunit levels and the known signaling pathways that alter these receptor levels after other types of cerebral injury (such as SE). Lowenstein and associates,[21] Toth and colleagues,[22] and Gupta can co-workers,[23] have shown that as early as 1 week after FPI there is enhanced excitability in the hippocampus, which indicates that TBI alters the balance of excitation and inhibition early post-injury. D'Ambrosio and associates,[24] and Kharatishvili and colleagues,[25]

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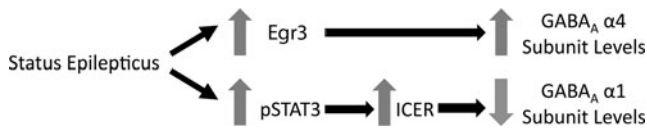
FIG. 1. Overview of signaling pathways activated after status epilepticus (SE) that contribute to GABA$_A$R subunit regulation (GABA$_A$R, gamma-aminobutyric acid type A receptor; Egr3, early growth response 3; ICER, inducible cAMP element repressor; pSTAT3, phosphorylated STAT3).

have shown that these animals can go on to develop epilepsy. The molecular mechanisms mediating early changes in excitability after TBI, as well as the contribution of early molecular and electrophysiological changes to later development of post-traumatic epilepsy (PTE), are not fully understood.

In this study we examined the levels of GABA$_A$R subunits after lateral FPI in the rat, and found that the $\alpha 1$, $\alpha 4$, $\gamma 2$, and δ subunit levels are decreased 1 week after injury, and that $\gamma 2$ and $\alpha 5$ subunit levels remain unchanged. We also found that two pathways known to regulate GABA$_A$R subunit composition, the JaK/STAT and Egr3 pathways, are activated after FPI.

Methods

Establishment of dural access and fluid percussion injury

Adult male Sprague-Dawley rats (250–300 g) were anesthetized with 3–5% isoflurane via nose cone and placed in a stereotaxic head frame. After scalp incision and reflection, a 3-mm-diameter craniotomy was created centered at -3 mm from the bregma and 3.5 mm left of the sagittal suture. For support, a steel screw was placed in the right parietal bone opposite the craniotomy site. A female Luer-Lok hub was centered over the craniotomy site and bonded to the skull with cyanoacrylate adhesive. Dental acrylic was poured around the Luer hub and support screw. After the acrylic hardened, antibiotic ointment was placed around the injury cap and the animal was removed from the stereotaxic frame and returned to its cage to recover.

Fifteen to 20 h after craniotomy and Luer hub implantation, the animals were anesthetized with isoflurane in an induction chamber. The animal was then removed from the chamber, immediately connected to the FPI apparatus, and received a 20-msec pulse of pressurized fluid (2.5–3.0 atm, moderate to severe impact) on the intact dural surface before awakening from anesthesia.[26] Sham-injured animals underwent establishment of dural access and were anesthetized and connected to the FPI apparatus, but the injury pulse was not triggered. A total of 81 animals underwent surgery, of which 34 were sham-injured animals and 47 were FPI animals. Out of the animals that underwent FPI, 37 animals lived, thus we had a survival rate of 79%. The University of Colorado Institutional Animal Care and Use Committee approved all the procedures described here.

After the FPI the animals were returned to their cages and allowed to recover for 6 h, 24 h, 48 h, or 1 week after injury. At these time points the animals were deeply anesthetized with isoflurane in an induction chamber, decapitated, and their brains were rapidly removed. The hippocampi were dissected and flash frozen for storage in a freezer at $-80°C$.

Western blot

Western blot was performed with modifications of published protocols.[17] Protein (25 μg) extracted from whole hippocampi was loaded into 8% SDS-polyacrylamide gels and run for 1.5 h at 115 V.

The blots were then transferred to nitrocellulose membranes and blocked in 5% milk/trisphosphate-buffered saline with Tween-20 (TBS-T). The membranes were incubated with rabbit polyclonal antibodies raised against STAT3 phosphorylated at Tyr705 (anti-pSTAT3) (1:1000; Cell Signaling Technologies, Danvers, MA), STAT3 (1:2000; Cell Signaling Technologies), $\alpha 1$ (1:5000; Millipore), $\alpha 2$ (1:2000; Millipore), $\alpha 4$ (1:2000; Millipore), $\alpha 5$ (1:1000; Millipore), δ (1:1000; Millipore), or β actin (1:40,000; Sigma-Aldrich, St. Louis, MO) overnight at 4°C in 5% bovine serum albumin/TBS-T (for pSTAT3 and STAT3), or 1% milk/TBS-T (for GABA$_A$R), then washed and incubated with anti-rabbit antibody (1:10,000) conjugated to horseradish peroxidase (HRP) for 1 h. Protein bands were detected with the use of chemiluminescent solution (Pierce Biotechnology, Rockford, IL), then the membranes were stripped and reprobed with rabbit polyclonal antibody raised against total STAT3 (anti-STAT3) (1:2000; Cell Signaling Technologies), or β-actin, and bands were quantified using Image J software (National Institutes of Health [NIH]), and expressed as percent change with respect to mean control values in the same run (defined as 100%).

RT-PCR

RNA was extracted from whole hippocampi with the use of a Trizol reagent protocol (Invitrogen, Carlsbad, CA). To synthesize complementary DNA (cDNA), 1 μg of RNA was separated and processed with the SuperScript II reverse transcription kit (Invitrogen), according to the manufacturer's instructions, and then diluted 1:4 for storage and subsequent RT-PCR. For RT-PCR reactions, each sample was run in triplicate and each 25-μL reaction contained 1.25 μL ICER, Egr3, GABA$_A$R subunit $\alpha 1$, GABA$_A$R subunit $\alpha 4$, or cyclophilin (ppia) Taqman gene expression primer/probe sets from Applied Biosystems (Foster City, CA), 12.5 μL of Taqman Master mix, and 10 μL of sample cDNA. RT-PCR was formed on the SDS-7500 PCR machine (Applied Biosystems). The RT-PCR runs 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. All values were normalized to cyclophilin expression in the same samples to control for loading variability, then expressed as fold change with respect to mean control values in the same run (defined as 1).

Statistical analysis

Statistical significance was set at $p < 0.05$. All calculations were done using InStat software. Mean amounts of pSTAT3 and each GABA$_A$R subunit protein assessed using Western blotting were compared between injured and sham-injured groups using the Student's t-test.

Mean RT-PCR expression of $\alpha 1$, $\alpha 4$, ICER, and Egr3 were compared between injured and sham-injured controls for the ipsilateral and contralateral brain regions (relative to FPI) using the Student's t-test, or the Mann-Whitney U test, as appropriate for non-parametric data.

Results

GABA$_A$R subunit levels change after FPI

Figure 2 shows GABA$_A$R subunit protein levels in hippocampi in sham-injured and FPI animals. Protein levels of the $\alpha 1$ subunit showed a significant decrease at 24 h, 48 h, and 1 week post-injury in the ipsilateral hippocampus (Fig. 2A and B). As shown in Figure 2C and D, $\alpha 4$ subunit levels were significantly increased at 24 h after injury, were not significantly different from controls at 48 h, and were significantly decreased compared to sham-injured controls at 1 week post-injury. There was no significant difference in $\alpha 1$ and $\alpha 4$ subunit levels at any time point in the hippocampus
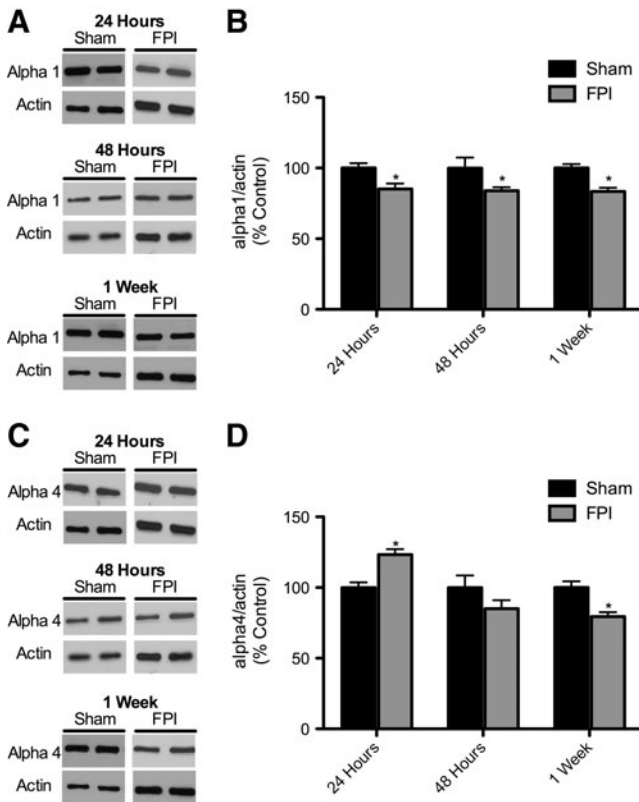
FIG. 2. Temporal profile of GABA$_A$ α1 and α4 receptor subunits after fluid percussion injury (FPI). (**A**) Representative Western blots from whole hippocampi of rats 24 h, 48 h, and 1 week after FPI probed with anti-GABA$_A$R α1 and β-actin antibodies. (**B**) Quantification of α1 blots showed a significant decrease at 24 h through 1 week after FPI injury relative to controls. α1 Levels were normalized to β-actin levels and expressed as percent change compared to sham-injured controls. (**C**) Representative Western blots from whole hippocampi of rats 24 h, 48 h, and 1 week after FPI probed with anti-GABA$_A$R α4 and β-actin antibodies. (**D**) Quantification of α4 blots showed a significant increase at 24 h after FPI, but at 1 week there was a significant decrease relative to controls. α4 Levels were normalized to β-actin levels and expressed as percent change compared to sham animals (24 h: $n = 7$ for sham, 6 for FPI; 48 h: $n = 6$ for sham, 8 for FPI; 1 week: $n = 7$ for sham, 7 for FPI; GABA$_A$R, gamma-aminobutyric acid type A receptor).

contralateral to the FPI compared to sham-injured controls (data not shown). To investigate whether the α1 and α4 subunit level changes at 24 h post-injury were due to transcriptional regulation we used quantitative RT-PCR on whole hippocampus samples 6 h after injury to determine the mRNA levels for these subunits. Figure 3 shows that in the ipsilateral hippocampus 6 h after FPI there was decreased expression of the α1 subunit (0.4-fold relative to controls), and an increase in the α4 subunit expression (1.7-fold relative to controls; Fig. 3A and B). There was no statistically significant difference in mRNA levels in the contralateral hippocampus compared to sham-injured controls (data not shown). Figure 4 shows that γ2 and δ subunit levels were significantly decreased 1 week after injury, but that the α2 and α5 subunits were unchanged compared to sham-injured controls. There was no significant difference in the γ2, δ, α2, or α5 subunit levels in the contralateral hippocampus compared to sham-injured controls (data not shown).
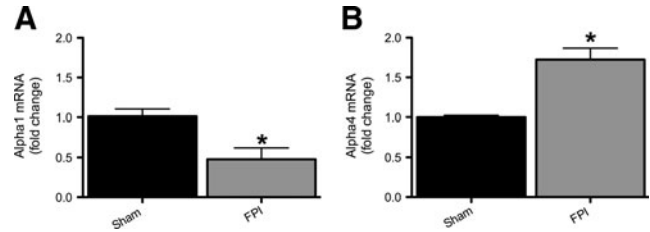
FIG. 3. α1 Expression is decreased and α4 expression is increased in injured hippocampi 6 h after fluid percussion injury (FPI). mRNA levels of α1 and α4 were quantified using RT-PCR analysis and represented as histograms showing the fold change of α1 (**A**) and α4 (**B**) 6 h after FPI and in sham-injured controls. α1 and α4 mRNA levels were normalized to cyclophilin mRNA levels in the same samples and expressed as fold change compared to sham animals (defined as 1; *$p < 0.05$; $n = 4$ for sham, 4 for FPI; RT-PCR, reverse transcriptase polymerase chain reaction).

FPI transiently activates the JaK/STAT pathway and affects downstream expression of ICER

As shown in Figure 5A and B, there was a statistically significant increase in the levels of pSTAT3 in the ipsilateral hippocampus compared to sham-injured controls at 6 h after injury. This increase declines temporally and at one week there is no difference in the levels of pSTAT3 when compared to controls. Figure 5C and D shows that the contralateral hippocampus there was a statistically significant increase in the pSTAT3 levels 6 hours after injury but at 24 hours after FPI the levels were not statistically different from sham injured controls. This suggests that the pathway is transiently being activated after FPI.

Figure 6A shows that ICER mRNA levels in the hippocampus ipsilateral to the injury were significantly increased 6 h after injury compared to sham-injured controls, although there was no difference between groups in the contralateral hippocampus (data not shown).

FPI increases Egr3 levels

The Egr3 pathway has been shown to be a critical mediator of α4 upregulation after SE.[27] Figure 6B shows that Egr3 mRNA levels in the hippocampus ipsilateral to injury were significantly increased 6 h after injury, compared to sham injured controls, although there was no difference between groups in the contralateral hippocampus (data not shown).

Discussion

In this study, we demonstrated that hippocampal injury after FPI alters the levels of protein and mRNA of several GABA$_A$R subunits, activates the JaK/STAT pathway, and elevates Egr3 mRNA levels. The GABA$_A$R α1 subunit level is decreased 24 h after injury, and remains lower than sham-injured controls for at least 1 week after FPI. The α4 subunit level is increased 24 h after injury, and then is lower than sham-injured controls by 1 week post-injury. We also found that mRNA expression of the α1 subunit was statistically significantly decreased, and that of the α4 subunit mRNA was statistically significantly increased, at 6 h after FPI, suggesting that the changes in the GABA$_A$R subunit levels are at least in part due to transcriptional regulation.

Several other GABA$_A$R subunits were analyzed to determine if their levels were also changed after injury. Our goal for looking at multiple GABA$_A$R subunits was to determine if there was an
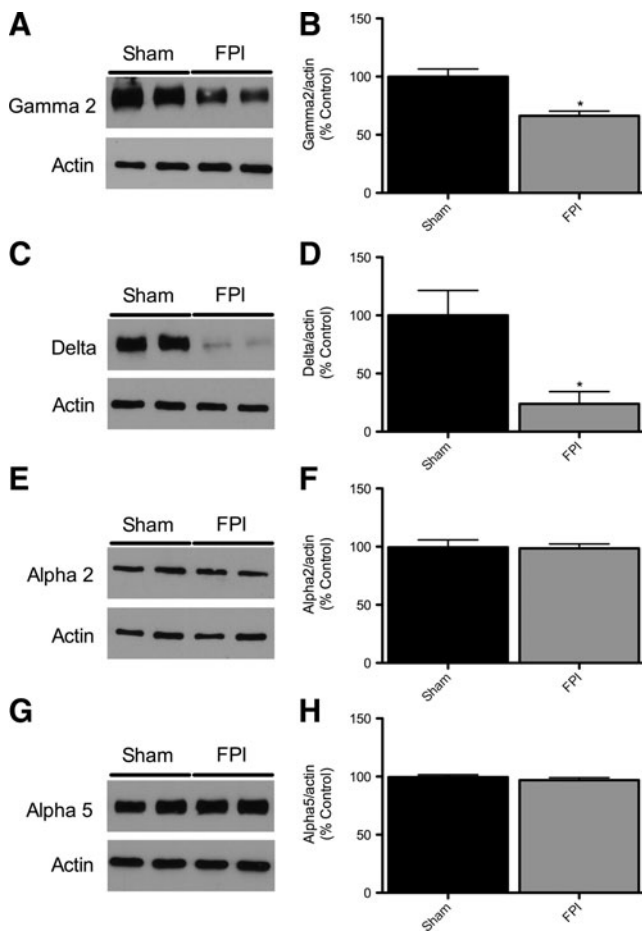
FIG. 4. GABA_A receptor subunit $\gamma 2$ is decreased, while $\alpha 2$ and $\alpha 5$ are unchanged, 1 week after fluid percussion injury (FPI). (**A**) Representative Western blots from whole hippocampi of rats 1 week after FPI probed with anti-GABA_AR $\gamma 2$ and β-actin antibodies. (**B**) Quantification of $\gamma 2$ blots shows that the $\gamma 2$ subunit is significantly decreased 1 week after FPI relative to sham-injured controls. (**C**) Representative Western blots from whole hippocampi of rats 1 week after FPI probed with anti-GABA_AR δ and β-actin antibodies. (**D**) Quantification of δ blots shows that the δ subunit is significantly decreased 1 week after FPI relative to sham animals. (**E**) Representative Western blots from whole hippocampi of rats 1 week after FPI probed with anti-GABA_AR $\alpha 2$ and β-actin antibodies. (**F**) Quantification of $\alpha 2$ blots shows that the $\alpha 2$ subunit is unchanged 1 week after FPI relative to sham animals. (**G**) Representative Western blots from whole hippocampi of rats 1 week after FPI probed with anti-GABA_AR $\alpha 5$ and β-actin antibodies. (**H**) Quantification of $\alpha 5$ blots shows that the $\alpha 5$ subunit is unchanged 1 week after FPI. GABA_AR subunit levels were normalized to β-actin levels and expressed as percent change compared to sham animals (*$p < 0.001$; $n = 7$ for sham and 7 for FPI; GABA_AR, gamma-aminobutyric acid type A receptor).

overall decrease in all subunits at 1 week, or if there was evidence to suggest subunit-specific regulatory processes occurring after FPI. Further, in some transgenic mice with GABA_AR subunit mutations, as well as after SE in the rat, it has been shown that changes in expression of one GABA_AR subunit can be associated with unanticipated regulation of other subunits.[14,28–31] While the $\gamma 2$ and δ subunit levels were found to decrease at 1 week post-injury, the $\alpha 2$ and $\alpha 5$ subunit levels showed no significant difference between FPI animals and sham-injured controls at 1 week post-injury.

These findings suggest that subunit changes are in part due to subunit-specific regulatory processes.

In a recent study using FPI in the rat, a reduction of approximately 55% was found in GABA_AR $\alpha 1$ subunit levels compared to sham-injured controls, with no change seen in the $\alpha 2$ and $\alpha 5$ subunits in the ipsilateral hippocampus 24 h and 1 week post-injury, results similar to ours.[18] These authors did not examine the $\alpha 4$ subunit or any of the signaling pathways that regulate GABA_AR subunits. Another study using the FPI model in the rat showed that after experimental TBI there is a progressive loss of phasic GABA_AR-mediated inhibition, which is suggestive of alterations in the GABA_AR. This study, however, only showed GABA_AR subunit mRNA expression changes in $\alpha 4$ (a $\sim 52\%$ increase in expression compared to controls), and $\beta 1$ (a $\sim 27\%$ increase in expression compared to controls).[32] The difference in the expression levels seen between that study and ours could be attributed to when the samples were collected, which was 6 h after injury in the current study, while the Pavlov group[32] measured GABA_AR mRNA levels 4 months after injury.[32]

Studies utilizing another injury model, controlled cortical impact (CCI) in the rat, showed a reduction in $\gamma 2$ subunit levels ~ 5–9 months after injury, similar to those found in the current study, but showed no change in $\alpha 1$ and $\alpha 4$ and an increase in δ.[33] These differences may be attributed to the difference in injury methods (CCI versus FPI), the time point of collecting the samples, or may be because the Kharlamov group[33] only analyzed the contralateral hippocampus, a brain region not known to be extensively affected in the CCI model. Electrophysiological studies done using the same injury model found that there is an increase in the GABA_AR-mediated tonic currents, a loss of diazepam potentiation, and an increase in furosemide inhibition of synaptic GABA_ARs in dentate granule cells (DGCs), ipsilateral to the injury site 90 days after injury.[34] These physiological changes are consistent with a decrease in $\alpha 1$-containing receptors, and an increase in $\alpha 4$-containing receptors in DGCs, similar to that seen in rats with epilepsy following SE.[14] In addition, the $\gamma 2$ and δ subunit levels have been shown to influence tonic and phasic synaptic inhibition and GABA_AR location.[20,35–44] Alterations of all of these levels, which we also saw after FPI, may result in altered GABAergic signaling, and could be a contributing factor to the development of TLE after head trauma.

Previous studies have shown that the JaK/STAT pathway mediates a decrease in $\alpha 1$ subunit levels after SE.[13,14] SE results in phosphorylation of STAT3, which upregulates expression of ICER, and subsequently binds to the *Gabra1* promoter and decreases transcription of *Gabra1* in the hippocampus of rats.[13] Multiple types of injuries to the hippocampus can lead to the development of TLE (including SE, ischemia, and head trauma). Interestingly, JaK/STAT pathway activation has been shown to occur in each of these types of injury models.[7,8,9,10,11,13]

In the current study we found that pSTAT3 levels were significantly increased in the injured hippocampus 6 h after FPI, and that there is a subsequent decline in pSTAT3 levels back to baseline levels by 1 week after injury. Our data are consistent with data from Oliva and associates,[7] suggesting that the JaK/STAT pathway is transiently activated in the ipsilateral hippocampus after cerebral injury.[7] Oliva and colleagues[7] also showed pSTAT3 co-localizing, mostly with glial fibrillary acidic protein (GFAP)-positive cells, and that pSTAT3 was transcriptionally regulating known STAT3-regulatory genes such as nitric oxide synthase 2.[7] However, they did not look for alterations in GABA_AR genes, subunit levels, or ICER or Egr3 expression. Zhao and associates performed Western
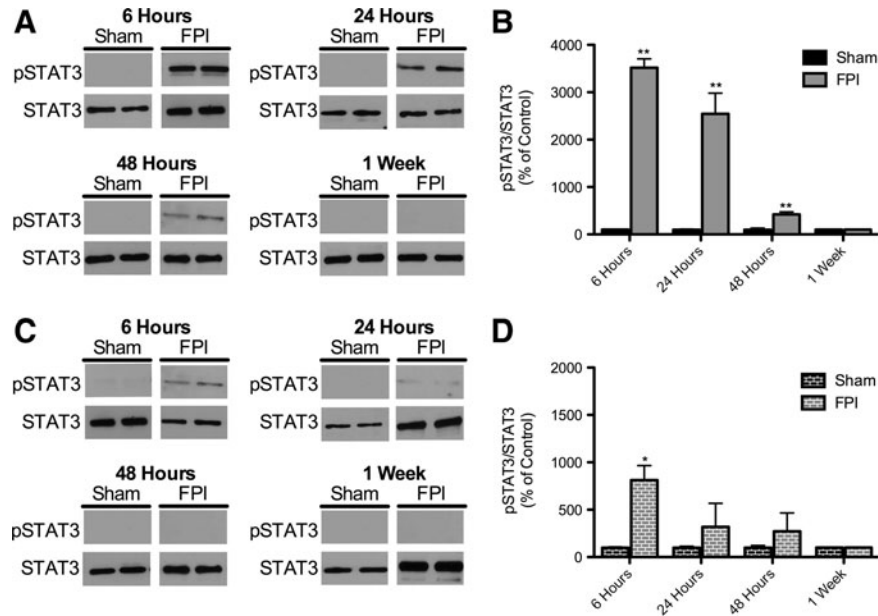
FIG. 5. Phosphorylated STAT3 (p STAT3) levels in injured hippocampus after fluid percussion injury (FPI). (**A**) Representative Western blots of protein homogenates from ipsilateral whole hippocampi (relative to FPI) of rats 6 h, 24 h, 48 h, and 1 week after FPI probed with pSTAT3 and STAT3 antibodies. (**B**) Quantification of pSTAT3 levels from FPI and sham-injured controls. (**C**) Representative Western blots of protein homogenates from contralateral whole hippocampi (relative to FPI) of rats 6 h, 24 h, 48 h, and 1 week after FPI probed with pSTAT3 and STAT3 antibodies. (**D**) Quantification of pSTAT3 levels from FPI and sham-injured controls. pSTAT3 levels were normalized to STAT3 levels and expressed as percent change compared to sham animals (*$p < 0.05$, **$p < 0.001$ for comparison of sham and FPI animals at the same time point; 6 h: $n = 5$ for sham, 7 for FPI; 24 h: $n = 7$ for sham, 6 for FPI; 48 h: $n = 6$ for sham, 8 for FPI; 1 week: $n = 7$ for sham, 7 for FPI; STAT3, signal transducer and activator of transcription 3).

blot analysis and immunohistochemistry, which showed that in the injured ipsilateral parietal cortex, pSTAT3 signal was enhanced at acute time points, and that pSTAT3 was co-localized with both NeuN- and GFAP-positive cells after TBI.[9] It remains to be determined if the activation of the JaK/STAT pathway after TBI directly alters the expression of GABA$_A$R subunits as has been shown after SE, and is a contributing factor in the development of TLE after TBI.

Last, we looked at the Egr3 mRNA levels 6 h after FPI because the α4 subunit was increased 24 h after injury, and previous studies in our lab have shown that the Egr3 pathway can regulate transcription of the α4 subunit.[27,45,46] We have shown that Egr3 mRNA levels were increased in the ipsilateral hippocampus after TBI, suggesting that this pathway may be regulating the increase seen in α4 subunit levels 24 h after injury. The mechanism mediating the reduction in α4 subunit levels 1 week post-injury remains uncertain. Future studies will be required to elucidate these mechanisms, as well as to understand the functional role of Egr3 changes after FPI.[46]

Our study does have several limitations. First, the whole hippocampus was used to determine GABA$_A$R subunit levels, as well as for the determination of pSTAT3 levels and expression of ICER and Egr3. Thus we are unable to determine where in the hippocampus these changes were occurring and in which cell type. Second, β-actin was used for normalization in this study to control for differences in protein loading, and β-actin levels can be affected by cell death and other regulatory processes. However, normalization of subunit densitometry to total protein loaded in each well produced results similar to those found with normalization to β-actin, and there was no difference in the expression of β-actin (normalized to total protein loaded) in the FPI samples compared to sham-injured controls (data not shown). Thus we could find no evidence that intergroup differences in β-actin levels were impacting our results. Further, we could find no studies in the literature suggesting that β-actin is actively regulated after FPI. Third, our data suggests an association, but not necessarily a causal relationship, between activation of the JaK/STAT and Egr3 pathways and GABA$_A$R alterations. A causal relationship has been shown more definitively in TLE after SE,[13,27,45,46] suggesting that a causal relationship between the two processes in TLE after TBI is plausible, if yet unproven. Fourth, we have only reported a set of molecular changes after TBI, but do not examine potential functional
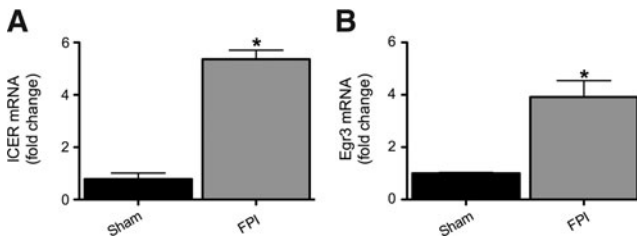
FIG. 6. Inducible cAMP element repressor (ICER) and early growth response 3 (Egr3) expression are increased in injured hippocampi 6 h after fluid percussion injury (FPI). mRNA levels of ICER and Egr3 were quantified using RT-PCR analysis and represented as histograms showing the fold change of ICER (**A**) and Egr3 (**B**), 6 h after FPI in injured and sham-injured controls. ICER and Egr3 mRNA levels were normalized to cyclophilin mRNA levels in the same samples and expressed as fold change compared to shams (defined as 1; *$p < 0.05$; $n = 4$ for sham, 4 for FPI; RT-PCR, reverse transcriptase polymerase chain reaction).

consequences of these changes. Results of previous studies examining inhibitory function utilizing similar injury models, however, are consistent with changes that we show.[32,34] Lastly, while the current findings demonstrate changes in the GABA$_A$R after TBI, they do not establish that these changes contribute to the later development of epilepsy. Establishing this connection will require future studies in which GABA$_A$R changes after TBI are prevented or reversed, and showing that subsequent epilepsy outcome is altered.

Conclusions

The current findings demonstrate that levels of the $\alpha1$, $\alpha4$, $\gamma2$, and δ GABA$_A$R subunits are decreased in injured hippocampus at 1 week after experimental TBI, and that $\alpha2$ and $\alpha5$ subunit levels remain unchanged, suggesting that there is subunit-specific regulation of the GABA$_A$R following TBI. We also found that mRNA expression of the $\alpha1$ subunit was decreased, and that the $\alpha4$ subunit mRNA was increased, suggesting that the changes in these GABA$_A$R subunits are at least in part due to transcriptional regulation. Lastly, we found that two pathways known to regulate GABA$_A$R subunit composition in other models of cerebral injury, the JaK/STAT and Egr3 pathways, are activated after FPI. Based on evidence from these other models, we can speculate that the activation of these pathways may mediate altered levels of GABA$_A$R subunits, and may contribute to the early hippocampal hyperexcitability previously reported after TBI, and may lead to the development of later epilepsy. Future studies are needed to determine if there is a causal relationship between JaK/STAT activation, Egr3 elevations, and changes in GABA$_A$R subunit composition, as well as to determine the contribution of these injury-related changes to the occurrence of experimental post-traumatic epilepsy.

Acknowledgments

Author Disclosure Statement

No conflicting financial interests exist.

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