Short communication

Effect of repeated seizures on spatial exploration and immediate early gene expression in the hippocampus and dentate gyrus

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ABSTRACT

Immediate early genes (IEGs) are coordinately activated in response to neuronal activity and can cause activation of secondary response genes that modulate synaptic plasticity and mediate long-lasting changes in behaviour. Excessive neuronal stimulation induced by epileptic seizures induce rapid and dramatic changes in IEG expression. Although the impact of acute seizure activity on IEG expression has been well studied, less is known about the long-term effects of chronic seizures on IEG induction during seizure free periods where behavioural and cognitive impairments are frequently observed in people with epilepsy and in animal models of epilepsy. The present study sought out to examine the impact of chronic pentylenetetrazole evoked seizures (PTZ kindling) on spatial exploration induced in IEG expression (c-Fos, ΔFosB, Homer1a, Egr1, Npas4, Nr4a1) in the hippocampus (CA1 and CA3 subfields) and dentate gyrus of rats. Male rats underwent two weeks of PTZ kindling (every 2 days) or received vehicle injections and were placed into a novel open field arena for 30 min either 24 hrs or 4 weeks after the last treatment. Although exploratory activity was similar between PTZ kindled and vehicle controls when examined 24 hrs after the last treatment, we observed a significant reduction in spatial exploration induced expression of c-Fos, Egr1, and ΔFosB in the hippocampus and dentate gyrus, and reduced expression of Nr4a1 in the dentate gyrus and Homer1a in the hippocampus only. When testing was conducted after a 4-week recovery period, only c-Fos continued to show reduced expression after exposure a novel environment in previously PTZ kindled animals. Interestingly, these animals also showed reduced activity in the center region of the open field suggestive of heightened anxiety-like behaviour. Collectively, these results suggest that repeated seizures may lead to longterm downregulation in hippocampal IEG expression that can extend into seizure free periods thereby providing a critical mechanism for the development of cognitive and behavioural deficits that arise during chronic epilepsy

1. Introduction

Immediate early genes (IEGs) are rapidly expressed in response to neuronal activity and contribute to long-lasting adaptations in brain function (Loebrich and Nedivi, 2009). The importance of these genes is based on evidence that they encode for late effector transcription factors that are critical in regulating cellular homeostasis, metabolism, and synaptic development (Madabhushi and Kim, 2018; West and Greenberg, 2011). Such regulatory cascades are also believed to be important in mediating long-term synaptic changes that result from an organism's experience (Gallo et al., 2018; Tyssowski et al., 2018; Guzowski, 2002).

Epilepsy is a disabling neurological disorder of recurrent seizures affecting up to 1% of people (Collaborators, 2019). Precisely how normal brain regions become and remain epileptogenic still remains

unclear. Despite this, multiple studies have shown robust IEG expression in the rat brain after experimentally induced seizures (Szyndler et al., 2013; Simonato et al., 1991; Kiessling and Gass, 1993; Duman et al., 1992; Burazin and Gundlach, 1996; Pereno et al., 2011; Murashima et al., 1996; Bing et al., 1997; Gass et al., 1993). The profile of IEG induction has been a helpful aid for identifying critical brain circuits involved in seizure propagation (Vendrell et al., 1998). There is also strong evidence that IEGs can mediate direct effects on neuronal excitability and function, as inhibition of IEG induction by antisense oligonucleotides can disrupt seizure development in several animal models of epilepsy (Suzukawa et al., 2003; Rocha et al., 1999; Chiasson et al., 1998; Rocha and Kaufman, 1998; Panegyres and Hughes, 1997). Furthermore, accumulating evidence suggests that IEG expression after seizures might provide an important functional pathway that links

transient events induced by epileptiform activity with the longer lasting molecular and structural changes associated with chronic epilepsy and epileptogenesis (Lasarge and Danzer, 2014).

While the impact of seizures on IEG expression during the minutes to hours after acute seizures has been well characterized, the extent of which these genes respond to chronic seizure stimulation or remain altered during seizure-free (or interictal) periods is unknown. This is especially important given that people with epilepsy often present with a history of multiple recurrent seizure events and the interictal period comprises more than 99% of most patients' lives. Despite the paucity of research, lasting decreases in hippocampal expression of IEGs, including Egr1 and Fos have been observed after repeated seizure episodes, with downregulation in the expression of these IEGs being larger following chronic seizures than acute seizures (Calais et al., 2013). Further support for persistent seizure-induced changes in IEG expression extending into the interictal period also comes from the work of Fournier and colleagues (Botterill et al., 2014; Fournier et al., 2013) who found that Fos protein expression in the dentate gyrus and CA1 hippocampal subfield of amygdala kindled rats was reduced following retrieval of a contextual fear memory. Interestingly, the loss of Fos expression was directly correlated with the extent of memory impairment associated with amygdala kindling.

These findings suggest that reduced expression of some IEGs after seizures might persist and extend into interictal periods thereby providing a potential mechanism for the emergence of behavioral deficits observed during chronic epilepsy. Whether these changes are observed for other IEGs or would occur in different regions of the hippocampus remains unclear. To investigate this, we examined the expression of several plasticity associated IEGs in the hippocampus and dentate gyrus following exploration of a novel spatial environment in pentylenetetrazole (PTZ) kindled rats.

2. Methods

Thirty-five male Sprague Dawley rats (Charles Rivers, QB, Canada) weighing between 200 and 300 g at the time of arrival were used in the experiment. Subjects were housed in standard laboratory cages with corn bedding on a 12:12 h light:dark cycle with lights on at 0700 h, and had ad libitum access to food and water. The facility was maintained at an ambient temperature of 21 °C. Animals were allowed to acclimate undisturbed in the animal colony for seven days. Following this, an experimenter handled all animal subjects for one week before the study began to minimize handling stress. All procedures were approved by Trent University Animal Care Committee and were in accordance with Canadian Council for Animal Care (CCAC). All efforts were taken to minimize the number of experimental animals used in this study.

2.1. PTZ treatment

The rats were randomly assigned into either vehicle (n = 16) or PTZ (n = 19) groups. PTZ was prepared fresh daily and was dissolved in 0.9% (w/v) physiological saline. For kindling, PTZ-treated rats received an initial sub-threshold dose of PTZ (35 mg/kg, Sigma Aldrich Canada) that was injected i.p. every 48 hrs for 2 weeks (resulting in 8 injections of PTZ). After the injection, the rats were placed in a glass observation chamber and seizure intensity over a 30 min period was recorded. The scoring of seizures was done according to a modified 5-point scale adapted from Racine (1972). Briefly, seizures were characterized as follows: 0 – no change in behaviour; 0.5 - orofacial automatisms and/or excessive sniffing/washing; 1 – myoclonic jerks; 2 – short myoclonic seizures or atypical/unilateral seizures; 3 – bilateral forelimb clonus with rearing; 4 – forelimb clonus with tonus and twist of body; 5 – tonic-clonic seizure with loss of posture. Vehicle-treated controls were handled in the exact manner with the exception that they were administered saline. These rats never displayed behavioural convulsions.

2.2. Open field testing

To induce IEG expression in hippocampus and dentate gyrus, rats were placed in a novel open field arena (60 ×60 x 60 cm) for 30 min either 24 hrs or 4 weeks after their last PTZ or vehicle treatment. The open field comprised peripheral (20 cm) and central (40 cm) zones where animals' movements were continuously tracked using AnyMaze software (ANY-maze 4.71, Stoelting Co, USA). Time spent in center, velocity, and distance travelled was recorded for off-line analysis of exploratory behaviour. Open field arenas were cleaned with Oxivir Five 16 Concentrate (Johnson Diversy, Canada) disinfectant between subjects and bedding refreshed to avoid olfaction-driven bias during exploration.

2.3. Tissue collection and microdissections

Thirty minutes after open field testing, each rat was briefly anesthetized with 5% isoflurane before decapitation for collection of tissue samples for RNA extraction. This time interval was chosen as previous studies observed a rapid induction of IEG transcription in the HPC within 30 min after exposure to a novel context (Lacar et al., 2016). The brain was rapidly removed 30 min after open field testing, and the hippocampus and dentate gyrus were separately extracted. The tissue was placed in nuclease-free tubes and flash frozen in liquid nitrogen and stored at − 80°C until RNA extraction for quantitative RT-PCR analysis.

For immunohistochemistry experiments, rats were deeply anesthetized 90 min after spatial exploration with sodium pentobarbital (340 mg/ml, Euthanosl, Merck Animal Health Canada) and then transcardially perfused with ice cold 0.1 M phosphate buffered saline (PBS; pH = 7.4) followed by 10% (v/v) buffered formalin. Following perfusion, the brains were extracted and postfixed in the same fixative for 48 hrs. After fixation, brains were cryoprotected in an ascending series of sucrose solutions (15- and 30% (w/v)) and then sectioned on a cryostat (SLEE Medical, Germany) at a thickness of 40 μm. Sections were stored at − 20 °C in a cryoprotectant solution comprised of 30% (w/v) sucrose, 1% (w/v) polyvinylpyrrolidone and 30% (v/v) ethylene glycol in PBS until used.

2.4. RNA Isolation and cDNA conversion

Total RNA from dissected tissues was extracted using QIAzol Lysis reagent (Qiagen, CA) and was further purified with RNeasy mini kit (Qiagen, CA) according to the manufacture's protocol. The quantity and quality of RNA was determined by measuring the absorbance at 260 nm (A260) using a NanoDrop spectrophotometer. The purity of RNA was determined based on the A_{260}/A_{280} ratio, which was 2.00–2.10 for all RNA preparations used for subsequent expression analyses. First-strand cDNA was generated with random primers using the QuantiTect Reverse Transcription (Qiagen, CA) kit.

2.5. Quantitative real-time polymerase chain reaction (qPCR)

Quantitative reverse transcriptase PCR (RT-qPCR) was used to quantify the mRNA levels of target genes and all reactions were carried out using Quantitect SYBR Green PCR kit (Qiagen, CA). Each 50 uL reaction contained 5 uL (10 ng) cDNA, 300 nM forward and reverse primers for each gene of interest, 25 ul SYBR Green qPCR Master Mix, and 10 uL of nuclease free water. Triplicate reactions were carried out for each experimental sample for each primer pair as well as an no reverse transcriptase, and no template control reactions. Thermal cycling was performed on a CFX Connect (Bio Rad, California, US) real-time PCR system with the following conditions: hot-start activation of HotStart TaqDNA polymerase (94 °C) for 15 min and 40 cycles of denaturation (95 °C, 15 s, annealing 30 s, 55 °C), and extension (72 °C, 30 s). Melting curve analysis was performed according to the dissociation stage data and reactions with a single peak at expected temperature

melting (T_m) were considered for further analysis. All primers were designed using Primer3 software and their specificity was verified using nucleotide blast software (BLAST Interface, www.ncbi.nlm.nih.gov/blast). The primers used in this study are listed in Table 1.

Relative gene expression was determined using the $2^{-\Delta\Delta Ct}$ method with GAPDH as the normalizer or reference gene. The relative expression of each gene of interest in the PTZ treated sample was determined by comparing it to the expression of the same gene in the control sample that is used as the calibrator.

2.6. Fos and Egr1 Immunohistochemistry

Sections were processed according to previously published methods (Eells et al., 2004). Briefly, sections were treated with primary rabbit anti-Fos polyclonal antibody (1:10,000; EMD Millipore Canada Ltd.) or rabbit anti-Egr1 polyclonal antibody (1:1000, Santa Crus Biotechnology USA) diluted in a solution containing 5% (v/v/) normal goat sera, 1% (w/v) bovine serum albumin, 0.3% (v/v) Triton X-100 dissolved in PBS. Following this, sections were incubated with a biotinylated goat anti-rabbit secondary antibody (1:500, room temperature, Vector Labs) and then incubated with avidin-biotin peroxidase complex (1:500, 1 h, Vectastain ABC Elite). Immunolabeling was visualized using 2.5 (w/v) nickel sulfate, 0.02% (w/v) DAB, and 0.000083% (v/v) H_2O_2 to produce a blue and black product.

Quantification of Fos and Egr1 was conducted on digital images collected from a Nikon Eclipse 80i light microscope (Nikon Instruments, USA). Cell counts were performed using ImageJ software and the Cell Counter plugin (Wayne Rasband, National Institute of Health). Only immunolabelled cells that appeared > 2X greater than background was quantified manually using the Cell Counter plugin thereby providing an estimate of the relative number of Fos+ or Egr1 + cells per mm^2.

3. Statistical analyses

Statistical analyses were performed in GraphPad Prism 6 (GraphPad Software, USA). Behavioural data were analyzed using a two-way analysis of variance (ANOVA), corrected for multiple comparisons (Tukey's HSD), with group (vehicle vs. PTZ) and testing (24 hrs vs. 4 weeks) as the between-subject factors. A series of independent t-tests were used to examine for group differences in IEG mRNA expression for each testing (24 hrs or 4 weeks) period separately. Finally, group differences in Egr1 and Fos cell counts for the hippocampus and dentate gyrus was examined using independent t-tests. The criterion for statistical significance was set at $P < .05$.

4. Results

Rats were kindled with an initial sub-convulsive dose of PTZ (35 mg/kg) administered intraperitoneally every 2 days for two weeks. As

Table 1
– List of PCR Primers Used.

| Gene	Primers (5' to 3')
c-Fos	Forward: TGGACTGGGATTCTCCTCTG
	Reverse: GCTGATTCCTGTGAAGGGGT
Egr1	Forward: CGCTGGTGGAGACAAGTTAT
	Reverse: GTCAGTGTTGGGAGTAGGAAAG
Nr4a1	Forward: CAGAAGATGGACAGAGAGAGAG
	Reverse: ATGGAAGGAGAGCGGAAGAG
ΔFosB	Forward: AGGCAGAGCTGGAGTCGGAGAT
	Reverse: GCCGAGGACTTGAACTTCACTCG
Npas4	Forward: AGCATTCCAGGCTCATCTGAA
	Reverse: GGCGAAGTAAGTCTTGGTAGGATT
Homer1a	Forward: TGGACTGGGATTCTCCTCTG
	Reverse: GCTGATTCCTGTGAAGGGGT
GAPDH	Forward: ACCACAGTCCATGCCATCAC
	Reverse: TCCACCACCCTGTTGCTGTA

expected, all rats showed a progressive increase in seizure severity and achieved Class 4 and 5 motor seizures with repeated administration of PTZ (Fig. 1). Two rats died during the course of kindling.

To induce IEG expression, rats were exposed to a novel environment for 30 min. One group underwent behavioural testing 24 hrs after receiving their last drug treatment (24 hrs: PTZ, n = 7; Vehicle, n = 6), while another group underwent behavioural testing 4 weeks after receiving their last drug treatment (4 weeks: PTZ, n = 4; Vehicle, n = 4). There were no differences in the total distance traveled in the arena between PTZ kindled and vehicle control groups ($P = .490$, Fig. 2a). However, there was a group (PTZ vs. vehicle) by testing (24 hrs vs. 4 weeks) interaction for the amount of time spent in the center compartment of the arena [$F_{(1,17)}= 4.67$, $P < .05$]. As shown in Fig. 2b, the source of the interaction came from the rats exposed to the open field arena 4 weeks after PTZ kindling spending significantly less time in the center compartment of the open field compared to those that underwent testing in the open field 24 hrs after kindling (Tukey's HSD, $P < .002$) or those that received vehicle (Tukey's HSD, $P < .025$). The time spent in the center compartment of the open field was comparable for the PTZ kindled and vehicle control (Tukey's HSD, $P = .125$) group tested 24 hrs after their last treatment. Within the center compartment of the open field, the average speed of movement (velocity) was significantly greater for the PTZ kindled rats that underwent behavioural testing 4 weeks later than all other groups [Group x Treatment Duration, $F_{(1,17)}= 5.42$, $P < .05$; Tukey's HSD, PTZ-4 weeks vs. PTZ-24 hrs, control, All Ps $< .002$, Fig. 2c]. Further examination found that the distance travelled within the central compartment was not significantly different between PTZ kindled rats and vehicle controls ($t_{(6)} = 0.153$, $P = .307$, Fig. 2d) exposed to the open field arena 4 weeks after their last treatment. And finally, there were no group differences in the average locomotor speed ($F_{(2,18)}= 0.405$, $P = .673$) during exploration of the peripheral compartment indicating that the change in locomotor activity in this group was specific to the center compartment of the open field arena.

To investigate the impact of PTZ kindling on IEG expression, we probed for mRNA changes in the hippocampus and dentate gyrus 30 min after placing rats into an unfamiliar environment. Compared to controls (n = 6), *Fos* ($t_{(11)} = 3.56$, $P < .006$), *Egr1* ($t_{(11)} = 2.20$, $P < .049$), *Homer1a* ($t_{(11)} = 2.95$, $P < .019$), and *ΔFosB* ($t_{(11)} = 2.52$, $P < .032$) mRNA levels in the hippocampus were reduced after spatial exploration in PTZ (n = 7) tested 24 hrs after their last treatment (Fig. 3a). In contrast, for the dentate gyrus, PTZ kindled rats showed downregulation in *Fos* ($t_{(11)} = 2.79$, $P < .017$), *Egr1* ($t_{(11)} = 3.21$, $P < .005$), *Nr4a1* ($t_{(11)} = 2.36$, $P < .042$) and *ΔFosB* ($t_{(11)} = 2.41$, $P < .039$) mRNA levels after spatial exploration (Fig. 3b). Interestingly, for kindled rats that underwent testing 4 weeks (PTZ, n = 4; Vehicle, n = 4) after their last
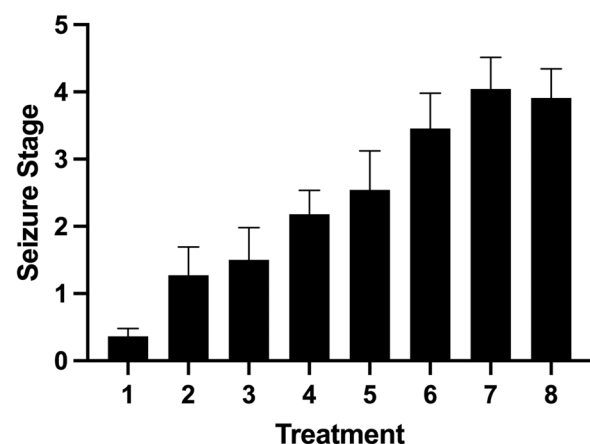
Fig. 1. Progression of seizures as measured on the Racine scale. Rats were injected with PTZ (35 mg/kg, i.p.) every 48 hrs for 2 weeks.
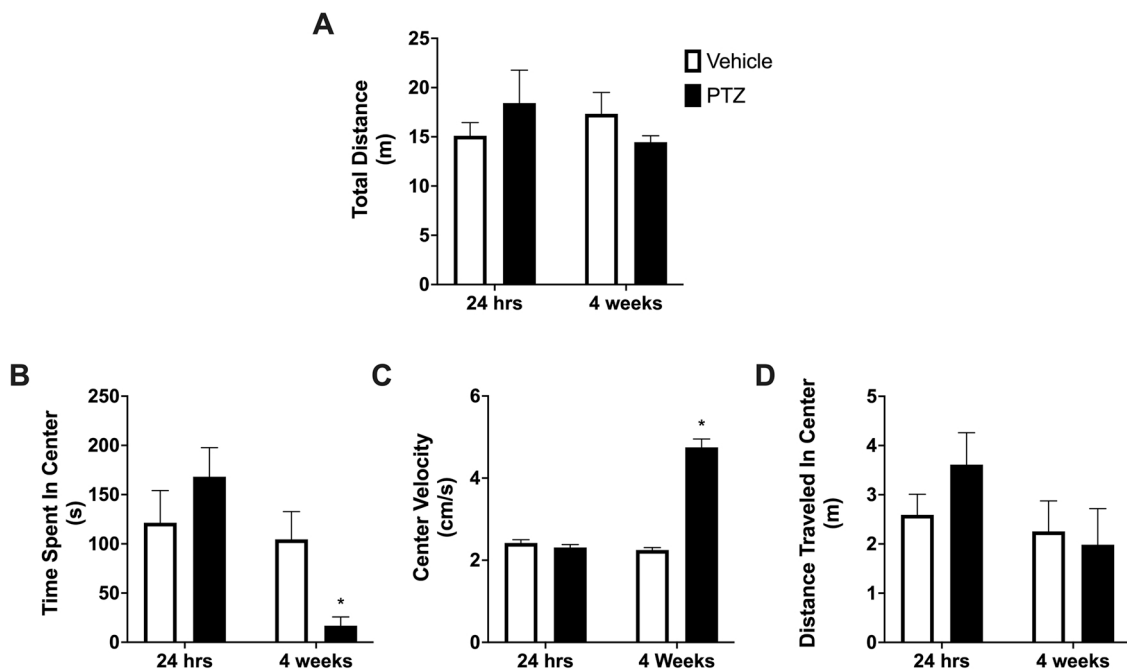
Fig. 2. Effect of PTZ kindled seizures on exploratory behaviour in a novel environment. (A) The mean distance travelled during a 30 min exposure session in the open field arena for PTZ kindled and vehicle control rats examined 24 hrs or 4 weeks after treatment. (B) The mean duration of time spent in the center compartment of the open field arena for PTZ kindled and vehicle control rats. (C) The mean velocity of movement in the center compartment of the open field arena for PTZ kindled and vehicle control rats. (D) The mean distance travelled in the center compartment of the open field arena for PTZ kindled and vehicle control rats. Data are presented as mean $\pm$ SEM. * $p < .025$, two-way ANOVA with Tukey's HSD post-hoc test, 4 week PTZ kindled vs. all other groups.
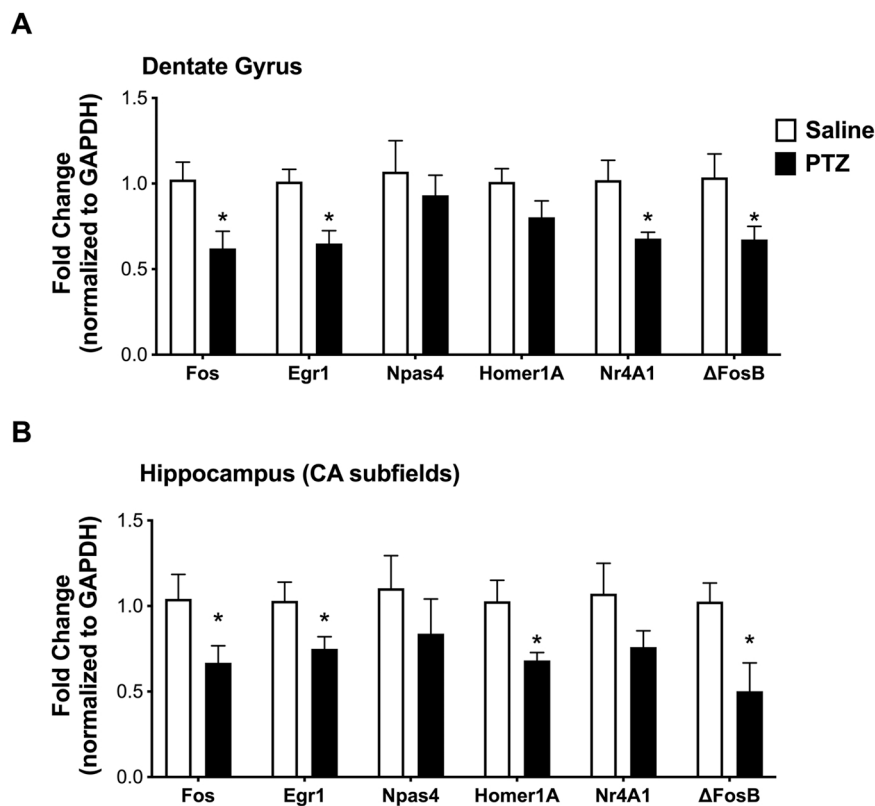
Fig. 3. Relative expression of each gene indicated on the x-axis was obtained by quantitative RT-PCR analysis from the RNA extracted from the dentate gyrus (A) and the hippocampus (B) of PTZ kindled rats and vehicle controls after exposure to a novel environment. Open field testing was conducted 24 hrs after the last treatment. The RT-qPCR data in each sample was normalized to the GAPDH gene. Gene expression in the PTZ treated samples were calibrated relative to the vehicle control sample. Data are presented as mean fold change in mRNA expression $\pm$ SEM, * $p < .05$, Student t-test, PTZ vs. control.

treatment, hippocampal *Fos* mRNA levels continued to be down-regulated after spatial exploration compared to non-kindled controls (61.4% decrease, $t(4) = 3.51$, $P < .008$). No additional differences in IEG mRNA expression were found in the hippocampus or dentate gyrus

after spatial exploration for PTZ kindled rats that underwent testing 4 weeks later (data not shown).

Finally, we wanted to expand on the analysis above to examine for potential regional variation in hippocampal IEG expression after spatial

exploration in PTZ treated animals. To accomplish this, we measured Fos (Fig. 4a) and Egr1 (Fig. 4c) immunoreactivity in the hippocampus and dentate gyrus in a subset of rats 90 min after exposure a novel open field arena (PTZ, n = 6; Vehicle, n = 6). Testing in the open field arena occurred twenty-four hours after rats received their final PTZ or vehicle treatments. These IEGs were specifically chosen as they showed robust differences at the mRNA level following PTZ treatment (see above). Following spatial exploration, the number of hippocampal (CA1 subfield, CA3 subfield) and dentate gyrus Fos+ cells was significantly reduced in the rats that underwent PTZ kindling compared to non-kindled controls (All $ts > 2.32$, All $Ps < .025$, Fig. 4b). There was a tendency for reduced hilar Fos+ cells after exploration for the PTZ rats compared to non-kindled controls, but this difference did not reach statistical significance [$t(10) = 2.05$, $P = .067$]. For Egr1 immunoreactivity, a similar pattern of altered expression was noted. Compared to non-kindled control, PTZ kindled rats had significantly fewer Egr1 + cells in the CA3 subfield ($t(10) = 4.17$, $P < .003$ CA1 subfield ($t(10) = 2.55$, $P < .029$), hilus ($t(10) = 2.65$, $P < .024$) and dentate granule cell layer ($t(10) = 3.06$, $P < .012$) after exposure to the novel environment (Fig. 4d).

5. Discussion

Robust induction of activity dependent IEGs has been widely reported in multiple animal models, including kainic acid, audiogenic seizures, picrotoxin, pilocarpine, and kindling (Kalinina et al., 2019; Simjee et al., 2012; Chiasson et al., 1995; Williams and Jope, 1994; Simler et al., 1999; Eells et al., 2004; Willoughby et al., 1995). However, IEG expression has been generally examined following acute seizure activity. Thus, there has been a paucity of research regarding how previously evoked seizures might impact the response of IEGs during the interictal or seizure-free period when behavioural-relevant experiences occur. This is especially important given that the interictal period comprises most patients' lives and the potential molecular and neurobiological adaptations that underlie behavioural and cognitive dysfunction observed during this period remains poorly understood (Ives-Deliperi and Butler, 2021; Devinsky, 2004).

The findings of this study reveal that seizures previously evoked by repeated PTZ treatment decrease the expression of several IEGs in the hippocampus and dentate gyrus following a 30 min exposure to a novel open field arena. More specifically, we found that despite showing similar levels of exploratory activity, kindled animals had reduced
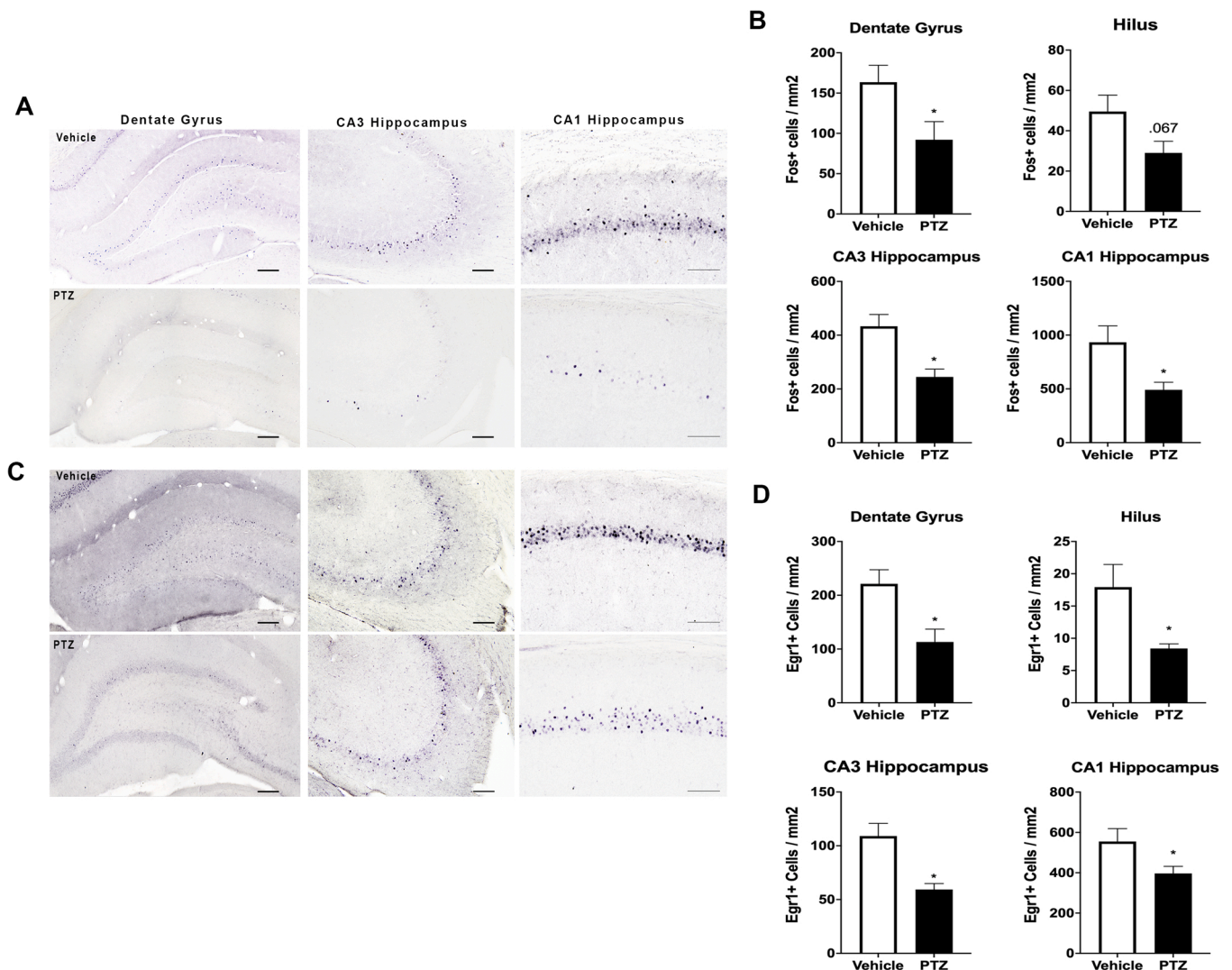
Fig. 4. Effect of PTZ on Fos and Egr1 immunohistochemical staining 90 min after exposure to a novel environment. Open field testing was conducted 24 hrs after the last treatment. (A) Representative hippocampal sections of PTZ kindled and control rats labeled with Fos directed antibodies. Scale bars 100 μm (B) Quantification of the mean number of Fos+ cells in the CA subfields of the hippocampus and dentate gyrus after spatial exploration. (C) Representative hippocampal sections of PTZ kindled and control rats labeled with Egr1 directed antibodies. Scale bars 100 μm (D) Quantification of the mean number of Egr1+ cells in the CA subfields of the hippocampus and dentate gyrus after spatial exploration. Data are presented as mean ± SEM, * $p < .05$, Student t-test, PTZ vs. control.

number of Fos+ and Egr1+ cells after exposure to the novel environment compared to control animals. These findings prompted us to conduct a larger analysis of additional IEGs markers that could be impacted by PTZ kindling. Our examination largely confirmed the above findings, but also revealed distinct patterns of IEG changes. For example, we found that *Egr1*, *Fos*, and *ΔFosB* mRNA levels were all significantly reduced in the hippocampus and dentate gyrus for kindled rats after exposure to the novel environment. In contrast, kindled rats also showed decreased levels of *Nr4a1* (Nur77) mRNA only in the dentate gyrus, whereas decreased *Homer1a* mRNA expression was confined to the hippocampus after spatial exploration suggesting that there may be brain regional specificity with respect to IEG changes in epileptic animals. Finally, although our study only examined for changes in IEG expression after a behavioural manipulation in kindled animals, it is possible that chronic seizures produced an overall reduction in basal IEG levels. We have recently addressed this possibility. In this study, we found that a group of behaviourally naïve home-cage PTZ and vehicle control rats showed similar numbers of Fos+ and Egr1+ cells in the hippocampus and dentate gyrus when euthanized twenty-four hours after receiving their last treatment (Kalinina et al., 2019) indicating that activity/experience-dependent, but not basal, IEG induction may be most adversely impacted by seizures.

The attenuation of hippocampal IEG levels we observed following chronic seizure stimulation raises the possibility that excessive neuronal activity might desensitize signaling cascades that induce IEG expression. In accordance with this idea, biphasic changes in hippocampal IEG expression have been regularly observed in variety of animal models of epilepsy, increasing after acute seizure activity but decreasing in chronically epileptic animals (Calais et al., 2013; Simler et al., 1999; Peng and Houser, 2005; Mello et al., 1996; Brecht et al., 1999; Winston et al., 1990). On the other hand, it is important to point out that the initial reduction in IEG expression after PTZ kindling may be transient, as the majority of IEG markers assessed appeared to recover to normal expression levels by 4 weeks after rats received their last PTZ treatment. There was, however, one notable exception noted for Fos expression. In contrast to what observed for the other IEGs, we found that levels of hippocampal *Fos* mRNA continued to be reduced in kindled animals even when behavioural testing was conducted 4 weeks after the last PTZ treatment. This in line with findings previously reported by Calais and colleagues (Calais et al., 2013) showing that hippocampal *Egr1, Arc,* and *Fos* mRNA levels are reduced initially after chronic electroconvulsive seizures (ECS). However, the reduction in hippocampal *Egr1* and *Arc* mRNA was found to be time-limited returning to baseline levels by 30 and 90 days after the last ECS treatment. In contrast, *Fos* mRNA expression continued to be decreased at both time points. The present results appear to corroborate these findings and suggest that PTZ kindling might also result in a potentially long-lasting disruption in Fos induction within the hippocampus.

The downstream signaling events that lead to desensitization of Fos induction after chronic seizures remain to be elucidated. However, chromatin remodeling has been increasingly recognized as a key contributor in modulating gene expression (Kobow and Blumcke, 2018). In support of this idea, previous work has shown that levels of acetylated histone H4 at the *Fos* gene increases initially after chronic seizures but then decreases substantially 24 h after the last seizure event (Tsankova et al., 2004). Deacetylation of surrounding histones is generally associated with attenuated gene promoter activity (Konsoula and Barile, 2012), thus histone modifications at the *Fos* promoter following chronic seizures could partly account for the sustained loss of Fos induction in PTZ kindled rats found in this experiment. However, Corbett and colleagues (Corbett et al., 2017) recently found that seizure-induced increases in hippocampal ΔFosB can induce epigenetic repression of key genes implicated in synaptic plasticity, including Fos. Given the unusually long half-life associated with ΔFosB mRNA (Nestler et al., 2001), acute neuronal activity may be sufficient to permit induction of both of Fos and ΔFosB, whereas protracted periods of high levels of neuronal

activity would lead to greater accumulation of ΔFosB and subsequent suppression of Fos expression (Corbett et al., 2017). In accordance with this, we found that both *Fos* and *ΔFosB* mRNA levels were similarly decreased after 2 weeks of PTZ kindling, however, *ΔFosB* returned to normal expression levels following a 4 week recovery period whereas Fos continued to be repressed. We hypothesize that the eventual recovery and normalization of ΔFosB might enable ongoing long-term repression of hippocampal Fos induction, a process that could have important implications for hippocampal-mediated behaviours.

What are the functional consequences associated with reduced IEG expression? Previous work has shown that cognitive impairments are frequently observed in conditions associated with lower hippocampal IEG expression, such as aging, depression, and neurodegenerative diseases (Gallo et al., 2018; Minatohara et al., 2015; Marrone et al., 2012). While we did not directly examine cognitive function after PTZ kindling in this study, we did observe that PTZ kindled rats exposed to a novel environment 4 weeks after their last treatment spent less time in the center compartment of the open field and exhibited higher overall movement velocity when travelling within this area compared to non-kindled controls. This effect appeared to be specific to the center compartment of the open field arena as there was no group differences in movement velocity in the peripheral compartment. Interestingly, we have seen a similar pattern of abnormal exploratory behaviour in novel environments for rats that have underwent amygdala kindling. Similar to the findings presented here, we found that amygdala kindled rats showed less exploration time in the center compartment than controls (Fournier et al., 2009, 2020). However, these animals also engaged in enhanced bursts of running and travelled at greater velocities when entering the center area compared to the peripheral compartment of the open field—a response not seen in control animals (Fournier et al., 2009; Fournier et al., 2020). These findings are suggestive of heightened emotional or anxiety-related behaviours (La-Vu et al., 2020; Crawley, 1985) and is consistent with past work showing that PTZ kindling can alter emotional behaviour in rodents on other behavioural tasks (Lamberty and Klitgaard, 2000; Hoeller et al., 2017; Erdogan et al., 2005). Behavioural alterations, such as anxiety, depression and impaired cognitive performance are frequently observed in people with epilepsy (Tramoni-Negre et al., 2017; Krishnan, 2020), and these impairments show correlation with reduced hippocampal IEG expression in animal models (Botterill et al., 2014; Fournier et al., 2013; Kalinina et al., 2019; Chawla et al., 2013). However, it is important to acknowledge that despite showing deficits in IEG induction, there were no apparent behavioural differences in open field exploration between PTZ treated and controls animals examined 24 hrs after their last treatment. Differences in emotional behaviour only emerged when a recovery period of 4 weeks was inserted between the last PTZ treatment and testing. While it is unclear why behavioural deficits in open field activity were not observed at a time point when multiple hippocampal IEGs showed robust downregulation after PTZ seizures, our findings raise the possibility that it may be the cumulative impact of long-term downregulation in IEGs, particularly Fos, that provide a molecular pathway for the emergence of behavioural impairments during chronic epilepsy. This view is not necessarily in contradiction with results from earlier rodent work showing that acute administration of anxiogenic drugs (Singewald et al., 2003) or stress manipulations (de Andrade et al., 2013) that enhance anxiety behaviour increase Fos expression in the hippocampus. Chronic epileptiform activity and seizures are abnormal events that induce long-lasting pathological changes in neuronal structure and intracellular signaling function (Bozzi et al., 2011). This could result in atypical patterns of gene expression that could differ from those observed in non-epileptic animals. Indeed, our observation of a long-term decrease in hippocampal Fos expression after PTZ kindling is consistent with findings from other animal models of epilepsy that also show deficits in cognition and emotional behaviours (Botterill et al., 2014; Simler et al., 1999; Mello et al., 1996; Winston et al., 1990; Corbett et al., 2017; Fournier et al., 2020; Kalynchuk et al., 2001) and

further underscore the importance of impaired induction of Fos as a possible correlate of epilepsy-related behavioral deficits.

In summary, our results found that rats with a history of chronic epileptic seizures show robust downregulation in hippocampal and dentate gyrus IEG expression after exposure to a novel environment. Furthermore, we observed that while other IEG markers returned normal expression levels, Fos expression continues to show long-term reduction up to 4 weeks after animals have experienced their last seizure. These findings may help shed some light on the potential molecular mechanisms that govern interictal behavioural changes and could provide a novel pathway for treatment.

CRediT authorship contribution statement

Alena Kalinina: performed experiments, analyzed data, Investigation, Visualization, Writing – original draft, Formal analysis. **Zakhar Krekhno:** Methodology and experiments. **Janet Yee:** Methodology, Resources and writing. **Hugo Lehmann:** Methodology, Resources, writing and assisted in writing. **Neil M. Fournier:** Methodology, Resources, Writing – original draft, Writing – review and editing, Project administration, Conceptualization, Funding acquisition, Supervision, and wrote the manuscript. All co-authors approved the submitted manuscript.

Data Availability

All data will be made available from the corresponding author upon reasonable request.

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Conflicts of interest

The authors have no conflicts of interest to disclose.

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