Fish and Shellfish Immunology 83 (2018) 26–36

Contents lists available at ScienceDirect

Fish and Shellfish Immunology

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

Full length article

Activation of BDNF-TrkB signaling pathway-regulated brain inflammation in pentylenetetrazole-induced seizures in zebrafish

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ARTICLE INFO

Keywords:
BDNF
TrkB
Seizures
Inflammatory responses
Zebrafish

ABSTRACT

Seizures are sustained neuronal hyperexcitability in brain that result in loss of consciousness and injury. Understanding how the brain responds to seizures is critical to help developing new therapeutic strategies for epilepsy, a neurological disorder characterized by recurrent and unprovoked seizures. However, the mechanisms underlying seizure-dependent alterations of biological properties are poorly understood. In this study, we analyzed gene expression profiles of the zebrafish heads that were undergoing seizures and identified 1776 differentially expressed genes. Gene-regulatory network analysis revealed that BDNF-TrkB signaling pathway positively regulated brain inflammation in zebrafish during seizures. Using K252a, a TrkB inhibitor to block BDNF-TrkB signaling pathway, attenuated pentylenetetrazole (PTZ)-induced seizures, which also confirmed BDNF-TrkB mediated inflammatory responses including regulation of *il1β* and *nfκb*, and neutrophil and macrophage infiltration of brain. Our results have provided novel insights into seizure-induced brain inflammation in zebrafish and anti-inflammatory related therapy for epilepsy.

1. Introduction

Epilepsy is a widespread neurological disorder, which is characterized by recurrent and unprovoked seizures and affects approximately 1% of the world's population [1]. Growing emphasis has been placed on understanding the underlying processes of epilepsy, including the molecular and structural changes that occur in brain [2,3]. Revealing the pathological mechanisms of seizures, a neurological condition characterized by sustained increase of electrical activity in brain, is important for development of new treatment strategies for epilepsy.

Different factors might be involved in the pathophysiology of epilepsy. Evidence from patients with epilepsy and experimental rodent models revealed that proinflammatory cytokines and inflammatory mediators are increased [4–8]. Gene expression profile analyses have shown that proinflammatory signals linked to the immune/inflammatory response are upregulated during seizures [9–13]. Blockade of specific inflammatory molecules and pathways significantly reduces seizures in experimental models of seizures and epilepsy [14]. In addition, the seizure-induced brain inflammation involves both the brain resident cells, such as glia and neurons, as well as cells of the innate immune systems such as granulocytes and macrophages [5]. However, the underlying mechanisms are not yet fully understood.

It is currently believed that changes in the pattern of the neurotrophic and inflammatory factors contribute to the epileptogenesis. Inhibition of leukocyte-vascular interactions markedly reduces seizures in mice [15]. It has been shown that seizures increase the production of interleukin-1β (IL1β) and brain-derived neurotrophic factor (BDNF) in Wistar Rats [16]. BDNF, a neurotrophin present in large amount in the brain, regulates neuronal survival, growth, differentiation, and synaptic plasticity by activating its specific receptor tropomyosin receptor kinase B (TrkB) signal transduction pathway [17]. Accumulating studies suggested that seizures induce drastic increases in BDNF expression and enhance activation of TrkB in both animal models and humans [18–23]. In turn, BDNF shows effects that facilitate seizures [24–27].

Although it has been reported that inflammatory responses occur during seizures provoked by chemoconvulsants or electrical stimulations, in brain regions where seizures are generated and spread, the underlying mechanisms remain unclear. Moreover, the relationship between neuronal hyperexcitability and brain inflammation in zebrafish model is lacking. In this study, we employed gene expression profiling and gene-regulatory network analysis in the head of zebrafish to characterize the pathophysiological pathways associated with brain inflammatory responses in seizures.

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https://doi.org/10.1016/j.fsi.2018.09.010
Received 31 May 2018; Received in revised form 24 August 2018; Accepted 5 September 2018
Available online 06 September 2018
1050-4648/

2. Materials and methods

2.1. Reagents and chemicals

Methylene blue, 1-phenyl-2-thiourea (PTU), tricaine, pentylenetetrazole (PTZ), K252a, and neutral red were purchased from Sigma (St. Louis, USA). The stock solutions were prepared in double-distilled water (ddH2O), and serial dilutions were made in normal bathing medium (reverse osmosis water equilibrated with instant ocean salts) before the experiments. All other chemicals and reagents utilized in this study were of analytical grade.

2.2. Animals and maintenance

Zebrafish (*Danio rerio*) of the wild-type AB and neutrophil-specific zebrafish line MPO: GFP strain [28] were maintained according to standard procedures. Fish were kept under a 14 h light/10 h dark cycle photoperiod and fed twice a day with commercial flake fish food supplemented with live brine shrimp. Zebrafish embryos were obtained from natural mating of adult zebrafish bred and maintained in bathing medium containing 2 mg/L methylene blue. To inhibit melanin formation in the leukocyte recruitment assays, 0.003% (w/v) PTU was added to the bathing medium after 10–12 h post fertilization (hpf).

2.3. Drug pretreatments and PTZ-induced seizures

Zebrafish larvae received the TrkB inhibitor K252a treatment 24 h before the PTZ exposure. The concentration of the K252a is 100 nM, which was selected based on preliminary studies conducted in our laboratory (Supplementary Figs. 1 and 2). To generate PTZ-induced seizures in zebrafish model, a common convulsant agent PTZ, was added to aquarium water. The final concentration of PTZ is 15 mM [29]. As described in Ref. [29], seizures were induced until stage III behavioral seizure activity was observed. If animals did not show both clonic movements and loss of posture, they were considered to be seizure-free and were not included in the following experiments.

2.4. Sample preparation and RNA isolation

Zebrafish larvae at 6 days post fertilization (dpf) were pretreated with K252a 24 h before the PTZ exposure. At 7 dpf, all groups were transferred to a 6-well plate (each group for per well) and subject to different treatments for 30 min. Then the heads of zebrafish were collected for RNA isolation. 50 heads of larval zebrafish were homogenized in 500 μl of TRIzol (Ambion, Austin, USA) using a pestle. Total RNA was extracted using the mirVana miRNA Isolation Kit (Ambion, Austin, USA) following the manufacturer's protocol.

2.5. RNA-seq

RNA integrity was evaluated using the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, USA). The samples with RNA Integrity Number (RIN) ≥ 7 were subject to the subsequent analysis. The libraries were constructed using TruSeq Stranded mRNA LTSample Prep Kit (Illumina, San Diego, USA) according to the manufacturer's instructions. Then these libraries were sequenced on the Illumina sequencing platform (HiSeqTM 2500).

2.6. RNA-seq preprocessing

Raw reads were processed using NGS QC Toolkit [30]. The reads containing ploy-N and the low quality reads were removed to obtain the clean reads. Then the clean reads were mapped to zebrafish reference genome using hisat2 [31]. FPKM value of each gene was calculated using cufflinks [32], and the read counts of each gene were obtained by htseq-count. Differentially expressed genes (DEGs) were identified using the DESeq R package functions estimateSizeFactors and nbinomTest. p-value < 0.05 and fold change > 2 or fold change < 0.5 was set as the threshold for significantly differential expression. GO enrichment and KEGG [33] pathway enrichment analysis were respectively performed using R based on the hypergeometric distribution.

2.7. Real-time quantitative PCR

Reverse transcription and qPCR were performed according to the manufacturer's protocols. Briefly, cDNA was generated using the PrimeScript™ RT Master Mix (Takara, Tokyo, Japan). qPCR was performed using SYBR® Premix DimerEraser™ (Takara, Tokyo, Japan) and the Bio-Rad CFX96 Real-Time System. Runs were carried out in triplicate using the housekeeping gene *rpl13a* to normalize the mRNA level of target genes. Data were analyzed using the Bio-Rad CFX Manager software to quantify the relative gene expression. Primer sequences are available on request.

2.8. Western blot analysis

The heads of zebrafish at 7 dpf (n = 50) receiving different treatments were collected in cold RIPA lysis buffer (Thermo Fisher Scientific). After centrifuge at 20000 g for 10 min at 4 °C, the supernatant was transferred to a new tube and ready for western blot analysis. Protein concentration was determined by bicinchoninic acid protein assay (Pierce, Rockford, USA). Samples were boiled in 5 × SDS-PAGE loading buffer (Beyotime, Beijing, China), and western blotting was carried out according to the Bio-Rad electrophoresis protocol with mouse anti-BDNF (1:1000, Proteintech), rabbit anti-TrkB (1:1000, Proteintech), and mouse anti-β-Actin (1:1000, Proteintech) antibodies.

2.9. Behavioral analysis

Zebrafish larvae at 7 dpf were transferred to 48-well plate (1 per well, with 300 μl of aquarium water) for 30 min to minimize any interference in the test. Following acclimation, aquarium water was disposed and 300 μl of 15 mM PTZ solution was added to each well. After that, the locomotor activity for each larva was recorded during 30 min using an automated computerized video-tracking system (Viewpoint, Lyon, France). The reason we recorded locomotion for first 30 min is that after 1 h of PTZ exposure there is no difference in locomotor activity between PTZ and the control group [34]. The detailed behavioral seizure profile characterization was performed during the same time frame each day (from 10:00 a.m. to 4:00 p.m.) and in a silent room. Zeblab software (Viewpoint, Lyon, France) was used to analyze the digital tracks and the average speed was analyzed every 60 s. A total of eight zebrafish larvae (n = 8) were used to compose each experimental group.

2.10. Latency score

The seizure latency period was defined as the time from initial exposure to PTZ until zebrafish reached each convulsion stage [35]. Stage I, dramatically increased swimming activity; Stage II, whirlpool swimming behavior; Stage III, wild jump, clonus-like seizures followed by loss of posture [29]. The video was observed to score the convulsion stages and to quantify the latency of each fish. Each set of experiments was repeated at least three times using animals from different batches and a minimum of 8 zebrafish per data point.

2.11. Zebrafish neutrophil recruitment assay

6 dpf zebrafish larvae of MPO: GFP were pretreated with K252a in the plate at 28.5 °C for 24 h. After treatment, PTZ solution was added to the plate to obtain a final concentration of 15 mM. The plate was incubated at 28.5 °C for 30 min. Neutrophil migration was recorded using

a microscope (Zeiss, Jena, Germany). The number of neutrophil at the head region was quantified.

2.12. Neutral red staining

Zebrafish larvae at 6 dpf were pretreated for 24 h with K252a. At 7 dpf, animals were exposed to 15 mM PTZ for 30 min. Then neural red staining was performed. Optimal staining of macrophages was achieved by incubating larvae in 2.5 μg/mL neutral red solution at 28.5 °C in the dark for 3–6 h [36]. After staining, the larval zebrafish were anesthetized with 0.03% tricaine and macrophage migration was recorded using a microscope (Zeiss, Jena, Germany). The number of macrophages at the head region was quantified.

2.13. Statistical analysis

The results were analyzed by one-way ANOVA followed by Dunnett's post-hoc test and expressed as mean ± SEM. $P < 0.05$ was considered as significant.

3. Results

3.1. Differentially expressed genes in the zebrafish head that displayed seizures

Zebrafish seizure models are now widely accepted for understanding the biological processes and molecular pathways underlying epilepsy [37–40]. To identify genes involved in PTZ-induced seizures in zebrafish, gene expression profiles from the head of zebrafish that displayed seizures were statistically analyzed (Fig. 1A). 1776 differentially expressed genes (DEGs) were identified (Table S1). Hierarchical cluster analysis was performed on these 1776 DEGs. As a result, transcriptional expression pattern of 3 control groups (Ctl) and the 3 PTZ treatment groups are distinct (Fig. 1B), suggesting that the datasets are sufficiently robust at the group level to distinguish PTZ-induced seizures from the control.

3.2. Enriched gene ontology terms and pathways

We performed Gene Ontology (GO) enrichment analysis on the DEGs and found they are strongly enriched in Biological Process GO terms related to “leukocyte migration involved in inflammatory response” and “inflammatory response” as well as in Cellular Component GO terms “I-κB/NF-κB complex” and “DNA binding” (Fig. 1C and Table S2). Pathway enrichment analysis identified the enriched pathways from the upregulated DEGs. The top 20 were listed in Fig. 2A and Table S3. One interesting pathway amongst this list is the MAPK signaling pathway. MAPK signaling pathway is widely reported to regulate “inflammatory response” that appeared amongst the enriched GO terms. Indeed, inflammatory-related genes, such as *il1β* and *nfκb* are present in this set. We name this gene set as “interesting set”. Genes in interesting set and their fold change are shown in Fig. 2B. *c-fos*, a neuronal activity marker which has been shown to be induced transiently in response to seizures, is also present in the interesting set.

3.3. Gene-regulatory network suggested BDNF-TrkB signaling pathway-regulated inflammation in seizures in zebrafish

To further investigate gene-regulatory network for the genes in interesting set, we used the STRING database, which provides co-localization, as well as direct (physical) and indirect (functional) associations [41]. The interconnectivity of proteins predicted by STRING were shown in Fig. 3A. We also performed PANTHER GO analysis on the genes in interesting set and found that they are overrepresented in Protein Class GO term “signaling molecule” (Fig. 3B). These findings suggested that the members in interesting set have strong associations and may act in the same signaling pathway to regulate inflammatory response. Notably, this set contains a neurotrophic ligand-receptor pair, BDNF and its receptor TrkB, which could probably act as upstream components of the inflammatory signaling pathway during PTZ-induced seizures in zebrafish. To verify this hypothesis, we used K252a, a broad-spectrum TrkB inhibitor to block BDNF-TrkB signaling pathway. Upregulation of BDNF and TrkB during seizures were significantly reversed by administration of K252a (Fig. 3C), suggesting that seizures in zebrafish result in the activation of BDNF-TrkB, which can be efficiently inhibited by K252a. This result is consistent with previous findings in mammals [18–23].

3.4. Seizure responses following blocking BDNF-TrkB activation

To investigate the roles of BDNF-TrkB during seizures in zebrafish, we analyzed locomotion plots by measuring the total distance moved (in cm) for each video recording. We found that pretreatment of K252a greatly inhibited the PTZ-induced increase in locomotor activity (Fig. 4A and C). Specifically, distance moved and swimming speed of the K252a pretreated group were similar to that found to the untreated group (Ctl group), suggesting that seizure behavior were suppressed by blocking BDNF-TrkB signaling pathway. In order to investigate seizure development following BDNF-TrkB inhibition, we monitored the latencies to first sign of seizure stage I, II, and III, which is another indicator of seizure behavior. As shown in Fig. 4B, the latencies to seizure stage II and III were significantly prolonged in larval zebrafish pretreated with K252a. Our results revealed that blockade of BDNF-TrkB extended the latency periods to the onset of seizure stages, especially stage II and III. This is the first report to demonstrate that blocking BDNF-TrkB signaling pathway decreases seizure-like behavior and extends the latency period to the onset of seizures in zebrafish. Moreover, relative mRNA expression levels of *c-fos* were downregulated after inhibition of BDNF-TrkB during seizures (Fig. 5). All these results together suggested that seizures induce the activation of BDNF-TrkB and blocking this activation attenuates seizures. In addition, zebrafish pretreated with 100 nM K252a without PTZ exposure (K252a alone) showed similar locomotor activity to the control group (Supplementary Fig. 1). However, the BDNF-TrkB regulated biological processes during seizures remain largely unknown. Based on our gene expression profiling analyses above, we hypothesize that zebrafish seizures could result in BDNF-TrkB regulated brain inflammation.

3.5. Effects of seizure-induced BDNF-TrkB activation on genes associated with neurotrophic and inflammatory responses

To investigate whether seizure-induced upregulation of BDNF-TrkB regulate inflammation in brain regions, relative mRNA expression levels of the inflammatory-related genes were measured. As shown in Fig. 5, expression of *il1β* and *nfκb* mRNA were low in untreated group, and significantly upregulated in animals that were exposed to PTZ. Blocking BDNF-TrkB signaling pathway was able to inhibit the seizure-induced increase of expression of inflammatory-related genes. All genes tested in Fig. 5 are in interesting set. As expected, while expression of *bdnf*, *trkB* and *c-fos* increased in PTZ-induced seizures, administration of K252a reversed this increase. Same phenomenon was observed for *hsp70.3*, whose proteins are reported to be upregulated in cells subject to stressful stimuli, including inflammation and oxidative stress [42–45]. Similarly, transcriptional levels of all above inflammatory-

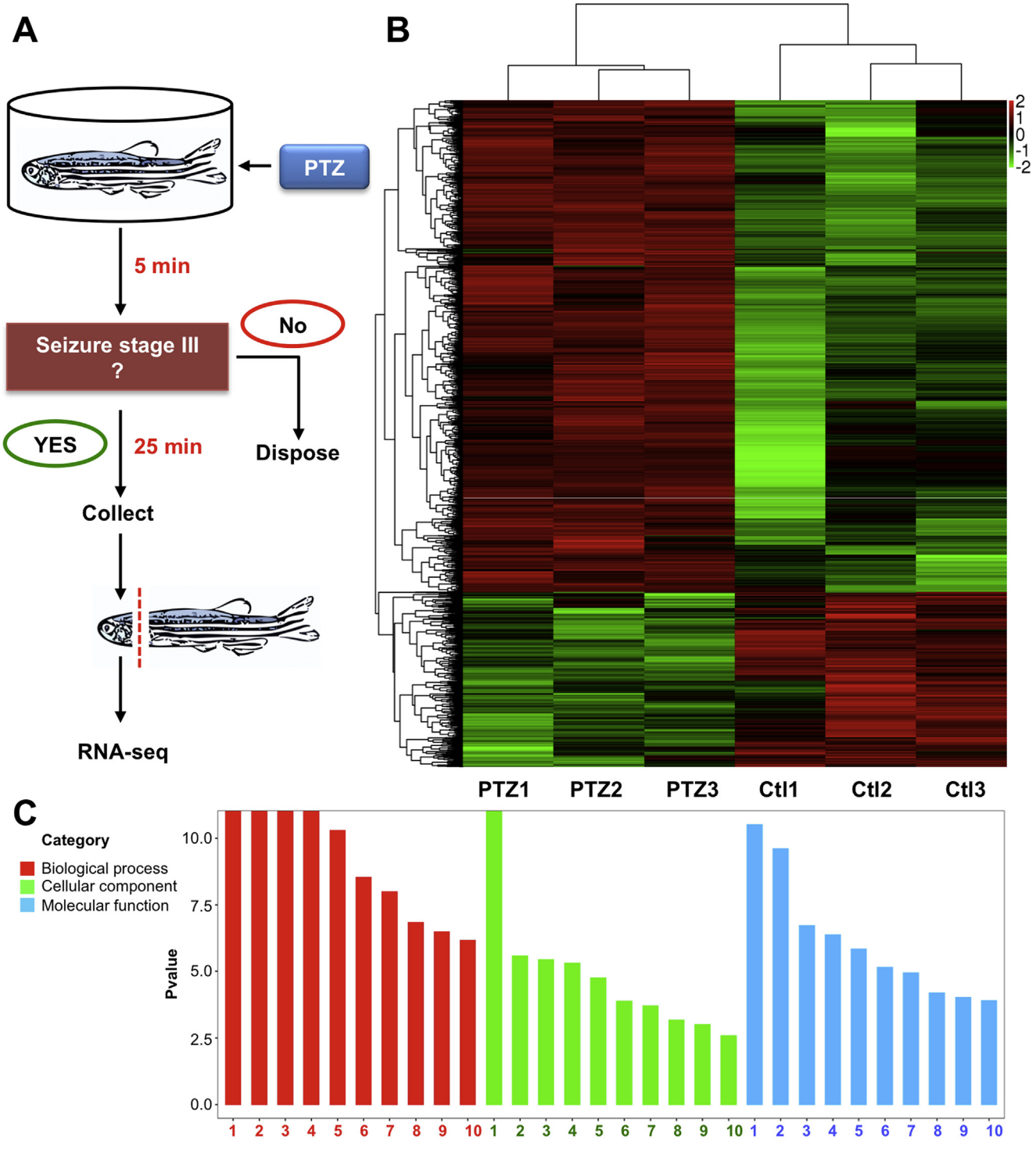

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Fig. 1. Gene expression profiling analyses in the zebrafish head that displayed seizures. (A) Experimental procedures of sample preparation for RNA-seq. Zebrafish larvae at 7 dpf were treated with 15 mM PTZ. Seizures were induced until stage III behavioral seizure activity was observed – it took about 5 min. Animals exhibited seizure behavior were collected after another 25 min. The heads of collected zebrafish were cut by a blade and subjected to RNA-seq. (B) Hierarchical clustering of the differentially expressed genes (DEGs). The heatmap shows the expression profiles of 1667 DEGs identified as potential genes involved in seizures. The color scale illustrates relative expression levels across all samples: red represents upregulated genes and green represents downregulated genes. The dendrograms on the left and top of the heatmap show the hierarchical clustering of the transcripts for gene names and for all different samples (3 PTZ-treated and 3 control groups), respectively. (C) Gene Ontology analysis of the DEGs, red: Biological process, green: Cellular component, blue: Molecular function. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

related genes assayed in our study were also decreased in K252a alone group, suggesting blockade of BDNF-TrkB dose play a role in modulating inflammation.

3.6. Activation of BDNF-TrkB signaling pathway during seizures triggers leukocyte infiltration into the head

In this study, gene expression profiling analyses and inflammatory-related genes expression assays suggested that BDNF-TrkB regulated inflammatory reaction is activated during seizures. To further validate this, leukocyte recruitment assays were performed since induction of various inflammatory pathways, and concomitant infiltration of inflammatory cells have been previously reported in patients with epilepsy [46]. We tracked neutrophil migration during PTZ-induced seizures by utilizing the MPO: GFP zebrafish, which expresses green fluorescent protein in neutrophils under the control of the myeloperoxidase promoter (MPO) [28]. Seizures triggered neutrophil infiltration into the head, whereas blocking BDNF-TrkB suppressed this effect (Fig. 6A and B and Supplementary Movies). Macrophage infiltration was also monitored to assess inflammatory responses during seizures [36]. As a result, macrophages efficiently migrated to the inflammatory foci, the head of zebrafish that was undergoing seizures. Inhibition of BDNF-TrkB caused a reduction in macrophage recruitment (Fig. 6A and C). The inhibitory effects of TrkB inhibitor on infiltration of neutrophils and macrophages into the head, a region associated with seizure initiation and propagation [47], further indicated that BDNF-TrkB signaling pathway regulates brain inflammatory responses during seizures.

Supplementary video related to this article can be found at https://doi.org/10.1016/j.fsi.2018.09.010.

4. Discussion

More efficacious investigation of pathological mechanisms underlying epilepsy is in desperate need as epilepsy is one of the most common chronic brain disorders [48]. Here, we used gene expression profiling followed by functional analyses, in combination with *in vivo* assays to investigate molecular and biological changes in the zebrafish head during seizures. Our results suggested that PTZ-induced seizures result in BDNF-TrkB regulated brain inflammation in zebrafish. By blocking BDNF-TrkB signaling pathway, we found that seizures were suppressed and seizure-induced brain inflammation was attenuated. This is the first report to show the involvement of BDNF-TrkB signaling pathway in activation of brain inflammation and infiltration of inflammatory cells into the zebrafish head in PTZ-induced seizures, offering new perspectives for the treatment of epilepsy.

4.1. Gene expression profiling studies in seizures and DEGs in our study

Gene expression profiling assays have been widely conducted to uncover the molecular mechanisms underlying epilepsy, and hence identify potential molecular targets for intervention, [49–53]. The differentially expressed genes reported in transcriptional studies may differ, possibly due to variability in profiling techniques, the models, the tissues, or time-points studied. However, these genes are associated with similar biological processes [54,55]. Consistent with previous findings, our studies revealed that the inflammatory response, a basic biological process, is common to those gene expression profiling assays conducted in mammalian seizure models.

In this study, we used the head instead of the whole body from the larval zebrafish displayed seizures to investigate transcriptional profiles since the head is highly associated with seizure development [47]. We found there was a direct response (upregulation) of *bdnf* and *trkB* in seizures. Previous studies show that overexpression of BDNF exhibits more severe seizures in mice [56]. Mice with a conditional knockout of TrkB, the receptor for BDNF, did not kindle [57]. All these results suggested the important roles of BDNF and TrkB in seizures. We also observed that transcription factors *c-fos* and c-*jun* were upregulated during seizures in zebrafish, which is consistent with previous studies using mammalian models. Both *c-fos* and *c-jun* have been shown to regulate neuronal hyperexcitability in the central nervous system of fish and mammals [52,58]. In addition, stress-related genes *hsp70* and *gadd45*, which have been reported to be induced under wide variety of stress conditions, were found to increase in PTZ-induced seizures. Increase in *hsp70* and *gadd45* results in modulation of inflammatory responses [42,59].

4.2. Inflammatory responses to seizures

Over the past decade, a large body of evidence strongly supports the role of inflammation in the pathophysiology of epilepsy [60]. Different inflammatory molecules and pathways have been shown to significantly contribute to the mechanisms of seizure progression in different experimental models [61–64]. Among the proinflammatory cytokines, IL1β is the most widely investigated [5,65–67]. Previous studies showed that blocking IL1β in the brain drastically reduces seizures and blockade of IL-1β biosynthesis causes powerful anticonvulsant effects. IL1β exerts its action by binding to the IL1 receptor (IL1R), which initiates a downstream signaling process that activates the transcription factor NFκB [68] and are in association with blood-brain barrier (BBB) leakage and neuronal damage [69]. Interestingly, both IL1β and NFκB are present in our designated interesting gene set, confirming the important roles of IL1β and NFκB in brain inflammatory responses during PTZ-induced seizures in zebrafish.

4.3. Relationship between neurotrophic and inflammatory factors

In addition to its role as a neurotrophic factor, accumulating evidence has shown that BDNF has the potential to play a role in the immune response. The release of BDNF leads to changes in cytokine profiles [70–73]. However, the causal relationship between BDNF and inflammatory factors during seizures remains unclear. Our study in the head of zebrafish showed that BDNF-TrkB is a positive regulator of proinflammatory molecules including IL1β and NFκB, major mediators of inflammation, which are capable of inducing changes in seizures

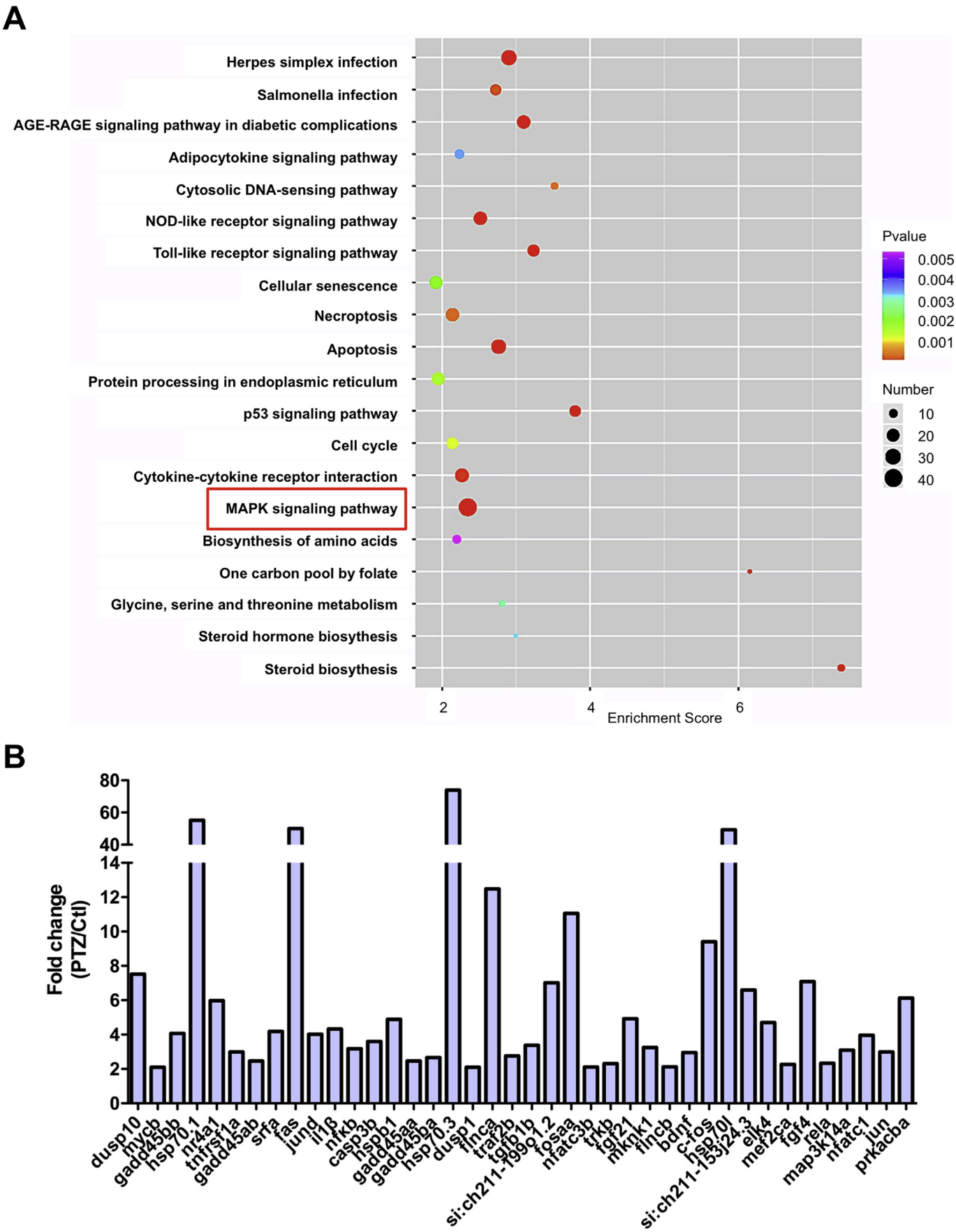

Fig. 2. KEGG pathway enrichment analysis of the DEGs. (A) Enriched pathways from the upregulated DEGs when the control was compared to zebrafish that were undergoing PTZ-induced seizures. The explanatory charts on right show the p-value and the number of genes that appear in each category. (B) Transcriptional fold change of the genes in interesting set.

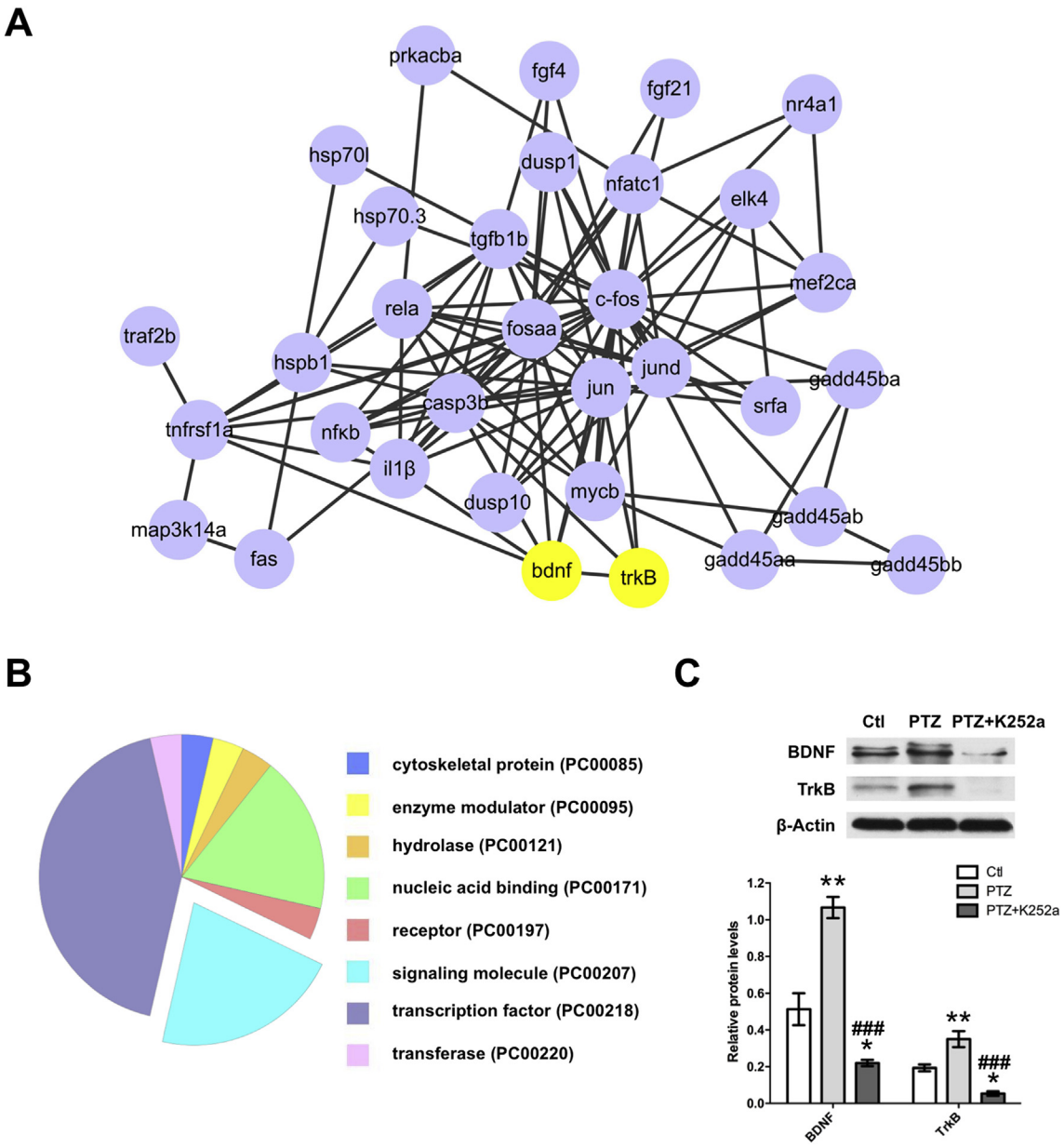

Fig. 3. Gene-regulatory network suggested BDNF-TrkB act as upstream components of the inflammatory signaling pathway during seizures. (A) The associations among the genes in interesting set were generated based on STRING analysis. *bdnf* and *trkB* were highlighted by yellow color. (B) GO enrichment in category Protein Class. The percentage of each section is proportional to the number of DEGs in GO categories. (C) Inhibitory effects of K252a. The upregulation in protein expression of BDNF and TrkB induced by PTZ was significantly reversed by administration of the TrkB inhibitor K252a. Quantitative measurements of the relative densities of BDNF and TrkB in control, PTZ-treated and K252a pretreated following PTZ exposure groups, respectively. $^{*}P < 0.05$, $^{**}P < 0.01$ vs Ctl, $^{###}P < 0.001$ vs PTZ. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

[74].

4.4. Neutrophil and macrophage infiltration of brain during PTZ-induced seizures

The role of the inflammatory responses in zebrafish seizure pathology is also highlighted by leukocyte infiltration of brain. Zebrafish larvae will be transparent following PTU treatment and allow imaging of leukocyte behavior *in vivo* during seizures [75,76]. Although PTU has been reported to interfere with thyroid hormone production [77], which may mediate BDNF expression [78]. PTU treatment step was included in both the experimental groups (PTZ and PTZ + K252a) and the control group in the leukocyte recruitment assays, therefore eliminating extraneous variables which could lead misinterpretation. We found infiltration of neutrophils and macrophages into the zebrafish head during seizures, which was suppressed by blockade of BDNF-TrkB signaling pathway. Our results are consistent with previous findings that epilepsy is typically accompanied by an increase of leukocytes, such as neutrophils, into the hippocampus, and the infiltration is thought to lead to higher levels of neurodegeneration. Leukocyte

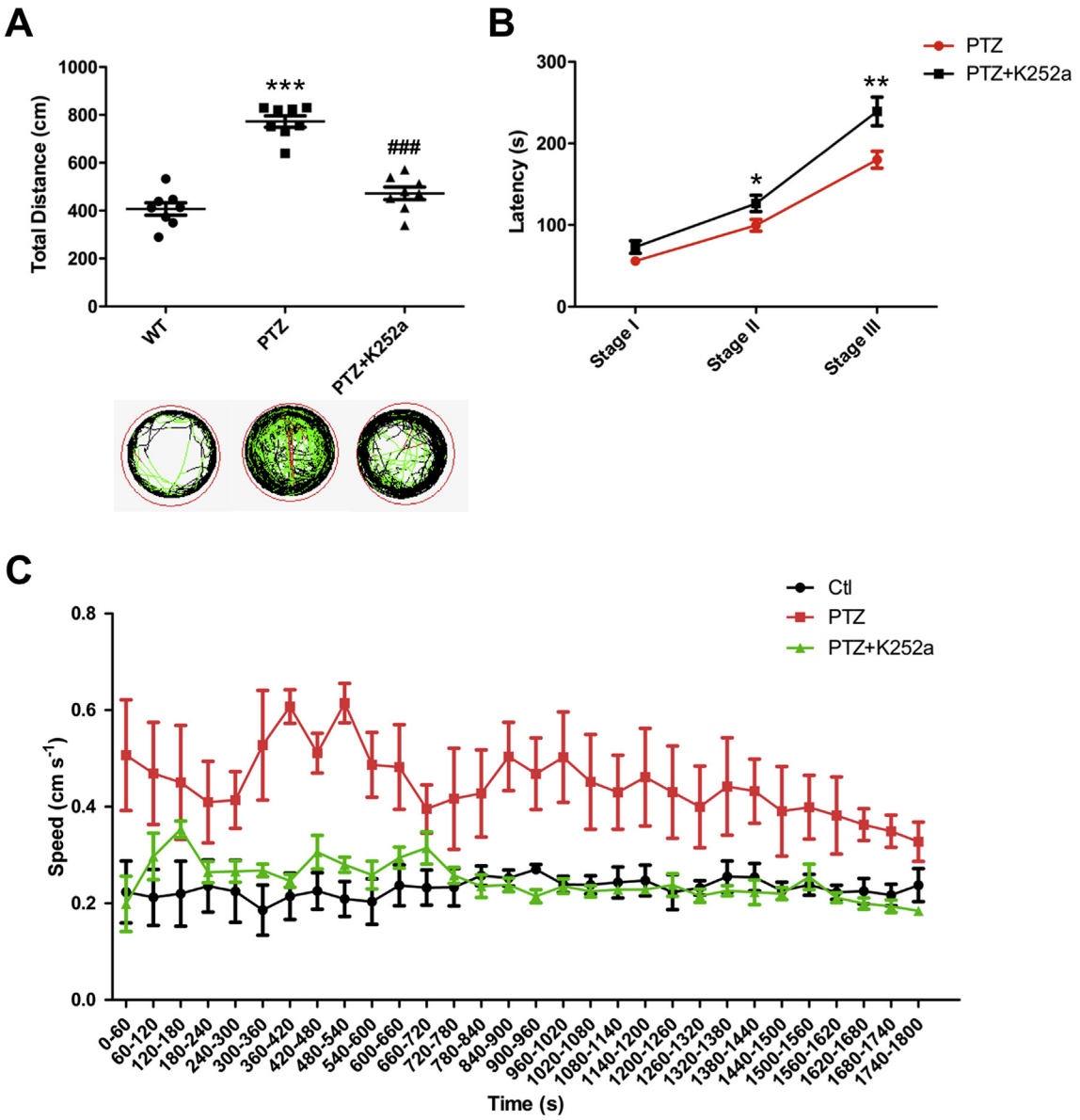

Fig. 4. Seizure responses following blockade of BDNF-TrkB activation. (A) The total distance moved. Animals were exposed to K252a for 24 h prior to 15 mM PTZ exposure. The distance moved for each larva from each group was analyzed using Zeblab software. n = 8 per group, $^{***}P < 0.001$ vs Ctl, $^{###}P < 0.001$ vs PTZ. In the digital tracks map, the red lines are associated with fast movement; green lines are associated with medium movement; and black lines indicate slow movement. (B) Latency to the first behavioral manifestation of seizure stage I, II, and III during PTZ exposure. Data were expressed as mean ± SEM of at least 8 animals for each group. $^{*}P < 0.05$, $^{**}P < 0.01$ vs PTZ. (C) The average speed of the larval zebrafish from different groups. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

infiltration of brain may be due to the seizure-induced brain inflammation as previous report showed that brain inflammation contributes to BBB breakdown [15]. Since BDNF can be synthetized and secreted by neurons and glial cells [79–82], PTZ-induced seizures is possibly to trigger BDNF expression in neurons and glial cells, thereby causes secretion of inflammatory molecules, such as IL1β [83,84]. Moreover, inflammatory mediators could be also released by macrophages and granulocytes entering the brain from the blood during seizures, promoting inflammation [85]. All these findings suggested that PTZ-induced seizures activate BDNF-TrkB signaling pathway regulated brain inflammation through upregulation of inflammatory molecules IL1β and NFκB and infiltration of neutrophils and macrophages into the zebrafish head (Fig. 7).

5. Conclusion

In summary, inflammatory and immune responses appear to be one of the major hallmarks in epilepsy. Our findings provide compelling evidence that BDNF-TrkB signaling pathway positively regulated brain inflammation in zebrafish during seizures, highlighting the possibility to inhibit seizures by interfering BDNF-TrkB regulated inflammatory mechanisms underlying the disease.

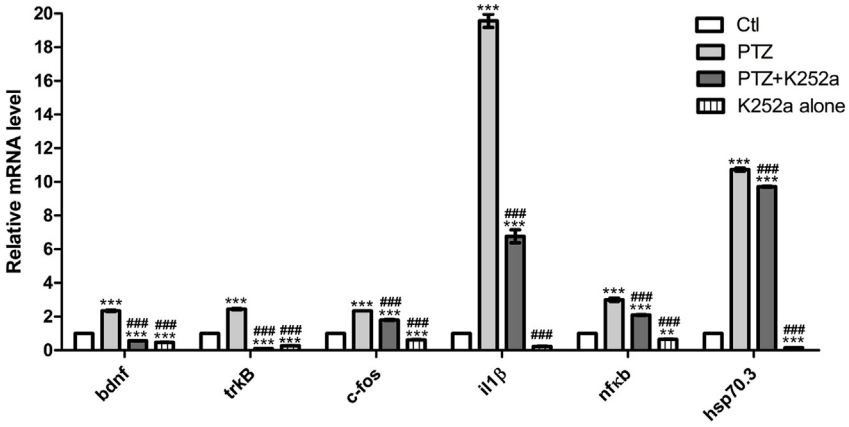

Fig. 5. Comparison of the transcription levels of the genes in interesting set involved in neurotrophic and inflammatory responses. Transcription levels of *bdnf*, *trkB*, *c-fos*, *il1β*, *nfκb*, and *hsp70.3* were measured by qPCR in 7 dpf larval zebrafish. The genes involved in neurotrophic and inflammatory responses significantly increased in PTZ-induced seizures while blocking BDNF-TrkB markedly suppressed this increase. $^{**}P < 0.01$, $^{***}P < 0.001$ vs Ctl, $^{###}P < 0.001$ vs PTZ.

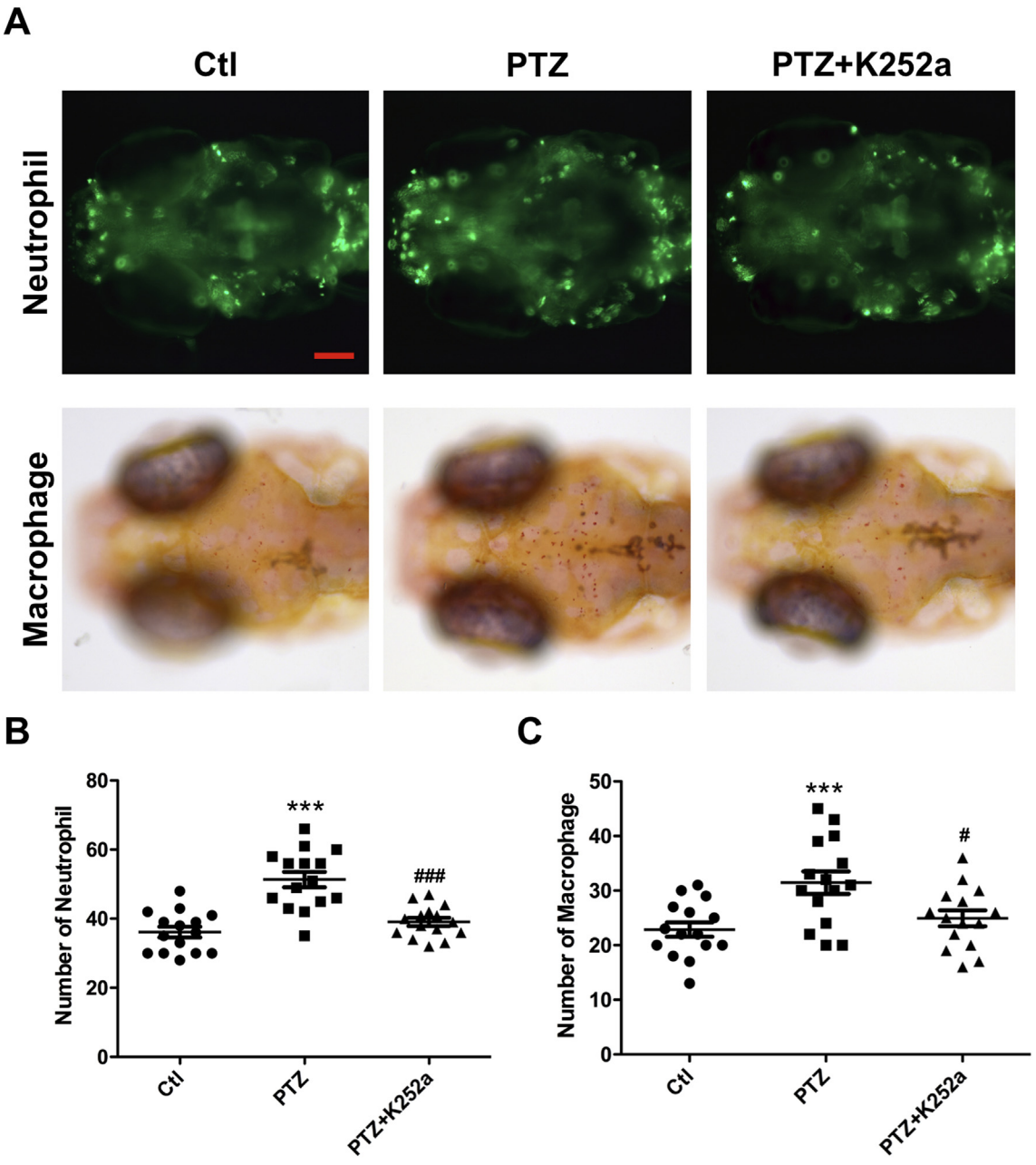

Fig. 6. Leukocyte infiltration of the head during seizures. (A) Infiltration of neutrophils (green dots) into the head of zebrafish displayed seizures was recorded by microscope. Infiltration of macrophages (orange dots) during seizures was visualized by neutral red staining. (B) Quantification of the number of neutrophils in the head. n = 15. (C) Quantification of the number of macrophages in the head. n = 15. $^{***}P < 0.001$ vs Ctl, $^{#}P < 0.05$, $^{###}P < 0.001$ vs PTZ, scale bar 100 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

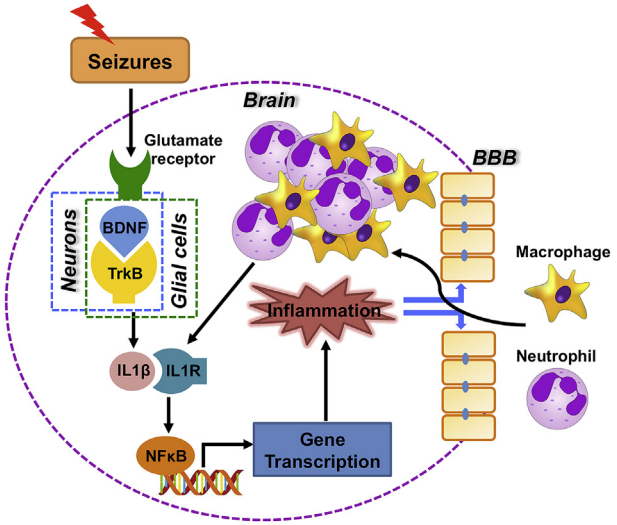

Fig. 7. Schematic of the activation of BDNF-TrkB regulated brain inflammation in PTZ-induced seizures. Following PTZ-induced seizures, there is instant increase of BDNF-TrkB in neurons and glial cells through glutamate receptor-regulated gene transcription. The activation of BDNF-TrkB causes upregulation of IL1β, which activates the transcription factor NFκB by binding to IL1 receptor (IL1R), further leading to brain inflammation. Brain inflammation results in leakage of blood-brain barrier (BBB), thereby triggers infiltration of neutrophils and macrophages into the head through BBB. In turn, accumulation of inflammatory cells in the head causes release of inflammatory mediators to promote brain inflammation during seizures.

Author contributions

MJ and KCL conceived the project and designed the experiments. MJ and XNJ performed the experiments and analyzed the data. WLS, LWH and QXH provided expertise on data analyses. MJ wrote the manuscript.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgments

We thank Ximin Wang for zebrafish maintenance. We are grateful to Dr. Ming Fa for critical reading and editing of manuscript, Fasheng Liu for technical help. This work was supported by the National Natural Science Foundation of China for Young Scientists (No. 81802629), Youth Fund of Shandong Academy of Sciences (No. 2018QN0024), and Chinese Academy of Sciences (Shenyang branch) - Shandong Academy of Sciences Partner Program for young scientists.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.fsi.2018.09.010.

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