

# Intracerebroventricular Administration of Streptozotocin as an Experimental Approach to Depression: Evidence for the Involvement of Proinflammatory Cytokines and Indoleamine-2,3-Dioxygenase

Leandro Cattelan Souza<sup>1</sup> · Cristiano R. Jesse<sup>1</sup> · Marcelo Gomes de Gomes<sup>1</sup> · Cristini Escobar Viana<sup>1</sup> · Etiara Mattos<sup>1</sup> · Neici Cáceres Silva<sup>1</sup> · Silvana Peterini Boeira<sup>1</sup>

Received: 18 May 2016 / Revised: 6 December 2016 / Accepted: 16 December 2016 / Published online: 2 February 2017  
© Springer Science+Business Media New York 2017

**Abstract** There is a lack of information about the molecular events underlying the depressive-like effect of an intracerebroventricular injection of streptozotocin (ICV-STZ) in mice. Elevated activity of the tryptophan-degrading enzyme indoleamine-2,3-dioxygenase (IDO) has been proposed to mediate depression in inflammatory disorders. In this study, we report that ICV-STZ activates IDO in the hippocampus of mice and culminates in depressive-like behaviors, measured by an increased duration in immobility time in the forced swimming test and decreased total time of grooming in the splash test. Indirect blockade of IDO activation with the cytokine inhibitor minocycline prevents the development of depressive-like behaviors and attenuates STZ-induced upregulation of proinflammatory cytokines in the hippocampus. Minocycline abrogates the increase in tryptophan and kynurenine levels as well as prevents serotonin dysfunction in the hippocampus of STZ-injected mice. These results suggest that hippocampal IDO activation in STZ-associated depressive-like behavior is mediated by proinflammatory cytokine-dependent mechanisms. Our study not only extends the evidence that IDO has a critical role in mediating inflammation-induced depression but also supports the notion that neuroinflammation and the kynurenine pathway are

important targets of novel therapeutic drugs for depression. In addition, our study provides new insights into the neurobiological mechanisms underlying ICV-STZ and indicates that this model could be employed in the preclinical research of depression.

**Keywords** Streptozotocin · Depressive-like behavior · Indoleamine-2,3-dioxygenase · Inflammation · Serotonin

## Introduction

Depression is a common psychological disorder affecting up to 15% of the population over a lifetime. It was the third leading cause of burden among all diseases in the year 2004 and is expected to be the greatest cause in high-income countries by 2030 (Mathers and Loncar 2006). This condition is associated with marked impairments in daily functioning and work and creates significant demands on service providers in terms of workload. Owing to the high prevalence and negative impact on the quality of life of the world population, a better understanding of the neurobiological mechanisms of depression is of paramount importance to develop new treatment drugs.

The pathophysiology of depression has not been fully elucidated, and numerous studies propose that neuroinflammation may play a role in the development of this disorder (Smith 1991; Dantzer et al. 2008; Anisman 2011; Xie et al. 2014). Specifically, it has been reported that patients with depression frequently show alterations in their immune system, such as elevated levels of proinflammatory cytokines in plasma and cerebrospinal fluid (Miller et al. 2009). Additionally, cytokine immunotherapy with interferon-alpha (IFN- $\alpha$ ) or interleukin-2 (IL-2) in the treatment of hepatitis C or cancer has been

**Electronic supplementary material** The online version of this article (doi:10.1007/s12640-016-9691-8) contains supplementary material, which is available to authorized users.

✉ Cristiano R. Jesse  
cristianoricardojesse@yahoo.com.br

<sup>1</sup> Laboratório de Avaliações Farmacológicas e Toxicológicas Aplicadas às Moléculas Bioativas – LaftamBio Pampa, Universidade Federal do Pampa, Itaqui, RS CEP 97650-000, Brazil

found to induce depression symptoms in otherwise psychiatrically normal individuals (Capuron et al. 2000; Capuron et al. 2001). Further evidence supporting an inflammatory basis of depression comes from studies reporting an induction of depressive-like behaviors in animals upon administration of inflammatory cytokines (Dunn and Welch 1991; Dantzer et al. 2008). Thus, it has been proposed that depression is an inflammatory-associated disorder.

It is well-established that an impaired central serotonin (5-hydroxytryptamine (5-HT)) system is implicated in the pathogenesis of depression (Cryan and Leonard 2000; Lee et al. 2010). Proinflammatory cytokines, including interleukin-6 (IL-6), interleukin-1beta (IL-1 $\beta$ ), tumor necrosis factor-alpha (TNF- $\alpha$ ), and interferon-gamma (IFN- $\gamma$ ) secreted by glial cells within the brain in response to injury and infection, have been found to impair the 5-HT system by activation of tryptophan (TRP)-metabolizing enzyme indoleamine-2,3-dioxygenase (IDO; Maes et al. 2011). IDO activation results in elevated kynurenine (KYN) production and several downstream metabolites that have been correlated with inflammation-associated depression (Christmas et al. 2011; Corona et al. 2013). In this way, the increased activity of IDO has been implicated as a critical molecular mediator of inflammation-induced depressive-like behavior (Da Silva Dias et al. 2016) (Table 1).

Streptozotocin (STZ), a glucosamine compound with beta-cytotoxic action, is commonly used to induce diabetes in laboratory animals. Recently, numerous studies have suggested that intracerebroventricular (i.c.v.) administration of a subdiabetogenic dose of STZ in rodents is a valid experimental model for the study of the pathophysiological alterations in sporadic Alzheimer's disease (AD; Salkovic-Petrisic et al. 2011; Shingo et al. 2012; Kalafatakis and Zarros 2014; Du et al. 2015). However, there is a lack of information on the noncognitive behavioral effects of intracerebroventricular injection of streptozotocin (ICV-STZ) in rodents, such as depressive-like states. A recent study conducted by our

research group (Souza et al. 2013) indicated for the first time that ICV-STZ-induced depressive-like behavior in the tail suspension test and anhedonia-like behavior, a core symptom of depression, in the sucrose preference test. The behavioral deficits were followed by an increase in TNF- $\alpha$  in the hippocampus, and treatment with fluoxetine and anti-TNF- $\alpha$  therapies prevented these alterations. Since animal models of depression with face and construct validity are generally responsive to antidepressant treatment (Cryan et al. 2002), we have reported that ICV-STZ could be an important mouse model of depression. Despite this information, the molecular events underlying the depressive-like effect of ICV-STZ are not well understood.

Thus, the objective of this study was to characterize the mechanisms by which ICV-STZ induces depressive-like behavior in mice secondary to its neuroinflammatory actions. The ability of ICV-STZ to induce the activation of tryptophan-degrading enzyme IDO and the impact on central 5-HT and KYN levels were investigated as potential mechanisms to link neuroinflammation and depressive-like behaviors.

## Materials and Methods

### Animals

Experiments were performed using male C57/BL6 mice (25–35 g, 60 days old). Animals were maintained at 22–25 °C with free access to water and food, under a 12:12-h light/dark cycle with lights on at 7:00 a.m. All manipulations were carried out during the light phase of the day. All experiments were performed in separate groups of animals, and each animal was used only once in each test. The procedures of this study were conducted according to the guidelines of the Committee on Care and Use of Experimental Animals Resources.

**Table 1** Effect of STZ and minocycline on TRP and 5-HT levels and their metabolites in hippocampus of mice

	(ng/g tissue)	Saline		STZ 6 h	
		Saline	Minocycline	Saline	Minocycline
TRP		3195 $\pm$ 80.42	3144 $\pm$ 220.0	4315 $\pm$ 174.6 <sup>b</sup>	34.73 $\pm$ 157.3 <sup>d</sup>
KYN		275.8 $\pm$ 13.58	234.8 $\pm$ 29.26	752.0 $\pm$ 54.72 <sup>b</sup>	395.0 $\pm$ 46.80 <sup>d</sup>
KYN/TRP		8.680 $\pm$ 0.66	7.71 $\pm$ 1.31	17.60 $\pm$ 1.82 <sup>b</sup>	11.31 $\pm$ 1.0 <sup>d</sup>
5-HT		1336 $\pm$ 79.37	1260 $\pm$ 69.66	647.3 $\pm$ 58.39 <sup>b</sup>	1221 $\pm$ 102.9 <sup>d</sup>
5-HIAA		1867 $\pm$ 115.5	1787 $\pm$ 67.14	2552 $\pm$ 156.0 <sup>b</sup>	1836 $\pm$ 75.63 <sup>d</sup>
5-HIAA/5HT		1.409 $\pm$ 0.131	1.535 $\pm$ 0.079	3.984 $\pm$ 0.505 <sup>b</sup>	1.548 $\pm$ 0.192

The values were analyzed by two-way ANOVA and Newman-Keuls multiple comparison test; each value is expressed as the mean  $\pm$  SEM ( $n = 6-8$ )

\* $P < 0.05$  when compared saline + saline with STZ + saline; \*\* $P < 0.05$  when compared STZ + minocycline with STZ + saline

## Drugs

The biochemicals STZ and minocycline were purchased from Sigma-Aldrich (USA) and were dissolved in saline solution before administration.

## Experimental Design

Two experiments were conducted. In the first experiment, mice ( $n = 6–8$  animals per group) received an ICV-STZ to investigate the depressive-like behavior and IDO activity over time (times: 1, 6, 24 h and 1 week after injection), similar to a previous study from our laboratory (Souza et al. 2013).

In the second experiment, mice were divided into four groups ( $n = 6–8$  animals per group): saline + saline (sham), saline + minocycline, STZ + saline, and STZ + minocycline. Six hours after the ICV-STZ (peak effect), mice were subjected to behavioral tests. Afterwards, they were euthanized and the hippocampus was removed for neurochemical experiments (Fig. 1).

## Intracerebroventricular Injection of Streptozotocin (ICV-STZ) and Minocycline Administration

STZ groups were administered an ICV-STZ (0.1 mg/site, total volume of 4  $\mu$ l), whereas the sham groups received an i.c.v. injection of saline solution (total volume of 4  $\mu$ l) as described earlier (Souza et al. 2013). Mice were anesthetized with an i.p. injection of sodium pentobarbital (0.067 mg/g). A single dose of ICV-STZ or saline was injected into the left ventricle of the brain using a stereotaxic apparatus. The bregma coordinates used for the injection were  $-1.0$  mm lateral,  $-0.3$  mm posterior, and  $-2.5$  mm below.

Minocycline was administered at a dose of 50 mg/kg (i.p.) once daily for 2 consecutive days prior to and on the same day as STZ injection, according to O'Connor et al. (2009).

## Behavioral Assessment

### Open-Field Test (OFT)

The OFT was carried out to evaluate if the drugs produced effects on locomotor activity. The animals were individually placed for a period of 5 min into an OFT apparatus (Insight model EP 154C). The total distance (unit: mm) was computed (Prut and Belzung 2003; Goes et al. 2014).

### Forced Swimming Test (FST)

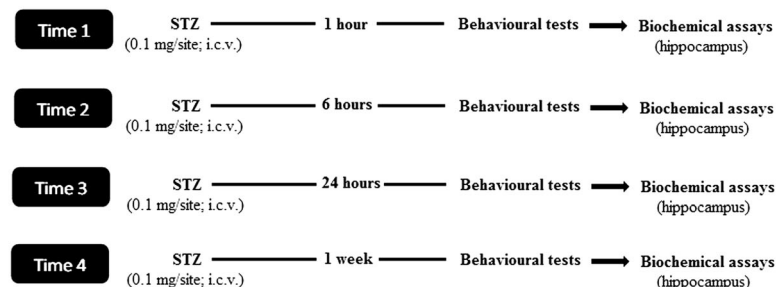
The test was conducted using the method described by Porsolt et al. (1977). Briefly, mice were individually forced to swim in open cylinders (25-cm height  $\times$  10-cm diameter) containing 19 cm of water at  $25 \pm 1$  °C. The duration of immobility was scored during the 6-min test period as described previously (Rodrigues et al. 2002). Each mouse was recorded as immobile when floating motionless or making only those movements necessary to keep its head above water.

### Splash Test

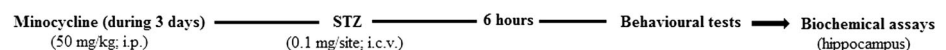
Ten minutes after the OFT, the splash test was carried out. This test consisted of squirting a 10% sucrose solution on the dorsal coat of a mouse placed individually in a clear Plexiglas box (9  $\times$  7  $\times$  11 cm) (Rosa et al. 2014). Because of its viscosity, the sucrose solution dirties the mouse fur, and animals initiate grooming behavior. After applying the sucrose solution, the total amount of time spent grooming was manually recorded for a period of 5 min as an index of self-care and motivational behavior, which are considered to reflect some symptoms of depression such as apathetic behavior (Willner 2005). The apparatus was cleaned with a solution of 10% ethanol between tests in order to hide animal cues.

**Fig. 1** Overview of study design. STZ streptozotocin, i.c.v. intracerebroventricula, IDO indoleamine-2,3-dioxygenase, i.p. intraperitoneally

### Experiment 1 (time-course curve)



### Experiment 2 (Cytokine inhibitor minocycline)



## Biochemical Assays

After behavioral tests, mice were euthanized, and blood and the hippocampus were removed. The hippocampus was homogenized in 50 mM Tris-Cl, pH 7.4. The homogenate was centrifuged at  $2400\times g$  for 15 min at 4 °C, and a low-speed supernatant fraction ( $S_1$ ) was used for assays. Blood samples were collected directly from the ventricle of the heart in anesthetized animals, using heparin as the anticoagulant, and plasma was separated by centrifugation ( $2400\times g$ ) for 15 min.

## Blood Glucose Determination

To confirm that ICV-STZ (0.1 mg/site) was a subdiabetogenic dose, the plasma glucose level was determined by enzymatic colorimetric methods using a commercial kit (LabTest Diagnostica, MG, Brazil). Glucose levels were expressed as mg/dl.

## Proinflammatory Cytokine Levels

The hippocampus ( $S_1$ ) was diluted in a solution containing bovine serum albumin (BSA 10 mg/ml), EGTA (2 mM), EDTA (2 mM), and PMSF (0.2 mM) in phosphate-buffered saline (PBS, pH 7.4). Levels of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interferon- $\gamma$  (IFN- $\gamma$ ) in the hippocampus were determined using commercially available ELISA assays, following the instructions supplied by the manufacturer (DuoSet Kits, R&D Systems, Minneapolis). The concentration of cytokines was normalized to the protein concentration contained in the samples. The results are shown as pg/mg tissue.

## Tryptophan (TRP) and Kynurenine (KYN) Levels

The tissue samples were diluted (1:5 *w/v* for high-performance liquid chromatography (HPLC) analysis) in buffer containing 20 mM  $\text{KH}_2\text{PO}_4$ , 0.1 mM PMSF, 10 mM EDTA, 0.4 M NaCl, protease inhibitors and 140 mM KCl. This tissue preparation was sonicated in an ultrasonic cooling bath for 15 min at 40 kHz, followed by centrifugation at  $16,000\times g$  for 10 min at 4 °C, and then 10  $\mu\text{L}$  of supernatant was used for HPLC to carry out the neurotransmitter measurements. The levels of TRP and its metabolite KYN in the hippocampus were measured in a Shimadzu LC-10A liquid chromatograph, according to Silva et al. (2002). The chromatographic separation was achieved using a 250 by 4.6-mm (inner diameter)  $\text{C}_{18}$  reverse-phase column (particle size, 4  $\mu\text{m}$ ; Aquapore RP-300 C-18). For the TRP measurement, the column was eluted isocratically at a flow rate of 1.0 ml/min with 0.015 M sodium acetate (pH 4.5) containing 15% methanol. For the KYN measurement, the column was eluted with acetonitrile at a 1:47 dilution in 0.1 M acetic acid–0.1 M ammonium acetate

(pH 4.65). The absorbance of the column effluent was monitored at 280 and 365 nm for TRP and KYN, respectively. The peaks of TRP or KYN were identified by comparison with the retention times of standard compounds (Sigma), and quantification was based on the ratios of the peak areas of the compound to the internal standard. The tissue levels were expressed in pg/mg tissue.

## Serotonin (5-HT) and 5-Hydroxyindoleacetic Acid (5-HIAA) Levels

The tissue samples were diluted (1:5 *w/v* for HPLC analysis) in buffer containing 20 mM  $\text{KH}_2\text{PO}_4$ , 0.1 mM PMSF, 10 mM EDTA, 0.4 M NaCl, protease inhibitors and 140 mM KCl. This tissue preparation was sonicated in an ultrasonic cooling bath for 15 min at 40 kHz, followed by centrifugation at  $16,000\times g$  for 0 min at 4 °C, and then 10  $\mu\text{L}$  of supernatant was used for HPLC to carry out the neurotransmitter measurements. The levels of 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in the hippocampus were analyzed by HPLC with electrochemical detection, as described by Ferraz et al. (2002). The mobile phase, used at a flow rate of 0.8 ml/min, consisted of 0.02 M phosphate/citrate buffer and 90/10 methanol (*v/v*), 0.12 mM  $\text{Na}_2\text{EDTA}$ , and 0.0556% heptane sulfonic acid as ion pair. The pH was adjusted to 2.64 with  $\text{H}_3\text{PO}_4$  at 22 °C. A 5- $\mu\text{m}$  ( $220 \times 4.6$ ) Spheri-5 RP-18 column from Brownlee Laboratory was used. Electrochemical detection was performed with a Shimadzu L-ECD-6A electrochemical detector with a potential of 0.75 V. The peak area of the internal standard (DHBA) was used to quantify the sample peaks. The tissue levels were expressed in pg/mg tissue.

## Indoleamine-2,3-Dioxygenase (IDO) Activity

IDO activity in the hippocampus was determined as previously described (Lestage et al. 2002). The supernatants (0.2 ml) were added to 0.8 ml of the reaction mixture containing 400  $\mu\text{M}$  L-tryptophan, 20 mM ascorbate, 10  $\mu\text{M}$  methylene blue, and 100  $\mu\text{g}$  catalase in 50 mM potassium phosphate buffer, pH 6.5. The reaction was carried out at 37 °C under agitation for 60 min. Then, it was blocked by adding 0.2 ml of 30% trichloroacetic acid and further incubated at 50 °C for 30 min to convert the N-formylkynurenine to L-kynurenine. Samples were centrifuged at  $13,000g$  for 10 min at 4 °C. The supernatants were filtered through microspin ultrafiltrates with a cutoff of 10,000  $\text{M}_r$ , before being taken for measurement of IDO.

The amount of L-kynurenine formed from tryptophan was determined by reversed phase HPLC. Here, 100  $\mu\text{l}$  of the reaction product was injected onto a Merck LiChrospher column (150 mm long, 4.6 mm diameter, packed with 5  $\mu\text{m}$  silica beads holding 18C long carbon chains). A cartridge guard



column containing the same material as the analytical column was used. The mobile phase consisted of 0.1 M ammonium acetate buffer (pH 4.65) with 5% acetonitrile. Flow rate was 1 ml/min. KYN was detected using a spectrometer measuring absorbency at a wavelength of 365 nm, and was quantified using known amounts of L-kynurenine. The retention time of KYN was approximately 5.35 min. All measurements were performed in duplicate. One unit of activity was defined as 1 pmol KYN/h/mg protein at 37 °C.

### Protein Determination

Protein concentration was measured by the Bradford (1976), using bovine serum albumin (1 mg/ml) as the standard (Sigma). The Bradford assay is a protein determination method that involves the binding of Coomassie Brilliant Blue G-250 to proteins (Bradford 1976). It is this blue protein form that is detected at 595 nm in the assay using a microplate reader.

### Statistical Analysis

Results are presented as the means  $\pm$  standard error medium (SEM). Comparisons between the experimental and control groups were performed by one-way (experiment 1) or two-way (experiment 2) analysis of variance (ANOVA), followed by Newman-Keuls test when appropriate. A value of  $p < 0.05$  was considered to be significant. All tests were carried out using the GraphPad software (San Diego, CA, USA).

## Results

### ICV-STZ Induced Depressive-like Behavior Without Causing Alterations in Spontaneous Locomotion

Injection of STZ 6 h, 24 h, and 1 week before the FST increased the immobility time of mice when compared to that of the saline-treated controls (sham group) [ $F(4,35) = 46.14$ ;  $p < 0.001$ ]. Post hoc comparisons demonstrated that the peak effect occurred 6 h after i.c.v. injection (Fig. 2a).

Injection of STZ before the splash test significantly decreased the total time mice spent grooming when compared to that of the sham group [ $F(4,35) = 12.62$ ;  $p < 0.001$ ]. Post hoc comparisons demonstrated that STZ induced a decrease in the total time of grooming 6 and 24 h after i.c.v. injection (Fig. 2b).

ICV-STZ did not cause significant alterations in locomotor activity in the OFT [ $F(4,35) = 0.196$ ;  $p = 0.937$ ] and glucose plasma levels [ $F(4,35) = 0.037$ ;  $p = 0.99$ ] (Fig. 2c, d, respectively).

### ICV-STZ Increased the Levels of Proinflammatory Cytokines in the Hippocampus

In response to STZ injection, a significant increase in TNF- $\alpha$  levels was found in the hippocampus when compared to that of the saline-treated controls [ $F(4,35) = 90.87$ ;  $p < 0.001$ ]. Post hoc comparisons showed that the injection of STZ increased TNF- $\alpha$  levels at 6 h, 24 h, and 1 week. The peak effect occurred at the 6-h time point (Fig. 3a).

When compared to the saline-treated controls, STZ-treated mice showed a significant increase in IFN- $\gamma$  in the hippocampus [ $F(4,35) = 49.10$ ;  $p < 0.001$ ]. Post hoc comparisons showed that the injection of STZ increased IFN- $\gamma$  levels at 6 and 24 h, returning to control levels at 1 week. The peak effect occurred at 6 h (Fig. 3b).

### STZ Caused IDO Activation Coupled with an Increase in TRP Levels and KYN Production in the Hippocampus

When compared to the saline-treated controls, STZ-treated mice showed a significant increase in IDO activity in the hippocampus [ $F(4,35) = 14.79$ ;  $p < 0.001$ ]. Post hoc comparisons showed that the injection of STZ increased IDO activity at 6 and 24 h, returning to baseline levels at 1 week (Fig. 4a).

Increased levels of TRP were found in the hippocampus after STZ injection [ $F(4,35) = 12.67$ ;  $p < 0.001$ ]. Post hoc comparisons showed that increased TRP was observed 6 and 24 h post-STZ injection, returning to control levels at 1 week (Fig. 4b).

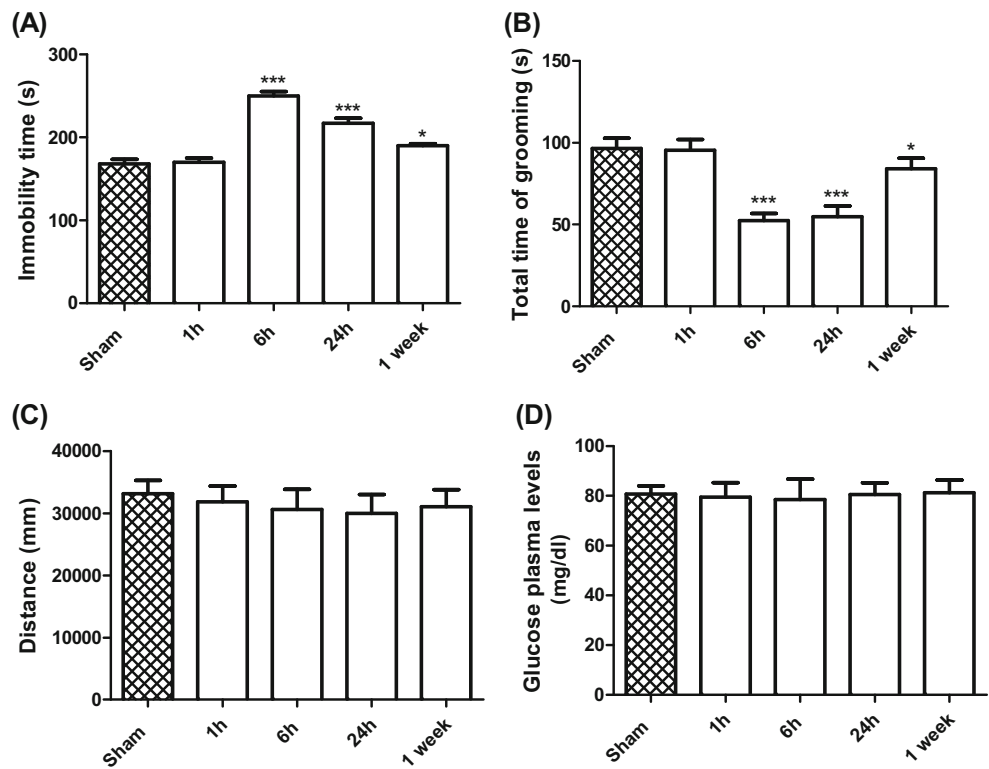
The injection of STZ also significantly increased KYN levels in the hippocampus [ $F(4,35) = 24.12$ ;  $p < 0.001$ ]. Post hoc comparisons showed the peak effect of STZ at 6 h. Moreover, increased KYN levels were maintained until the 1-week time point (Fig. 4c). This translated to an increase in the KYN/TRP ratio post-STZ injection [ $F(4,35) = 5.93$ ;  $p < 0.01$ ] at 6 h (peak effect), maintaining the significantly elevated level at the 24-h and 1-week time points (Fig. 4d).

### STZ Induced Serotonergic Dysfunction in Hippocampus

In response to STZ, a significant reduction in 5-HT levels was found in the hippocampus when compared to that in the sham group [ $F(4,35) = 9.75$ ;  $p < 0.001$ ] (Fig. 5a). Post hoc comparisons revealed that the injection of STZ decreased 5-HT levels at 6 and 24 h. The peak effect occurred at the 6-h time point.

Increased levels of the 5-HT metabolite 5-HIAA were also found in response to STZ injection [ $F(4,35) = 6.79$ ;  $p < 0.01$ ], indicative of increased 5-HT metabolism. Post hoc comparisons showed that the STZ injection increased 5-HIAA levels at 6 and 24 h, returning to control levels at 1 week (Fig. 5b). An increase in the 5-HIAA/5-HT ratio was observed after STZ injection

**Fig. 2** Effect of ICV-STZ (0.1 mg/site) on immobility time in the FST (a), total time of grooming in the splash test (b), total distance in the OFT (c), and plasma glucose levels (d) in groups tested 1, 6, 24 h and 1 week after an STZ injection. Values are mean ± SEM (*n* = 6–8). \**P* < 0.05; \*\**P* < 0.01; \*\*\**P* < 0.001 compared with the sham group



[*F*(4,35) = 12.65; *p* < 0.001]. Post hoc comparisons showed that this increase was significant at the 6- and 24-h time points (Fig. 5c).

**STZ-Induced Depressive-Like Behavior Is Blocked by Minocycline Pretreatment**

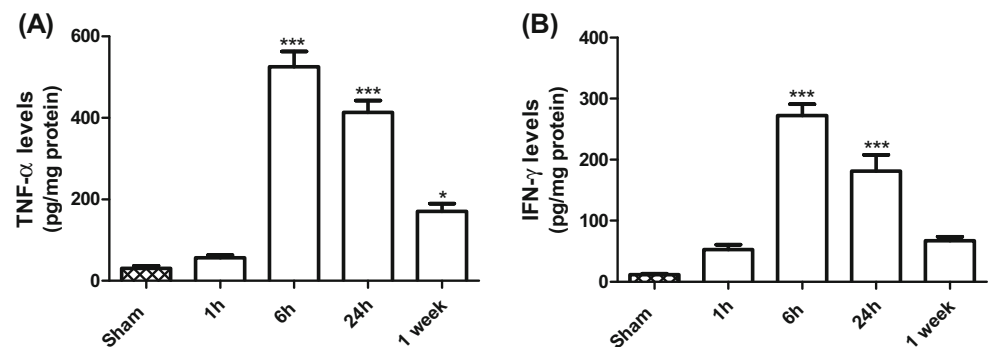
In experiment 2, mice were pretreated with minocycline during the 2 weeks before STZ injection. The depressive-like behavior of ICV-STZ-treated mice reached its peak at 6 h (Fig. 2a). Thus, the 6-h time point with the maximum effect of STZ was chosen for all further studies.

A two-way ANOVA of immobility time in the FST revealed a significant STZ × minocycline interaction [*F*(1,24) = 16.93; *p* < 0.01]. Post hoc comparisons showed that minocycline pretreatment significantly blocked the increase in immobility time induced by STZ (Fig. 6a).

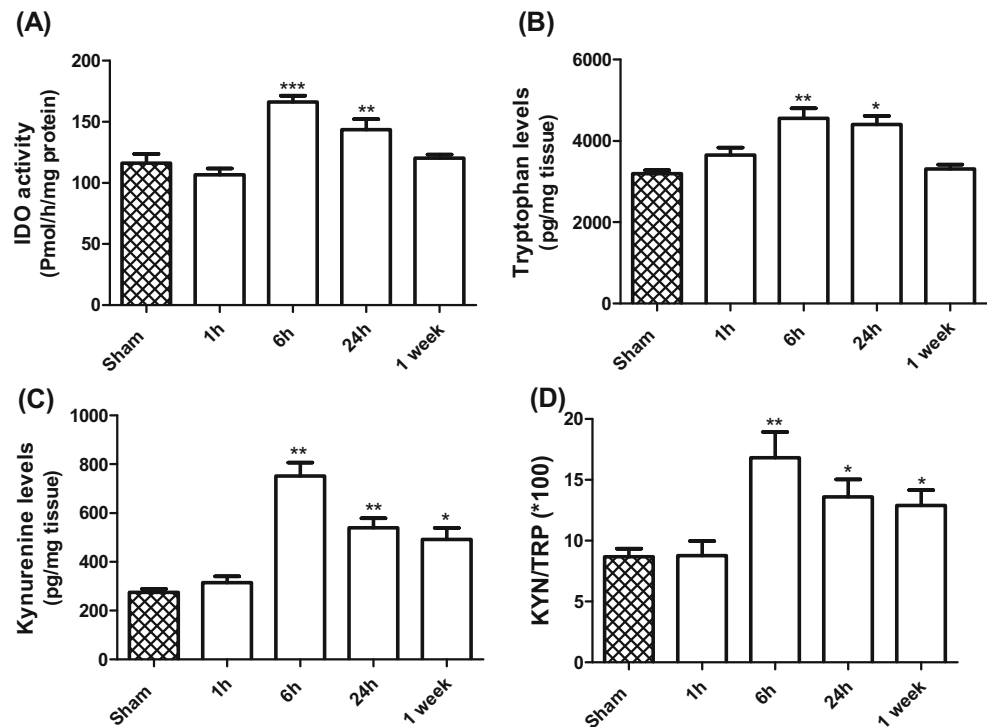
Statistical analysis of grooming latency in the splash test yielded a significant STZ × minocycline interaction [*F*(1,24) = 23.11; *p* < 0.001]. Post hoc comparisons demonstrated that minocycline pretreatment significantly protected against the decrease in total time of grooming caused by STZ (Fig. 6b).

Two-way ANOVA showed that locomotor activity in the OFT was not significantly altered by STZ injection

**Fig. 3** Effect of ICV-STZ (0.1 mg/site) on the levels of TNF-α (a) and IFN-γ (b) in hippocampus of mice in groups tested 1, 6, 24 h and 1 week after an STZ injection. Values are mean ± SEM (*n* = 6–8). \**P* < 0.05; \*\**P* < 0.01; \*\*\**P* < 0.001 compared with the sham group



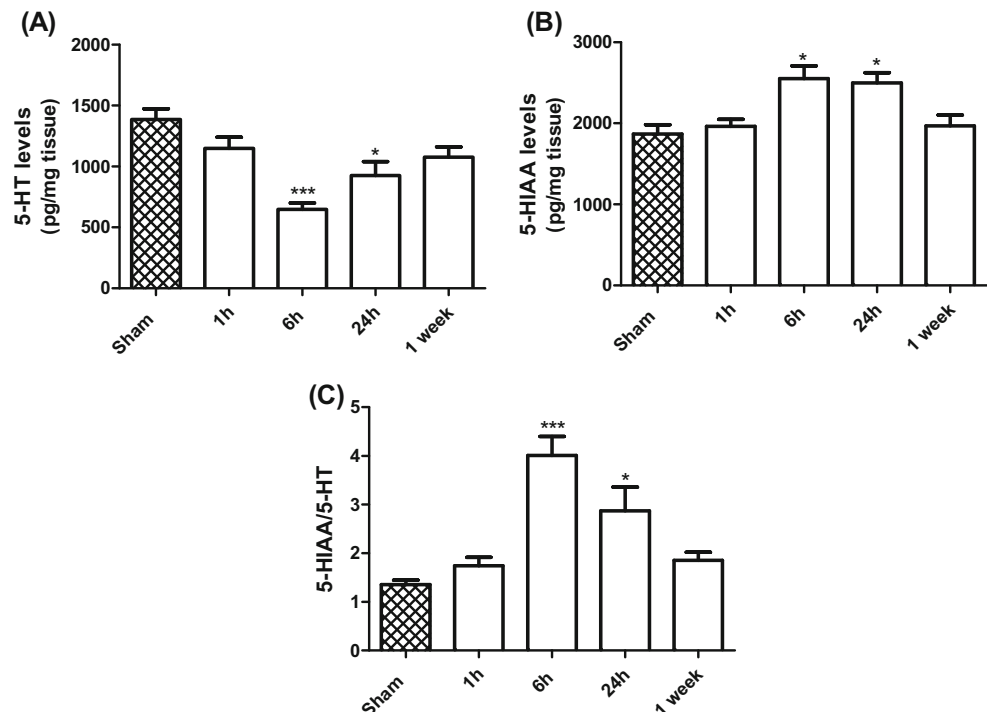
**Fig. 4** Effect of ICV-STZ (0.1 mg/site) on the IDO activity (a), TRP levels (b), KYN levels (c), and KYN/TRP ratio (d) in hippocampus of mice in groups tested 1, 6, 24 h and 1 week after an STZ injection. Values are mean  $\pm$  SEM ( $n = 6-8$ ). \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$  compared with the sham group



[ $F(1,24) = 0.09$ ;  $p = 0.76$ ], minocycline pretreatment [ $F(1,24) = 0.03$ ;  $p = 0.86$ ] or their interaction [ $F(1,24) = 0.14$ ;  $p = 0.71$ ] (Fig. 6c).

Plasma glucose levels were also not significantly altered by STZ injection [ $F(1,24) = 0.22$ ;  $p = 0.64$ ], minocycline pretreatment [ $F(1,24) = 0.10$ ;  $p = 0.75$ ], or their interaction [ $F(1,24) = 0.01$ ;  $p = 0.92$ ] (Fig. 6d).

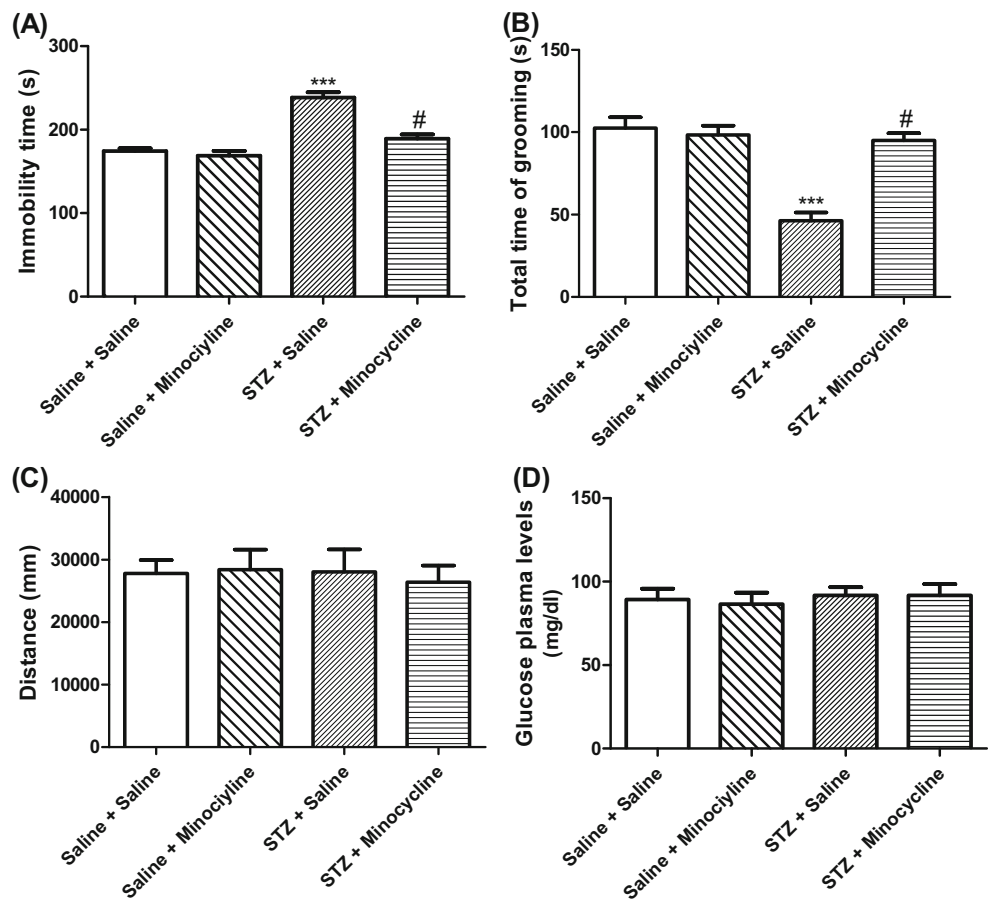
**Fig. 5** Effect of ICV-STZ (0.1 mg/site) on the 5-HT levels (a), 5-HIAA levels (b), and 5-HIAA/5-HT ratio (c) in hippocampus of mice in groups tested 1, 6, 24 h and 1 week after an STZ injection. Values are mean  $\pm$  SEM ( $n = 6-8$ ). \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$  compared with the sham group



#### STZ-Induced Neuroinflammation in the Hippocampus Is Attenuated by Minocycline

A two-way ANOVA of expression levels of TNF- $\alpha$  in the hippocampus demonstrated a significant STZ  $\times$  minocycline interaction [ $F(1,24) = 127.13$ ;  $p < 0.001$ ]. Post hoc comparisons revealed that STZ

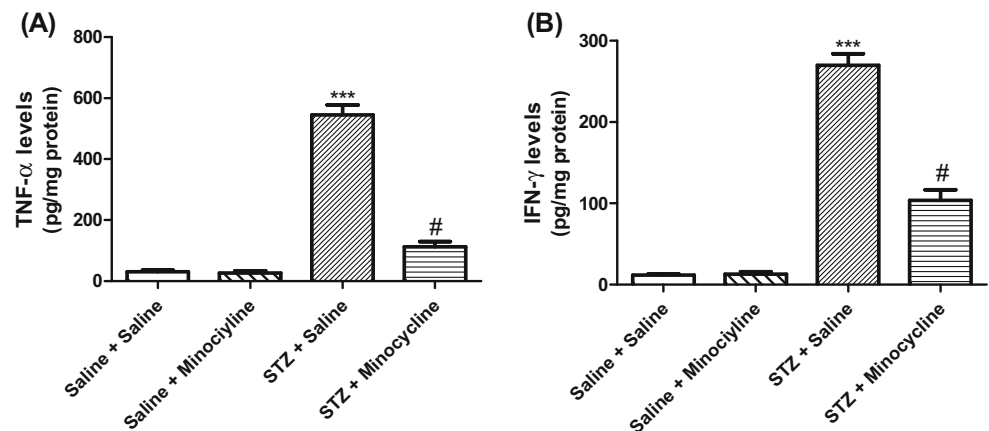
**Fig. 6** Effects of minocycline (50 mg/kg; i.p.) on the immobility time in the FST (a), total time of grooming in the splash test (b), total distance in the OFT (c), and plasma glucose levels (d) 6 h after ICV-STZ (0.1 mg/site). Values are mean ± SEM (*n* = 6–8). \*\*\**P* < 0.01 when compared STZ + saline with saline + saline. #*P* < 0.01 when compared STZ + minocycline with STZ + saline



significantly increased TNF- $\alpha$  levels in the hippocampus of mice compared to that of the sham group. Minocycline pretreatment attenuated the increase in TNF- $\alpha$  caused by STZ (Fig. 7a).

Statistical analysis of IFN- $\gamma$  in the hippocampus revealed a significant STZ  $\times$  minocycline interaction [ $F(1,24) = 73.37; p < 0.001$ ]. Post hoc comparisons demonstrated that the increase in IFN- $\gamma$  induced by STZ was significantly attenuated by minocycline pretreatment (Fig. 7b).

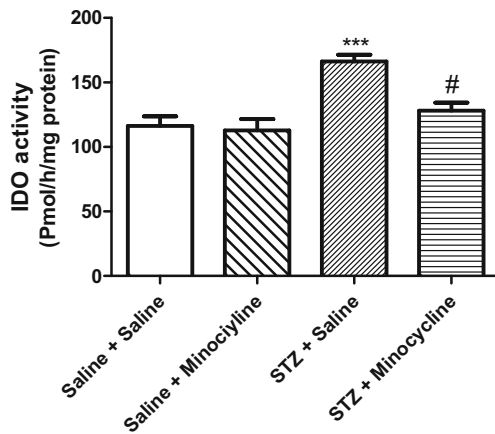
**Fig. 7** Effects of minocycline (50 mg/kg; i.p.) on the levels of TNF- $\alpha$  (a) and IFN- $\gamma$  (b) in the hippocampus of mice 6 h after ICV-STZ (0.1 mg/site). Values are mean ± SEM (*n* = 6–8). \*\*\**P* < 0.001 when compared STZ + saline with saline + saline. #*P* < 0.001 when compared STZ + minocycline with STZ + saline



**Minocycline Inhibits the Activation of the KYN Pathway Induced by ICV-STZ**

Statistical analysis of IDO activity in the hippocampus demonstrated a significant STZ  $\times$  minocycline interaction [ $F(1,24) = 6.08; p < 0.05$ ]. Post hoc comparisons revealed that STZ significantly increased IDO activity in the hippocampus of mice compared to that of the sham group. Minocycline pretreatment blocked the increase in IDO activity caused by STZ (Fig. 8).

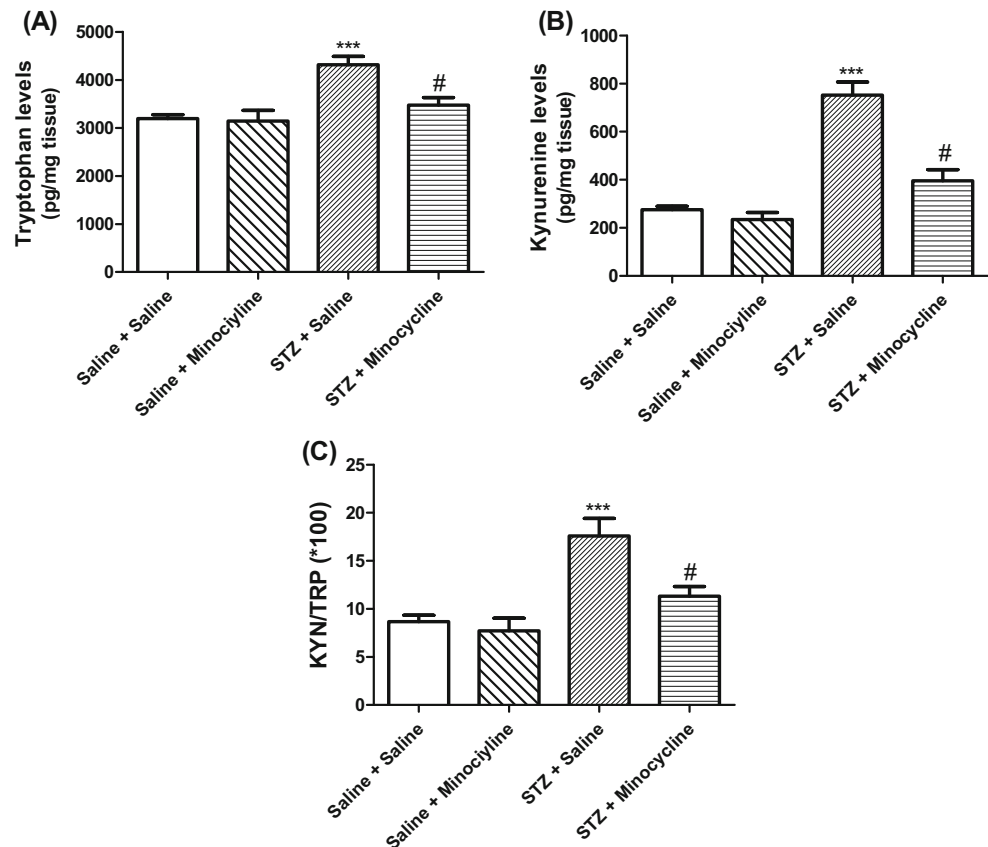




**Fig. 8** Effects of minocycline (50 mg/kg; i.p.) on the IDO activity in hippocampus of mice 6 h after ICV-STZ (0.1 mg/site). Values are mean  $\pm$  SEM ( $n = 6-8$ ). \* $P < 0.01$  when compared 1-MT + saline with saline + saline. \*\*\* $P < 0.01$  when compared STZ + saline with saline + saline. # $P < 0.001$  when compared STZ + minocycline with STZ + saline

A two-way ANOVA of TRP levels in the hippocampus demonstrated a significant STZ  $\times$  minocycline interaction [ $F(1,24) = 5.68$ ;  $p < 0.05$ ]. Post hoc comparisons revealed that STZ significantly increased TRP levels in the hippocampus of mice compared to the levels of the sham group. Minocycline pretreatment normalized the TRP levels (Fig. 9a).

**Fig. 9** Effects of minocycline (50 mg/kg; i.p.) on the TRP levels (a), KYN levels (b), and KYN/TRP ratio (c) in hippocampus of mice 6 h after ICV-STZ (0.1 mg/site). Values are mean  $\pm$  SEM ( $n = 6-8$ ). \* $P < 0.01$  when compared 1-MT + saline with saline + saline. \*\*\* $P < 0.01$  when compared STZ + saline with saline + saline. # $P < 0.001$  when compared STZ + minocycline with STZ + saline



A two-way ANOVA of KYN levels in the hippocampus demonstrated a significant STZ  $\times$  minocycline interaction [ $F(1,24) = 16.04$ ;  $p < 0.01$ ]. Post hoc comparisons revealed that STZ significantly increased KYN levels in hippocampus of mice compared to that of the sham group. Minocycline pretreatment significantly attenuated this effect (Fig. 9b).

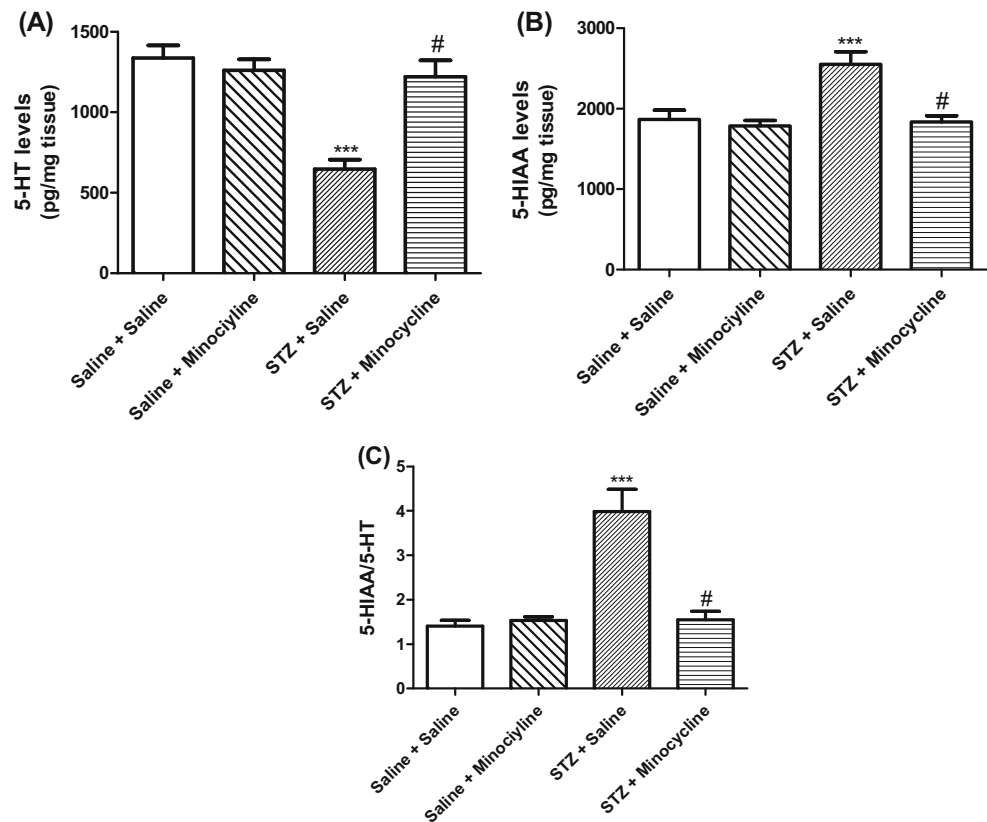
Statistical analysis of the KYN/TRP ratio in the hippocampus revealed a significant STZ  $\times$  minocycline interaction [ $F(1,24) = 4.33$ ;  $p < 0.05$ ]. Post hoc comparisons demonstrated that the increase in the KYN/TRP ratio induced by STZ was attenuated by minocycline pretreatment (Fig. 9c).

### Minocycline Prevented STZ-Induced Serotonergic Dysfunction in the Hippocampus

Statistical analysis of 5-HT levels in the hippocampus revealed a significant STZ  $\times$  minocycline interaction [ $F(1,24) = 16.77$ ;  $p < 0.01$ ]. Post hoc comparisons revealed that STZ significantly decreased 5-HT levels in the hippocampus of mice compared to that of the sham group. Minocycline pretreatment significantly protected against the decrease in 5-HT levels induced by STZ (Fig. 10a).

A two-way ANOVA of 5-HIAA levels in the hippocampus demonstrated a significant STZ  $\times$  minocycline interaction [ $F(1,24) = 8.44$ ;  $p < 0.05$ ]. Post hoc comparisons revealed that STZ significantly increased 5-HT levels in the

**Fig. 10** Effects of minocycline (50 mg/kg; i.p.) on 5-HT levels (a), 5-HIAA levels (b), and 5-HIAA/5-HT ratio (c) in hippocampus of mice 6 h after ICV-STZ (0.1 mg/site). Values are mean  $\pm$  SEM ( $n = 6-8$ ). \* $P < 0.01$  when compared 1-MT + saline with saline + saline. \*\*\* $P < 0.01$  when compared STZ + saline with saline + saline. # $P < 0.001$  when compared STZ + minocycline with STZ + saline



hippocampus of mice compared to that of the sham group. Minocycline pretreatment normalized the decrease in 5-HT levels induced by STZ (Fig. 10b).

Statistical analysis of the 5-HIAA/5-HT ratio in the hippocampus revealed a significant STZ  $\times$  minocycline interaction [ $F(1,24) = 20.74$ ;  $p < 0.001$ ]. Post hoc comparisons revealed that STZ significantly increased the 5-HIAA/5-HT ratio in the hippocampus of mice compared to that of the sham group. Minocycline pretreatment failed to protect against the increase in 5-HIAA/5-HT levels induced by STZ (Fig. 10c).

## Discussion

In the present study, we used an ICV-STZ to investigate the link between neuroinflammation and depression in mice and determined the impact of the STZ-induced inflammatory response on the KYN/5-HT axis, two systems implicated in the pathogenesis of depression.

### ICV-STZ Induced Depressive-Like Behavior and Apathetic Behavior

We have previously published that ICV-STZ induced a depressive-like effect (Souza et al. 2013). We have shown that STZ-injected mice displayed increased immobility time in the

tail suspension test and an anhedonia-like response characterized by reduced sucrose intake in the sucrose preference test.

In the present study, we have found similar results. In response to STZ, depressive-like behaviors in the FST and apathetic behavior in the splash test were observed at the 6-h, 24-h, and 1-week time points, with the peak effect at 6 h. As expected, ICV-STZ did not alter plasma glucose levels or the locomotor activity in the OFT. Depressive-like behavior induced by STZ has already been reported by other laboratories (Hirano et al. 2007; Ho et al. 2012). However, these studies used an STZ-induced diabetes (metabolism disease and metabolic stressor) model in rodents, in which STZ is administered by an intraperitoneal route. In contrast, our studies are the first to show depression-like effects when STZ is administered directly into the brain. Based on our findings, we also confirmed that ICV-STZ (0.1 mg/site) has the capacity to elicit a depression-like state in mice.

### STZ Induced a Neuroinflammatory Response

Our previous work has demonstrated that the depressive-like behavior induced by ICV-STZ was accompanied by an increase in levels of the proinflammatory cytokine TNF- $\alpha$  in the hippocampus of mice (Souza et al. 2013). To confirm the interrelationship between neuroinflammation and STZ-induced depression states, we sought to assay the levels of

proinflammatory cytokines TNF- $\alpha$  and IFN- $\gamma$  in the hippocampus, a brain region that constitutes part of the cortical-limbic neural circuits implicated in depression (Piser 2010). Therefore, we suggest that the upregulation of inflammatory cytokines observed in the present study is likely one of the mechanisms that mediates the behavioral effects of ICV-STZ.

### STZ Induced IDO Activation, Kynurenine Production, and Serotonergic Alterations

It has been proposed that IDO plays a pivotal role in mediating the depression-like behaviors in response to immune activation (Xie et al. 2014). It is known that the increase in proinflammatory cytokines, such as TNF- $\alpha$  and IFN- $\gamma$  may trigger IDO activation in the hippocampus (Lawson et al. 2013). Consequently, the proinflammatory cytokine-induced activation of IDO leads to depletion of TRP and reduced synthesis of 5-HT in the hippocampus, which may then induce depressive symptoms (Neumeister 2003; Myint and Kim 2003). In addition, the induction of IDO causes activation of the KYN pathway, which results in increased KYN generation and its neuroactive metabolites, including the free-radical generator, 3-hydroxyanthranilic acid, and the excitotoxin and N-methyl D-aspartate receptor agonist, quinolinic acid (Stone and Darlington 2002). A large body of evidence has shown that these neurotoxic metabolites are related to the development of depressive symptomatology in both laboratory animals and depressed patients (Dantzer et al. 2008; Raison et al. 2010; Dantzer et al. 2011; Salazar et al. 2012).

In the present study, we demonstrated that STZ induced an increase in IDO activity in the hippocampus of mice 6–24 h post-administration. This activation of IDO coincides with the appearance of depression-like behaviors in the same time course, confirming the key role of this enzyme in the initiation of depression symptoms. Therefore, here, we demonstrated for the first time that ICV-STZ may cause IDO activation in the hippocampus of mice similar to that found with depression-like behavior induced by immune activating drugs.

In our study, the increase in IDO activity induced by STZ was followed by increased levels of both TRP and its metabolite KYN. These findings are somewhat counterintuitive and certainly argue against the hypothesis that IDO activation depletes TRP bioavailability for 5-HT synthesis. Importantly, we demonstrated that central STZ injection precipitated depression-like behaviors coupled with increased KYN levels and KYN/TRP ratio, supporting the role for brain KYN metabolism in driving depression. Increased IDO enzymatic activity and elevated KYN levels have been correlated with inflammation-associated depression. Thus, our data indicate that the activation of the KYN pathway may be necessary for ICV-STZ to induce depressive-like behaviors.

It is well-recognized that depression is associated with impaired central 5-HT metabolism (Cryan and Leonard 2000). Here, we found a marked depletion of 5-HT levels in the hippocampus of mice at 6 and 24 h after ICV-STZ. Corroborating this finding, an increase in the 5-HT metabolite 5-HIAA was observed in the hippocampus of mice following STZ injection. Here, we also observed that the 5-HIAA/5-HT ratio, an indicator of 5-HT turnover, was increased in the hippocampus of mice following STZ injection. This is relevant because increased 5-HT turnover may reflect a reduction in 5-HT bioavailability (Haroon et al. 2012). Moreover, elevated 5-HT turnover in the brain is often seen in depressed patients (Barton et al. 2008). Thus, our data suggest that, although ICV-STZ did not affect 5-HT synthesis, it may promote 5-HT depletion in the hippocampus, mainly due to increasing 5-HT breakdown, thereby contributing to depressive symptoms.

Together, our data demonstrated that ICV-STZ induced depressive-like behavior in mice related to a neuroinflammatory response, the activation of the KYN pathway, and serotonergic alterations in the hippocampus. Thus, our study provides evidence that the central administration of STZ mimics some characteristics of a depressive-like phenotype, supporting the hypothesis that this may be a useful experimental model for studying depression in mice.

### Minocycline Blocked Depressive-Like Behaviors via Attenuation of the Neuroinflammatory Response and Inhibition of IDO Activation in the Hippocampus of STZ-Injected Mice

To examine the feasible link between IDO activation and inflammation-associated depression, we determined whether the inhibition of IDO indirectly (by a treatment that targets proinflammatory cytokines that induce IDO) abrogates depressive-like behavior in mice. For this approach, we administered the tetracycline derivative minocycline, a cytokine inhibitor agent, during the 3 days before ICV-STZ (experiment 2).

Here, we demonstrated that the indirect blockade of hippocampal IDO activation with minocycline prevented the development of depressive-like behaviors in STZ-injected mice. The data obtained in the present study suggest a novel mechanistic link between STZ and depression via IDO activation in the hippocampus. Minocycline has a potent anti-inflammatory effect. It is well-known that minocycline blocks macrophage and microglial activation in various *in vivo* animal models of peripheral or brain inflammation (O'Connor et al. 2009). Similar to previous reports (O'Connor et al. 2009; Corona et al. 2013), the current study demonstrated that minocycline treatment attenuated the upregulation of TNF- $\alpha$  and IFN- $\gamma$  levels in the hippocampus of STZ-treated mice. This result is relevant since it has been reported that these cytokines are

the main inducers of IDO activation (Takikawa et al. 1999; Fujigaki et al. 2006). From a mechanistic point of view, we indicate that the inhibition of proinflammatory cytokines released in the hippocampus could be due to the ability of this tetracycline to downregulate microglial activation and, therefore, downregulate the increased hippocampal TNF- $\alpha$  and IFN- $\gamma$  levels induced by IVC-STZ. Thus, our study suggests that the ICV-STZ induction of IDO is mediated by TNF- $\alpha$  and IFN- $\gamma$ -dependent mechanisms.

In the present study, we also demonstrated that minocycline pretreatment abrogated KYN production and normalized the KYN/TRP ratio in the hippocampus of STZ-injected mice, a result that is consistent with a previous report (Gibney et al. 2013). These findings showed that increased levels of KYN and the KYN/TRP ratio in the hippocampus were regulated through IDO activity. Therefore, we indicate that increased KYN levels act as a potential precipitant of neuropsychiatric-like behaviors induced by ICV-STZ.

Another interesting finding from the present study was that minocycline pretreatment abrogated the depletion of 5-HT levels and normalized the 5-HIAA production and 5-HIAA/5-HT in the hippocampus of STZ-treated mice. The increased 5-HT turnover induced by ICV-STZ may be a direct result of IDO activation because IDO can use 5-HT as a substrate (Corona et al. 2013). Another plausible explanation for 5-HT dysfunction in the hippocampus of STZ-injected mice may be due to increased cytokine exposure. In support of this premise, altered cytokine secretion has been reported to play an important role in the termination of serotonergic neurotransmission by 5-HT uptake into presynaptic neurons (Tsao et al. 2006). We speculate that proinflammatory cytokines might be the cause for an increase in 5-HT transporter expression or the modulation of specific 5-HT receptor subtypes.

In summary, our study supports the idea that ICV-STZ may cause depressive-like behaviors in mice through IDO activation in response to the upregulation of proinflammatory cytokines. Moreover, increased KYN levels may activate neurodegenerative pathways that ultimately contribute to the depression symptoms observed. Future studies are required to determine any exact causal relationship between the depressive complications observed and the STZ-induced changes in the KYN and 5-HT pathways.

## Conclusions

The results of the present study clearly confirmed that ICV-STZ has the capability to mimic core components of inflammation-associated depression in mice, with similar findings to other depression modeling paradigms. Our study provides new insights in the neurobiological mechanisms underlying the effects of ICV-STZ. Thus, we indicate that this

model could be employed in the preclinical research of depression.

Of particular importance, our data suggest that hippocampal IDO activation in STZ-associated depressive-like behavior is mediated by proinflammatory cytokine-dependent mechanisms. Thereby, our study not only extends the evidence that IDO plays a critical role in mediating inflammation-induced depression but also supports the notion that neuroinflammation and the kynurenine pathway are important targets of novel therapeutic drugs for depression.

**Acknowledgements** The financial support by CNPQ Research Grant No. 474397/2013-0. C.R.J. is recipient of CNPQ fellowships. L.C.S. is recipient of FAPERGS fellowships.

## Compliance with Ethical Standards

**Conflict of Interest** The authors declare that they have no conflict of interest.

## References

- Anisman H (2011) Inflaming depression. *J Psychiatry Neurosci* 36:291–295
- Barton DA, Esler MD, Dawood T, Lambert EA, Haikerwal D, Brechley C, Socratous F, Hastings J, Guo L, Wiesner G, Kaye DM, Bayles R, Schlaich MP, Lambert GW (2008) Elevated brain serotonin turnover in patients with depression: effect of genotype and therapy. *Arch Gen Psychiatry* 65:38–46
- Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principles of protein-dye binding. *Anal Biochem* 72:248–254
- Capuron L, Ravaud A, Dantzer R (2000) Early depressive symptoms in cancer patients receiving interleukin 2 and/or interferon alfa-2b therapy. *J Clin Oncol* 18:2143–2151
- Capuron L, Ravaud A, Gualde N, Bosmans E, Dantzer R, Maes M, Neveu PJ (2001) Association between immune activation and early depressive symptoms in cancer patients treated with interleukin-2-based therapy. *Psychoneuroendocrinology* 26:797–808
- Christmas DM, Potokar J, Davies SJ (2011) A biological pathway linking inflammation and depression: activation of indoleamine 2,3-dioxygenase. *Neuropsychiatric Dis Treat* 7:431–439
- Corona AW, Norden DM, Skendelas JP, Huang Y, O'Connor JC, Lawson M, Dantzer R, Kelley KW, Godbout JP (2013) Indoleamine 2,3-dioxygenase inhibition attenuates lipopolysaccharide induced persistent microglial activation and depressive-like complications in fractalkine receptor (CX(3)CR1)-deficient mice. *Brain Behav Immun* 31:134–142
- Cryan JF, Leonard BE (2000) 5-HT1A and beyond: the role of serotonin and its receptors in depression and the antidepressant response. *Hum Psychopharmacol* 15:113–135
- Cryan JF, Markou A, Lucki I (2002) Assessing antidepressant activity in rodents: recent developments and future needs. *Trends Pharmacol Sci* 23:238–245
- Da Silva Dias IC, Carabelli B, Ishii DK, de Moraes H, de Carvalho MC, Rizzo de Souza LE, Zanata SM, Brandão ML, Cunha TM, Ferraz AC, Cunha JM, Zanoveli JM (2016) Indoleamine-2,3-dioxygenase/kynurenine pathway as a potential pharmacological target to treat depression associated with diabetes. *Mol Neurobiol* 53:6997–7009



- Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW (2008) From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat Rev Neurosci* 9:46–56
- Dantzer R, O'Connor JC, Lawson MA, Kelley KW (2011) Inflammation associated depression: from serotonin to kynurenine. *Psychoneuroendocrinology* 36:426–436
- Du LL, Chai DM, Zhao LN, Li XH, Zhang FC, Zhang HB, Liu LB, Wu K, Liu R, Wang JZ, Zhou XW (2015) AMPK activation ameliorates Alzheimer's disease-like pathology and spatial memory impairment in a streptozotocin-induced Alzheimer's disease model in rats. *J Alzheimers Dis* 43:775–784
- Dunn AJ, Welch J (1991) Stress- and endotoxin-induced increases in brain tryptophan and serotonin metabolism depend on sympathetic nervous system activity. *J Neurochem* 57:1615–1622
- Ferraz AC, Anselmo-Franci JA, Perosa SR, de Castro-Neto EF, Bellissimo MI, de Oliveira BH, Cavalheiro EA, Naffah-Mazzacoratti Mda G, Da Cunha C (2002) Amino acid and monoamine alterations in the cerebral cortex and hippocampus of mice submitted to ricinine-induced seizures. *Pharmacol Biochem Behav* 72:779–786
- Fujigaki H, Saito K, Fujigaki S, Takemura M, Sudo K, Ishiguro H, Seishima M (2006) The signal transducer and activator of transcription 1 $\alpha$  and interferon regulatory factor 1 are not essential for the induction of indoleamine 2,3-dioxygenase by lipopolysaccharide: involvement of p38 mitogen-activated protein kinase and nuclear factor- $\kappa$ B pathways, and synergistic effect of several proinflammatory cytokines. *J Biochem (Tokyo)* 139:655–662
- Gibney SM, McGuinness B, Prendergast C, Harkin A, Connor TJ (2013) Poly I:C-induced activation of the immune response is accompanied by depression and anxiety-like behaviours, kynurenine pathway activation and reduced BDNF expression. *Brain Behav Immun* 28:170–181
- Goes AT, Souza LC, Filho CB, Del Fabbro L, De Gomes MG, Boeira SP, Jesse CR (2014) Neuroprotective effects of swimming training in a mouse model of Parkinson's disease induced by 6-hydroxydopamine. *Neuroscience* 256:61–71
- Haroon E, Raison CL, Miller AH (2012) Psychoneuroimmunology meets neuropsychopharmacology: translational implications of the impact of inflammation on behavior. *Neuropsychopharmacology* 37:137–162
- Hirano S, Miyata S, Kamei J (2007) Antidepressant-like effect of leptin in streptozotocin-induced diabetic mice. *Pharmacol Biochem Behav* 86:27–31
- Ho N, Balu DT, Hilario MR, Blendy JA, Lucki I (2012) Depressive phenotypes evoked by experimental diabetes are reversed by insulin. *Physiol Behav* 105:702–708
- Kalafatakis K, Zarros A (2014) Intracerebroventricular administration of streptozotocin as an experimental approach to Alzheimer's disease. *Int J Neurosci* 124:944–946
- Lawson MA, Parrott JM, McCusker RH, Dantzer R, Kelley KW, O'Connor JC (2013) Intracerebroventricular administration of lipopolysaccharide induces indoleamine-2,3-dioxygenase-dependent depression-like behaviors. *J Neuroinflammation* 18:10–87
- Lee S, Jeong J, Kwak Y, Park SK (2010) Depression research: where are we now? *Molecular Brain* 3:8
- Lestage J, Verrier D, Palin K, Dantzer R (2002) The enzyme indoleamine 2,3-dioxygenase is induced in the mouse brain in response to peripheral administration of lipopolysaccharide and superantigen. *Brain Behav Immun* 16:596–601
- Maes M, Leonard BE, Myint AM, Kubera M, Verkerk R (2011) The new '5-HT' hypothesis of depression: cell-mediated immune activation induces indoleamine 2,3-dioxygenase, which leads to lower plasma tryptophan and an increased synthesis of detrimental tryptophan catabolites (TRYCATs), both of which contribute to the onset of depression. *Prog Neuro-Psychopharmacol Biol Psychiatry* 35:702–721
- Mathers CD, Loncar D (2006) Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Med* 3:e442
- Miller AH, Maletic V, Raison CL (2009) Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. *Biol Psychiatry* 65:732–741
- Myint AM, Kim YK (2003) Cytokine-serotonin interaction through IDO: a neurodegeneration hypothesis of depression. *Med Hypotheses* 61:519–525
- Neumeister A (2003) Tryptophan depletion, serotonin, and depression: where do we stand. *Psychopharmacol Bull* 37:99–115
- O'Connor JC, Lawson MA, Andre C, Moreau M, Lestage J, Castanon N, Kelley KW, Dantzer R (2009) Lipopolysaccharide-induced depressive-like behavior is mediated by indoleamine 2,3-dioxygenase activation in mice. *Mol Psychiatry* 14:511–522
- Piser TM (2010) Linking the cytokine and neurocircuitry hypotheses of depression: a translational framework for discovery and development of novel anti-depressants. *Brain Behav Immun* 24:515–524
- Porsolt RD, Le Pichon M, Jalfre M (1977) Depression: a new animal model sensitive to antidepressant treatments. *Nature* 266:730–732
- Prut L, Belzung C (2003) The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. *Eur J Pharmacol* 463:3–33
- Raison CL, Dantzer R, Kelley KW, Lawson MA, Woolwine BJ, Vogt G, Spivey JR, Saito K, Miller AH (2010) CSF concentrations of brain tryptophan and kynurenines during immune stimulation with IFN- $\alpha$ : relationship to CNS immune responses and depression. *Mol Psychiatry* 15:393–403
- Rodrigues ALS, Da Silva GL, Mateussi AS, Fernandes ES, Miguel OG, Yunes RA, Calixto JB, Santos AR (2002) Involvement of monoaminergic system in the antidepressant-like effect of the hydroalcoholic extract of *Siphocampylus verticillatus*. *Life Sci* 70:1347–1358
- Rosa PB, Ribeiro CM, Bettio LE, Colla A, Lieberknecht V, Moretti M, Rodrigues AL (2014) Folic acid prevents depressive-like behavior induced by chronic corticosterone treatment in mice. *Pharmacol Biochem Behav* 127:1–6
- Salazar A, Gonzalez-Rivera BL, Redus L, Parrott JM, O'Connor JC (2012) Indoleamine 2,3-dioxygenase mediates anhedonia and anxiety-like behaviors caused by peripheral lipopolysaccharide immune challenge. *Horm Behav* 62:202–209
- Salkovic-Petrisic M, Osmanovic-Barilar J, Brückner MK, Hoyer S, Arendt T, Riederer P (2011) Cerebral amyloid angiopathy in streptozotocin rat model of sporadic Alzheimer's disease: a long-term follow up study. *J Neural Transm* 118:765–772
- Shingo AS, Kanabayashi T, Murase T, Kito S (2012) Cognitive decline in STZ-3 V rats is largely due to dysfunctional insulin signalling through the dentate gyrus. *Behav Brain Res* 229:378–383
- Silva NM, Rodrigues CV, Santoro MM, Reis LF, Alvarez-Leite JL, Gazzinelli RT (2002) Expression of indoleamine 2,3-dioxygenase, tryptophan degradation, and kynurenine formation during in vivo infection with *Toxoplasma gondii*: induction by endogenous gamma interferon and requirement of interferon regulatory factor 1. *Infect Immun* 70:859–868
- Smith RS (1991) The macrophage theory of depression. *Med Hypotheses* 35:298–306
- Souza LC, Filho CB, Fabbro LD, de Gomes MG, Goes AT, Jesse CR (2013) Depressive-like behaviour induced by an intracerebroventricular injection of streptozotocin in mice: the protective effect of fluoxetine, antitumour necrosis factor- $\alpha$  and thalidomide therapies. *Behav Pharmacol* 24:79–86
- Stone TW, Darlington L (2002) Endogenous kynurenines as targets for drug discovery and development. *Nat Rev Drug Discov* 1:609–620
- Takikawa O, Tagawa Y, Iwakura Y, Yoshida R, Truscott RJ (1999) Interferon-gamma-dependent/independent expression of indoleamine 2,3-dioxygenase. Studies with interferon-gamma-knockout mice. *Adv Exp Med Biol* 467:553–557



- Tsao CW, Lin YS, Chen CC, Bai CH, SR W (2006) Cytokines and serotonin transporter in patients with major depression. *Prog Neuro-Psychopharmacol Biol Psychiatry* 30:899–905
- Willner P (2005) Chronic mild stress (CMS) revisited: consistency and behavioural–neurobiological concordance in the effects of CMS. *Neuropsychobiology* 52:90–110
- Xie W, Cai L, Yu Y, Gao L, Xiao L, He Q, Ren Z, Liu Y (2014) Activation of brain indoleamine 2,3-dioxygenase contributes to epilepsy-associated depressive-like behavior in rats with chronic temporal lobe epilepsy. *J Neuroinflammation* 4:11–41