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ORIGINAL RESEARCH

Severe Uncontrolled Maternal Hyperglycemia Induces Microsomia and Neurodevelopment Delay Accompanied by Apoptosis, Cellular Survival, and Neuroinflammatory Deregulation in Rat Offspring Hippocampus

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Abstract

Maternal diabetes constitutes an unfavorable intrauterine environment for offspring development. Although it is known that diabetes can cause brain alterations and increased risk for neurologic disorders, the relationship between neuroimmune activation, brain changes, and neurodevelopment deficits in the offspring remains unclear. In order to elucidate the short- and long-term biological basis of the developmental outcomes caused by the severe uncontrolled maternal hyperglycemia, we studied apoptosis, neurogenesis, and neuroinflammation pathways in the hippocampus of neonates and young rats born to diabetic dams. Diabetes was induced on gestational day 5 by an injection of streptozotocin. Evaluations of milestones, body growth, and inhibitory avoidance were performed to monitor the offspring development and behavior. Hippocampal modifications were studied through cellular survival by BrdU in the dentate gyrus, expression of apoptosis-regulatory proteins (procaspase 3, caspase 3, and Bcl-2), BDNF, and neuroinflammatory modulation by interleukins, MHC-I, MHC-II, Iba-1, and GFAP proteins. Severe maternal diabetes caused microsomia and neurodevelopmental delay in pups and decrease of Bcl-2, procaspase 3, and caspase 3 in the hippocampus. Moreover, in a later stage of development, it was found an increase of TNF-α and a decrease of procaspase 3, caspase 3, MHC-I, IL-1β, and BDNF in the hippocampus, as well as impairment in cellular survival in the dentate gyrus. This study showed significant short- and long-term commitments on the development, apoptosis, cell survival, and neuroinflammation in the offspring hippocampus induced by severe uncontrolled maternal hyperglycemia. The data reinforce the need for treatment of maternal hyperglycemic states during pregnancy and breast-feeding.

Keywords Gestational diabetes · Caspase 3 · Bcl-2 · Microglia · Astrocytes · BrdU

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Introduction

Maternal diabetes is known to exert detrimental effects on offspring health, both in clinic (Aberg and Westbom 2001; DeBoer et al. 2005; Ornoy et al. 2001; Perna et al. 2015) and experimental conditions (Chandna et al. 2015; Kim et al. 2014; Ramos-Rodriguez et al. 2017; Sadeghi et al. 2018; Vuong et al. 2017). Classically, maternal hyperglycemia can cause oxidative stress, hyperinsulinemia, chronic tissue hypoxia, and decreased levels of iron in the fetus, which are inversely proportional to the degree of maternal glycemic control (Hami et al. 2015; Ornoy et al. 2001). Moreover, of particular interest is the fact that maternal diabetes profoundly changes the normal trajectory of fetal brain development, thereby predisposing the offspring to neurodevelopmental disorders possibly by neuroinflammation (Chandna et al. 2015; Van Lieshout and Voruganti 2008; Xiang et al. 2015).

According to the literature, two models have been proposed to study diabetes in rodents during pregnancy and its implications on the offspring. Moderate diabetes model causes mild hyperglycemia in mothers and macrosomia in the offspring, being similar to repercussions of diabetes type 2 and gestational diabetes mellitus. On the other hand, severe diabetes model causes high hyperglycemia in mothers and microsomia in the offspring, as a result of intrauterine growth restriction, being similar to repercussions of uncontrolled clinic diabetes (Ornoy 2011; Rudge et al. 2013; Van Assche et al. 2001). Both macrosomic and microsomic infants may have long-term consequences at childhood, adolescence, and adulthood. Paradoxically, many of the long-term consequences are similar, especially in metabolism (Ornoy 2011). Although moderate diabetes is more frequent in clinically pregnant patients, the prevalence of severe gestational hyperglycemia in developed countries should be a concern, for contributing to the 7–8% of all liveborn infants with a birth weight below normal (Ornoy 2011) and 5–10% of diabetes-induced birth defect (Ornoy et al. 2015; Reece 2012).

The cross-talk between the nervous and immune systems has been well described in the context of physiology and disease. In this way, recent advances in the understanding of immune cell ontogeny have revealed a notable interplay between neurons and glia during prenatal and postnatal emergence of functional circuits, maintenance of life-long hippocampal neurogenesis, brain development, and behavior (Bilbo and Schwarz 2012; Thion et al. 2018; Ziv and Schwartz 2008). The balance in the production of neurotrophins like BDNF (brain-derived neurotrophic factor), pro- or anti-inflammatory cytokines and chemokines by neurons, astrocytes and microglia cells, under healthy or severe/chronic stressful conditions, seems modulate adult hippocampal neurogenesis, being detrimental or beneficial to cognition (Kohman and Rhodes 2013; Yirmiya and Goshen 2011). Indeed, compelling evidence indicates that prolonged maternal hyperglycemia during critical periods of development induces inflammation by increasing the formation of advanced glycation end-products (AGEs) and its receptor in the offspring hippocampus, deregulates apoptosis levels and affects hippocampal neurogenesis, decreasing cell proliferation and survival (Chandna et al. 2015; Haghir et al. 2017; Kim et al. 2014; Kruse et al. 2012; Lotfi et al. 2016; Ramos-Rodriguez et al. 2017; Sadeghi et al. 2018). Although isolated data reveal candidate mechanisms by which severe maternal hyperglycemia predisposes the offspring to neurodevelopmental disorders, the pre- and postnatal effects underlying maternal diabetes on offspring's immune system, nerve cells, behavior, and physical parameters remain poorly understood.

Thus, this study was designed to describe the short- and long-term effects of the untreated severe maternal diabetes on the development, aversive memory, and neuroimmune activation, affecting the astrocytic and microglial response, cytokines, and the expression of MHC-I and MHC-II immune regulatory proteins in the offspring's hippocampus. These changes may be evident by somatic and neurodevelopment parameters, the expression of apoptosis-regulatory proteins (procaspase 3, caspase 3, and Bcl-2), and cell survival by BrdU in the dentate gyrus, as well as BDNF and the neuroinflammatory modulation in two different stages of the offspring life, postnatal day (PND) 1 and PND 40. The hypothesis was that maternal diabetes could induce neuroimmune and developmental changes in the offspring soon after delivery (an isolated effect of the maternal hyperglycemic intrauterine environment) and that these alterations could remain in the long-term, covering the maturation time of the nervous system (effect of both maternal gestational diabetes and postnatal maternal breast-feeding).

Materials and Methods

Experimental Animals

Procedures for the care and handling of animals were in compliance with current international laws and policies (NIH Guide for the Care and Use of Laboratory Animals, NIH Publication No. 85–23, 1985, revised 1996) and with the AROUCA Brazilian Laws for animal care and ethical use of animals. The study was approved by the Ethical Committee from UFRGS (2015/28221) and all efforts were made to minimize animal suffering and reduce the number of animals needed. Animals were maintained with free access to food and water, at a temperature around 22 °C, in a 12-h light:dark cycle.

Seventy-five-day-old female Wistar rats from our local breeding colony (CREAL, Universidade Federal do Rio Grande do Sul, Brazil) weighing 250 g ($n = 18$) were placed overnight in cages with males of the same strain. Vaginal smears were examined the next morning and the presence of spermatozoa was considered as day 0 of gestation. Pregnant rats were randomly divided into 2 groups: control and streptozotocin (STZ)-treated groups ($n = 8$–10 in each group). After 6-h fasting, diabetes was induced pre-implantation on gestational day (GD) 5 by a single intravenous injection into the tail vein of 40 mg/Kg/mL Streptozotocin (Sigma–Aldrich, USA) diluted in 0.1 M sodium-citrate buffer pH 4.3 (Kim et al. 2014; Piazza et al. 2014; Salazar García et al. 2015). Vehicle-treated rats served as control (1 mL/Kg). Blood glucose levels were determined in pregnant rats from the tail vein 48 h after STZ injection and only STZ-treated dams with fasting glycemia > 180 mg/dL and symptoms of polyuria and polydipsia were considered diabetic and selected for the present study (Kruse et al. 2012). The glycemic levels of all the pregnant rats were also verified on GD 13 and 19. In all cases, a commercial strip and portable glucometer were used (On Call Plus, ACON Laboratories, USA).

Parturition day was designated as postnatal day (PND) 0. After birth, the number of pups/litter was culled to 8. Some of the male offspring that exceeded the litter in eight pups of both maternal groups were decapitated on PND 1, and the remaining pups (males and females) were left together with their mothers until weaning and decapitated on PND 40 (Chandna et al. 2015; Kim et al. 2014). The pups of rats were divided into the following four groups: control group (PND 1), maternal STZ-injection group (PND 1), control group (PND 40), and maternal STZ-injection group (PND 40). The glycemic levels of the offspring were determined from the trunk blood on PND 1 immediately after decapitation, or from the tail vein on PND 40 pups before death.

Data from all offspring of litters (males and females) were used for analysis. When there was statistical difference between the offspring sex, the data were shown. Only the male offspring (1–2 chosen randomly per litter) were used in biochemical and immunohistochemical analysis.

Neonatal Developmental Evaluations

In order to monitor the offspring neonatal development from PND 1 to 21, the somatic parameters (weight, length, and cephalic measurements) were evaluated weekly by a digital pachymeter, as previously described by Silva et al. (2005). The main developmental milestones (surface righting, cliff aversion, negative geotaxis, hindlimb proprioceptive placing, forelimb grasp, auditory startle, activity, and eye opening) were assessed daily, as previously described (Marcuzzo et al. 2010; Stigger et al. 2013) and indicated by the average postnatal day in which the group performed positively the task.

BrdU Administration

Control and maternal STZ-injection pups received seven intraperitoneally BrdU injections (5-bromo-2′-deoxiuridine; Sigma, 50 mg/kg, dissolved in 0.1 M NH_4OH, 10 mg/mL) from PND 9 to 15, 24 h apart (Kim et al. 2014; Qiu et al. 2007). All animals were submitted to euthanasia approximately 4 weeks after the last BrdU injection (PND 40) to study the effect of severe maternal diabetes on the survival of newborn cells in the dentate gyrus (DG) of hippocampus (Piazza et al. 2014; Qiu et al. 2007; Taupin 2007).

Offspring Oral Glucose Tolerance Test

Oral glucose tolerance test (OGTT) was carried out in the offspring on PND 40. After 10-h fasting, blood was collected from the tail vein for the 0 time point. Immediately, the rats were fed with an oral dose of 70% glucose solution (1 g glucose/kg body weight) using a gavage syringe, and the blood was collected at 7.5, 15, 30, and 60 min following glucose administration. The glycemic levels were determined by glucometer (Zhao and Weiler 2010).

Inhibitory Avoidance Test

The step-down inhibitory avoidance test was performed in the offspring as previously described by Izquierdo et al. (2000) and Katche et al. (2012). The rats received a 0.7 mA, 3.0-s footshock. Each animal was tested for two retentions, one at 1.5 h later to measure short-term memory (STM) and another at 24 h later to measure long-term memory (LTM). The behavioral test was performed immediately before euthanasia. For more details, see Supplementary Materials.

Euthanasia and Sample Collection

The offspring was decapitated on PND 1 or PND 40. The liver, adrenal, and thymus were weighed and the whole brain (PND 1) or hippocampi (PND 40) rapidly dissected, frozen on liquid nitrogen, and stored at − 80 °C for biochemical analyses.

Western Blotting

Homogenates from offspring frozen brains/hippocampi were obtained by sonication in ice-cold lysis buffer containing protease inhibitor cocktail and PMSF as previously described by Kruse et al. (2012). Then samples were sonicated and centrifuged. The supernatants were collected and protein concentrations were determined by Bradford (Bradford 1976). Procaspase 3, Caspase 3, GFAP, Iba-1, MHC-I, MHC-II, and Bcl-2 were quantified incubating the blocked membranes with primary antibodies: B-cell lymphoma 2 (Bcl-2) (1:400, Santa Cruz Biotech, SC-492, in TBS-T), Caspase 3 (1:200, Santa Cruz Biotech., SC-7148, in 1% BSA), Glial Fibrillary Acid Protein (GFAP) (1:200, Sigma–Aldrich, in TBS-T), Ionized calcium binding adapter molecule 1 (Iba-1) (1:500, Abcam, ab5076, in TBS-T containing 5% of fat-free milk), MHC class I RT1A (1:200, Serotec, MCA51R, in 1% BSA), MHC class II RT1B (1:50, Serotec, MCA46R, in TBS-T), or F-actin (1:1.000, Santa Cruz Biotech, SC-1616, in TBS-T) (Dixon-Salazar et al. 2014; Kruse et al. 2009, 2012). Immunoblots were incubated with the respective HRP-conjugated secondary antibodies (anti-rabbit 1:5.000, anti-goat 1:2.000, anti-mouse 1:5000 in TBS-T, GE Healthcare Life Sciences, Argentina). For more details on the protocol, see Supplementary Materials.

Cytokine and BDNF Analyses

The whole brain (PND 1) or hippocampi (PND 40) of offspring were homogenized with lysis buffer (1 g tissue/10 mL), the lysates were centrifuged for 10,000 rpm, 5 min, and the supernatant collected for analyses. The levels of tumor necrosis factor (TNF-α), IL-1β, IL-6, and IL-10 were determined using Milliplex MAP Kit (Rat Cytokine/Chemokine Magnetic Bead Panel, Cat.#RECYTMAG-65K, Millipore, USA) according to the manufacturer's instructions.

BDNF analysis was done by ChemiKine Sandwich ELISA kit (Cat. No. CYT306, Millipore, USA), according to the manufacturer's instructions for the specific lysis buffer and centrifugation. For the standard curve, a serial dilution of the BDNF protein standard (included in the kit) was also performed with the indicated lysis buffer (0–1000 pg/mL) (Greisen et al. 2005). All samples and standards were prepared in duplicate. The cytokines and BDNF levels were expressed as pg/ml. Protein concentration of each sample was measured by Pierce BCA Protein Assay Kit (Thermo Scientific, Cat.No. 23225).

Histological Procedures

Other group of animals was transcardially perfused on PND 40. The offspring brains were dissected, post-fixed, and the coronal sections of the dorsal hippocampus (40 µm thick) were obtained using a cryostat. Every fifth section (200 µm apart) was processed for the BrdU immunohistochemistry (Piazza et al. 2014). For more details, see Supplementary Materials.

Immunohistochemical Procedures and BrdU-Labeled Cell Quantification

The BrdU immunostaining was performed as previously described by Piazza et al. (2014).

Digitalized images from the dentate gyrus (DG) of the dorsal hippocampus were obtained with a Nikon Optiphot-2 microscope (×10, Tokyo, Japan) coupled to a Micrometric camera (Accu Scope, Commack, NY, USA). With Image Pro Plus 6.0 software and 100% zoom, BrdU-labeled cells were counted in the granule cell layer (GCL) and the subgranular zone (SGZ) of the DG, defined as a two-nucleus-wide band between the apparent border of the GCL and the hilus. The cells in the outermost focal plane were omitted. The quantification was evaluated by two blinded observers. Six sections per animal, from both left and right side (5–6 animals per group) were analyzed. The total number of BrdU-labeled cells per section was determined and multiplied by the section periodicity (5) to obtain the total number of cells per DG (Malberg et al. 2000; Piazza et al. 2014).

Statistical Analysis

The data were analyzed using unpaired Student's t test, two-way ANOVA (Analysis of variance), or repeated measures ANOVA for parametric statistics and represented by mean ± standard error of mean (SEM). When there were statistically significant F values ($p \leq 0.05$), the Student–Newman–Keuls post hoc test was used. Latency to step-down platform in the inhibitory avoidance of both groups on PND 40 required the use of non-parametric statistics, Friedman analysis of variance (analysis within the groups) followed by Mann–Whitney U-test (analysis between the groups), and was expressed as median latencies and interquartile ranges of each group. The Statistica 6.0 software package (StatSoft, Inc., USA) was used for the statistical analysis.

Results

Effects of Streptozotocin (STZ) Treatment on Dams and Pups

Pregnant dams were treated with either vehicle or STZ on GD 5. Glycemic levels prior to STZ injection were not different between groups of dams. However, those treated with STZ increased blood glucose levels 48 h after treatment (222.8 ± 13.21 mg/dL) compared to vehicle (87.5 ± 11.82 mg/dL), indicating that the diabetes induction was effective ($p < 0.001$). During the pregnancy, STZ-treated dams continued to show high and progressive levels of blood glucose compared to controls on GD 13 and GD 19 ($p < 0.001$) (Fig. 1a).

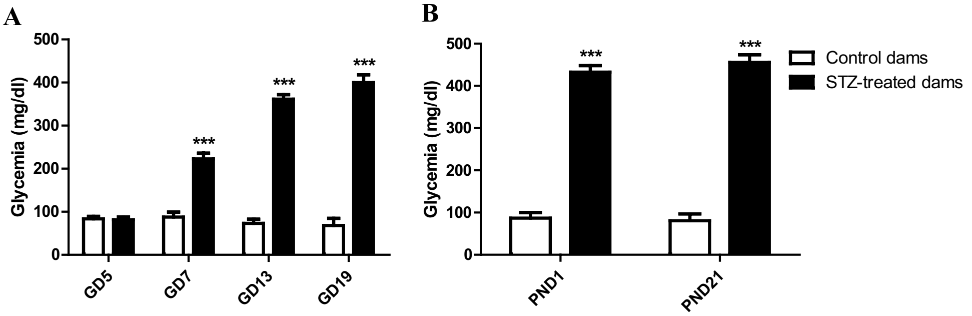

Fig. 1 Effects of STZ treatment on glycemic levels in the pregnant dams. **a** Blood glucose levels taken from the dams before (on gestational day—GD5) and 48 h after STZ injection (on GD 7), on GD13 and GD19. Repeated measures ANOVA: Treatment effect (*F* (1,16) = 438.6, $p < 0.001$), Time effect (*F* (3,48) = 64.73, $p < 0.001$), and time versus treatment interaction effect (*F* (3,48) = 79.17, $p < 0.001$). **b** Glycemic levels taken from the dams on postnatal day (PND) 1 and 21 (Treatment effect: *F* (1,16) = 521.89, $p < 0.001$). Control, $n = 10$ dams; STZ, $n = 8$ dams; ***$p < 0.001$ versus respective control dams. Data were expressed as mean ± SEM

Glycemia was also measured in dams on days 1 and 21 after delivery (Fig. 1b). The effects of STZ treatment on blood glucose observed during pregnancy continued postnatally with the STZ-treated dams showing significantly higher glycemic levels than controls ($p < 0.001$).

The average number of pups born per litter was no different between groups ($p > 0.05$) (Table 1). The average body weight for the pups of each group, from PND 1 to 40, is summarized in Table 1. On PND 1, there were no significant differences in pup weight between groups, despite pups of STZ-treated dams being born weighing less (5.64 ± 0.10 g) than those from control dams (7.38 ± 0.10 g). However, pups of STZ-treated dams gained significantly less weight than those from control dams during the postnatal development ($p < 0.01$).

For a more thorough analysis, the pup body weights on PND 1 were classified according to the mean ± 2.0 × standard deviation (SD) of the body weight obtained from the control group, to identify possible fetal macrosomia or microsomia (Ornoy 2011; Rudge et al. 2013). In this way, 60.4% of pups from STZ-treated dams were born microsomic (body weight below 6.03 g) (7.38–2.0 × 0.6745 = 6.03 g).

Offspring of maternal STZ-injection group decapitated on PND 1 showed higher blood glucose levels (104.6 ± 9.97 mg/dL) than control (84.43 ± 3.66 mg/dL) (t (33) = − 2.179, $p < 0.05$). On the other hand, on PND 40, maternal STZ-injection group showed lower blood glucose levels (78.15 ± 2.18 mg/dL) than control (87.36 ± 1.25 mg/dL) (t (43) = − 3.85, $p < 0.001$). Nevertheless, none of them were in the diabetic range.

Offspring Oral Glucose Tolerance Test (OGTT)

In order to evaluate the effect of severe maternal hyperglycemia on the long-term glucose metabolism of offspring, the OGTT was performed on PND 40. The glucose overload increased blood glucose levels over time in both groups ($p < 0.001$). No difference was observed in glucose metabolism between groups and sexes ($p > 0{\cdot}05$). However, the maternal STZ-injection group showed lower blood glucose

Table 1 Effects of maternal STZ-injection on the offspring body weight (g) during the development and weaning from PND 1 to 21, and on PND 40

Treatment	Average pups per litter	Average body weight per group				
		PND 1	PND 7	PND 14	PND 21	PND 40
Control dams	9.9 ± 0.43	7.38 ± 0.10	18.23 ± 0.29	32.43 ± 1.35	50.41 ± 0.72	167.67 ± 2.34
STZ-treated dams	11.63 ± 0.75	5.64 ± 0.10	10.56 ± 0.31**	19.46 ± 1.44***	25.88 ± 0.77***	110.71 ± 2.50***

The average number of pups/litter/group is indicated in the table

Average number of pups/litter: Unpaired Student's *t* test (*t* (16) = − 2.08, $p > 0.05$)

Body weight: repeated measures ANOVA (day × maternal treatment) showed day effect (*F* (4,352) = 4040.93, $p < 0.001$), maternal treatment effect (*F* (1,88) = 403.44, $p < 0.001$), and day versus maternal treatment interaction effect (*F* (4,352) = 165.60, $p < 0.001$)

**$p < 0.01$, ***$p < 0.001$ versus respective offspring of control dams. Data were expressed as mean ± SEM from animals of both sexes. *STZ* streptozotocin, *PND* postnatal day levels during all the points detected in the OGTT on PND 40 comparing to control group ($p < 0.05$) (Fig. 2).

Liver, Adrenal, and Thymus Weights

Diabetes is a common metabolic disorder accompanied by an increased secretion of glucocorticoid corticosterone and cognitive deficits in rats (Beauquis et al. 2008; Piazza et al. 2014). Thus, the relative weight of the adrenal glands, thymus, and liver was used as an indirect measure of immunomodulation induced by corticosterone levels in the offspring (Piazza et al. 2011; Revsin et al. 2009). The relative thymus and liver weight increased with age in both offspring groups, while relative adrenal weight decreased ($p < 0.001$). Moreover, maternal STZ-injection group had greater relative thymus weight on PND1 and on PND40, when compared to the respective controls ($p < 0.05$ and $p < 0.001$, respectively). In contrast, a lower relative liver weight was found in the maternal STZ-injection group than in the control on PND40 ($p < 0.001$) (Table 2).

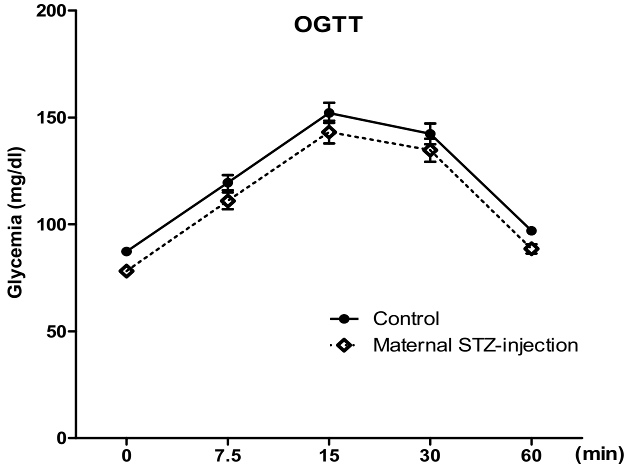

Fig. 2 Offspring oral glucose tolerance test (OGTT) on PND40. Blood glucose levels at different times after oral glucose overload (1 g/kg body weight). Repeated measures ANOVA: Time effect (F (4,172) = 167.85, $p < 0.001$), Maternal Treatment effect (F (1,43) = 5.25, $p < 0.05$), time versus maternal treatment and offspring sex interaction effects, both n.s., $p > 0{\cdot}05$. There was no difference in glucose metabolism between groups. Data were collected from males and females. (n = 20–25 per group) and expressed as mean ± SEM

Neonatal Developmental Evaluations

Evaluations of weight, height, and cephalic perimeter relating to age are amply used in the clinic to evaluate the growth and the development, as well as the nutritional state. Similar measures are also used to assess the somatic development in rat (Silva et al. 2005). In the present study, somatic parameters like length and cephalic measurements were estimated weekly, from PND 1 to 21, to study the effect of severe maternal hyperglycemia on the physical development of the offspring. On PND 1, only latero-lateral axis of skull parameter was lower in maternal STZ-injection group ($p < 0.001$), whereas from PND 7 to 21, all somatic parameters were lower compared to control ($p < 0.001$) (Table 3).

Main developmental milestones were also assessed daily, from PND 1 to 21, to study the effect of severe maternal diabetes on the neurological development of the offspring. Maternal STZ-injection group presented a delay in the postnatal day of the emergence of the developmental milestones of cliff aversion ($p < 0.001$), auditory startle ($p < 0.001$), activity ($p < 0.001$), and eye opening ($p < 0.001$) compared to control group (Table 4). Besides, males were more affected than females by maternal STZ-injection in some tasks (negative geotaxis: males 9.82 ± 0.85, females 6.79 ± 0.39, $p < 0.001$; auditory startle: males 14.53 ± 0.47, females 13.28 ± 0.25, $p < 0.01$; eye opening: males 17.65 ± 0.46, females 16.14 ± 0.27, $p < 0.001$).

Table 2 Effects of maternal STZ-injection on the relative organ weights in offspring (in mg)

	Control PND1	Maternal STZ-injection PND1	Control PND40	Maternal STZ-injection PND40
Adrenal/body weight ratio	0.43 ± 0.02	0.40 ± 0.04	$0.27 \pm 0.01^{***}$	$0.25 \pm 0.009^{***}$
Thymus/body weight ratio	2.01 ± 0.08	$2.38 \pm 0.19^{\#}$	$3.68 \pm 0.08^{***}$	$4.24 \pm 0.09^{***\#\#\#}$
Liver/body weight ratio	40.35 ± 0.77	40.68 ± 1.21	$50.72 \pm 0.51^{***}$	$44.29 \pm 0.60^{**\#\#\#}$

Two-way ANOVA (Maternal Treatment and offspring Age): Maternal Treatment effect on thymus/ body weight ratio (F (1,104) = 18.82, $p < 0.001$) and liver/body weight ratio (F (1,108) = 16.52, $p < 0.001$); Age effect on Adrenal /body weight ratio (F (1,106) = 75.61, $p < 0.001$), thymus/ body weight ratio (F (1,104) = 272.98, $p < 0.001$) and liver/body weight ratio (F (1,108) = 86.92, $p < 0.001$); and Maternal Treatment versus offspring Age interaction effect on liver/body weight ratio (F (1,108) = 20.31, $p < 0.001$)

$^{**}p < 0.01$ versus respective PND1 group, $^{***}p < 0.001$ versus respective PND1 group; $^{\#}p < 0.05$ versus control PND1 group, $^{\#\#\#}p < 0.001$ versus control PND40 group. Data were expressed as mean ± SEM from animals of both sexes. *PND* postnatal day, *STZ* streptozotocin

Table 3 Effects of maternal STZ-injection on the offspring somatic parameters from PND 1 to 21

	Control	Maternal STZ-injection
Latero-lateral axis of skull PND 1 (mm)	11.31 ± 0.09	10.20 ± 0.08***
Latero-lateral axis of skull PND 7 (mm)	14.54 ± 0.15	12.66 ± 0.12***
Latero-lateral axis of skull PND 14 (mm)	16.55 ± 0.15	14.52 ± 0.13***
Latero-lateral axis of skull PND 21 (mm)	17.45 ± 0.12	15.30 ± 0.10***
Antero-posterior axis of skull PND 1 (mm)	18.97 ± 0.19	18.39 ± 0.16
Antero-posterior axis of skull PND 7 (mm)	26.96 ± 0.31	23.68 ± 0.26***
Antero-posterior axis of skull PND 14 (mm)	33.84 ± 0.37	29.08 ± 0.31***
Antero-posterior axis of skull PND 21 (mm)	37.83 ± 0.29	32.76 ± 0.24***
Longitudinal axis PND1 (mm)	52.84 ± 0.42	49.33 ± 0.35
Longitudinal axis PND 7 (mm)	68.64 ± 0.70	58.93 ± 0.58***
Longitudinal axis PND 14 (mm)	88.53 ± 0.90	69.55 ± 0.75***
Longitudinal axis PND 21 (mm)	107.24 ± 1.30	84.86 ± 1.08***
Length of tail PND 1 (mm)	18.41 ± 0.30	16.63 ± 0.25
Length of tail PND 7 (mm)	34.52 ± 0.73	27.71 ± 0.61**
Length of tail PND 14 (mm)	56.98 ± 1.11	41.37 ± 0.92***
Length of tail PND 21 (mm)	78.09 ± 1.43	56.20 ± 1.19***

Repeated measures ANOVA (day × maternal treatment): day effect (Latero-lateral axis of skull: *F* (3,171) = 1665.38, $p < 0.001$; Antero-posterior axis of skull: *F* (3,165) = 4697.67, $p < 0.001$; Longitudinal axis: *F* (3,171) = 3704.87, $p < 0.001$; Length of tail: *F* (3,171) = 3371.70, $p < 0.001$); maternal treatment effect (Latero-lateral axis of skull: *F* (1,57) = 180.83, $p < 0.001$; Antero-posterior axis of skull: *F* (1,55) = 100.98, $p < 0.001$; longitudinal axis: *F* (1,57) = 180.57, $p < 0.001$; Length of tail: *F* (1,57) = 107.1, $p < 0.001$); and day versus maternal treatment interaction effect (Latero-lateral axis of skull: *F* (3,171) = 14.5, $p < 0.001$; Antero-posterior axis of skull: *F* (3,165) = 93, $p < 0.001$; longitudinal axis: *F* (3,171) = 182.42, $p < 0.001$; Length of tail: *F* (3,171) = 145.38, $p < 0.001$)

**$p < 0.01$, ***$p < 0.001$ versus control group. Data were expressed as mean ± SEM from animals of both sexes. *PND* postnatal day; *STZ* streptozotocin

Table 4 Effects of maternal STZ-injection on the offspring developmental milestones from PND 1 to 21

	Control	Maternal STZ-injection
Surface righting	1.17 ± 0.05	1.35 ± 0.10
Cliff aversion	2.69 ± 0.11	3.65 ± 0.25***
Negative geotaxis	7.67 ± 0.23	7.91 ± 0.45
Hindlimb placing	5.11 ± 0.43	4.30 ± 0.34
Forelimb grasp	7.92 ± 0.29	8.15 ± 0.46
Auditory startle	12.21 ± 0.10	13.74 ± 0.25***
Activity	12.38 ± 0.26	14.74 ± 0.32***
Eye opening	14.58 ± 0.08	16.71 ± 0.26***

The average postnatal day that the group performed positively the task

Two-way ANOVA (offspring sex × maternal treatment): maternal treatment effect on cliff aversion (*F* (1,90) = 11.90, $p < 0.001$), auditory startle (*F* (1,90) = 40.67, $p < 0.001$), activity (*F* (1,90) = 34.85, $p < 0.001$), eye opening (*F* (1,89) = 78.4, $p < 0.001$); offspring Sex effect on negative geotaxis (*F* (1,90) = 12.08, $p < 0.001$), auditory startle (*F* (1,90) = 6.27, $p < 0.05$), Eye opening (*F* (1,89) = 9.45, $p < 0.01$); and maternal treatment versus offspring sex interaction on negative geotaxis (*F* (1,90) = 7.74, $p < 0.01$), auditory startle (*F* (1,90) = 4.67, $p < 0.05$), Eye opening (*F* (1,89) = 6.9, $p < 0.01$)

***$p < 0.001$ versus control group. Data were expressed as mean ± SEM from animals of both sexes. *PND* postnatal day, *STZ* streptozotocin

Inhibitory Avoidance Test

The latency to step-down platform in the inhibitory avoidance in both groups on PND 40 was analyzed to verify the effect of severe maternal hyperglycemia on the aversive memory retention in the offspring. Friedman's ANOVA followed by Dunn's Multiple Comparison Test showed that animals of both groups learned and did not show impairment in memory retention after 1.5 h and 24 h of the training ($p < 0.0001$). So, there were no differences induced by maternal STZ injection on the short- and long-term memory of the offspring (Fig. 3). Mann–Whitney *U*-test showed no difference neither in the latencies to step-down the platform in the training session ($p > 0.05$) nor in the performances at 1.5 h ($p > 0.05$) and 24 h ($p > 0.05$) in the test sessions between groups.

Cytokine and BDNF Levels

BDNF and immune system molecules have the ability to influence neural plasticity, adult hippocampal neurogenesis, and memory either in pathological conditions or in the healthy uninjured brain (Kohman and Rhodes 2013; Yirmiya and Goshen 2011). In our study, maternal STZ injection did not change significantly the cytokines and BDNF levels in

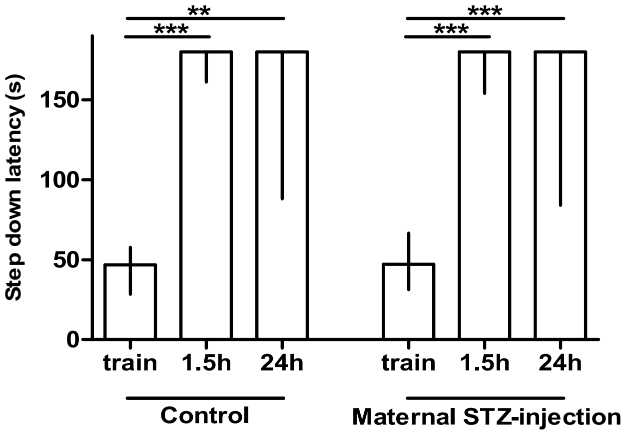

Fig. 3 Latency to step-down platform in the inhibitory avoidance test on PND40. There were no differences induced by maternal STZ injection on the short- and long-term memory of the offspring between groups. Friedman's ANOVA followed by Dunn's multiple comparison test and Mann–Whitney *U*-test. $n = 18–28$ per group. Data from males and females. STZ, streptozotocin. $^{**}p < 0.01$, $^{***}p < 0.001$. Data were expressed as median latencies and interquartile ranges of each group

pup brains on PND 1 ($p > 0.05$). Nevertheless, maternal STZ-injection PND 40 group presented a decrease in IL-1β ($p < 0.001$) and BDNF levels ($p < 0.001$), besides an increase in TNF-α levels ($p < 0.05$) compared to control PND40 group in hippocampus. Except for TNF-α, all interleukin and BNDF levels increased with age in control animals ($p < 0.001$) (Table 5).

Expression of Neuroinflammatory and Apoptotic Proteins

The expression of Procaspase 3, Caspase 3, Bcl-2, Iba-1, GFAP, MHC-I, and MHC-II was then evaluated by Western blot in the hippocampus of animals on PND1 and PND40.

Procaspase 3 is precursor of the active cleaved caspase 3, the major effector protein in the apoptotic pathway, whereas Bcl-2 is an anti-apoptotic protein of the Bcl-2 family, which controls and regulates apoptotic mitochondrial events (Haghir et al. 2017). In our study, the expression of the Bcl-2, Procaspase 3 and Caspase 3 proteins decreased along with age in both groups ($p < 0.001$) (Fig. 4a–c). Maternal diabetes decreased the expression of Procaspase 3 and Caspase 3 on PND1 ($p < 0.05$) and PND40 ($p < 0.05$) (Fig. 4b, c), and of Bcl-2 levels only on PND1 ($p < 0.05$) (Fig. 4a).

GFAP (glial fibrillary acid protein) is an intracellular intermediate filament protein, essential for the formation of stable astrocytic processes in response to neuronal damage or physiologic demands, being used as an astrocytic marker (de Senna et al. 2017). Iba-1 (ionized calcium binding adapter molecule 1) protein expressed on the cell surface is used predominantly to study the activated states in microglia and is up-regulated during inflammation and neuronal injury. Additionally, the antigen presentation function of the activated microglia is verified through major histocompatibility complex (MHC) class II positivity, since it specifies the harmful form of microglia activation (Kettenmann et al. 2011; Nagayach et al. 2014). Here, the expression of the MHC-II protein decreased along with age in both groups ($p < 0.01$) (Fig. 5b). Pups born to diabetic dams on PND1 showed a non-significant increase in MHC-II expression compared to the control PND1 group (121.14 ± 12.76% vs. 100.00 ± 6.82%; $p > 0.05$) that could suggest a tendency to induce the activation of microglia (Fig. 5b). The expression

Table 5 Effects of maternal STZ injection on the cytokine and BDNF levels in offspring hippocampus

	Control PND1	Maternal STZ-injection PND1	Control PND40	Maternal STZ-injection PND40
IL-1β	17.26 ± 1.96	12.92 ± 1.22	119.3 ± 10.10***	80.06 ± 7.16***$^{\#\#\#}$
IL-6	3.80 ± 1.09	2.66 ± 0.47	19.18 ± 3.25***	27.63 ± 3.91***
TNF-α	0.24 ± 0.03	0.19 ± 0.03	0.18 ± 0.04	0.38 ± 0.08*$^{\#}$
IL-10	0.70 ± 0.44	0.30 ± 0.08	16.01 ± 2.23***	11.10 ± 2.11***
BDNF	162.02 ± 11.06	180.32 ± 29.96	327.96 ± 9.79***	156.48 ± 45.66$^{\#\#\#}$

PND postnatal day, *STZ* streptozotocin

Two-way ANOVA (offspring age × maternal treatment): maternal treatment effect on IL-1β (F (1,22) = 11.19, $p < 0.01$) and BDNF (F (1,22) = 10.23, $p < 0.01$); age effect on IL-1β (F (1,22) = 168.75, $p < 0.001$), IL-6 (F (1,23) = 65.52, $p < 0.001$), IL-10 (F (1,20) = 66.53, $p < 0.001$), BDNF (F (1,22) = 8.81, $p < 0.01$); and maternal treatment versus age interaction effect on IL-1β (F (1,22) = 7.18, $p < 0.05$), TNF-α (F (1,21) = 7.35, $p < 0.05$) and BDNF (F (1,22) = 15.71, $p < 0.001$).

$^{*}p < 0.05$ versus respective PND1 group, $^{***}p < 0.001$ versus respective PND1 group; $^{\#}p < 0.05$ versus control PND40 group, $^{\#\#\#}p < 0.001$ versus control PND40 group. $n = 5–8$ male offspring per group. Data were expressed as mean of pg/mg protein ± SEM

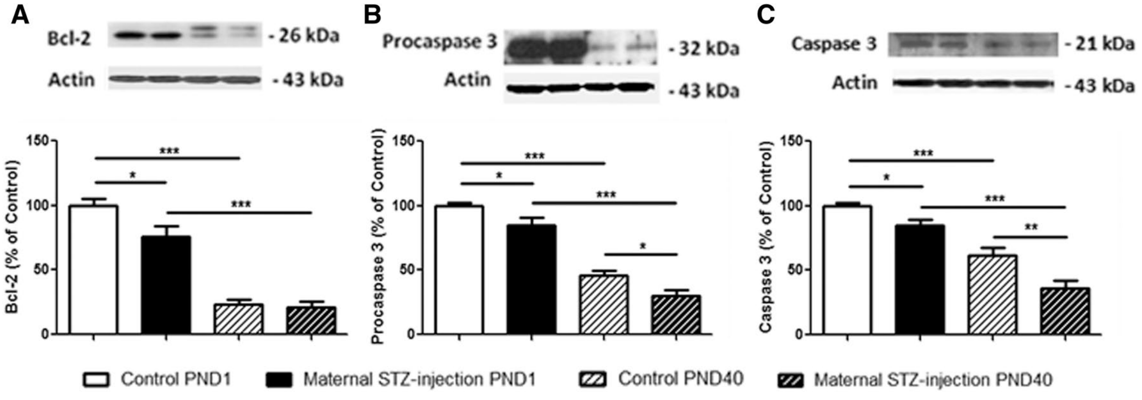

Fig. 4 Effects of maternal STZ injection on the Bcl-2 (**a**), Procaspase 3 (**b**), and Caspase 3 (**c**) levels in offspring hippocampus by Western blotting. Representative pictures of Bcl-2, Procaspase 3, and Caspase 3 and the loading control F-actin are shown in the upper panel. PND, postnatal day; STZ, streptozotocin. Two-way ANOVA (offspring age × maternal treatment): age effect on Procaspase 3 (*F* (1,16) = 142.93, $p < 0.001$), Caspase 3 (*F* (1,19) = 83.70, $p < 0.001$) ,and Bcl-2 levels (*F* (1,15) = 125.71, $p < 0.001$); maternal treatment effect on Procaspase 3 (*F* (1,16) = 11.48, $p < 0.01$), Caspase 3 (*F* (1,19) = 17.45, $p < 0.001$), and Bcl-2 levels (*F* (1,15) = 5.07, $p < 0.05$). $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. Data were expressed as mean ± SEM from at least two independent experiments ($n = 5$–6 male offspring per group)

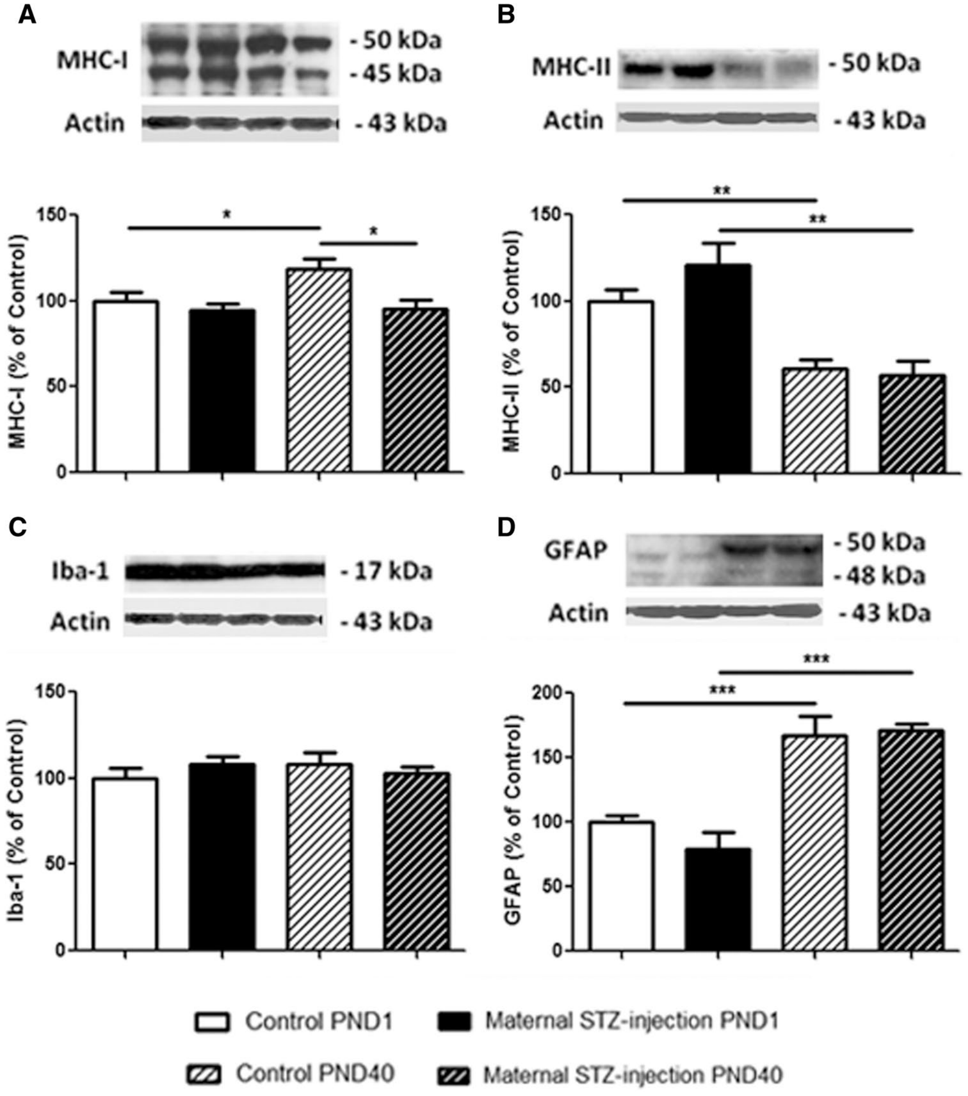

Fig. 5 Effects of maternal STZ-injection on the MHC-I (**a**), MHC-II (**b**), Iba-1 (**c**), and GFAP (**d**) levels in offspring hippocampus by Western blotting. Representative pictures of MHC-I, MHC-II, Iba-1, GFAP, and the loading control F-actin are shown in the upper panel. *PND* postnatal day, *STZ* streptozotocin. Two-way ANOVA (offspring age × maternal treatment): age effect on GFAP (*F* (1,16) = 58.55, $p < 0.001$), MHC-II (*F* (1,14) = 31.31, $p < 0.001$); Maternal Treatment effect on MHC-I (*F* (1,16) = 7.40, $p < 0.05$). $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. Data were expressed as mean ± SEM from at least two independent experiments ($n = 5$–6 male offspring per group)

of GFAP only increased with age in both groups ($p < 0.001$), but was not modified by maternal hyperglycemia (Fig. 5d). Moreover, no change in Iba-1 expression was observed between groups ($p > 0.05$) (Fig. 5c).

On the other hand, MHC-I is expressed in almost every nucleated cell of the body, including hippocampal neurons, neural progenitors, and glial cells, playing a role in immune and non-immune functions, as for activity-dependent refinement of neuronal connections, synaptic plasticity, and normal neurodevelopment (Bilbo and Schwarz 2012; Chacon and Boulanger 2013; Elmer and McAllister 2012). Furthermore, levels and/or patterns of MHC-I expression in the prenatal brain may contribute to the pathogenesis of neurodevelopmental disorders (Chacon and Boulanger 2013). In the present study, the expression of MHC-I increased with age in control animals ($p < 0.05$) (Fig. 5a). In the offspring subjected to maternal hyperglycemia, MHC-I expression decreased on PND 40 compared to control group ($p < 0.05$) (Fig. 5a).

Quantification of BrdU-Labeled Cells

Postnatal neurogenesis in the dentate gyrus (DG) of hippocampus is a complex multi-step process that includes proliferation, survival, migration, and differentiation of progenitor cells into mature neurons or glial cells that later integrate into the synaptic network, playing an important role in learning and memory functions (Lucassen et al. 2010). In our study, BrdU injections were administrated in pups from PND 9 to 15, when the rodent brain is growing rapidly and formation of axons, dendrites, synapses, and myelin is maximal (Qiu et al. 2007). Thus, to evaluate the effect of severe maternal diabetes on the survival of BrdU-labeled cells in the DG of the offspring hippocampus, the analysis was performed approximately 4 weeks after the last BrdU injection (Piazza et al. 2014; Qiu et al. 2007). Maternal STZ-injection group presented a decrease in the number of BrdU + cells compared to the control group ($p = 0.05$) on PND 40, indicating a relevant impairment in cell survival phase of hippocampal neurogenesis (Fig. 6).

Discussion

In the present study, we reported the short- (on PND 1) and long-term (on PND 40) effects of severe uncontrolled maternal hyperglycemia induced by STZ on the development, apoptosis, cell survival, and neuroinflammation in the offspring hippocampus. The studied animal model mimicked the consequences of maternal diabetes observed in previous experimental studies, being a reproducible model. Compelling evidence have shown that this maternal type 1 diabetes model can induce microsomia in the offspring, possibly as a result of intrauterine growth restriction with placental commitment (Gabbay-Benziv and Baschat 2015; Rudge et al. 2013; Van Assche et al. 2001; Volpato et al. 2015), leading to long-term effects, especially in metabolic and neurologic parameters in the infants (Hami et al. 2015; Ornoy 2005; Perna et al. 2015; Reece et al. 2009).

In fact, in our study, 60% of pups of maternal STZ-injection group were born microsomic (see "Effects of Streptozotocin (STZ) Treatment on Dams and Pups"), although

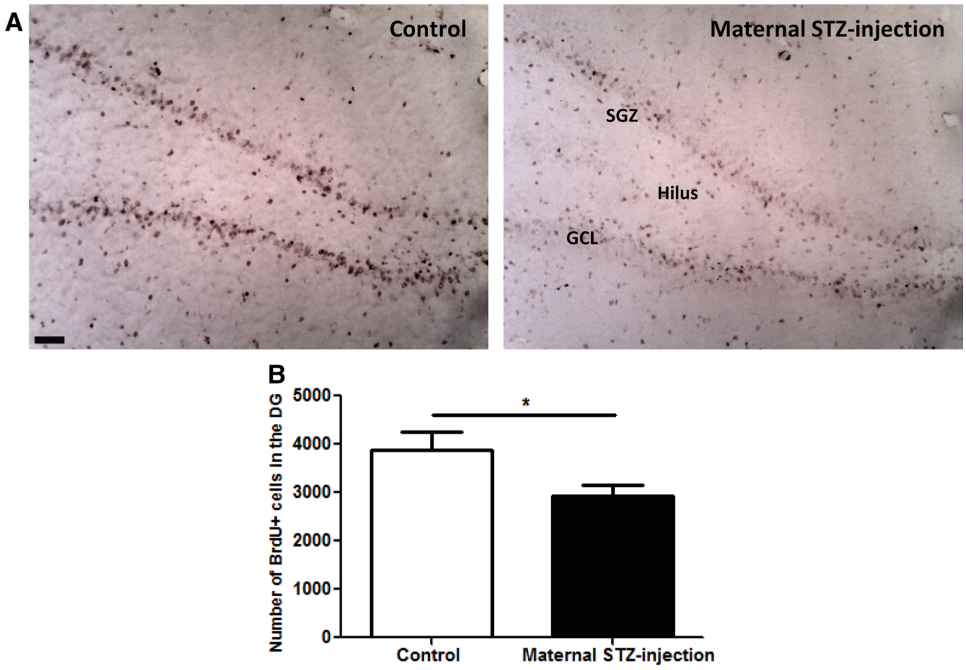

Fig. 6 Effects of maternal STZ-injection on survival of BrdU-labeled cells in the DG of the hippocampus on PND 40. **a** Digitalized images of the DG stained for BrdU showing cell survival in the SGZ and GCL between groups. **b** Quantitative analysis of the immunocytochemistry for BrdU in different groups. *DG* dentate gyrus, *GCL* granule cell layer, *SGZ* subgranular zone, *PND* postnatal day, *STZ* streptozotocin. $*p = 0.05$ (Unpaired Student's t test: $t(9) = 2.20$). Data were expressed as mean ± SEM ($n = 5$–6 male offspring per group). Scale bar = 50 µm

a non-significant statistical difference was observed in pup body weight and pachymetric measurements on PND1, as previously reported by Salazar García et al. (2015). Nevertheless, maternal STZ injection did not affect the average number of pups per litter. From PND 7 to 21, the somatic development compromise was more evident, showing the prenatal and postnatal effects of maternal STZ injection on the offspring. The continuation of the hyperglycemic status in the STZ-treated dams after parturition seemed to contribute to the reduced growth during suckle, and pups remained small up to youth (PND 40), as previously described by Chandna et al. (2015). Accordingly, the observed effects on the PND 40 may not be only due to the gestational/prenatal diabetes, but also to the malnutrition at early stages of offspring development, since that maternal type 1 diabetes can delay lactogenesis onset by affecting prolactin secretion and lactose, citrate, and total nitrogen concentrations in the milk (Hartmann and Cregan 2001; Neubauer et al. 1993; Ostrom and Ferris 1993). On the other hand, in the clinic, diabetic mother's breast-feeding is recommended and should be highly encouraged due to maternal benefits, including increased insulin sensitivity, and the decreased prolactin secretion can be reversed by increased breast-feeding frequency and by insulin administration (Feldman and Brown 2016).

As expected, the offspring of maternal STZ-injection group presented higher blood glucose levels on PND 1, similar to that observed in newborn babies of gestational diabetic mothers (Rudge et al. 2013). However, maternal STZ-injection group did not demonstrate change in glucose metabolism in the OGTT on PND 40, instead, it showed lower blood glucose levels during all the test points detected, compared to the control group. In accordance, Zhao and Weiler (2010) also did not detect glucose metabolism impairment in offspring of STZ-treated dams with glucose controlled or poorly controlled at 8 and 12 weeks of age. Notwithstanding, this situation changes later in life with the appearance of glucose intolerance at the age of 5 months in both female and male offspring of diabetic mothers (Kruse et al. 2014). In addition, the relative liver weight in the offspring on PND 1 was not affected by maternal prenatal hyperglycemia, although it was decreased in maternal STZ-injection group on PND 40. We did not aim to analyze metabolism; however, this finding may be related to long-term alterations in liver lipid accumulation or in glucose/insulin metabolism in the offspring, and the mechanisms should be investigated in future studies. Moreover, in our study, the relative thymus weight was increased in neonates of maternal STZ-injection group and persisted up to PND 40, but no differences were found in the relative adrenal weight of the offspring. The thymus is a primary lymphoid organ of lymphocytic maturation (Zdrojewicz et al. 2016). Thus, the increase of relative thymus weight observed in maternal STZ-injection group may be related to immunomodulation induced by severe maternal hyperglycemia in the offspring immune system pre- and postnatally.

As short-term consequences, severe maternal diabetes caused microsomia and neurodevelopmental delay in pups, accompanied by a significant decreasing in the expression of apoptosis-regulatory proteins (procaspase 3, caspase 3, and Bcl-2) and a non-significant increasing (20%) in the MHC-II protein in newborn developing brain on PND 1. On the other hand, the long-term effects were revealed by a decreasing in the expression of procaspase 3, caspase 3, MHC-I, IL-1β, and BDNF, and by an increasing in TNF-α levels, as well as an impairment in cellular survival in the DG of hippocampus in maternal STZ-injection group on PND40. This study complements the results found by Chandna et al. (2015), in which maternal diabetes created a proinflammatory state mediated by increased RAGE signaling and a non-significant increased relative expression of NF-kB in the brains of pups of STZ-treated dams on PND1, affecting the hippocampal excitability and offspring behavior later in life. Taken together, the data reinforce the neuroinflammatory bases underlying neurodevelopmental commitments in the offspring of diabetic mothers.

Developmental milestones play an important role in assessing the maturation of neonatal neurologic reflexes and serve as predictors of behavioral changes in adults (Marcuzzo et al. 2010; Stigger et al. 2013). In our study, the offspring of maternal STZ-injection group presented neurodevelopment delay in the cliff aversion task, auditory startle, activity, and eye opening, according to and complementing the cognitive impairments reported by the clinic (DeBoer et al. 2005; Ornoy et al. 2001; Ornoy 2005; Perna et al. 2015; Rizzo et al. 1995; Sells et al. 1994). Furthermore, male offspring were more affected than females by maternal diabetes in some tasks (see "Neonatal Developmental Evaluations"), like previously reported by Kruse et al. (2014). As long-term effects on the cognition, the offspring of maternal STZ-injection group did not show impairment in aversive memory retention at 1.5 h and 24 h of the training in the inhibitory avoidance test on PND40. Differently, Kim et al. (2014) demonstrated, with the same experimental model, impaired memory in step-down avoidance task on PND42. Nevertheless, the animals were evaluated later than ours, 48 h after the training session. Actually, we cannot affirm if the aversive memory deficit would manifest later in the life of the offspring of maternal STZ-injection group.

The apoptosis occurrence can be defined by the balance between pro- and anti-apoptotic regulators (Elmore 2007; Haghir et al. 2017). Bcl-2 is an anti-apoptotic protein of the Bcl-2 family, which regulates negatively caspase activation (Haghir et al. 2017). Nevertheless, in our study, a significant decrease in the expression of both Bcl-2, procaspase 3, and caspase 3 was observed on PND 1 and persisted until youth

(PND 40) in the offspring hippocampus of maternal STZ-injection group. Therefore, we postulate that maternal diabetes could induce disturbances in the process of physiological synaptic pruning in pups, which is especially important to the formation of neuronal networks during development (Elmore 2007). Our results are supported by Haghir et al. (2017) that also showed decreased levels of Bcl-2 protein in the hippocampus of rat neonates of diabetic mothers, but in contrast with other previous studies that reported non-significant changes in protein levels of procaspase 3 and caspase 3 (Kruse et al. 2012) or showed an increase in the expression of caspase 3 in rat embryos and pups exposed to maternal hyperglycemia (Kim et al. 2014; Reece et al. 2006). Besides, this imbalance in apoptotic pathway was accompanied by an impairment in cell survival phase of postnatal neurogenesis in the offspring hippocampus of maternal STZ-injection group on PND 40, possibly justifying the reduction in the number of granular cells in the DG of rat offspring's born to diabetic mothers previously reported by Kafshgiri et al. (2014). The decrease in the cell proliferation phase of hippocampal neurogenesis in rat offspring of severe diabetic mothers on PND 42 had already been described by Kim et al. (2014), while Sadeghi et al. (2018) demonstrated a significant down-regulation in neurogenesis by the expression of a specific neuronal nuclear protein (NeuN) in the pup developing hippocampus of diabetic mothers on PND14.

In fact, BDNF and immune system molecules can influence neural plasticity, adult hippocampal neurogenesis, and memory (Kohman and Rhodes 2013; Yirmiya and Goshen 2011). Even though IL-Iβ is considered a proinflammatory interleukin, normal/physiological IL-1β levels are required to adult hippocampal neurogenesis maintenance and play an important role in learning and memory processes, while high levels of BDNF promote/enhance neurogenesis, and high levels of TNF-α seem to be detrimental (Kohman and Rhodes 2013; Yirmiya and Goshen 2011). Moreover, other immune molecule that was also found to be important for normal neurodevelopment and synaptic functioning is MHC-I (Bilbo and Schwarz 2012; Yirmiya and Goshen 2011). Indeed, in the present study, the expression of MHC-I protein was decreased in the offspring hippocampus of maternal STZ-injection group on PND 40. These data fortify that a deregulation in the apoptosis levels, MHC-I signaling, and postnatal cell survival in the hippocampus, possibly through the modulation of cytokines and BDNF, may be also implicated in the offspring's neurodevelopmental and neurocognitive disorders of diabetic mothers.

This was the first study that reported microsomia and neurodevelopment delay, through analysis of the main developmental milestones, accompanied by apoptosis, cellular survival, and neuroinflammatory deregulation in rat offspring hippocampus of the maternal model of severe uncontrolled hyperglycemia. We call attention to that all the results observed in the offspring on PND 1 are due to maternal prenatal/gestational diabetes, whereas on PND 40, they are due to both maternal gestational diabetes and maternal postnatal nutrition. From this screening, some points should be emphasized for future studies: to create another group with insulin replacement in the diabetic dams to control blood glucose levels during pregnancy and/or after delivery to isolate the pre- and postnatal effects of hyperglycemia on the pup development, or use surrogate mothers to raise the pups of STZ-treated dams, and to study only the prenatal effects of untreated gestational hyperglycemia on offspring parameters.

Conclusion

Taken together, as an exploratory study, we showed alterations in various levels of biological organization in the offspring induced by maternal diabetes. There is a very strong data reinforcing the commitment of the progeny health caused to maternal hyperglycemia. The present study can contribute to the understanding of (1) the significant gestational risk of the hyperglycemia poorly controlled, (2) how it can reprogram the offspring brain and, more importantly, (3) consolidate prevention strategies, improving the management of prenatal and postnatal diabetes, as well as, (4) it can point to potential biological substrates to pharmacological and neurobehavioral interventions.

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Author Contributions All the authors listed have participated sufficiently in the study, in the research design, execution, and analyses reported in this manuscript. The responsibility for writing the article rests with FVP, MSK, HC, and SM, but every coauthor had the opportunity to review it and all approved the final version. All the authors have read and followed the ethical standards statement for manuscripts submitted to Cellular and Molecular Neurobiology.

Compliance with Ethical Standards

Conflict of interest The authors declare no conflicts of interest regarding the authorship and/or publication of this article.

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