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Prenatal stress and KCl-induced depolarization modulate cell death, hypothalamic-pituitary-adrenal axis genes, oxidative and inflammatory response in primary cortical neurons

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ABSTRACT

Maternal stress has been described as an important component in the offspring's cerebral development, altering the susceptibility to diseases in later life. Moreover, the postnatal period is essential for the development and integration of several peripheral and central systems related to the control of homeostasis. Thus, this study aimed to evaluate the effects of prenatal stress on the activation of cortical neurons, by performing experiments both under basal conditions and after KCl-induced depolarization. Female mice were divided in two groups: control and prenatal restraint stress. Cortical neurons from the offspring were obtained at gestational day 18. The effects of prenatal stress and KCl stimulations on cellular mortality, autophagy, gene expression, oxidative stress, and inflammation were evaluated. We found that neurons from PNS mice have decreased necrosis and autophagy after depolarization. Moreover, prenatal stress modulated the HPA axis, as observed by the increased GR and decreased 5HTr1 mRNA expression. The BDNF is an important factor for neuronal function and results demonstrated that KCl-induced depolarization increased the gene expression of BDNF I, BDNF IV, and TRκB. Furthermore, prenatal stress and KCl treatment induced significant alterations in oxidative and inflammatory markers. In conclusion, prenatal stress and stimulation with KCl may influence several markers related to neurodevelopment in cortical neurons from neonate mice, supporting the well-known long-term effects of maternal stress.

1. Introduction

Maternal stress has been described as an important component in the offspring's cerebral development, altering the susceptibility to physiological and neurobehavioral changes in later life (Harris and Seckl, 2011). The hypothalamic-pituitary-adrenal (HPA) axis regulates the production and secretion of corticosteroids in response to stress. The release of glucocorticoids is mainly controlled by a negative feedback mechanism through the activation of glucocorticoid (GR) and mineralocorticoid (MR) receptors (de Kloet et al., 2005). However, studies have demonstrated that excessive exposure to maternal glucocorticoids, caused by an adverse prenatal environment, promotes a reduction in the expression of GR and MR, enhancing HPA axis activity (Seckl, 2004). In many species, the perinatal and postnatal periods are characterized by intense neurotransmitter and neuromodulatory system maturation. These processes are regulated by the action of glucocorticoids, which are important for fetal development, although when excessively stimulated may be prejudicial to both fetal growth and maturation (Walker et al., 2004). Although less studied, evidence has shown that prenatal stress promotes a decrease in birth weight (Choe et al., 2011), reduction in cell proliferation in the brain (Lemaire et al., 2006; Van den Hove et al., 2006) and alterations in dendritic development (Bock et al., 2011) in

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neonatal rodents. Thus, the postnatal period is essential for the development and integration of several peripheral and central systems related to the control of offspring homeostasis.

The fetal "programming" hypothesis proposes that an adverse intrauterine environment during critical periods of organogenesis and tissue growth could permanently alter organ structure and function (Barker et al., 1989). This concept has been used widely to explain the associations between prenatal environmental events, altered fetal growth and disease development (Harris and Seckl, 2011). Therefore, altered expression of GR and MR, in addition to influencing the control of the stress response, may also play an important role in neuronal homeostasis (Anacker et al., 2013), along with several other factors. For example, the rodent gene brain-derived neurotrophic factor (BDNF) is a compound of nine 5′ non-coding exons and a 3' protein-coding region, essential for neuronal survival, growth and differentiation, acting through its receptor tropomyosin receptor kinase B (TRκB) (Aid et al., 2007). In immature neurons, the exposure to glucocorticoids decreases the BDNF gene expression (Kino et al., 2010) and inhibits the synaptic formation and BDNF-dependent dendritic growth (Kumamaru et al., 2008). Similarly, serotonin (5HT) is a neurotransmitter that acts as a brain growth factor during brain development (Brummelte et al., 2017). Accumulating evidence indicates a direct association between changes in the HPA axis and the expression of 5HT (Ancelin et al., 2017; Goel et al., 2014; Sorenson et al., 2013), including altered development of 5HT neurons induced by prenatal stress in mice (Miyagawa et al., 2015). Moreover, the exposure to restraint stress during pregnancy decreases the serotonergic metabolism in the cortex of adult mice (Gur et al., 2019).

Preclinical studies have already identified an important relationship between prenatal stress and the brain activation of the immune system (Enayati et al., 2020; Sowa et al., 2017; Zhang et al., 2016) and oxidative stress response (Song et al., 2009; Zhu et al., 2004), contributing to the development of neurological diseases throughout life. The activation of an immunosuppressive phenotype is characterized by the increased exposure to glucocorticoids, promoting an imbalance in the levels of inflammatory pathways, lipid peroxidation and antioxidant defenses (Coutinho and Chapman, 2011).

Studies with different prenatal stress protocols have been used to demonstrate the long-term changes in the offspring's HPA axis regulation and behavior. However, to date, very few studies investigated the effects of maternal stress exposure in the neonatal brain. Working towards this goal, we have tested the effects of prenatal stress on cellular mortality, autophagy, expression of HPA axis genes, oxidative stress, and inflammation in primary cortical neurons. Moreover, we have also evaluated its effects on the activation of cortical neurons, by performing experiments both under basal conditions and after KCl exposure. KCl is known to promote membrane depolarization and to activate transcriptional factors, allowing us to evaluate the effects of depolarization on activity-dependent gene regulation and biochemical changes.

2. Materials and methods

2.1. Animals

Male and female Balb/c mice (8 weeks of age) were obtained from the Center for Experimental Biological Models (CeMBE) from the Pontifical Catholic University of Rio Grande do Sul (PUCRS). Mice were kept in an environment with controlled temperature (22 ± 2 °C), 12h/12 h light-dark cycle, and with free access to water and food. All animals were kept in the vivarium without any intervention for at least 10 days prior to the beginning of the experimental protocols. For the study, the estrous cycle was monitored and, on the night of the pro-estrus, two females and one male were mated. Pregnant mice were divided into two experimental groups: control (CON, $n = 6$ dams) and prenatal stress (PNS, $n = 5$ dams). In the first day of gestation (G1), male mice were moved to another cage and pregnant females were kept together until the gestational day 8 (G8).

All animals were maintained according to the guidelines of the Brazilian Association for Laboratory Animal Sciences and the study was approved by the University Research Ethics Committee for Animal Use (protocol number 8465).

2.2. Prenatal stress

Prenatal stress was performed from G8, every other day, until G18. Briefly, females were kept immobile in a ventilated crystal acrylic container (Insight, Brazil) for 30 min. The restraint stress protocol was performed between 10 h and 12 h and the control group (CON) was not submitted to any intervention during pregnancy (Luft et al., 2020).

2.3. KCl-induced depolarization in primary cortical neuron culture

At G18, pregnant females were euthanized under sterile conditions and offspring (embryonic day 18) cortical neurons were dissociated in 0.025% trypsin at 37 °C for 5 min. For all experiments, cells were plated (5×10^5 per well) on six-well plates coated with Poly-D-lysine (Sigma-Aldrich, USA), in 2 mL Neurobasal (Gibco, Life-technologies, USA) medium with 2% B27 supplement (Invitrogen, USA), penicillin-streptomycin (Invitrogen, USA) and L-glutamine 2 mM (Invitrogen, USA). Primary cortical neurons were maintained at 37 °C in a humidified incubator at 5% CO_2 for 7 days *in vitro*. On days 3 and 5, 1 mL of medium was changed for fresh medium. In order to evaluate the neuronal activity, cortical neurons were treated with 50 mM KCl for 7 h on day 7 and collected for the analyzes, following a previous protocol (Ratnu et al., 2014).

2.4. Cell death

Apoptosis and necrosis were analyzed in the primary cortical neurons using Annexin-V and Propidium Iodide (PI), respectively, according to the manufacturer's instructions (QuatroG, Porto Alegre, Brazil). Briefly, neurons were washed with ice-cold phosphate-buffered saline (PBS) and stained with Annexin V-FITC and PI, at room temperature away from light for 10 min. Cell death was assessed by flow cytometry (FACS Canto II, BD Bioscience, USA) and analyzed with FlowJo software v. 7.2.5 (Tree Star Inc., USA).

2.5. Autophagy

Autophagy was investigated by Acridine Orange staining (Sigma-Aldrich, USA), an autophagic marker of acidic vacuolar organelles (AVO's). Briefly, cells were washed with ice-cold PBS and stained with Acridine Orange staining (500 μg/mL) for 15 min at room temperature. In order to quantify the percentage of AVO's and the intensity of orange fluorescence, the Acridine Orange stained cells were assessed by flow cytometry (FACS Canto II, BD Bioscience, San Jose, CA). Data were analyzed with FlowJo software v. 7.2.5 (Tree Star Inc., Ashland, OR).

2.6. mRNA levels

Total RNA was extracted by the Trizol method (ThermoFisher – Scientific, USA) and converted to complementary deoxyribonucleic acid (cDNA) (GoScript™ Reverse Transcription System Protocol – Promega, USA), according to the protocol specified by the manufacturer. The cDNA concentration was measured using the NanoDrop spectrophotometer (Model 1000, Thermo Scientific). Messenger RNA (mRNA) gene expression was performed in real-time quantitative PCR (Step One Plus - Applied Biosystems) using SYBR® Green fluorescence marker (ThermoFisher – Scientific, USA). A total of 64 ng of cDNA from each sample was used. The samples were prepared in duplicate and the relative expression of mRNA was calculated by the Delta-Delta Ct method (ΔΔCt). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was

used as the reference endogenous gene. Table 1 shows the set of specific primers for each gene.

2.7. Oxidative stress

In order to evaluate the oxidative stress parameters, the supernatant was immediately separated and used for the measurements of catalase (CAT), reduced glutathione (GSH), and thiobarbituric acid reactive substance (TBARS).

2.7.1. Catalase assay (CAT)

Catalase converts H_2O_2 into water and oxygen. For the analysis, 102 μL of H_2O_2 30% and 100 μL of Triton X-100 was added to 10 μL of the cells supernatant and read at 240 nm in a semi-automatic spectrophotometer (Genesis 8/Spectronic). One CAT unit is defined as 1 μmol of H_2O_2 consumed per minute and the results were represented as CAT units/500.000 cells.

2.7.2. Reduced glutathione (GSH)

Reduced glutathione reacts with the superoxide radical (O_2), producing an increase in oxygen consumption and formation of oxidized glutathione, both inhibited by superoxide dismutase. For the test, 250 μL of acid metaphosphoric was added to 50 μL of cells supernatant and centrifuged. After, 650 μL of Na_2HPO_4 and 100 μL of the color reagent (5,5′-Dithiobis-2-Nitrobenzoic Acid) were added to 250 μL of the supernatant. The compound formed was measured in a semi-automatic spectrophotometer at 412 nm (Genesis 8/Spectronic).

2.7.3. Thiobarbituric acid reactive substance (TBARS)

TBARS is induced by lesions in the cell membrane and leads to the formation of malondialdehyde and other substances. For the test, 10 μL of the cell supernatant was collected and added to 10 μL of sodium dodecyl sulfate (SDS 12,4 mM) and 400 μL of thiobarbituric acid. The mixture was heated for 30 min and centrifuged at 750 g for 10 min at 25 °C. The compound formed was measured in a semi-automatic spectrophotometer at 532 nm (Genesis 8/Spectronic).

Table 1
Primer sequences for real-time PCR analysis.

Gene	Primer sequences
5HTr1	Forward: 5′ GCGTTGTTGGGTGCCATAAT 3′ Reverse: 5′ CCGGATTGAGCAGGGAGTT 3′
BDNF I	Forward: 5′ GCGTTGAGAAAGCTGCTTCAG 3′ Reverse: 5′ GAATGAGCGAGGTTACCAATGA 3′
BDNF IV	Forward: 5′ GCAGCTGCCTTGATGTTTAC 3′ Reverse: 5′ CCGTGGACGTTTACTTCTTTC 3′
GAPDH	Forward: 5′ GGGGAGCCAAAAGGGTCATC 3′ Reverse: 5′ GACGCCTGCTTCACCACCTTCTTG 3′
GR	Forward: 5′ GGAATAGGTGCCAAGGGTCT 3′ Reverse: 5′ GAGCACACCAGGCAGAGTTT 3′
IL-6	Forward: 5′ TGGAGTCACAGAAGGAGTGGCTAAG 3′ Reverse: 5′ CTGACCACAGTGAGGAATGTCCAC 3′
IL-1β	Forward: 5′ GCCCATCCTCTG TGACTCAT 3′ Reverse: 5′ AGGCCACAGGTATTTTGTCG 3′
MR	Forward: 5′ CCAGTTCTCCGTTCTCTGTA 3′ Reverse: 5′ CTTGAGCACCAATCCGGTAG 3′
TNF-α	Forward: 5′ ATAGCTCCCAGAAAAGCAAGC 3′ Reverse: 5′ CACCCCGAAGTTCAGTAGACA 3′
TRκB	Forward: 5′ TGGTGCATTCCATTCACTGT 3′ Reverse: 5′ CGTGGTACTCCGTGTGATTG 3′

5HTr1: serotonin receptor 1; BDNF I: brain-derived neurotrophic factor exon I; BDNF IV: brain-derived neurotrophic factor exon IV; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; GR: glucocorticoid receptor; IL-6: interleukin-6; IL-1β: interleukin-1β; MR: mineralocorticoid receptor; TNF-α: tumor necrosis factor-α; TRκB: tropomyosin receptor kinase B.

2.8. Statistical analysis

The normality of data was verified using the Shapiro-Wilk test. Outliers were excluded from analyses. Data were expressed using mean and standard error of the mean (SEM). Comparisons between groups were performed using two-way ANOVA followed by the Fisher's LSD post-test. Effect sizes were also calculated and values are presented as partial eta squared (η_p^2), and categorized as small (0.01), moderate (0.06), and large (0.14). In all cases, the level of significance was set at 5% ($p \leq 0.05$). Data were analyzed and graphs were made using both Prism GraphPad (version 8.0.1, GraphPad Software Inc., USA) and SPSS version 18.0 (SPSS Inc., USA).

3. Results

3.1. Prenatal stress and KCl-induced depolarization decreases necrosis in primary cortical neurons

Possible effects of stress and depolarization on neuronal cell death were evaluated through flow cytometry (Fig. 1). A significant main effect of both prenatal stress ($F_{(1,8)} = 11.970$; $p = 0.008$; $\eta_p^2 = 0.59$) and KCl ($F_{(1,8)} = 13.160$; $p = 0.006$; $\eta_p^2 = 0.62$) was observed, indicating a decrease on necrotic cells (Fig. 1B). In addition, although significance was not reached ($F_{(1,8)} = 3.611$; $p = 0.093$; $\eta_p^2 = 0.31$), a large effect size was seen for increased apoptosis as an effect of prenatal stress (Fig. 1C). No significant differences in late apoptosis were found (Fig. 1D).

3.2. Acidic vesicular organelles are altered in prenatally stressed primary cortical neurons

The effects of early-life stress and depolarization in cellular autophagy was evaluated using acridine orange staining. Two-way ANOVA revealed a significant effect for both prenatal stress ($F_{(1,8)} = 5.393$; $p = 0.048$; $\eta_p^2 = 0.40$) and KCl treatment ($F_{(1,8)} = 6.894$; $p = 0.030$; $\eta_p^2 = 0.46$) on autophagy (Fig. 2B). Large effect sizes indicating decreased autophagy were shown for both prenatal stress and KCl treatment.

3.3. Prenatal stress influences HPA axis regulation in primary cortical neurons

The effects of prenatal stress and the influence of KCl treatment on HPA axis markers gene expression were investigated. A significant effect of prenatal stress indicating increased GR mRNA ($F_{(1,15)} = 6.319$; $p = 0.023$; $\eta_p^2 = 0.30$) expression was observed (Fig. 3A). No significant effects for MR mRNA expression were found (Fig. 3B).

3.4. Prenatal stress decreases 5HTr1 in primary cortical neurons

A significant effect of prenatal stress on serotonin receptor 1 - 5HTr1 - ($F_{(1,15)} = 8.748$; $p = 0.009$; $\eta_p^2 = 0.37$) gene expression was observed (Fig. 4A). Large effect sizes indicating decreased 5HTr1 (Fig. 4A) in PNS neurons were shown.

3.5. BDNF and TRκB mRNA expression increases in an activity-dependent manner

Two-way ANOVA revealed a significant effect of KCl treatment on BDNF I ($F_{(1,17)} = 8.288$; $p = 0.010$; $\eta_p^2 = 0.33$), BDNF IV ($F_{(1,18)} = 14.430$; $p = 0.001$; $\eta_p^2 = 0.45$), and TRκB ($F_{(1,18)} = 10.860$; $p = 0.004$; $\eta_p^2 = 0.38$) gene expression (Fig. 4B, C and 4D, respectively). No significant effects for prenatal stress were found.

3.6. Prenatal stress increases antioxidant defense in primary cortical neurons

The effects of prenatal stress on the oxidative response in neuronal

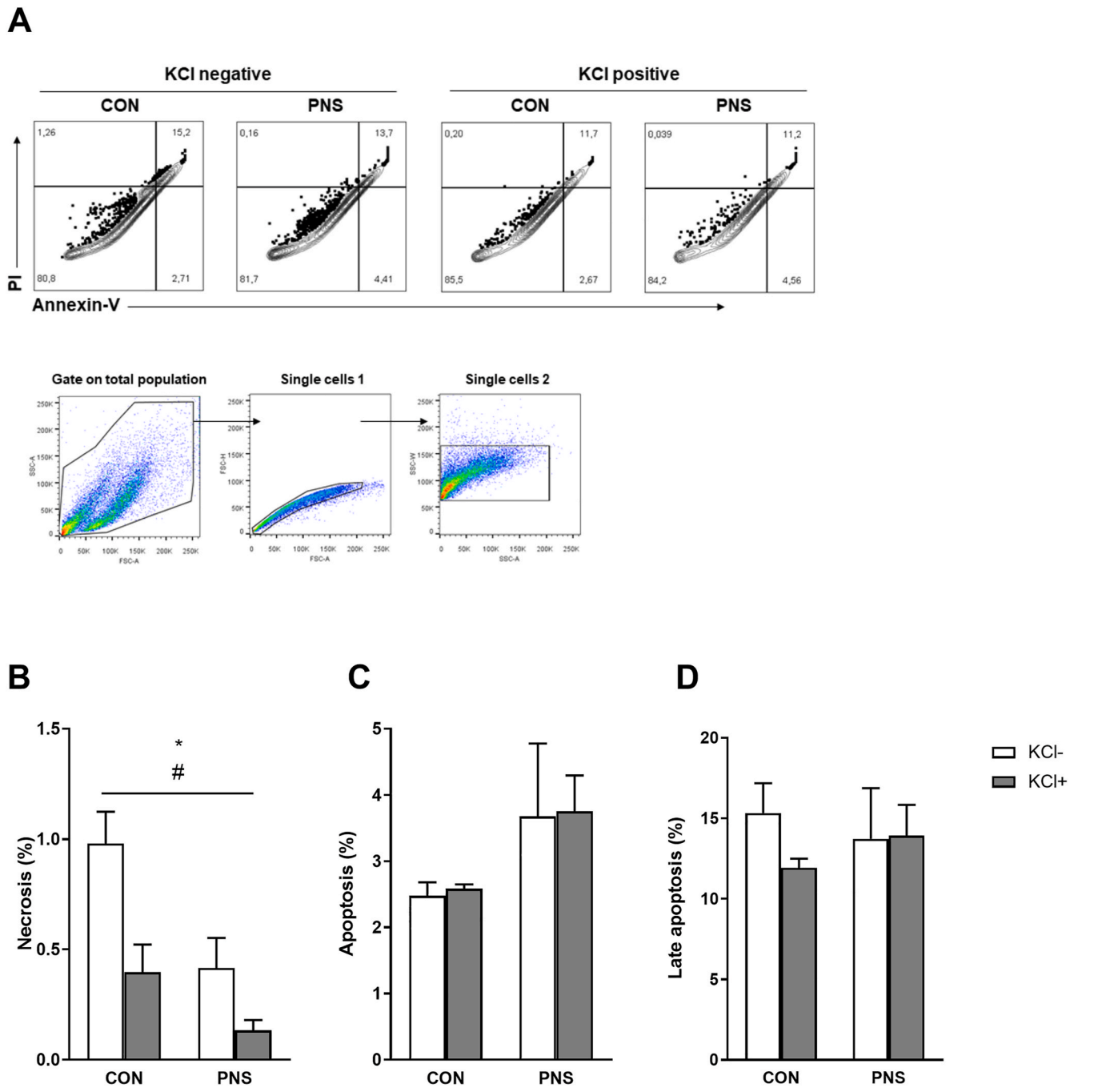

Fig. 1. Analyses of cell death in primary cortical neurons. Representative flow cytometric plots of CON and PNS at baseline and following KCl-induced depolarization (A). The lower left quadrant represents the negative cell cluster (Annexin V-/PI-, live cells), the upper left quadrant represents the positive cell population for one parameter (Annexin V+/PI-, apoptosis), the lower right quadrant represents cells positive for the second parameter (Annexin V-/PI+, necrosis), and the upper right quadrant (Q2) represents cells that express both parameters (Annexin V+/PI+, late apoptosis). Data are expressed as a percentage of necrosis (B), apoptosis (C), and late apoptosis (D). Results are shown as mean and standard error of the mean and were analyzed using two-way ANOVA. $^{*}p < 0.05$ indicates significant main effect of prenatal stress; $^{\#}p < 0.05$ indicates significant main effect of KCl. CON: control; PNS: prenatal restraint stress.

cell culture supernatant were evaluated. Results have shown a significant main effect of prenatal stress on GSH content ($F_{(1,18)} = 8.429$; $p = 0.009$; $\eta_p^2 = 0.32$) with a large effect size indicating increased GSH (Fig. 5B). No significant differences were found for CAT (Fig. 5A) and TBARS (Fig. 5C).

3.7. Prenatal stress alters the inflammatory response in primary cortical neurons

The inflammatory response in cortical neurons was also explored. Two-way ANOVA revealed a significant effect for KCl treatment ($F_{(1,13)} = 8.084$; $p = 0.013$; $\eta_p^2 = 0.38$) on the IL-6 mRNA expression (Fig. 6A), indicating a reduction after stimulation with KCl. Although significance was not reached ($F_{(1,15)} = 3.767$; $p = 0.071$; $\eta_p^2 = 0.28$), a large effect size was seen for increased TNF-α as an effect of prenatal stress (Fig. 6B). Moreover, data showed a significant interaction between prenatal stress and KCl treatment ($F_{(1,14)} = 5.014$; $p = 0.041$; $\eta_p^2 = 0.26$) on IL-1β mRNA expression (Fig. 6C). Post-hoc analysis revealed a decrease in IL-1β gene expression ($p = 0.056$) of PNS KCl^+ primary cortical neurons when compared to control KCl^+ (Fig. 6C).

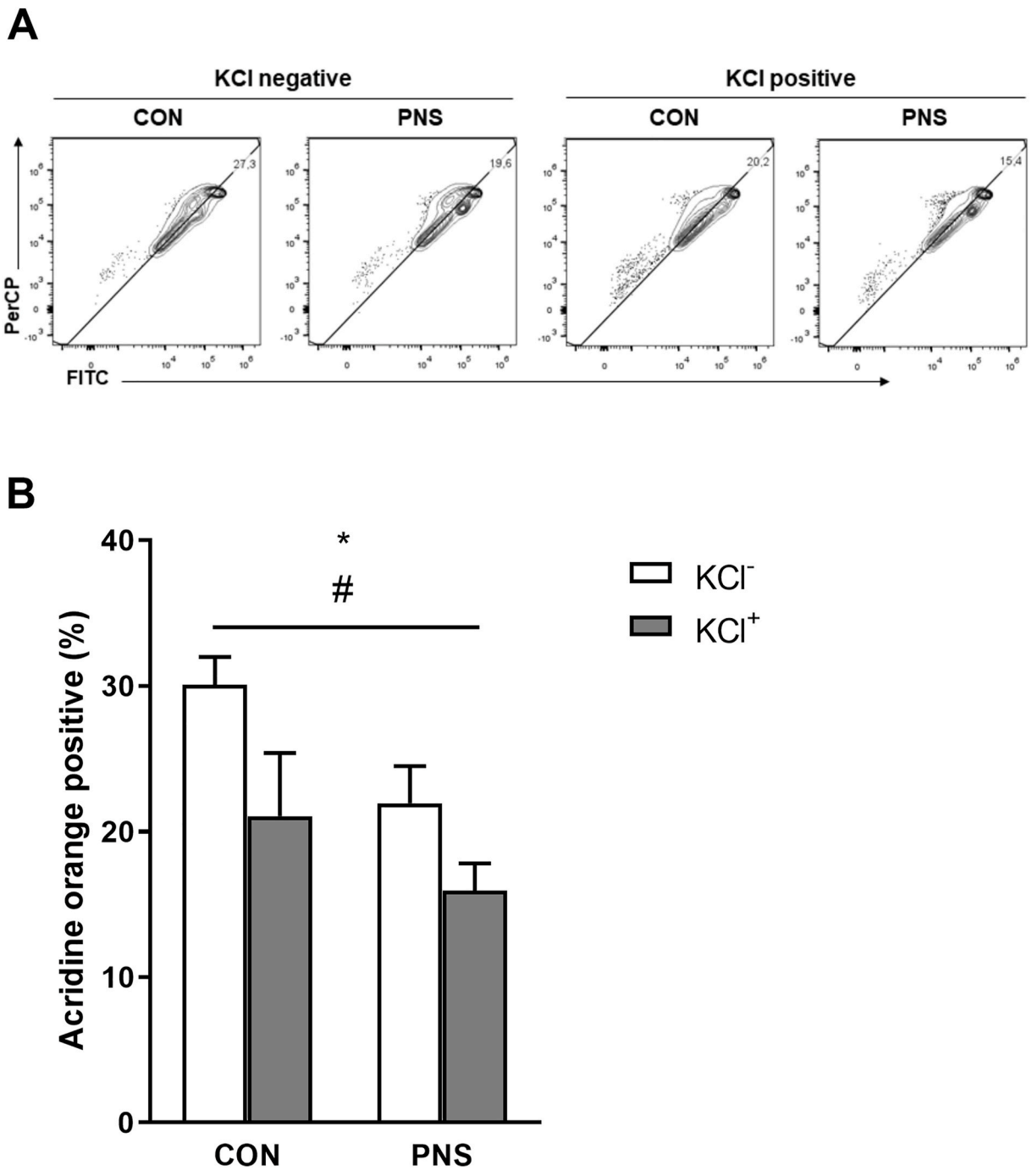

Fig. 2. Analyses of acidic compartments in primary cortical neurons. Representative flow cytometric plots from CON and PNS at baseline and following KCl-induced depolarization (A). Percentage of acridine orange positive cells (B). Results are shown as mean and standard error of the mean and were analyzed using two-way ANOVA. $^{*}p < 0.05$ indicates significant main effect of prenatal stress; $^{\#}p < 0.05$ indicates significant main effect of KCl. CON: control; PNS: prenatal restraint stress.

4. Discussion

Prenatal stress promotes permanent physiological changes increasing the susceptibility of the offspring to develop neurological diseases throughout life. Particularly, our group has demonstrated that prenatal stress promotes long-term effects on anxiety (Luft et al., 2020) and memory behavior (Luft et al., 2021) in adult mice. However, the evaluation of maternal stress and its effects on specific cells, such as cortical neurons, during the neonatal period have been little explored. The present study has shown, for the first time, the effects of prenatal stress on post-mitotic cortical neurons from embryonic mice and their activity-dependent responses.

Our results have shown that stress *in utero* is able to alter neuronal cell death in the offspring. Cell death and its regulatory mechanisms are essential to maintain homeostasis and neuronal circuitry throughout life (Fricker et al., 2018), although excessive cell death has been linked to neurodegenerative diseases (Caccamo et al., 2017; Guo et al., 2012). The present data revealed a decrease in neuronal necrosis and a trend towards increased cell death by apoptosis in the cortical neurons from animals stressed *in utero*. This effect may be a result of several compensatory mechanisms involved in the neuronal response to stress. Prenatal stress has been associated with changes in the expression of pro

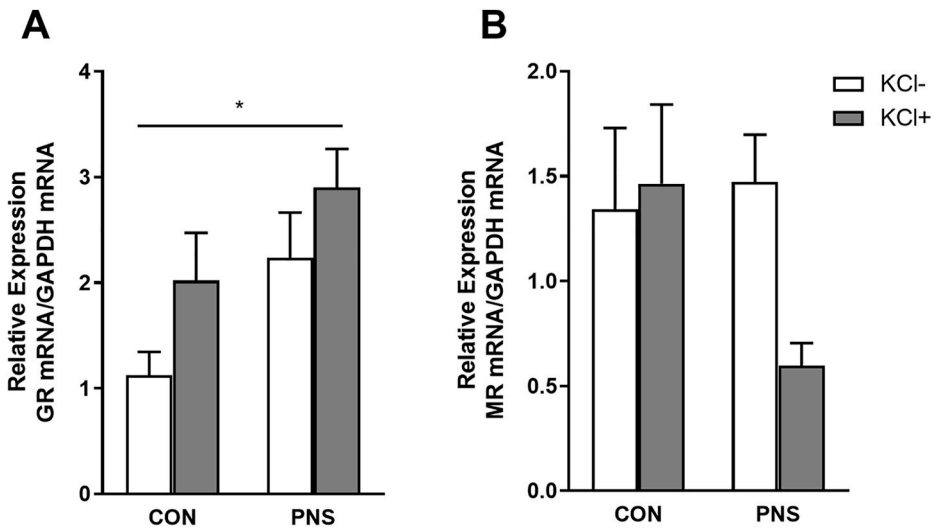

Fig. 3. GR and MR mRNA expression in primary cortical neurons. The gene expression of GR (A) and MR (B) were assessed at baseline and following KCl-induced depolarization. Results are shown as mean and standard error of the mean and were analyzed using two-way ANOVA. $^{*}p < 0.05$ indicates significant main effect of prenatal stress. GR: glucocorticoid receptor; MR: mineralocorticoid receptor; CON: control; PNS: prenatal restraint stress.

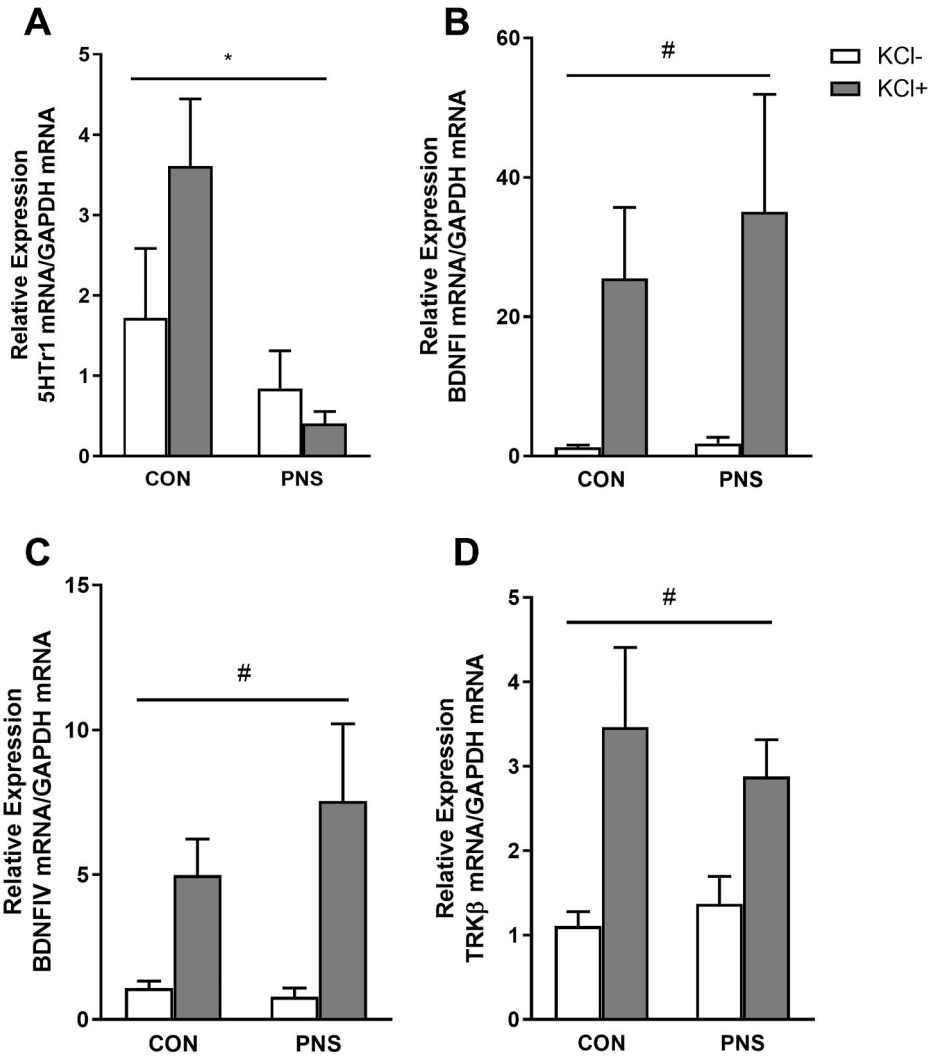

Fig. 4. 5HTr1, BDNF I, BDNF IV, and TRκB mRNA expression in primary cortical neurons. The gene expression of 5HTr1 (A), BDNF I (B), BDNF IV (C), and TRκB (D) were assessed at baseline and following KCl-induced depolarization. Results are shown as mean and standard error of the mean and were analyzed using two-way ANOVA. $^{*}p < 0.05$ indicates significant main effect of prenatal stress. $^{\#}p < 0.05$ indicates significant main effect of KCl. 5HTr1: serotonin receptor 1; BDNF I: brain-derived neurotrophic factor exon I; BDNF IV: brain-derived neurotrophic factor exon IV; TRκB: tropomyosin receptor kinase B; CON: control; PNS: prenatal restraint stress.

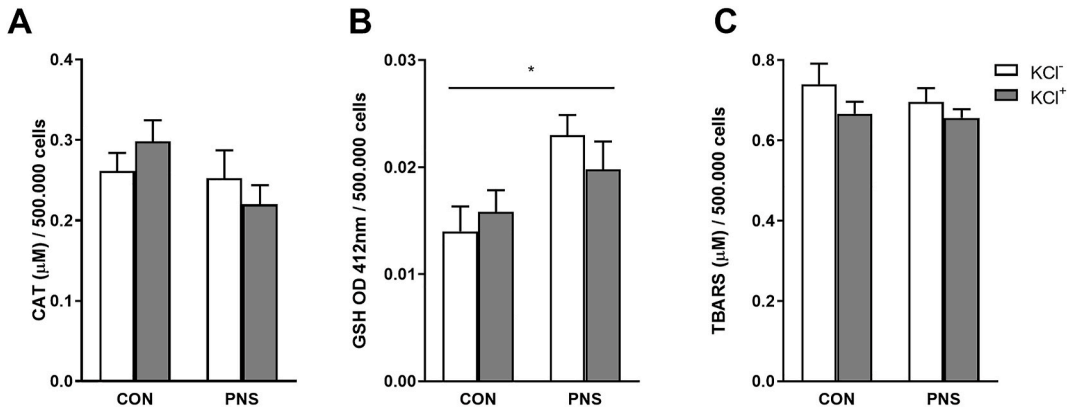

Fig. 5. Evaluation of oxidative stress markers in primary cortical neurons. CAT (A), GSH (B), and TBARS (C) content were assessed at baseline and following KCl-induced depolarization. Results are shown as mean and standard error of the mean and were analyzed using two-way ANOVA. $^{*}p < 0.05$ indicates significant main effect of prenatal stress. CAT: catalase; GSH: reduced glutathione; TBARS: thiobarbituric acid reactive substance; CON: control; PNS: prenatal restraint stress.

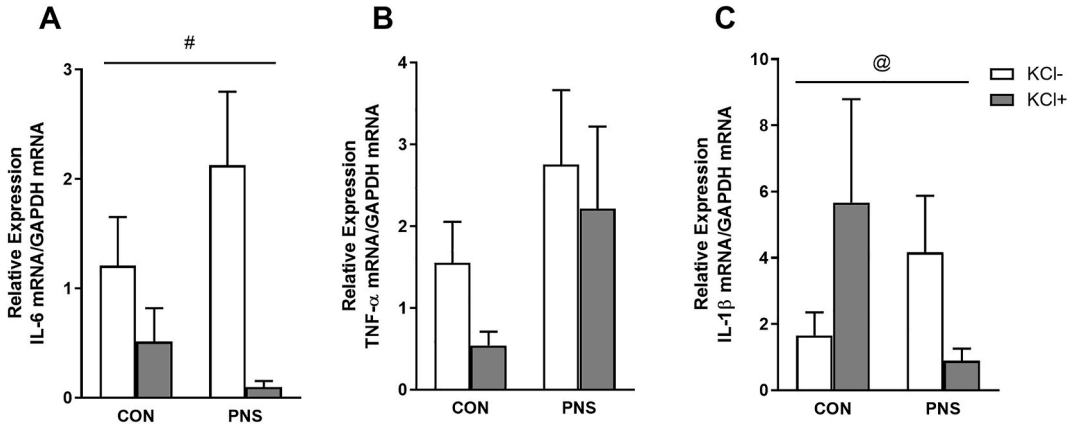

Fig. 6. IL-6, TNF-α, and IL-1β mRNA expression in primary cortical neurons. The gene expression of IL-6 (A), TNF-α (B), and IL-1β (C) were assessed at baseline and following KCl-induced depolarization. Results are shown as mean and standard error of the mean and were analyzed using two-way ANOVA. $^{\#}p < 0.05$ indicates significant main effect of KCl; $^{@}p < 0.05$ indicates significant interaction between prenatal stress and KCl treatment. IL-6: interleukin-6; TNF-α: Tumor necrosis factor α; IL-1β: interleukin-1β; CON: control; PNS: prenatal restraint stress.

and anti-apoptotic proteins in the brain (Kim et al., 2015) and neuronal apoptosis has previously been linked to the development of altered behavior in rodents (Bachis et al., 2008). Autophagy is another essential mechanism for neuronal survival, as it is involved in the degradation of cellular components to maintain cellular homeostasis (Kulkarni et al., 2018). Our findings showed that PNS and KCl-stimulated neurons have decreased acidic vesicular organelles, which may indicate a possible attenuation in lysosomal content and, thus, autophagy. The effects of stress on autophagy are controversial and dependent on the model and brain region evaluated. In a prenatal stress model, adolescent rats presented increased autophagy in the hippocampus (Zhang et al., 2017). In contrast, chronic stress decreased autophagy in the cortex of adult mice in association with depressive-like behavior (Zhou et al., 2021). In addition, studies have shown that autophagy has a pro-survival function and has been associated with increased longevity in animals (Nixon and Yang, 2012). The impairment of autophagy during the neonatal period may influence prenatal stress programming mechanisms, resulting in deficiency of neuroprotective cellular functions.

Maternal stress has been largely related to the expression of GR and MR in the brain of adult offspring, contributing to an attenuated negative feedback response controlled by glucocorticoids and, consequently, increased HPA axis activity and cortisol/corticosterone levels (Seckl, 2004). As a result of altered HPA axis regulation after early life stress, brain development is impaired at cell differentiation and maturation levels, impacting neural and behavioral responses (van Bodegom et al., 2017). Moreover, studies *in vitro* have demonstrated that GR impairment may contribute to altered cell proliferation and differentiation (Anacker et al., 2013; Kim et al., 2004). Our findings in neonates demonstrate a significantly increased GR gene expression in cortical neurons exposed to prenatal stress. This increase was independent of neuronal activation with KCl. The cortical region is a site of high GR expression and alterations in this receptor could influence the development of neuronal dysfunctions (Herman, 1993). However, to the best of our knowledge, there is no data reporting the effects of stress during pregnancy on this brain region in neonates. GR are widely distributed throughout the brain and, when glucocorticoid levels increase in response to a stressful event, it saturates MR and promotes GR activation (De Kloet et al., 1998; Reul and de Kloet, 1985). It is possible that an extensive binding to GR as a consequence of the exposure to maternal glucocorticoids may be upregulating its expression, resulting in the increase seen in our findings.

5HT is a neurotransmitter that participates in fetal and neonatal neurodevelopment (Hanswijk et al., 2020) through its receptor 1 (5HTr1), which has been implicated in behavioral changes related to cognition and mood (Drevets et al., 2000; Elvander-Tottie et al., 2009). Adult rodents submitted to prenatal restraint stress have decreased serotonin levels in both hippocampus (Soares-Cunha et al., 2018) and whole brain (Enayati et al., 2020), which is associated with depression and anxiety. Likewise, in rats, early life exposure to exogenous corticosteroids has been shown to alter the expression of tryptophan hydroxylase 2 (TPH2), which plays a key role in regulating the

serotonergic neurotransmission (Hiroi et al., 2016). Our findings also revealed a decreased 5HTr1 gene expression in stimulated and unstimulated primary cortical neurons submitted to prenatal stress, in an similar effect as demonstrated by (Akatsu et al., 2015). Taken together, these results suggest that maternal stress is able to program the synthesis of serotonin in the cortex during the embryonic period, which could be associated to an increased risk of developing neuropsychiatric diseases throughout life.

Similar to the serotonergic system, BDNF performs several essential functions for neuronal homeostasis (Kowianski et al., 2018). The induction of depolarization by KCl stimulates the action potential and induces neuronal activity (Sohya et al., 2007). As previously demonstrated (Ratnu et al., 2014), this stimulation of cortical neurons induces an increase in BDNF exons I and IV, in addition to its receptor TRκB, showing an activity-dependent gene expression. Moreover, our study demonstrated that exposure to glucocorticoids *in utero* did not alter this response. In a study *in vitro* with cortical neurons, it was shown that the administration of aldosterone (MR agonist) increases BDNF mRNA/protein expression, while treatment with dexamethasone (GR agonist) promotes BDNF reduction (Kino et al., 2010). Direct exposure to corticosteroids may lead to decreased BDNF expression in the hippocampus, and prefrontal cortex of adult rats, as well as in primary cortical neurons (Donoso et al., 2019; Wu et al., 2018). The decreased mRNA expression of BDNF exons I and IV in the frontal cortex has been associated with reduced social interaction in prenatally stressed mice during adulthood (Dong et al., 2015). These previous findings on BDNF demonstrate that dysregulation of the HPA axis, via the participation of corticosteroid receptors, may influence essential neuronal functions, such as survival and synaptic plasticity (Aid et al., 2007). Although the exons I and IV have an activity-dependent modulation (Metsis et al., 1993), the lack of differences in our study between prenatal stress and the control group indicates that these effects may be multifactorial and dependent on species, age and experimental model used.

Our findings have also demonstrated that maternal stress may alter antioxidant defenses in the neonatal offspring, as an increase in the GSH levels from cortical neurons was observed, with no effects of KCl stimulation. Studies demonstrate an important role for GSH in cell proliferation and apoptosis (Armstrong et al., 2004; Shih et al., 2006), and its imbalance has been associated with reactive oxygen species (ROS) and development of neuropsychiatric and neurodegenerative disorders (Johnson et al., 2012; Rosa et al., 2014). Conversely, the activation of the HPA axis and subsequent exposure to glucocorticoids has already been linked to an attenuation of GSH levels in both the whole brain (Samarghandian et al., 2017) and frontal cortex (Atif et al., 2008) from adult rodents submitted to restraint stress. In the embryonic brain mouse, a study has shown that prenatal stress disrupted glutathione levels (Bittle et al., 2019). However, little from these effects is known during early developmental stages, and our data shows a possible primary compensatory response to restore GSH levels and reduce cellular damage. Nevertheless, further studies are needed to elucidate the mechanisms related to this hypothesis.

The immune system has been also connected to stress and the modulation of neuroendocrine systems (Menard et al., 2017). Cortical depolarization with KCl has been shown to induce the expression of pro-inflammatory genes. For example, a study reported that depolarization during 4 h increases the expression of IL-1β mRNA (Jander et al., 2001). Conversely, our data has demonstrated a decreased IL-6 gene expression in cortical neurons after depolarization. Moreover, PNS stimulated neurons showed decreased IL-1β mRNA expression compared to the stimulated controls. It is possible that stress during pregnancy may alter the effects of exposure to KCl and attenuate the transcription of genes related to cytokine signaling, such as IL-1β, although further studies are needed to investigate the mechanisms involved in this response. It is well established that stress increases central and peripheral levels of pro-inflammatory cytokines (Hantsoo et al., 2019). Preclinical findings in adult animals exhibited an increase in IL-1β, IL-6 and TNF-α in the hippocampus and frontal cortex, which was associated with depressive-like behavior in prenatally stressed animals (Diz-Chaves et al., 2012; Szczesny et al., 2014). Interestingly, as the immune response differs between neonates and adults, during brain development, cytokines are important mediators to neural and synaptic maturation (Deverman and Patterson, 2009). IL-1β promotes cell migration (Ma et al., 2014) and differentiation (Park et al., 2018), and is an astroglial growth factor during neurodevelopment. Likewise, during development, studies have shown that IL-6 has neurotrophic properties in order to promote tissue repair (Gadient and Otten, 1997), in addition to participating in neural growth. Therefore, a decrease in both IL-6 and IL-1β may be related to impaired development as a result of maternal exposure to glucocorticoids. To the best of our knowledge, this is the first study to evaluate this response in neonatal cortical neurons.

In conclusion, results indicate that prenatal stress and stimulation with KCl influence cell death, and gene expression of 5HTr1 and regulatory markers of the HPA axis in cortical neurons from neonate mice. In addition, exposure to stress *in utero* appears to be able to induce changes in the oxidative and inflammatory responses. These findings indicate that early-life stress has effects during neurodevelopment that support the well-known long-term effects of maternal stress.

Author contributions

Carolina Luft conceived the work, acquired data, drafted the paper, performed data analysis, and approved the final version. Gabriela Viegas Haute, Luís Eduardo Wearick, Krist Helen Antunes, and Mariana Severo da Costa acquired data, revised the article and approved the final version. Jarbas Rodrigues de Oliveira conceived the work, revised the paper, and approved the final version. Márcio Vinícius Fagundes Donadio conceived the work, acquired funding, performed data analysis, revised the article, and approved the final version.

Ethics approval

All the experiments were performed in agreement with the international ethical standards and following the local animal protection guidelines. The experimental protocol was approved by the Ethics Research Committee (protocol number 8465) of the Pontifical Catholic University of Rio Grande do Sul (PUCRS).

Data availability statement

The data used to support the findings of this study are available from the corresponding author upon request.

Declaration of competing interest

The authors have no conflict of interests related to the present study to declare.

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References

Aid, T., Kazantseva, A., Piirsoo, M., Palm, K., Timmusk, T., 2007. Mouse and rat BDNF gene structure and expression revisited. J. Neurosci. Res. 85, 525–535.

Akatsu, S., Ishikawa, C., Takemura, K., Ohtani, A., Shiga, T., 2015. Effects of prenatal stress and neonatal handling on anxiety, spatial learning and serotonergic system of male offspring mice. Neurosci. Res. 101, 15–23.

Anacker, C., Cattaneo, A., Luoni, A., Musaelyan, K., Zunszain, P.A., Milanesi, E., Rybka, J., Berry, A., Cirulli, F., Thuret, S., Price, J., Riva, M.A., Gennarelli, M., Pariante, C.M., 2013. Glucocorticoid-related molecular signaling pathways regulating hippocampal neurogenesis. Neuropsychopharmacology 38, 872–883.
Ancelin, M.L., Scali, J., Norton, J., Ritchie, K., Dupuy, A.M., Chaudieu, I., Ryan, J., 2017. Heterogeneity in HPA axis dysregulation and serotonergic vulnerability to depression. Psychoneuroendocrinology 77, 90–94.
Armstrong, J.S., Whiteman, M., Yang, H., Jones, D.P., Sternberg Jr., P., 2004. Cysteine starvation activates the redox-dependent mitochondrial permeability transition in retinal pigment epithelial cells. Invest. Ophthalmol. Vis. Sci. 45, 4183–4189.
Atif, F., Yousuf, S., Agrawal, S.K., 2008. Restraint stress-induced oxidative damage and its amelioration with selenium. Eur. J. Pharmacol. 600, 59–63.
Bachis, A., Cruz, M.I., Nosheny, R.L., Mocchetti, I., 2008. Chronic unpredictable stress promotes neuronal apoptosis in the cerebral cortex. Neurosci. Lett. 442, 104–108.
Barker, D.J., Winter, P.D., Osmond, C., Margetts, B., Simmonds, S.J., 1989. Weight in infancy and death from ischaemic heart disease. Lancet 2, 577–580.
Bittle, J., Menezes, E.C., McCormick, M.L., Spitz, D.R., Dailey, M., Stevens, H.E., 2019. The role of redox dysregulation in the effects of prenatal stress on embryonic interneuron migration. Cerebr. Cortex 29, 5116–5130.
Bock, J., Murmu, M.S., Biala, Y., Weinstock, M., Braun, K., 2011. Prenatal stress and neonatal handling induce sex-specific changes in dendritic complexity and dendritic spine density in hippocampal subregions of prepubertal rats. Neuroscience 193, 34–43.
Brummelte, S., Mc Glanaghy, E., Bonnin, A., Oberlander, T.F., 2017. Developmental changes in serotonin signaling: implications for early brain function, behavior and adaptation. Neuroscience 342, 212–231.
Caccamo, A., Branca, C., Piras, I.S., Ferreira, E., Huentelman, M.J., Liang, W.S., Readhead, B., Dudley, J.T., Spangenberg, E.E., Green, K.N., Belfiore, R., Winslow, W., Oddo, S., 2017. Necroptosis activation in Alzheimer's disease. Nat. Neurosci. 20, 1236–1246.
Choe, H.K., Son, G.H., Chung, S., Kim, M., Sun, W., Kim, H., Geum, D., Kim, K., 2011. Maternal stress retards fetal development in mice with transcriptome-wide impact on gene expression profiles of the limb. Stress 14, 194–204.
Coutinho, A.E., Chapman, K.E., 2011. The anti-inflammatory and immunosuppressive effects of glucocorticoids, recent developments and mechanistic insights. Mol. Cell. Endocrinol. 335, 2–13.
de Kloet, E.R., Joels, M., Holsboer, F., 2005. Stress and the brain: from adaptation to disease. Nat. Rev. Neurosci. 6, 463–475.
De Kloet, E.R., Vreugdenhil, E., Oitzl, M.S., Joels, M., 1998. Brain corticosteroid receptor balance in health and disease. Endocr. Rev. 19, 269–301.
Deverman, B.E., Patterson, P.H., 2009. Cytokines and CNS development. Neuron 64, 61–78.
Diz-Chaves, Y., Pernia, O., Carrero, P., Garcia-Segura, L.M., 2012. Prenatal stress causes alterations in the morphology of microglia and the inflammatory response of the hippocampus of adult female mice. J. Neuroinflammation 9, 71.
Dong, E., Dzitoyeva, S.G., Matrisciano, F., Tueting, P., Grayson, D.R., Guidotti, A., 2015. Brain-derived neurotrophic factor epigenetic modifications associated with schizophrenia-like phenotype induced by prenatal stress in mice. Biol. Psychiatr. 77, 589–596.
Donoso, F., Ramirez, V.T., Golubeva, A.V., Moloney, G.M., Stanton, C., Dinan, T.G., Cryan, J.F., 2019. Naturally derived polyphenols protect against corticosterone-induced changes in primary cortical neurons. Int. J. Neuropsychopharmacol. 22, 765–777.
Drevets, W.C., Frank, E., Price, J.C., Kupfer, D.J., Greer, P.J., Mathis, C., 2000. Serotonin type-1A receptor imaging in depression. Nucl. Med. Biol. 27, 499–507.
Elvander-Tottie, E., Eriksson, T.M., Sandin, J., Ogren, S.O., 2009. 5-HT(1A) and NMDA receptors interact in the rat medial septum and modulate hippocampal-dependent spatial learning. Hippocampus 19, 1187–1198.
Enayati, M., Mosaferi, B., Homberg, J.R., Diniz, D.M., Salari, A.A., 2020. Prenatal maternal stress alters depression-related symptoms in a strain - and sex-dependent manner in rodent offspring. Life Sci. 251, 117597.
Fricker, M., Tolkovsky, A.M., Borutaite, V., Coleman, M., Brown, G.C., 2018. Neuronal cell death. Physiol. Rev. 98, 813–880.
Gadient, R.A., Otten, U.H., 1997. Interleukin-6 (IL-6)–a molecule with both beneficial and destructive potentials. Prog. Neurobiol. 52, 379–390.
Goel, N., Innala, L., Viau, V., 2014. Sex differences in serotonin (5-HT) 1A receptor regulation of HPA axis and dorsal raphe responses to acute restraint. Psychoneuroendocrinology 40, 232–241.
Guo, Z., Rudow, G., Pletnikova, O., Codispoti, K.E., Orr, B.A., Crain, B.J., Duan, W., Margolis, R.L., Rosenblatt, A., Ross, C.A., Troncoso, J.C., 2012. Striatal neuronal loss correlates with clinical motor impairment in Huntington's disease. Mov. Disord. 27, 1379–1386.
Gur, T.L., Palkar, A.V., Rajasekera, T., Allen, J., Niraula, A., Godbout, J., Bailey, M.T., 2019. Prenatal stress disrupts social behavior, cortical neurobiology and commensal microbes in adult male offspring. Behav. Brain Res. 359, 886–894.
Hanswijk, S.I., Spoelder, M., Shan, L., Verheij, M.M.M., Muilwijk, O.G., Li, W., Liu, C., Kolk, S.M., Homberg, J.R., 2020. Gestational factors throughout fetal neurodevelopment: the serotonin link. Int. J. Mol. Sci. 21.
Hantsoo, L., Kornfield, S., Anguera, M.C., Epperson, C.N., 2019. Inflammation: a proposed intermediary between maternal stress and offspring neuropsychiatric risk. Biol. Psychiatr. 85, 97–106.
Harris, A., Seckl, J., 2011. Glucocorticoids, prenatal stress and the programming of disease. Horm. Behav. 59, 279–289.
Herman, J.P., 1993. Regulation of adrenocorticosteroid receptor mRNA expression in the central nervous system. Cell. Mol. Neurobiol. 13, 349–372.
Hiroi, R., Carbone, D.L., Zuloaga, D.G., Bimonte-Nelson, H.A., Handa, R.J., 2016. Sex-dependent programming effects of prenatal glucocorticoid treatment on the developing serotonin system and stress-related behaviors in adulthood. Neuroscience 320, 43–56.
Jander, S., Schroeter, M., Peters, O., Witte, O.W., Stoll, G., 2001. Cortical spreading depression induces proinflammatory cytokine gene expression in the rat brain. J. Cerebr. Blood Flow Metabol. 21, 218–225.
Johnson, W.M., Wilson-Delfosse, A.L., Mieyal, J.J., 2012. Dysregulation of glutathione homeostasis in neurodegenerative diseases. Nutrients 4, 1399–1440.
Kim, J.B., Ju, J.Y., Kim, J.H., Kim, T.Y., Yang, B.H., Lee, Y.S., Son, H., 2004. Dexamethasone inhibits proliferation of adult hippocampal neurogenesis in vivo and in vitro. Brain Res. 1027, 1–10.
Kim, T.W., Ji, E.S., Lee, S.W., Lee, C.Y., Lee, S.J., 2015. Postnatal treadmill exercise attenuates prenatal stress-induced apoptosis through enhancing serotonin expression in aged-offspring rats. J Exerc Rehabil 11, 12–19.
Kino, T., Jaffe, H., Amin, N.D., Chakrabarti, M., Zheng, Y.L., Chrousos, G.P., Pant, H.C., 2010. Cyclin-dependent kinase 5 modulates the transcriptional activity of the mineralocorticoid receptor and regulates expression of brain-derived neurotrophic factor. Mol. Endocrinol. 24, 941–952.
Kowianski, P., Lietzau, G., Czuba, E., Waskow, M., Steliga, A., Morys, J., 2018. BDNF: a key factor with multipotent impact on brain signaling and synaptic plasticity. Cell. Mol. Neurobiol. 38, 579–593.
Kulkarni, A., Chen, J., Maday, S., 2018. Neuronal autophagy and intercellular regulation of homeostasis in the brain. Curr. Opin. Neurobiol. 51, 29–36.
Kumamaru, E., Numakawa, T., Adachi, N., Yagasaki, Y., Izumi, A., Niyaz, M., Kudo, M., Kunugi, H., 2008. Glucocorticoid prevents brain-derived neurotrophic factor-mediated maturation of synaptic function in developing hippocampal neurons through reduction in the activity of mitogen-activated protein kinase. Mol. Endocrinol. 22, 546–558.
Lemaire, V., Lamarque, S., Le Moal, M., Piazza, P.V., Abrous, D.N., 2006. Postnatal stimulation of the pups counteracts prenatal stress-induced deficits in hippocampal neurogenesis. Biol. Psychiatr. 59, 786–792.
Luft, C., Levices, I.P., da Costa, M.S., de Oliveira, J.R., Donadio, M.V.F., 2021. Effects of running before pregnancy on long-term memory and hippocampal alterations induced by prenatal stress. Neurosci. Lett. 746, 135659.
Luft, C., Levices, I.P., da Costa, M.S., Haute, G.V., Grassi-Oliveira, R., de Oliveira, J.R., Donadio, M.V.F., 2020. Exercise before pregnancy attenuates the effects of prenatal stress in adult mice in a sex-dependent manner. Int. J. Dev. Neurosci. 80, 86–95.
Ma, L., Li, X.W., Zhang, S.J., Yang, F., Zhu, G.M., Yuan, X.B., Jiang, W., 2014. Interleukin-1 beta guides the migration of cortical neurons. J. Neuroinflammation 11, 114.
Menard, C., Pfau, M.L., Hodes, G.E., Russo, S.J., 2017. Immune and neuroendocrine mechanisms of stress vulnerability and resilience. Neuropsychopharmacology 42, 62–80.
Metsis, M., Timmusk, T., Arenas, E., Persson, H., 1993. Differential usage of multiple brain-derived neurotrophic factor promoters in the rat brain following neuronal activation. Proc. Natl. Acad. Sci. U. S. A. 90, 8802–8806.
Miyagawa, K., Tsuji, M., Ishii, D., Takeda, K., Takeda, H., 2015. Prenatal stress induces vulnerability to stress together with the disruption of central serotonin neurons in mice. Behav. Brain Res. 277, 228–236.
Nixon, R.A., Yang, D.S., 2012. Autophagy and neuronal cell death in neurological disorders. Cold Spring Harb Perspect Biol 4.
Park, S.Y., Kang, M.J., Han, J.S., 2018. Interleukin-1 beta promotes neuronal differentiation through the Wnt5a/RhoA/JNK pathway in cortical neural precursor cells. Mol. Brain 11, 39.
Ratnu, V.S., Wei, W., Bredy, T.W., 2014. Activation-induced cytidine deaminase regulates activity-dependent BDNF expression in post-mitotic cortical neurons. Eur. J. Neurosci. 40, 3032–3039.
Reul, J.M., de Kloet, E.R., 1985. Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation. Endocrinology 117, 2505–2511.
Rosa, A.R., Singh, N., Whitaker, E., de Brito, M., Lewis, A.M., Vieta, E., Churchill, G.C., Geddes, J.R., Goodwin, G.M., 2014. Altered plasma glutathione levels in bipolar disorder indicates higher oxidative stress; a possible risk factor for illness onset despite normal brain-derived neurotrophic factor (BDNF) levels. Psychol. Med. 44, 2409–2418.
Samarghandian, S., Azimi-Nezhad, M., Farkhondeh, T., Samini, F., 2017. Anti-oxidative effects of curcumin on immobilization-induced oxidative stress in rat brain, liver and kidney. Biomed. Pharmacother. 87, 223–229.
Seckl, J.R., 2004. Prenatal glucocorticoids and long-term programming. Eur. J. Endocrinol. 151 (Suppl. 3), U49–U62.
Shih, A.Y., Erb, H., Sun, X., Toda, S., Kalivas, P.W., Murphy, T.H., 2006. Cystine/glutamate exchange modulates glutathione supply for neuroprotection from oxidative stress and cell proliferation. J. Neurosci. 26, 10514–10523.
Soares-Cunha, C., Coimbra, B., Borges, S., Domingues, A.V., Silva, D., Sousa, N., Rodrigues, A.J., 2018. Mild prenatal stress causes emotional and brain structural modifications in rats of both sexes. Front. Behav. Neurosci. 12, 129.
Sohya, K., Kitamura, A., Akaneya, Y., 2007. Chronic membrane depolarization-induced morphological alteration of developing neurons. Neuroscience 145, 232–240.
Song, L., Zheng, J., Li, H., Jia, N., Suo, Z., Cai, Q., Bai, Z., Cheng, D., Zhu, Z., 2009. Prenatal stress causes oxidative damage to mitochondrial DNA in hippocampus of offspring rats. Neurochem. Res. 34, 739–745.
Sorenson, A.N., Sullivan, E.C., Mendoza, S.P., Capitanio, J.P., Higley, J.D., 2013. Serotonin transporter genotype modulates HPA axis output during stress: effect of stress, dexamethasone test and ACTH challenge. Transl Dev Psychiatry 1, 21130.

Sowa, J.E., Slusarczyk, J., Trojan, E., Chamera, K., Leskiewicz, M., Regulska, M., Kotarska, K., Basta-Kaim, A., 2017. Prenatal stress affects viability, activation, and chemokine signaling in astroglial cultures. J. Neuroimmunol. 311, 79–87.

Szczesny, E., Basta-Kaim, A., Slusarczyk, J., Trojan, E., Glombik, K., Regulska, M., Leskiewicz, M., Budziszewska, B., Kubera, M., Lason, W., 2014. The impact of prenatal stress on insulin-like growth factor-1 and pro-inflammatory cytokine expression in the brains of adult male rats: the possible role of suppressors of cytokine signaling proteins. J. Neuroimmunol. 276, 37–46.

van Bodegom, M., Homberg, J.R., Henckens, M., 2017. Modulation of the hypothalamic-pituitary-adrenal Axis by early life stress exposure. Front. Cell. Neurosci. 11, 87.

Van den Hove, D.L., Steinbusch, H.W., Scheepens, A., Van de Berg, W.D., Kooiman, L.A., Boosten, B.J., Prickaerts, J., Blanco, C.E., 2006. Prenatal stress and neonatal rat brain development. Neuroscience 137, 145–155.

Walker, C.D., Deschamps, S., Proulx, K., Tu, M., Salzman, C., Woodside, B., Lupien, S., Gallo-Payet, N., Richard, D., 2004. Mother to infant or infant to mother? Reciprocal regulation of responsiveness to stress in rodents and the implications for humans. J. Psychiatry Neurosci. 29, 364–382.

Wu, T., Li, X., Li, T., Cai, M., Yu, Z., Zhang, J., Zhang, Z., Zhang, W., Xiang, J., Cai, D., 2018. Apocynum venetum leaf extract exerts antidepressant-like effects and inhibits hippocampal and cortical apoptosis of rats exposed to chronic unpredictable mild stress. Evid Based Complement Alternat Med 5916451, 2018.

Zhang, H., Shang, Y., Xiao, X., Yu, M., Zhang, T., 2017. Prenatal stress-induced impairments of cognitive flexibility and bidirectional synaptic plasticity are possibly associated with autophagy in adolescent male-offspring. Exp. Neurol. 298, 68–78.

Zhang, X., Wang, Q., Wang, Y., Hu, J., Jiang, H., Cheng, W., Ma, Y., Liu, M., Sun, A., Li, X., 2016. Duloxetine prevents the effects of prenatal stress on depressive-like and anxiety-like behavior and hippocampal expression of pro-inflammatory cytokines in adult male offspring rats. Int. J. Dev. Neurosci. 55, 41–48.

Zhou, Y., Yan, M., Pan, R., Wang, Z., Tao, X., Li, C., Xia, T., Liu, X., Chang, Q., 2021. Radix Polygalae extract exerts antidepressant effects in behavioral despair mice and chronic restraint stress-induced rats probably by promoting autophagy and inhibiting neuroinflammation. J. Ethnopharmacol. 265, 113317.

Zhu, Z., Li, X., Chen, W., Zhao, Y., Li, H., Qing, C., Jia, N., Bai, Z., Liu, J., 2004. Prenatal stress causes gender-dependent neuronal loss and oxidative stress in rat hippocampus. J. Neurosci. Res. 78, 837–844.