Behavioural Brain Research 376 (2019) 112221

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Acute neuroinflammation elicited by TLR-3 systemic activation combined with early life stress induces working memory impairments in male adolescent mice

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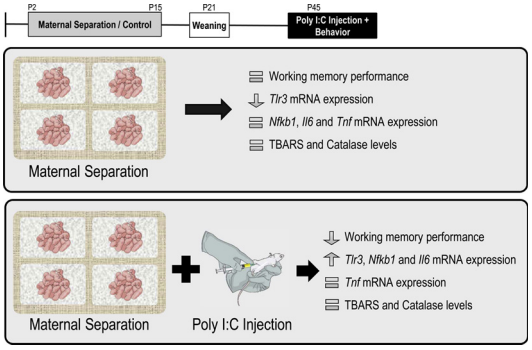
GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Early life stress
Cognition
Working memory
Inflammation
Toll-like receptors
Neuroimmunomodulation

ABSTRACT

Toll-like Receptors (TLRs) are implicated with the pathogenesis of cognitive impairment induced by inflammation. Early life stress is associated with altered trajectories of neuroimmune signaling with implications for cognitive development. However, effects of TLR-3 activation on early life stress-related cognitive outcomes are understudied. We investigated the effects of maternal separation (MS) during postnatal development and a viral immune challenge during adolescence on working memory performance. BALB/c mice exposed to MS were separated from their dams daily for 180-min from postnatal day (PND) 2 to 15. At PND 45, animals were challenged with a single i.p. injection of either Poly (I:C) or sterile saline, and then subjected to a spatial working memory test in a Y-maze apparatus. Gene expression was determined by qPCR. Protein levels of oxidative stress markers were also assessed. A single peripheral administration of a TLR-3 agonist was able to induce working memory impairments in adolescent mice exposed to MS. At a molecular level, exposure to MS was associated with lower mRNA levels of *Tlr3* in the medial prefrontal cortex (mPFC). However, when MS animals were exposed to Poly (I:C), a more robust activation of *Tlr3, Il6* and *Nfkb1* gene transcription was observed in these mice compared with control animals. These modifications did not result in oxidative stress. Finally, higher

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https://doi.org/10.1016/j.bbr.2019.112221
Received 2 July 2019; Received in revised form 6 September 2019; Accepted 8 September 2019
Available online 09 September 2019
0166-4328/

mRNA levels of *Nfkb1* in the mPFC were correlated with lower working memory performance, suggesting that altered NF-κB signaling might be related with poor cognitive functioning. These results have implications for how ELS affects neuroimmune signaling in the mPFC.

1. Introduction

Toll-like Receptors (TLRs) are the first line of defense against exogenous and endogenous pathogens and are responsible for triggering innate immune responses [1]. These receptors are expressed in a variety of immune cells, while in the brain, they are identified predominantly on microglia, astrocytes, and with limited expression levels on neurons [2]. Upon stimulation, TLRs can impact numerous aspects of central nervous system homeostasis by the production of inflammatory mediators, such as cytokines and chemokines, particularly through activation of the transcription factor nuclear factor kappa B (NF-κB) [3]. Neuroinflammatory responses elicited by TLRs stimulation are recognized to play a role in the pathophysiology of cognitive impairment [4,5].

Among the family of TLRs, neuroinflammatory signaling mediated by TLR-2 and TLR-4, which are receptors that recognize infections of bacterial origin have been the most widely investigated [4]. For instance, administration of a TLR-4 agonist that induces peripheral and central inflammation, also results in increased hippocampal amyloid-beta, tumor necrosis factor (TNF)-α, and interleukin (IL)-6 levels with concomitant cognitive deficits in contextual fear-related memory [6,7], suggesting that TLRs activation can be detrimental to brain functioning during acute inflammatory response. Another consequence of TLRs activation is the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS). Both ROS and RNS have been implicated with the onset or as the result of neuroinflammation [8]. In addition, when ROS and/or RNS production is exacerbated and antioxidants components are unable to compensate for such increase, this result in oxidative stress (OS) and related adverse effects (e.g. oxidative damage in brain tissue) [9,10].

However, some TLRs are implicated in the recognition of viral infections, such as the TLR-3, TLR-7, TLR-8 and TLR-9 [11]. As a sensor for double-stranded RNAs of viral origin, TLR-3 is predominantly expressed intracellularly [12], and recent evidence demonstrated its functions on the regulation of hippocampal glial excitability [13]. Although TLRs that respond to bacteria or viral ligands have important differences regarding their immune functions, the majority of evidence suggests that upon activation these receptors have similar effects on brain functioning, in particular related to hippocampal-dependent spatial memory deficits [4,14]. However, intracerebral infusion of a TLR-3 agonist - Polyinosinic: polycytidylic acid [(Poly (I:C)] into the lateral ventricle was shown to induce working memory impairments in mice [15]. Even though systemic activation or ventricular infusions could be impacting several neocortical and limbic regions, working memory is a key function of the medial prefrontal cortex (mPFC). This short-term memory process is engaged during active maintenance and manipulation of information in order to guide behavior [16,17].

Moreover, compelling evidence suggests that the nature and severity of cognitive deficits later in life depend upon many factors involved with brain development, including the effect of experiences that occur during the sensitive period of postnatal life [18–20]. Stress exposure during this critical period has been related to cognitive impairments including poor working memory performance [21]. Beyond behavioral outcomes, early life stress is associated with a chronic inflammatory state [22], oxidative damage [23,24], and neuronal and glia cells abnormalities [25]. This supports the potential role of altered neuroimmune signaling as a candidate pathway for mediating the consequences of postnatal stress on brain and cognitive functioning. Classic early life stress experiments using the maternal separation (MS) model have indicated persistent higher levels of inflammatory markers in the brain, including TLR-4 and NF-κB levels [26]. In addition, NF-κB gene expression was upregulated in the mPFC of adolescent mice exposed to MS early in life, and this effect was associated with higher levels of cocaine-contextual memory during adolescence [27]. In contrast, effects of TLR-3 activation on early life stress-related cognitive outcomes are not well understood.

Given the potential relationship between early life stress, altered cognitive functioning and TLR-3 mediated neuroinflammation, the current study investigated the effects of MS associated with a viral immune challenge during adolescence on working memory performance of mice. The cumulative effect of both factors was also assessed at the molecular level in the mPFC by measuring mRNA levels of NF-κB, IL-6, TNF-α and TLR-3. Also, two oxidative stress biomarkers were quantified: Thiobarbituric Acid Reactive Substances (TBARS) that estimates the product of lipid peroxidation (Malondialdehyde – MDA) [28] and Catalase enzymatic activity, involved in the hydrogen peroxide (H2O2) detoxification [29]. Considering that early life stress *per se* is associated with biological and cognitive impairments, we hypothesized that Poly (I:C) administration would enhance stress-mediated working memory deficits as an immunological “second hit”, increasing the expression of the immune-related genes and oxidative stress markers in the mPFC.

2. Methods

2.1. Animals

This study was performed with male BALB/c mice. All animals were housed under a 12 h/12 h light–dark cycle in ventilated cages with temperature maintained at 21 ± 1 °C. Food and water were available ad libitum. The experiments were conducted in accordance with the NIH laboratory animal care guidelines and approved by the Ethical Committee on the Use of Animals of the Pontifical Catholic University of Rio Grande do Sul, Brazil.

2.2. Maternal separation model

The early life stress model consisted of exposing infant animals to daily episodes of MS during the first days of life. Pregnant females were visually checked daily for the presence of pups. On the day of birth, the litters were randomly assigned to one of two groups: MS or animal facility rearing (AFR) control animals. The AFR litters were left undisturbed until weaning, except for cage cleaning at postnatal day (PND) 10. The MS litters were subjected to a procedure that was used in previous studies with BALB/c mice [27,30]. In this procedure, pups were separated from their dams daily for 180 min (15:00–18:00), from PND 2 to PND 15. To do this, first, the dam was transferred to another cage. Then, the whole litter was transferred to another clean cage with bedding material and placed in different room, to prevent vocal communication between the dam and pups. The temperature of pups’ cage (33 ± 2 °C) was controlled using a digital heating pad placed under the cage to compensate for the dams’ body heat. After MS period, pups were returned to their home cage, followed by the dam. All pups were weaned at PND 21 and remained together with their same-sex littermates (two or three animals per cage) under standard housing conditions. A total of 18 litters were used (10 AFR and 8 MS), and to avoid any potential litter effects, no more than 2 animals per litter were used. Exceeded males and females from these litters were assigned to different ongoing research projects.

2.3. Working memory test

At the PND 45 animals were subjected to a spatial working memory test in a black Plexiglas Y-maze apparatus [31]. The Y-maze apparatus consists of three symmetrical arms (30 cm x 5 cm x 10 cm, each arm), and it uses the natural tendency of mice to explore novel over familiar arms. This working memory test consisted of two phases, called the sample and test phases. The allocation of arms (start, familiar and novel arm) to a specific spatial location was counterbalanced across the experimental groups.

In the sample phase, animals were allowed to explore two arms (referred to as 'start arm' and 'familiar arm'), while the access to the remaining arm ('novel arm') was obstructed by a removable barrier wall. To begin a trial, the animal was introduced at the end of the start arm and was allowed to freely explore both the start and the familiar arms. After 5 min of exploration, the animal was removed from the apparatus and kept in a holding cage for 1 min, while the barrier wall was removed.

In the test phase, the animal was reintroduced to the maze and could freely explore all arms of the maze for 2 min. After the test, the animal was removed from the apparatus and placed back in their home cage. The wood shavings that covered the floor of the apparatus was changed in preparation for the next animal. On each trial, the time spent in each of the three arms was recorded. The relative time spent in the novel arm during the choice phase was calculated by the formula [time spent in the novel arm/[time spent in all arms]) × 100 and used as the index for working memory performance. In addition, total distance moved on the entire maze was recorded and analyzed in order to assess general locomotor activity. Behavioral measurements were performed in the ANY-maze software (Version 5.3; Stoelting Co., Wood Dale, Il)

2.4. TLR-3 agonist administration

Poly (I:C) was used to mimic the acute phase of a viral infection, and it was prepared for injection by re-suspension in sterile saline and administered at a dose of 7.5 mg/kg (i.p.). This mild to moderate dose was chosen based on previous studies showing that this concentration of the TLR-3 agonist could induce behavioral and cognitive alterations in mice [32–34]. Therefore, experimental groups were challenged with a single injection of either Poly I:C or sterile saline at PND 45, specifically 5 h before behavioral test.

2.5. Gene expression analysis

Six hours after the Poly (I:C) or saline injection animals were euthanized, specifically 30 min after behavioral test. We selected this time point since previous evidence indicated enhanced gene expression of brain pro-inflammatory genes after 6 h of Poly (I:C) administration [34]. The tissue from the mPFC was rapidly hand-dissected with a scalpel and stored at -80°c until molecular analysis. Total RNA was isolated from 6 samples per group using QIAzol (Qiagen; Hilden, Germany) and chloroform standard protocols. RNA concentration was measured using the NanoDrop spectrophotometer. Total of 500 ng of RNA from each sample was reverse transcribed using the miScript II RT Kit (Qiagen). The following primers (IDT) were designed, tested and used: *Il6* Forward (CCCCAATTTCCAATGCTCTCC), *Il6* Reverse (GACC ACAGTGAGGAATGTCCA), *Tnf* Forward (CCTGTAGCCCACGTCGTAG), *Tnf* Reverse (GGGAGTAGACAAGGTACAACCC), *Tlr3* Forward (GTGAG ATACAACGTAGCTGACTG), *Tlr3* Reverse (TCCTGCATCCAAGATAGCA AGT), *Pgk* Forward (TGCACGCTTCAAAAGCGCACG), *Pgk* Reverse (AAGTCCACCCTCATCACGACCC). The Quantitect primer (QT00154091) for *Nfkb1* was purchased from Qiagen. Each SYBR Green PCR reaction was run in duplicate for each sample using a Rotor Gene Real-Time PCR machine (Qiagen). The fold change relative expression was calculated using the ΔΔCt method with the AFR-vehicle group as a

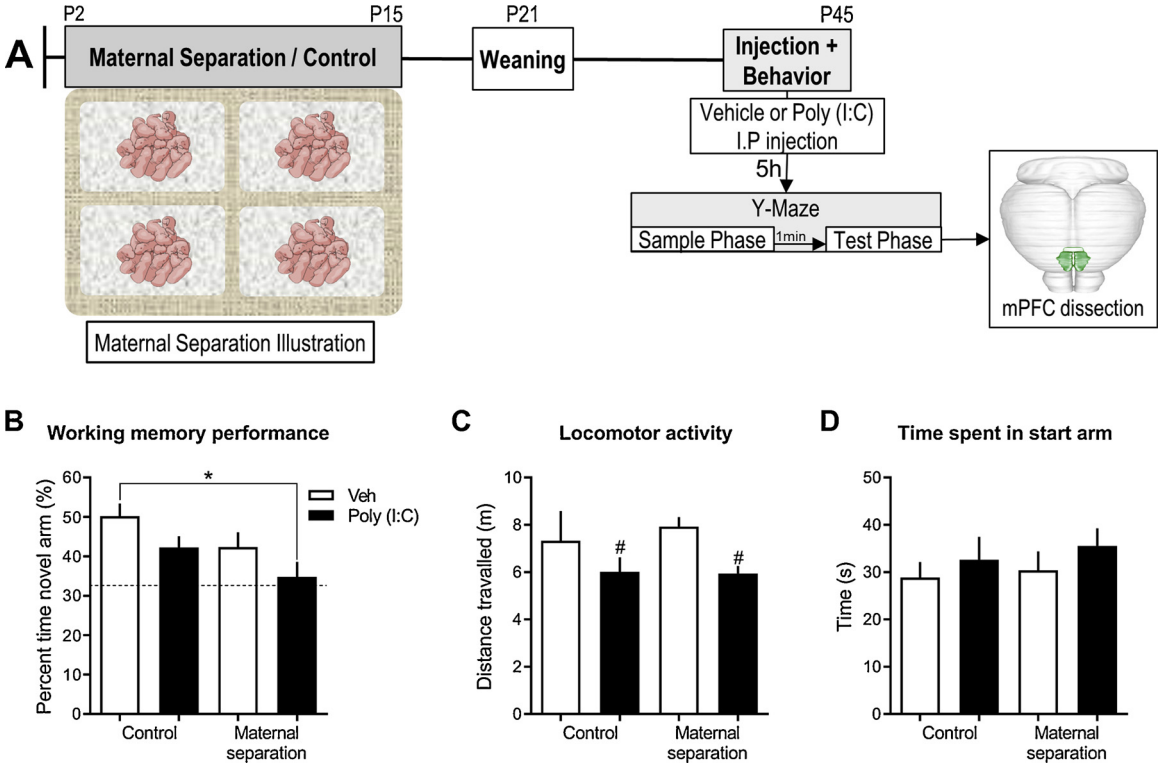

Fig. 1. legend. A, Experimental design. B, Percent time in novel arm in the Y-maze working memory test. C, Locomotor activity in the Y-maze working memory test. *, $p < 0.05$ - Tukey post-hoc tests. # represents treatment effect. Control, animal facility rearing. Early life stress, maternal separation. Dotted line represents "chance" performance. $n = 10/12$ per group.

reference. PGK ct values were used as endogenous control for mRNA analysis. To verify primer specificities, melting curve analyses and agarose gels were performed.

2.6. Oxidative stress biomarkers

Total protein from 9 to 12 tissues per group were homogenate in 300 ⌈M of cold RIPA buffer, centrifugated at 10.000 g for 15 min at 4 °C. The supernatant was removed and used to quantify the Thiobarbituric Acid Reactive Substances (TBARS) and Catalase (CAT) enzymatic activity using commercial kits purchased from Cayman Chemical (MI, USA). The assays were conducted according to the manufacture's protocol. TBARS fluorescence was read at an excitation wavelength of 530 nm and emission wavelength of 550 nm using a plate reader. CAT absorbance was read at 540 nm using a plate reader.

2.7. Statistical analysis

All statistical analyses were performed using the SPSS 20.0 (IBM – New York, USA) and the graphs were constructed using the Prism GraphPad 6.0 (La Jolla, USA). Group differences were assessed by two-way ANOVAs (group and treatment effects). The ANOVAs were followed by Tukey post-hoc tests. Pearson's correlation analysis was used to evaluate the association between behavioral and gene expression data. All the groups were collapsed to perform correlation analysis. Statistical significance was defined as $p < 0.05$ and results are expressed as the mean ± SEM.

3. Results

3.1. Effects of early life stress and TLR-3 activation on working memory performance

The experimental design of the study is illustrated in Fig. 1A. Significant group [$F(3,39) = 4.58$, $p < 0.05$] and treatment [$F(3,39) = 4.69$, $p < 0.05$] effects were detected on the percentage of time spent in the novel arms relative to time spent in all arms when looking at performance of mice in the Y-maze (Fig. 1B). Post-hoc analysis revealed that only animals exposed to both MS and to Poly (I:C) presented significant deficits in working memory performance compared with AFR-vehicle group ($p < 0.05$), suggesting that the combination of MS with Poly (I:C) was able to induce working memory impairments. A significant treatment [$F(3,39) = 5.49$, $p < 0.05$] effect was observed on the distance traveled by mice during the working memory test, showing that Poly (I:C) administration reduced locomotor activity (Fig. 1C). However, exposure to MS did not affect locomotor activity given that no group differences were observed [$F(3,39) = 0.14$, $p > 0.05$]. In addition, post-hoc analysis revealed no significant group differences. Additionally, regarding time spent in the start arm, no significant group [F(3,32) = 0,27, $p = 0.6$] or treatment [F(3,32) = 1,12, $p = 0.29$, Fig. 1D] effects were observed. Finally, no statistically significant correlation was observed between discrimination index and distance travelled (data not shown), indicating that locomotor activity played a minor role on working memory performance in the Y-maze.

3.2. Effects of early life stress and TLR-3 activation on gene expression levels

Significant group [$F(3,20) = 12.89$, $p < 0.01$] and treatment [$F(3,20) = 36.06$, $p < 0.01$] effects were detected on mRNA levels of *Tlr3* (Fig. 2A). Post-hoc analysis revealed the MS-vehicle group had significant less *Tlr3* expression compared with the AFR-vehicle group ($p < 0.01$). In addition, the AFR-Poly (I:C) group ($p < 0.05$) and the MS-Poly (I:C) group ($p < 0.01$) had significant higher *Tlr3* expression compared with their vehicle conditions. This suggests that while MS exposure reduced, Poly (I:C) exposure increased *Tlr3* expression in the mPFC.

A significant treatment [$F(3,20) = 11,48$, $p < 0.01$] effect was observed on mRNA levels of *Nfkb1,* showing that Poly (I:C) exposure increased *Nfkb1* expression in the mPFC, while no group differences were detected [$F(3,20) = 2.71$, $p > 0.05$]. However, post-hoc analysis revealed that only animals exposed to both MS and to Poly (I:C) presented significant higher *Nfkb1* expression compared with the MS-vehicle group ($p < 0.01$), while no significant differences were detected regarding AFR groups (Fig. 2B).

A significant treatment [$F(3,20) = 26.75$, $p < 0.01$] effect was observed on mRNA levels of *Il6,* showing that Poly (I:C) exposure increased *Il6* expression in the mPFC, while no group differences were detected [$F(3,20) = 0.50$, $p > 0.05$]. However, post-hoc analysis

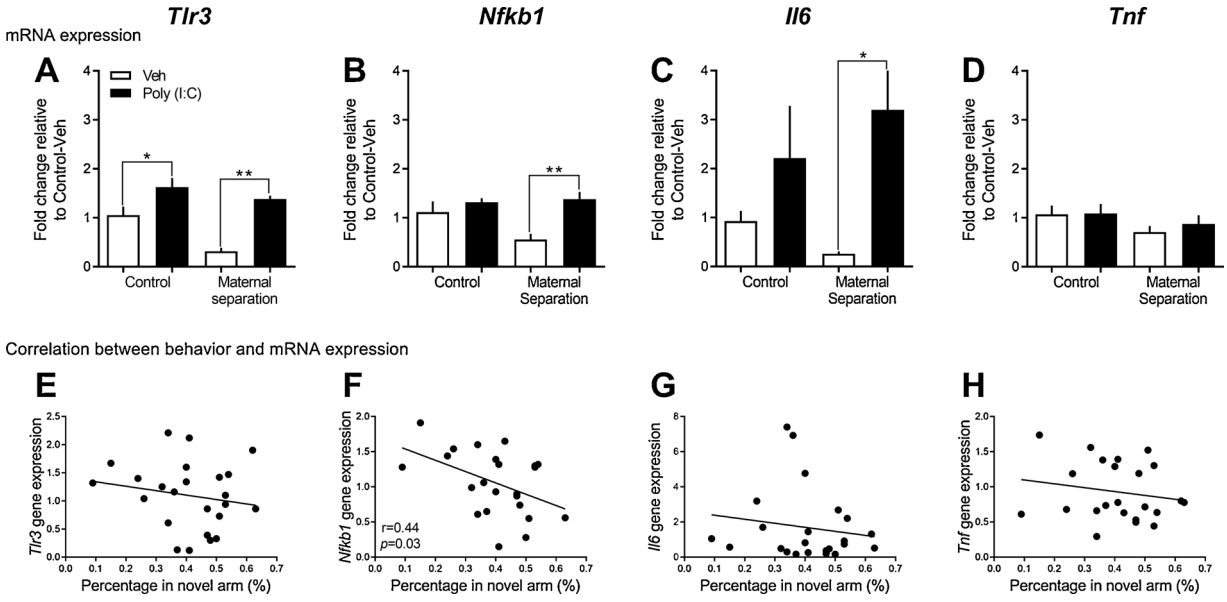

Fig. 2. legend. A, *Tlr3* gene expression levels in medial prefrontal cortex (mPFC). B, *Il6* gene expression levels in the mPFC. C, *Tnf-α* gene expression levels in the mPFC. D, *Nfkb1* gene expression levels in the mPFC. E, F, G and H, correlation analysis between gene expression and working memory performance score. *, $p < 0.05$, or **, $p < 0.01$ - Tukey post-hoc tests. Control, animal facility rearing. Early life stress, maternal separation. $n = 6$ per group.

revealed that only animals exposed to both MS and to Poly (I:C) presented significant higher *Il6* expression compared with the MS-vehicle group ($p < 0.05$), while no significant differences were detected regarding AFR groups (Fig. 2C). No significant treatment [$F(3,20) = 0.28$, $p > 0.05$] or group [$F(3,20) = 2.84$, $p > 0.05$] effects were detected regarding mRNA levels of *Tnf-α* (Fig. 2D).

Correlation analysis revealed a significant negative association between *Nfkb1* gene expression and the percentage of time exploring the novel arm during the Y-maze test ($R = 0.44$; $p < 0.05$), showing that higher mRNA levels of *Nfkb1* in the mPFC was associated lower working memory performance (Fig. 2F). No significant correlations were observed for *Tl3, Il6* and *Tnf-α* mRNA levels with behavior.

3.3. Effects of early life stress and TLR-3 activation on oxidative stress markers

No significant treatment [$F(3,37) = 0.01$, $p > 0.05$] or group [$F(3,20) = 2.71$, $p > 0.05$] effect was observed on TBARS levels (Fig. 3A). Similarly, no significant treatment [$F(3,37) = 0.99$, $p > 0.05$] or group [$F(3,20) = 2.24$, $p > 0.05$] effect was observed on Catalase enzymatic activity (Fig. 3B).

4. Discussion

The current study provided evidence that a single peripheral administration of a TLR-3 agonist can modulate early life stress induced working memory impairments in adolescent mice. At a molecular level, exposure to MS was associated with lower mRNA levels of *Tlr3* in the mPFC. However, when MS animals were exposed to Poly (I:C) during adolescence, a more robust activation of *Il6*, *Nfkb1* and *Tlr3* gene transcription was observed in these mice compared with standard reared animals. Additionally, higher mRNA levels of *Nfkb1* in the mPFC were correlated with lower working memory performance, suggesting that altered NF-κB signaling might be related to poor cognitive functioning. Despite that, early life stress combined or not with acute Poly (I:C) exposure did not trigger oxidative stress in the mPFC.

Our behavioral results are consistent with previous findings showing that either early life stress [35] or acute inflammation [15] could produce cognitive deficits that include working memory performance. Specifically, we demonstrated that only following a viral immune challenge the effect of MS on cognition reached statistical significance in comparison to normally reared animals without acute inflammation. Therefore, these data support the hypothesis that an immunological “second hit” could not only aggravate the effects of postnatal stress on working memory, but it could also be a pathological trigger for cognitive decline in vulnerable individuals due to postnatal stress exposure [36]. This combinatory effect has already been documented regarding viral diseases, such as HIV infection, given that HIV induced neurocognitive impairments are more pronounced in individuals with a history of early life stress [37], particularly on cognitive flexibility and working memory processes [38].

Although there is little information on brain and behavioral responses to TLR-3 in vivo, previous evidence demonstrated that direct activation of TLR-3 by intracerebroventricular infusion of Poly (I:C) impaired working memory [15], and that TLR-3 signaling can suppress neuronal plasticity and induce neuroglial immune responses by driving gene expression of pro-inflammatory cytokines [13]. In our experiments we observed increased mRNA levels of *Il6*, *Nfkb1* and *Tlr3* in the mPFC after 5 h of a single injection of Poly (I:C) in a mild to moderate dose. Although we did not provide direct evidence of cellular specificity, it is possible that our gene expression data is closely involved with the response of glia cells. We hypothesized that because previous findings showed that astrocytes and microglia cells express TLR-3, and that these cells can synthesize pro-inflammatory cytokines in response to Poly (I:C) stimulation [39]. Furthermore, when cortical glia cells are activated upon TLRs stimulation, neuroinflammatory toxicity can be extended to primary cortical neurons, an effect that has been shown to be involved with cognitive impairment [13]. For instance, activated microglia cells produce IL-6, IL-1β, and TNF-α, and when these cytokines are released extracellularly they directly affect synaptic plasticity and working memory performance [40].

Additionally, ROS and RNS have an important role in the defense against viral infection by participating in inflammatory responses. However, overproduction of reactive species combined with failure of repair mechanisms can cause oxidative damage in cells and tissues [9]. Monte et al. [41], showed that chronic neonatal Poly (I:C) administration combined or not with peripubertal unpredictable stress increased levels of lipid peroxidation and decreased levels of glutathione in the PFC, and this imbalance was associated with oxidative stress [41]. In our study, we did not observe alterations in TBARS and catalase oxidative stress biomarkers. Given that lipid peroxidation affect the cell membrane and function, resulting in cell death via apoptosis [42], the current data support the absence of such oxidative damage. The H2O2 can interact with iron ion and form hydroxyl radicals (•OH), considered the most cytotoxic ROS [43]. The catalase enzyme is able to protect cells from H2O2 reactivity through decomposing this molecule in water and dioxygen, thus high catalase activity provide cells with greater resistance against ROS generation [44]. The stability in catalase quantification in our study may indicate low generation of ROS by both MS and Poly (I:C) treatment. Therefore, these data suggest that MS-induced working memory deficits during adolescence occurs via neuroinflammation, but not via oxidative stress. In other words, the combinatory effect of MS and Poly (I:C) is capable of dynamically induce gene transcription that correlates with poor working memory performance, without resulting in cortical tissue damage attributed to oxidative stress. This hypothesis is supported by previous evidence

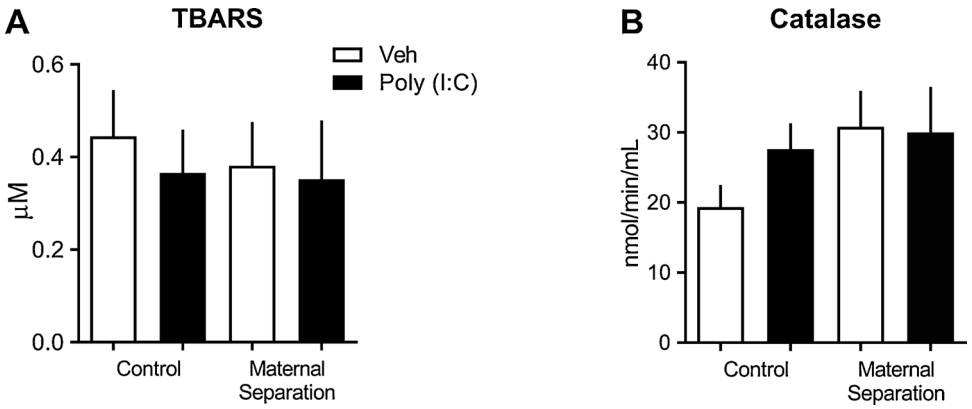

Fig. 3. legend. A, Thiobarbituric acid reactive substance (TBARS) levels (Malondialdehyde – MDA) in the medial prefrontal cortex (mPFC). B, Catalase activity in the mPFC. Control, animal facility rearing. Early life stress, maternal separation. $n = 9/12$ per group.

showing that MS was able to increase cortical pro-inflammatory cytokines levels without altering markers of oxidative stress [45]. However, it is possible that MS may yield oxidative damage if combined with a higher dose of Poly (I:C), or with chronic treatment of this TLR-3 agonist.

We also observed that regardless of Poly (I:C) administration, exposure to MS was associated with lower mRNA levels of *Tlr3* in the mPFC of adolescent animals, suggesting that early life stress has persistent effects on the transcription of this gene. However, following Poly (I:C) administration, animals exposed to MS presented a significant enhancement in the expression of *Il6*, *Nfkb1* and *Tlr3* compared to the MS-vehicle group, an effect that was not significant when analyzing gene expression changes in standard reared animals. These findings are in accordance with the concept of "glial priming" [46,47]. Previous studies demonstrated that "primed" glia cells are characterized by an activated morphology with enlarged cell bodies, accompanied by persistent lower expression levels of pro-inflammatory cytokines [40]. However, the inflammatory response produced by primed glia to a subsequent immune challenge is significantly exaggerated when compared to typical glia cells that receive a similar challenge, and this aberrant response is associated with detrimental brain functioning and cognitive impairment [48]. Previous evidence supports this idea since infant maternal care deprivation was associated with increased number, density, and surface area of glia cells in the brain [49].

Interestingly, we also found that higher mRNA levels of *Nfkb1* in the mPFC was correlated with lower working memory performance, even though we only observed gene expression group differences between animals exposed to MS. This link between NF-κB signaling and cognition is consistent with previous findings showing that enhanced cortical activation of NF-κB signaling pathway is accompanied by impairment of working memory functioning, particularly due to the deleterious effect of neuroinflammation on cognitive abilities [50]. NF-κB is a critical transcription factor implicated in the regulation of inflammatory responses, as it enhances the transcription of pro-inflammatory cytokines, including TNF-α, IL-1β and IL-6, as well as the oxidative stress related enzyme cyclooxygenase 2 [51]. In this sense, our data fits well with the hypothesis that by controlling NF-κB signaling in the brain, it would be possible to prevent working memory impairment in cognitively-impaired individuals [50], including those affected by early life stress. However, it still remains to be revealed whether the blockage of cortical NF-κB gene expression would rescue the cognitive deficits induced by the exposure to TLR-3 activation combined with MS exposure.

Our behavioral results should be interpreted having in light some limitations. First, we did not measure sickness behavior induced by systemic Poly (I:C). However, we showed that Poly (I:C) decreased locomotor activity in both MS and control animals. This effect is consistent with a previous study [52], that showed decreased activity of mice in the open field 8 h following Poly (I:C) injection with a dose similar to that used in our study. This same study did not show changes in sickness behavior such as body weight and altered temperature 8 h after injection. Given that locomotor activity could bias the working memory analysis, we ran additional analysis comparing the time that animals spent in the start arm, and no differences between groups were observed. In addition, correlation analysis did not show a significant association between distance travelled in the Y-maze and working memory performance. Although we cannot exclude the effects of Poly (I:C) on locomotor behavior, it seems that that locomotor activity played a minor role on working memory performance in the Y-maze in our experiment.

In conclusion, the present study provides further evidence to support the idea that early life stress is a major risk factor to cognitive impairment, and that one possible pathway involved with this phenotype is through altered neuroinflammation [36]. In particular, we demonstrated that the systemic activation of TLR-3 during adolescence is capable of induce working memory impairments following MS exposure, and that this effect is accompanied by modifications in cortical gene expression of *Il6*, *Nfkb1* and *Tlr3*, while these alterations are not followed by oxidative stress. In this sense, our study adds further weight to the notion that interventions targeting anti-inflammatory pathways may be highly promising for the treatment of neurocognitive disorders [53], especially those that early life stress is accounted as a major risk factor.

Funding

This study was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) [454429/2014-2]; [306271/2014-1]; [400850/2014-1] and by scholarships from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

Ethical approval

The experiments were conducted in accordance with the NIH laboratory animal care guidelines and approved by the Ethical Committee on the Use of Animals of the Pontifical Catholic University of Rio Grande do Sul, Brazil.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Acknowledgements

The authors would like to thank the staff of Center for Experimental Biological Models (Cembe) for providing helpful technical assistance during the development of this study.

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