Acute Methylglyoxal-Induced Damage in Blood–Brain Barrier and Hippocampal Tissue

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Abstract
Methylglyoxal (MG) is a reactive dicarbonyl compound formed mostly via the glycolytic pathway. Elevated blood glucose levels can cause MG accumulation in plasma and cerebrospinal fluid in patients with diabetes mellitus and Alzheimer’s disease. Under these disease conditions, the high reactivity of MG leads to modification of proteins and other biomolecules, generating advanced glycation end products (AGEs), which are considered mediators in neurodegenerative diseases. We investigated the integrity of the blood–brain barrier (BBB) and astrocyte response in the hippocampus to acute insult induced by MG when it was intracerebroventricularly administered to rats. Seventy-two hours later, BBB integrity was lost, as assessed by the entry of Evans dye into the brain tissue and albumin in the cerebrospinal fluid, and a decrease in aquaporin-4 and connexin-43 in the hippocampal tissue. MG did not induce changes in the hippocampal contents of RAGE in this short interval, but decreased the expression of S100B, an astrocyte-secreted protein that binds RAGE. The expression of two important transcription factors of the antioxidant response, NF-κB and Nrf2, was unchanged. However, hemeoxigenase-1 was upregulated in the MG-treated group. These data corroborate the idea that hippocampal cells are targets of MG toxicity and that BBB dysfunction and specific glial alterations induced by this compound may contribute to the behavioral and cognitive alterations observed in these animals.

Keywords Astrocytes · Aquaporin-4 · Blood–brain barrier · Methylglyoxal · S100B · Transcription factors

Introduction
The blood–brain barrier (BBB) is a complex cellular structure comprising endothelial cells, pericytes, and astrocytes, which together regulate the transport of specific molecules into the brain and limit the entry of blood-derived substances such as immunoglobulins and albumin (Hawkins and Davis 2005; Engelhardt and Sorokin 2009).

Astrocytes contain proteins that are essential for ensuring BBB functionality, such as aquaporin 4 (AQP4), which plays a critical role in cellular water content and flow (Niermann et al. 2001; Badau et al. 2014), and connexin 43 (Cx43), which is a predominant gap-junction protein in astrocytes, regulating the passage of ions and metabolites (Lapato and Tiwari-Woodruff 2018; Xing et al. 2019). Alterations in AQP4 and Cx43 expression are associated with several physiological and pathological conditions (Sáez et al. 2003; Zhang et al. 2015; Verkman et al. 2017; Mader and Brimberg 2019).

Methylglyoxal (MG) is a reactive dicarbonyl compound formed mainly via the glycolytic pathway (Phillips and Thornalley 1993; Angeloni et al. 2014). The chronic elevation of blood glucose results in MG accumulation in plasma and cerebrospinal levels, that are encountered in patients with diabetes mellitus (DM) and Alzheimer’s disease (AD) (Phillips and Thornalley 1993; Kuhla et al. 2005). The high reactivity of MG leads to the modification of proteins and other biomolecules, generating advanced...
glycation end products (AGEs) (Ahmed and Thornalley 2003; Rabbani and Thornalley 2008). Studies on diabetes models in rodents have shown that MG induces brain endothelial cell dysfunction through barrier-protein glycation (Li et al. 2015), impairing BBB function and integrity (Beeri et al. 2011; Rabbani and Thornalley 2015; Hussain et al. 2016), triggering inflammatory responses, increasing permeability, causing cellular morphology changes (Hussain et al. 2016; Tóth et al. 2014a), and disrupting gap junctions (Li et al. 2013; Tóth et al. 2014b).

The cellular accumulation of AGEs alters the protein machinery, including nuclear factors (Pugliese 2008) and contributes to abnormal cross-linking of proteins, which causes functional changes typical of chronic diabetic complications (Sell et al. 1992). Activation of the receptor for AGEs (RAGE) leads to cellular signaling involving many pathways, including nuclear factor κB (NF-κB), which is mainly associated with the inflammatory response (Piarulli et al. 2013; Saleh et al. 2019). Together with NF-κB, the nuclear factor erythroid-2 related factor (Nrf2) regulates the inflammatory response beyond modulating the metabolic oxidative response (Bellezza et al. 2018). Nrf2 is a protein involved in restoring the intracellular balance between oxidants and antioxidants after oxidative insult and in regulating numerous genes and enzymes (e.g., heme oxygenase 1 (HO-1)) (Minelli et al. 2009; Jian et al. 2011; Zhang et al. 2013; Choi et al. 2016; Wang et al. 2019). Nrf2 deletion may contribute to an increased sensitivity to oxidative stress and inflammation (Jakel et al. 2007; Ichihara et al. 2010; Taki-Nakano et al. 2014; Zhao et al. 2019). Nrf2 mediates a signaling pathway against MG toxicity (Oliveira et al. 2015) and prevents the damage caused by high concentrations of glucose in animal models of diabetes (Choi et al. 2016; Zhao et al. 2019).

Chronically elevated levels of MG were shown to alter the BBB and contribute to the brain changes observed in DM and neurodegenerative diseases (Hussain et al. 2016; Fang et al. 2015; Kim et al. 2020). However, few studies have addressed the acute effects of MG, which accompanies blood glucose peaks, particularly in vivo, on barrier integrity and neuroglial functions. In previous studies by our group, we observed that acute intracerebroventricular (ICV) administration of elevated concentrations of MG impaired short-and long-term learning and memory processes and glial function (Lissner et al. 2021). Seeking a better understanding of these findings, we aimed to investigate how high concentrations of MG in vivo affected BBB function and integrity, and if this could modulate inflammatory pathways and cellular stress responses in the hippocampus, which is a sensitive brain area that is affected in neurodegenerative diseases.

### Material and Methods

#### Reagents

MG solution (40% in H₂O), Triton X-100, o-phthaldialdehyde, S100B protein, and anti-S100B (SH-B1) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Polyclonal anti-S100B was purchased from DAKO (São Paulo, SP, Brazil) and Evans blue dye (EBD) from Neon Comercial, São Paulo, Brazil. Immunoblots were performed using the following antibodies: rabbit polyclonal (anti-AQP4 and anti-Cx43), mouse monoclonal (anti-RAGE) (Millipore, Darmstadt, Germany), and HRP-conjugated β-actin antibody (Proteintech, Rosemont, IL, USA). Peroxidase secondary antibodies were obtained from GE (Little Chalfont, UK). All the other chemicals were purchased from local commercial suppliers.

#### Animals

Male Wistar rats (90 days old) were obtained from our breeding colony (Department of Biochemistry, UFRGS, Porto Alegre, Brazil). The rats were maintained under controlled light and environmental conditions (12 h light/12 h dark cycle at a constant temperature of 22 ± 1 °C) and had free access to commercial chow and water.

#### Surgical Procedure

MG was unilaterally infused into the lateral ventricle, as was described in previous studies (Lissner et al. 2021; Rodrigues et al. 2019). The rats were divided into two groups: SHAM-operated and MG-treated. Briefly, the rats were anesthetized with ketamine/xylazine (75 and 10 mg/kg, respectively, i.p.) and positioned in a stereotaxic apparatus. A midline sagittal incision was made on the scalp, and burr holes were drilled in the skull on the right side of the lateral ventricle. The right ventricle was accessed using the following coordinates: 0.9 mm posterior to the bregma; 1.5 mm lateral to the sagittal suture; and 3.6 mm beneath the surface of the brain. The animals received 5 µL of MG ICV unilaterally at a concentration of 3 µmol/µL. The final concentration of MG in the cerebrospinal fluid (CSF) was assumed to be equal to the injected concentration (3 µmol/µL) multiplied by the injected volume (5 µL), divided by the CSF volume. Considering a static CSF volume of 305 (300 µL basal + 5 µL injected volume), we can calculate a maximum final concentration of 49 nmol/µL at the time of injection, which should fall considering the CSF flow (Chiu et al. 2012). This high initial MG value contrasts with the value described in the CSF of patients with AD (approximately 22 pmol/µL) (Kuhla et al. 2005). However, this allowed us to envision a direct effect...
of high concentrations of MG, which could occur at determined sites in extreme situations, during the hyperglycemia peaks associated with insulin resistance, found both in patients with diabetes and AD. The SHAM group received an equal volume of Hank's balanced salt solution (HBSS–containing in mM: 137 NaCl, 5.36 KCl, 1.26 CaCl2, 0.41 MgSO4, 0.49 MgCl2, 0.63 Na2HPO4·7H2O, 0.44 KH2PO4, 4.17 NaHCO3, and 5.6 glucose, adjusted to pH 7.2). After the surgical procedure, the animals were kept warm until they recovered from anesthesia. Approximately 72 h after MG injection, the rats were euthanized, and biochemical parameters were evaluated.

**Biochemical Analysis**

**Obtaining Cerebrospinal Fluid, Serum, and Hippocampal Slices**

Animals were anesthetized with ketamine/xylazine (i.p.) and positioned in a stereotoxic holder to collect 100 µL (approximately) of CSF from the cisterna magna. The puncture was performed using an insulin syringe (27-gauge × 1/2" length). The rats were then removed from the stereotoxic apparatus and placed on a flat surface. Whole blood was obtained through an intracardiac puncture using a 0.37-mm diameter needle, collected into clot activator tubes, and centrifuged (3000 rpm, 10 min, 4 °C) to separate the serum. The animals were killed by decapitation. The hippocampi were dissected, and transverse 0.3 mm were obtained using a McElwain Tissue Chopper. The samples were frozen at − 80 °C until further analysis (Netto et al. 2006).

**Determination of CSF/Serum Albumin Ratio**

The CSF/serum albumin ratio was analyzed using the bromocresol green assay kit from Doles (Goiânia, Brazil); the methodology reported by Durgawale et al. was modified for this procedure (Durgawale et al. 2005). Serum (10 µL) or CSF (50 µL) was mixed with 2 mL of color reagent. The samples were incubated for 10 min at 37 °C, and the absorbance was measured at 546 nm against a reagent blank.

**In Vivo EBD Injection and Dye Extraction**

BBB permeability was evaluated according to the distribution in the rat brain of EBD administered at a dose of 2 mg/kg by intracarotid injection (Manaenko et al. 2011; Wang and Lai 2015). After a 2 h period to enable uniform EBD distribution, the rats were anesthetized using ketamine/xylazine and perfused through the left cardiac ventricle with 200 mL of saline solution. The brains were removed, weighed, and homogenized in phosphate-buffered saline (PBS)/TCA 60%. The solutions were centrifuged at 10,000 g for 14 min to remove precipitates and tissue debris. The supernatants were added to a 96-well plate (30 µL per well, each plate supplemented with 90 µL of ethanol (to provide consistent optical path length spectroscopic measurements)). The standard EBD curve ranged from (0.05 to 10 µg/ml) (Manaenko et al. 2011; Wang and Lai 2015), modified. The brains and the content of the dye extracted from each brain were determined using a spectrophotometer (at 620 nm).

**Western Blot**

Proteins from the hippocampal sample were homogenized in a sample buffer [62.5 mM Tris–HCl pH 6.8, 2% (w/v) SDS, 5% (w/v) β-mercaptoethanol, 10% (v/v) glycerol, 0.002% (w/v) bromophenol blue] and separated using SDS-PAGE on 12% sodium dodecyl sulfate polyacrylamide and electro transferred onto nitrocellulose membranes. The membranes were blocked overnight at 4 °C with 2% chicken egg albumin in Tris-buffered saline with Tween 20 (TTBS). After overnight incubation at 4 °C with primary antibodies (anti-AQP4, anti-Cx43, and anti-RAGE at dilution 1:5000), the membranes were incubated for 1 h at room temperature and exposed to horseradish peroxidase-linked anti-IgG antibodies. Chemiluminescent bands were detected using an image analyzer (Image Quant LAS4000 from GE), and the optical density was quantified using ImageJ software. To quantify the WB, we used the ratio of the intensity of the band of interest on the intensity of the signal for the revelation to the reference protein (β-Actin). To gels image saturation, we used the automatic tone of Adobe Photoshop. The results are expressed as percentages of SHAM.

**Quantification of S100B**

Hippocampal S100B contents were measured by an enzyme-linked immunosorbent assay (ELISA) as described previously (Leite et al. 2008). Fifty microliters of sample (previously diluted when necessary) plus 50 µL of Tris buffer were incubated for 2 h on a microtitre plate previously coated overnight with monoclonal anti-S100B (SH-B1) antibody. Polyclonal anti-S100 and peroxidase-conjugated anti-rabbit antibodies were incubated at the same time for 60 min at 37 °C. A colorimetric reaction with o-phenylenediamine was performed using an insulin syringe (27-gauge × 1/2" length). The rats were then removed from the stereotaxic apparatus and placed on a flat surface. Whole blood was obtained through an intracardiac puncture using a 0.37-mm diameter needle, collected into clot activator tubes, and centrifuged (3000 rpm, 10 min, 4 °C) to separate the serum. The animals were killed by decapitation. The hippocampi were dissected, and transverse 0.3 mm were obtained using a McElwain Tissue Chopper. The samples were frozen at − 80 °C until further analysis (Netto et al. 2006).

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High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Thermo Fisher Scientific) in a 20 μL reaction according to the manufacturer’s instructions. The messenger RNA (mRNA) encoding HMGB1 (#Rn02377062_g1), HO-1 (#Rn01536933_m1), Nrf2 (#Rn00582415_m1), and p50 NF-κB (#Rn01399572_m1) were quantified using the TaqMan real-time RT-PCR system with inventory primers and probes purchased from Applied Biosystems (Bobermin et al. 2019). The target mRNA levels were normalized to β-actin (#Rn00667869_m1) levels. The results were analyzed using the $2^{-\Delta\Delta C_t}$ method (Bobermin et al. 2020) adapted from Livak and Schmittgen (2001) and expressed relative to the levels in the SHAM group.

**Protein Determination**

Protein content was measured by Lowry’s method, using bovine serum albumin as the standard (Peterson 1977).

**Statistical Analysis**

Data are reported as mean ± standard error and were analyzed statistically using Student’s $t$ test. Differences were considered significant at $p \leq 0.05$. All analyses were performed using the Prism 6.0 (GraphPad).

**Results**

For all techniques performed, the samples were obtained 72 h after ICV administration of MG.

**MG Infusion Disrupted BBB Permeability and Increased Albumin Levels**

Initially, we evaluated the effect of MG-ICV on the functional permeability of the BBB using Evans blue staining assay and CSF/serum albumin ratio. The concentration of EBD extracted from the total brain increased in the MG group (Fig. 1A, $p = 0.001$), and concomitantly, the CSF/serum albumin ratio increased drastically in the MG-treated group when compared with the SHAM group (Fig. 1B, $p < 0.0001$).

**Decreased AQP4 and Cx43 Levels in MG-Treated Group**

To identify alterations in astroglial connectivity related to the BBB, we measured the levels of AQP4 and Cx43 proteins using western blotting. We observed a decrease in AQP4 (Fig. 2A, $p = 0.04$) and Cx43 (Fig. 2B, $p = 0.01$) levels.

**RAGE Signaling Was Altered in MG-Treated Group**

To understand whether RAGE signaling was involved, we measured the levels of RAGE and S100B, a RAGE ligand secreted by astrocytes. No changes were observed in RAGE (Fig. 3A, $p = 0.89$) or S100B (Fig. 3B, $p = 0.19$). We then evaluated the mRNA levels of S100B and HMGB1, which are RAGE ligands. In the MG-treated groups, we observed a decrease in the mRNA levels of S100B (Fig. 3C, $p = 0.04$), but not HMGB1 (Fig. 3D, $p = 0.16$).

![Fig. 1](image1.png) **Effect of ICV administration of MG on function and integrity of the BBB.** All measurements were made 72 h after surgery. Concentration of EBD was extracted from total brain and measured by spectroscopy (absorbance at 620 nm) (A) and CSF/serum albumin ratio was measured by bromocresol green assay kit (B). Data expressed as means ± S.E.M. ($N = 4–6$ animals per group). Data were analyzed using Student’s $t$ test assuming $p < 0.05$, and the statistical significance represents difference from the SHAM group.
MG Exposure Increased HO-1 mRNA Expression in the Hippocampus

We then investigated the hippocampal inflammatory pathways that may be affected by MG exposure. No changes were observed in the mRNA levels of NF-κB (Fig. 4A, $p = 0.74$) and Nrf2 (Fig. 4B, $p = 0.82$) in the hippocampal tissue of animals exposed to MG. However, an increase in mRNA levels were observed for HO-1 in the MG group (Fig. 4C, $p = 0.03$).

Discussion

Carbonyl stress is triggered by the accumulation of reactive species such as MG (Ramasamy et al. 2006). Additionally, the risk of glycation levels in the cardiovascular and cerebrovascular systems of patients with diabetes and Alzheimer’s disease is elevated (Tóth et al. 2014b; Vander Jagt and Hunsaker 2003; Lapolla et al. 2003). Under hyperglycemic conditions, MG induces endothelial barrier microvascular dysfunction (Hussain et al. 2016; Li et al. 2013; Irshad et al. 2019), reduced integrity, increased BBB permeability (Tóth et al. 2014a), and contributed to a higher progression of vascular complications and cerebrovascular injury (Fang et al. 2015). Vascular alterations in diabetes were linked to the breakdown of BBB functioning (Hawkins et al. 2007), and these alterations contributed functionally and structurally to brain changes and cognitive impairment observed in patients and experimental models (Serlin et al. 2011; Zanotto et al. 2017). We have previously shown that acute insult with MG affects astroglial activity (Lissner et al. 2021), and considering the involvement of these cells in BBB, we decided to evaluate this possibility and evaluated the involvement of these cells in the BBB.

MG and/or AGEs induce oxidative stress and inflammatory pathway activation by interacting with RAGE (Choi et al. 2016; Kalaposs 2008; Ramasamy et al. 2011; Liu et al. 2013; MacLean et al. 2019). Exacerbated expression of RAGE has been implicated in many neurodegenerative disorders, which can be activated by AGEs and the ligands HMGB1 and S100B (MacLean et al. 2019; Sagheddu et al. 2018).

In this acute model of brain lesions, mRNA levels of S100B (but not HMGB1) were reduced in the hippocampus, without a corresponding decrease in S100B content. Changes in S100B mRNA levels are not necessarily accompanied by changes in protein content (Zimmer et al. 1997; Vizuete et al. 2021). Considering the rapid degradation of MG in organisms (Rabbani and Thornalley 2015), it is important to mention here that we measured S100B parameters only at 72 h and, in further studies, shorter time points in this interval could allow us to better understand such variations. In the central nervous system, S100B was predominantly expressed and secreted by astrocytes and can act as a neurotrophic factor or an inflammatory cytokine by binding to RAGE (Gonçalves et al. 2008; Donato et al. 2009). We did not observe any differences in the hippocampal immune content of RAGE. These data corroborate those of a previous study by our group (Hansen et al. 2016a), which suggests that RAGE is not involved when hippocampal slices are treated with a high concentration of MG. Although it has been observed that high levels of MG can affect astrocyte activity (Hansen et al. 2016a), our suggestion is that more studies are required to confirm that MG could trigger an inflammatory process independent of RAGE (Vlassara 2001; Ott et al. 2014). Changes in protein glycation due to high levels of MG can occur in intracellular signaling pathways, not only in the ligand (S100B and HMGB1) and/or receptor (RAGE). Considering such changes, it is possible to conceive...
an alteration in S100B/RAGE signaling in this acute model, which could occur in situations of hyperglycemic peaks.

Considering the importance of understanding the regulation of the astrocytic inflammatory response in this model, we also investigated the levels of two main transcription factors, possibly activated, NF-κB and Nrf2, and homeoxygenase-1 (HO-1), an enzyme related to neuroprotection (Nitti et al. 2018). Activated Nrf2 has been associated with modulating the expression of antioxidant and detoxifying enzymes, including HO-1 (Syapin 2009; Calkins et al. 2009; Niture et al. 2014), and its absence is related to cellular damage due to an increase in oxidative stress molecules and inflammation (Ichihara et al. 2010; Taki-Nakano et al. 2014). Oxidative stress can also activate NF-κB and concomitantly release proinflammatory cytokines (Patel and Santani 2009).

We observed no changes in the expression of Nrf2 or NF-κB following acute MG exposure. However, the mRNA levels for HO-1 were increased. It should be noted that, in astrocytes, variations in the expression of mRNA levels for HO-1 commonly correspond to variations in their protein content (Schipper et al. 2009; Liddell 2017). These data suggest that upregulation of HO-1 is a protective response against the acute insult generated by MG. These data confirm the importance of HO-1 in MG toxicity and indicate that other transcription factors, in addition to Nrf2 and NF-κB, must be involved in the initial antioxidant response (Nitti et al. 2018).

In addition, we observed acute BBB rupture measured by a higher EBD concentration in the brain tissue and an increased CSF/serum albumin ratio in the MG group. It is known that long-term incubation with high MG concentrations leads to a dramatic change in the permeability of BBB endothelial cells,
possibly due to glycation of tight junction proteins (Hussain et al. 2016). MG has been shown to bind to specific basic sites of proteins, causing endothelial cell detachment in the brain tissue (Tóth et al. 2014a). Although we did not characterize the glycated proteins of the BBB in this acute MG-induced model of damage, the functional impairment in the BBB is clear, as evidenced by the greater permeability of serum albumin and Evans blue dye.

BBB disruption may affect the balance of water and ion homeostasis as well as impair intercellular communication in astrocytic cells (Hussain et al. 2016; Chu et al. 2016). Astroglial impairment induced by MG has been described in cell cultures (Chu et al. 2016; Hansen et al. 2017) and hippocampal slices directly exposed (Hansen et al. 2016a) and hippocampal slices after ICV administration (Hansen et al. 2016b). Considering the importance of astrocytes in BBB function, we investigated whether glial parameters related to the activity of this barrier were altered in this model of acute injury produced by MG.

These results showed that the MG-treated group exhibited decreased hippocampal AQP4 and Cx43 levels. Cx43 is an important connexin for BBB functionality because it is involved in gap communication between endothelial cells and astrocytes (Kovács et al. 2012; Johnson et al. 2018). AGE-albumin treatment of aortic endothelial cells reduced gap communication (Wang et al. 2011). These data suggest that impaired Cx43-dependent gap communication caused by MG exposure affects BBB functionality and the astroglial syncytium in the hippocampus.

Moreover, we observed a decrease in hippocampal AQP4. This water channel is highly expressed in astrocytes, including the end-feet surrounding the endothelium in the BBB. Although we do not know whether these proteins are direct targets of MG-mediated glycation, these data show, for the first time, the acute impairment of BBB cell connectivity to this glucose derivative. Changes in Cx43 and AQP4 revealed an acute failure in BBB connectivity due to MG exposure, which could compromise aqueous and ionic homeostasis.

**Fig. 4** Effect of ICV administration of MG on the Nrf2/HO-1 pathway. All measurements were made 72 h after surgery. Hippocampal slices of rats were dissected out, and mRNA expression of NF-κB (A), Nrf2 (B), and HO-1 (C) were measured. Data expressed as means ± S.E.M (N=5–8 animals per group) assuming SHAM value as 100% and analyzed using Student’s t test, assuming p < 0.05, and the statistical significance represents difference from the SHAM group.
This study has some limitations. First, the MG concentration under pathological conditions (e.g., Alzheimer’s disease) was much lower than that used in this study. However, this concentration was based on previous studies of ICV administration, and it seemed appropriate to study acute MG insult in the BBB (see (Lisnner et al. 2021; Hansen et al. 2016b; Hamsch et al. 2010)). Furthermore, our previous results showed that glial cells and hippocampal slices exposed to high concentrations of MG induced neurochemical alterations without compromising cellular viability or integrity (Hansen et al. 2016a, 2017, 2012). Second, euthanasia was performed 72 h after the administration of MG, and shorter times would allow for a better temporal correlation between functional and neurochemical changes. However, at 72 h, the free circulating presence of MG can be disregarded, because the endogenous metabolism by the glyoxalase system lasts approximately 10 min. Finally, we used commercial MG and did not analyze the purity of this compound. The possibility that impurities may have contributed to the observed effects cannot be excluded.

**Conclusions**

Our findings demonstrated that elevated levels of MG administered by ICV infusion could induce loss of BBB integrity in a short time, based on the increase in permeability and decrease in AQP4 and Cx43 levels. This reinforces the idea that the astrocytic parameters evaluated in this study are susceptible targets of this compound, and that BBB dysfunction may contribute to the behavioral and cognitive alterations observed in these animals. The decrease in S100B mRNA also emphasized glial susceptibility to damage caused by MG. Finally, the defense against acute MG-induced oxidative stress involves upregulation of HO-1 in hippocampal cells, whose initial expression does not appear concomitantly with Nrf2 and/or NF-kB expression.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s12640-022-00571-x.

**Author Contribution** C.A.G. and L.J.L. designed the study. L.J.L., K.M.W., L.R., L.D.B., E.B., and V.G.D. performed the laboratory experiments and collected data. L.J.L. and K. M. W. performed statistical analyses. C.A.G., L. J. L., K.M.W., and L.R. wrote the manuscript. All authors have edited and approved the manuscript.

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**Data Availability** Data used to support the findings of this study are available from the corresponding author upon request.

**Declarations**

**Ethics Approval** All animal experiments were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80–23), and all procedures were approved by the local Animal Care Ethical Committee (CEUA-UFRGS; project number 33663). All efforts were made to minimize animal suffering and to reduce the number of animals used.

**Conflict of Interest** The authors declare no competing interests.

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