Enriched environment prevents oxidative stress in zebrafish submitted to unpredictable chronic stress

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ABSTRACT

Background. The enriched environment (EE) is a laboratory housing model that emerged from efforts to minimize the impact of environmental conditions on laboratory animals. Recently, we showed that EE promoted positive effects on behavior and cortisol levels in zebrafish submitted to the unpredictable chronic stress (UCS) protocol. Here, we expanded the characterization of the effects of UCS protocol by assessing parameters of oxidative status in the zebrafish brain and reveal that EE protects against the oxidative stress induced by chronic stress.

Methods. Zebrafish were exposed to EE (21 or 28 days) or standard housing conditions and subjected to the UCS protocol for seven days. Oxidative stress parameters (lipid peroxidation (TBARS), reactive oxygen species (ROS) levels, non-protein thiol (NPSH) and total thiol (SH) levels, superoxide dismutase (SOD) and catalase (CAT) activities were measured in brain homogenate.

Results. Our results revealed that UCS increased lipid peroxidation and ROS levels, while decreased NPSH levels and SOD activity, suggesting oxidative damage. EE for 28 days prevented all changes induced by the UCS protocol, and EE for 21 days prevented the alterations on NPSH levels, lipid peroxidation and ROS levels. Both EE for 21 or 28 days increased CAT activity.

Discussion. Our findings reinforce the idea that EE exerts neuromodulatory effects in the zebrafish brain. EE promoted positive effects as it helped maintain the redox homeostasis, which may reduce the susceptibility to stress and its oxidative impact.

Subjects Neuroscience, Zoology

Keywords Environmental enrichment, Unpredictable chronic stress, Oxidative stress, Zebrafish

INTRODUCTION

Currently, the issue of housing condition of animals in the laboratory is widely acknowledged in scientific discussions (*Kempermann, Kuhn & Gage, 1997*; *National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011*; *Kim et al., 2017*). *Sherwin (2004)*, for example, reviewed the

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effects of standard laboratory cages design and husbandry in rodents. The author argues that validity of research data is another reason to improve housing conditions of experimental animals, besides the more often mentioned welfare aspect. Different husbandry conditions could contribute to the observed data variability and irreproducibility among different laboratories. For zebrafish, however, studies comparing the impact of housing conditions on research outcomes are scarce. The standard laboratory condition for this species consists of housing the animal in shoals in barren tanks only with a recirculation system, heater thermostat (temperature control), and water in ideal conditions including physical, chemical and biological characteristics (pH, salinity, alkalinity, hardness, dissolved oxygen and nitrogen residues) (*Lawrence & Mason, 2012*). However, this housing environment is very far from the natural habitat conditions to zebrafish, that lives in shallow water with aquatic vegetation and gravel substrates (*Arunachalam et al., 2013*).

In this context, the practice of enriched environment (EE) arose from efforts to minimize the impact of environmental conditions on laboratory animals (*Diamond, Krech & Rosenzweig, 1964*; *Kempermann, Kuhn & Gage, 1997*; *Young et al., 1999*; *Van Praag, Kempermann & Gage, 2000*; *Bennett et al., 2006*). EE is a laboratory housing model that aims to approximate the housing condition to the natural habitat of the animals. This form of housing includes interventions that contributes to increase stimulation of sensory, motor and cognitive neuronal systems of the brain, and it allows or facilitates the animals to develop natural and species-specific behaviors (*Van Praag, Kempermann & Gage, 2000*; *Lazarov et al., 2005*; *Meshi et al., 2006*; *Nithianantharajah & Hannan, 2006*; *Tanti et al., 2013*).

A growing body of evidence reports the beneficial effects of EE for several species of laboratory animals (*Nilsson et al., 1999*; *Brown et al., 2003*; *Sale et al., 2007*; *Sztainberg & Chen, 2010*; *Toth et al., 2011*; *Gapp et al., 2016*). Studies in rodents report that EE has positive effects on the maintenance of the redox state, promoting protection against oxidative stress. For example, it was demonstrated that rats housed in EE presented reduced oxidative stress biomarkers, such as thiobarbituric acid reactive substances (TBARS), protein oxidation, superoxide anion ($O_2^{\bullet -}$) activity and higher values for antioxidant parameters, such as the total radical antioxidant parameter, catalase (CAT) and superoxide dismutase (SOD) when compared to animals housed in standard laboratory conditions (*Mármol et al., 2015*) . A report showed that EE attenuated the upregulation of biomarkers of ROS production, such as levels of oxidase 2 (NOX2) and 8-hydroxy-2-deoxyguanosine (8-OH-dG) induced by a rat model of post-traumatic stress disorder (*Sun et al., 2016*). In addition, EE promoted neuroprotection through epigenetic mechanisms because it increased levels of DNA methylation and reduced levels of hydroxymethylation, as well as increased histone acetylation levels of H3 and H4. This resulted in increased expression of genes encoding oxidative machinery proteins, such as Hmox1, Aox1, and Cox2, and reduced expression of inflammatory genes such as IL-6 E Cxcl10 (*Griñan Ferré et al., 2016*).

Lastly, although some evidence suggests promising results for EE on oxidative stress in rodents, studies that report the effects of EE on this parameter in zebrafish are still scarce. Considering that in our previous study we demonstrated that EE prevented the increase of ROS in zebrafish submitted to the unpredictable chronic stress (UCS) protocol (*Marcon et al., 2018*), we hypothesized that zebrafish submitted to UCS protocol housed in EE would

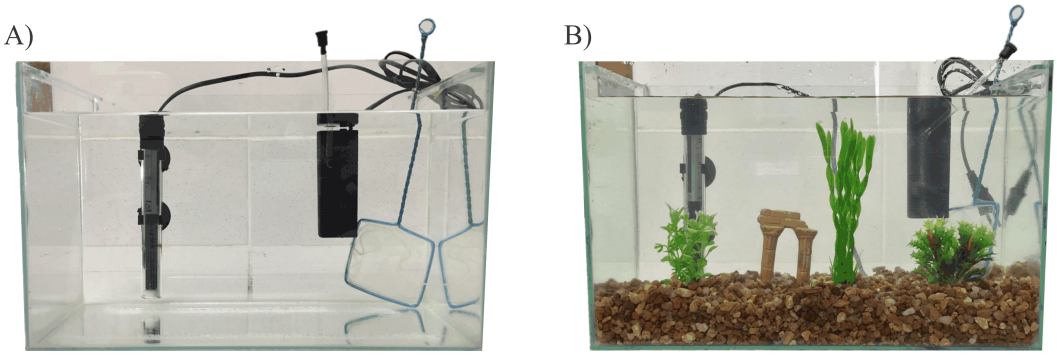

Figure 1 Housing conditions. (A) Barren tank; (B) Enriched environment tank. Image credit/source: (A and B) Matheus Marcon.

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be less vulnerable to oxidative stress. Therefore, in this study we tested the effects of EE in zebrafish submitted to UCS on a range of oxidative stress parameters including lipid peroxidation (TBARS), reactive oxygen species (ROS), non-protein thiol (NPSH) levels, total thiol (SH) levels, superoxide dismutase (SOD) activity, and catalase (CAT) activity.

MATERIAL AND METHODS

Animals

A total of 150 short fin wild-type (WT) adult zebrafish (*Danio rerio*) 50:50 male/female ratio over 6-month-old were purchased from Delphis aquariums (Porto Alegre, Brazil). The fish were kept in a closed acclimation tank system of 16 L (40 × 20× 24 cm, <2 fish per liter) for two weeks. Tanks were filled with non-chlorinated tap water, well-aerated in appropriate conditions as previously reported by *Marcon et al. (2018)*. The illumination of the room was 14/10 h light/dark photoperiod cycle (lights on at 06:00 am). The fish were fed twice a day with a commercial flake fish food (Alcon BASIC®, Alcon, Brazil) and nauplii of brine shrimp (*Artemia salina*). The amount of food was calculated based on the number of fish per tank and followed the instructions of the Zebrafish Book (*Westerfield, 2000*). All experiments were approved by the Ethics Committee of Universidade Federal do Rio Grande do Sul (#30992/2015).

Experimental procedures

EE methodology followed that described by *Marcon et al. (2018)* and is shown in Figs. 1 and 2. After the acclimation period (two weeks), zebrafish were randomly assigned to one of two experimental housing environments: barren tank (BARREN) or enriched environment (EE). BARREN condition consists of standard laboratory tank as described above and containing only water, heater, filter, and aeration system while EE condition consists the same BARREN condition plus tank gravel in the bottom (English sea stones, 4–9 mm, 3 cm high from the bottom of the tank), a ruin-like plastic object, and three submerged plastic plants (two 10 cm tall and one 20 cm tall) (Fig. 1). All tanks of both experimental acclimation conditions were kept in a horizontal plane at the same room, so

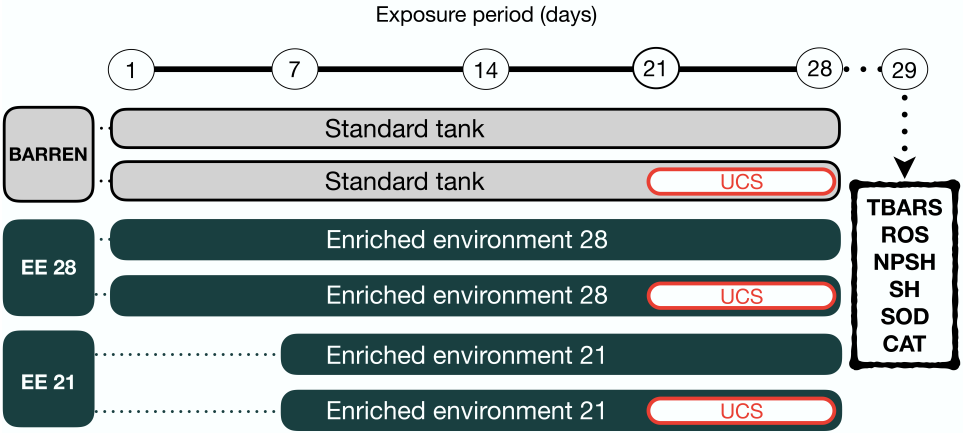

Figure 2 Experimental design. The fish were housed in barren tank (BARREN) or enriched environment tank (EE) for 21 or 28 days. In the last seven days of the experimental protocol, they were submitted to the unpredictable chronic stress (UCS) protocol or remained unchanged (Control). The day after the last stressor, at 08:00 a.m., the fish were euthanized for biochemical analyzes of oxidative stress (the brain was used for the dosage of the brain was used for the dosage of lipid peroxidation (TBARS), reactive oxygen species (ROS) levels, non-protein thiol (NPSH) and total thiol (SH) levels, superoxide dismutase (SOD) and catalase (CAT) activities.

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we used a white frosted cardboard (30 × 60 cm) placed only in between tanks to prevent visual contact of fish from different tanks.

Zebrafish were kept in barren tank (BARREN) or enriched environment tank for 21 (EE 21) or 28 (EE 28) days. In both housing conditions, in the last seven days of the experimental protocol the animals were again divided into two experimental subgroups (non-stressed or stressed, respectively, S− and S+). The S+ groups were submitted to unpredictable chronic stress (UCS) protocol detailed bellow. At the end of the experimental protocol on day 29th (24 h of the last intervention), the animals were removed from their tanks by using a net and immediately anesthetized by rapid cooling (immersion in water at 2–4 °C). After cessation of opercular movements zebrafish were euthanized by decapitation. The brain was used for analysis of oxidative stress.

Unpredictable chronic stress (UCS) protocol

To induce stress in the zebrafish a UCS protocol was used which is already well established and described in our previous studies (*Piato et al., 2011*; *Marcon et al., 2016*; *Rambo et al., 2017*). UCS protocol was based on the following stressors (1) heating tank water up to 33 °C (30 min); (2) cooling tank water to 23 °C (30 min); (3) crowding of 12 animals in a 300-mL beaker (50 min); (4) transferring the animals to other tank with low water level exposing the dorsal body wall (2 min); (5) tank change, three consecutive times with 30-min interval; and (6) chasing with a net (8 min) that were randomly presented twice a day for 7 consecutive days (day 21 to day 28) to the animals of the stressed groups. The non-stressed (S−) animals were maintained in the same room and did not undisturbed throughout the experiments.

Oxidative stress analysis

For brain tissue preparation, immediately after euthanasia, fish were dissected out in ice and to each sample used five brains were pooled that were gently homogenized in ice-cold phosphate buffered saline (PBS; Sigma Aldrich, St. Louis, MO, USA) pH 7.4 and centrifuged at 10,000× g for 5 min at 4 °C to remove cellular debris. The supernatants were collected and used for estimation biomarkers associated with oxidation mechanisms and induction of oxidative stress described herein.

Lipid peroxidation (TBARS)

Lipid peroxidation (*Draper & Hadley, 1990*) was estimated by monitoring thiobarbituric acid reactive substance (TBARS) production. Briefly, a volume equal to 50–70 μg protein of brain homogenate were added to 150 μL of 2% trichloroacetic acid (TCA, Sigma Aldrich®) and centrifuged (10,000× g, 10 min). The supernatants were collected, mixed with 150 μL of 0.5% thiobarbituric acid (TBA; Sigma Aldrich, St. Louis, MO, USA) and then heated at 100 °C for 30 min. The reading of TBARS levels occurred in the microplate reader in absorbance at 532 nm, using 1,1,3,3-tetramethoxypropane (TMP; Sigma Aldrich, St. Louis, MO, USA) as a standard. Results were expressed as nanomoles (nmol) MDA/mg protein ($n = 5$).

Reactive oxygen species (ROS) assay

To evaluate the free radical content (*LeBel et al., 1990*; *Ali, LeBel & Bondy, 1992*), the fluorescent probe 2′, 7′-dichlorofluorescin diacetate (DCFH-DA; Sigma Aldrich, St. Louis, MO, USA) was used. Briefly, 25 μL of brain homogenate was incubated with of 1 mM DCFH-DA and PBS buffer at 37 °C for 30 min. ROS levels was estimated in the microplate reader in fluorescence at 520 nm of emission and 480 nm of excitation using dichlorofluorescein (DCF) as standard. Results were expressed as relative fluorescence unit (RFU) ($n = 5$).

Non-protein thiol (NPSH) levels

To estimate NPSH levels (*Ellman, 1959*), equal volumes (30 μL) of brain preparation and 6% trichloroacetic acid (Sigma Aldrich, St. Louis, MO, USA) was mixed and centrifuged (3,000× g, 10 min at 4 °C). Subsequently, an aliquot of supernatant (50 μg protein) was further mixed with 10 mM 5,5-dithio-bis-2-nitrobenzoic acid (DTNB; Sigma Aldrich, St. Louis, MO, USA) dissolved in ethanol and the intense yellow color developed was measured in the microplate reader at 412 nm after 1 h of incubation at room temperature. Results were expressed as μmol NPSH/mg of protein ($n = 5$).

Total thiol (SH) levels

To estimate SH levels (*Ellman, 1959*), a volume equal to 50 μg protein of brain homogenate was mixed with 10 mM 5,5-dithio-bis-2-nitrobenzoic acid (DTNB; Sigma Aldrich, St. Louis, MO, USA) dissolved in ethanol (Sigma Aldrich, St. Louis, MO, USA). The intense yellow color developed was measured in the microplate reader at 412 nm after 1 h of incubation at room temperature. Results were expressed as μmol SH/mg of protein ($n = 5$).

Superoxide dismutase (SOD) activity

SOD activity (*Misra & Fridovich, 1972*) was estimated by quantifying the inhibition of superoxide-dependent adrenaline auto-oxidation. Adrenochrome formation rate was observed at 480 nm in the microplate reader in a reaction medium containing glycine-NaOH (50 mM, pH 10, Sigma Aldrich®), epinephrine (60 mM, pH 1.7; Sigma Aldrich, St. Louis, MO, USA), and homogenate brain (15–30–60 μg of protein). Results were expressed in Units/mg protein ($n = 5$).

Catalase (CAT) activity

CAT activity (*Aebi, 1984*) was estimated by measuring the rate of decrease in hydrogen peroxide (H_2O_2) absorbance at 240 nm. Assay mixture consisted of potassium phosphate buffer (Sigma Aldrich®), H_2O_2 (1 M; Sigma Aldrich, St. Louis, MO, USA) and brain homogenate (30 μg protein). The results were expressed in Units/mg protein ($n = 5$).

Protein determination

Protein was determined by the Coomassie blue method (*Bradford, 1976*) using bovine serum albumin (Sigma Aldrich, St. Louis, MO, USA) as standard. Absorbance of samples was measured at 595 nm.

Statistical analysis

Kolmogorov–Smirnov and Levene tests were used to determine the normal distribution of the data and homogeneity of variance, respectively. Results were analyzed by two-way ANOVA (stress and enriched environment as independent factors) followed by Tukey post hoc test for comparisons within groups and between housing conditions. Differences were considered significant at $p < 0.05$. The data were expressed as a mean + standard error of the mean (S.E.M.).

RESULTS

Figure 3 shows the effects of EE on biochemical parameters associated with oxidative stress (TBARS and ROS) in zebrafish submitted to UCS and summarizes the two-way ANOVA analyzes. Regarding TBARS (Fig. 3A), two-way ANOVA revealed that UCS interacted with EE: stress only increased TBARS levels when fish were housed in barren tanks, but not when they were housed for 21 or 28 days of EE. Regarding ROS (Fig. 3B), two-way ANOVA also revealed an interaction between UCS and EE: increased ROS levels were observed only in stressed fish from barren tanks, but not from enriched tanks.

Figures 4 and 5 show the effects of EE on biochemical parameters associated with antioxidant mechanisms (NPSH and SH levels, SOD and CAT activity) in zebrafish submitted to UCS. In Fig. 4A, two-way ANOVA revealed an interaction between UCS and EE for NPSH levels: stress decreased NPSH levels only in fish from barren tanks, while EE for 21 or 28 days prevented this effect of stress. Regarding SH levels (Fig. 4B), two-way ANOVA revealed an interaction but no main effects of UCS and EE; post hoc tests, however, did not reach significance for multiple comparisons between groups.

In Fig. 5A, two-way ANOVA for SOD activity revealed an interaction between UCS and EE: stress decreased SOD activity, which was prevented only when fish were housed in

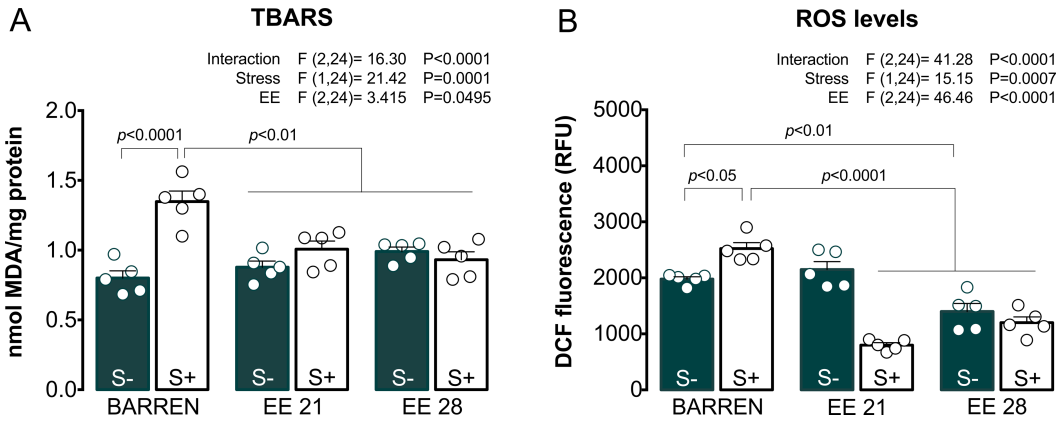

Figure 3 Effects of enriched environment for 21 (EE 21) or 28 days (EE 28) on biochemical parameters associated with oxidative stress in zebrafish brain submitted to unpredictable chronic stress (S+) or not (S−). Lipid peroxidation (TBARS) and reactive oxygen species (ROS) levels. BARREN: barren tank. Data are expressed as a mean + S.E.M. $n = 5$. Two-way ANOVA/Tukey.

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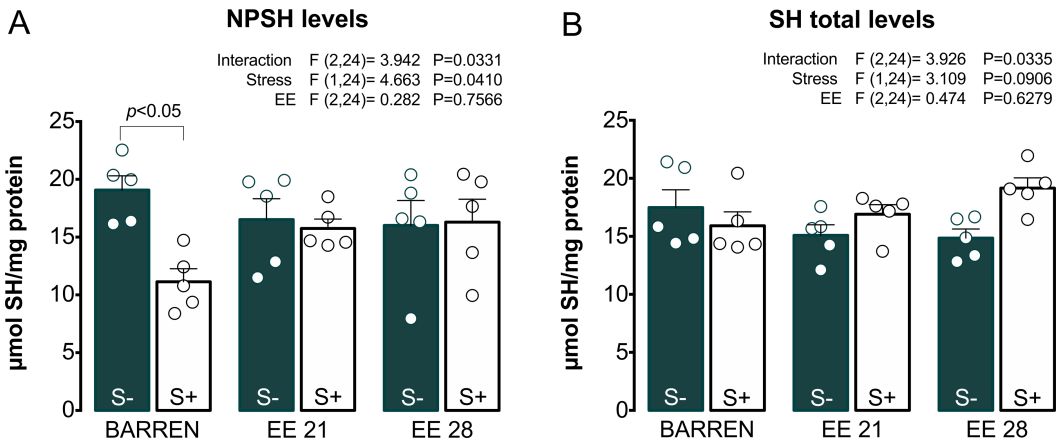

Figure 4 Effects of enriched environment for 21 (EE 21) or 28 days (EE 28) on antioxidant mechanisms in zebrafish brain submitted to unpredictable chronic stress (S+) or no (S−). Non-protein thiols (NPSH) and Total thiol (SH) levels. BARREN: barren tank. Data are expressed as a mean + S.E.M. $n = 5$. Two-way ANOVA/Tukey.

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EE for 28, but not 21 days. Regarding CAT activity (Fig. 5B), two-way ANOVA revealed main effects for UCS and EE, but no interaction between these factors; overall, stress decreased while EE increased CAT activity. Previously, some studies had already reported the protective potential of EE on maintenance of redox homeostasis. EE showed to prevent DNA oxidation (*Kang et al., 2016*; *Sun et al., 2016*), the increase of carbonyl protein (*Herring et al., 2008*), the increase of total free radicals content (*Cechetti et al., 2012*) and the increase of lipid peroxidation (*Muhammad et al., 2017*) in rodents.

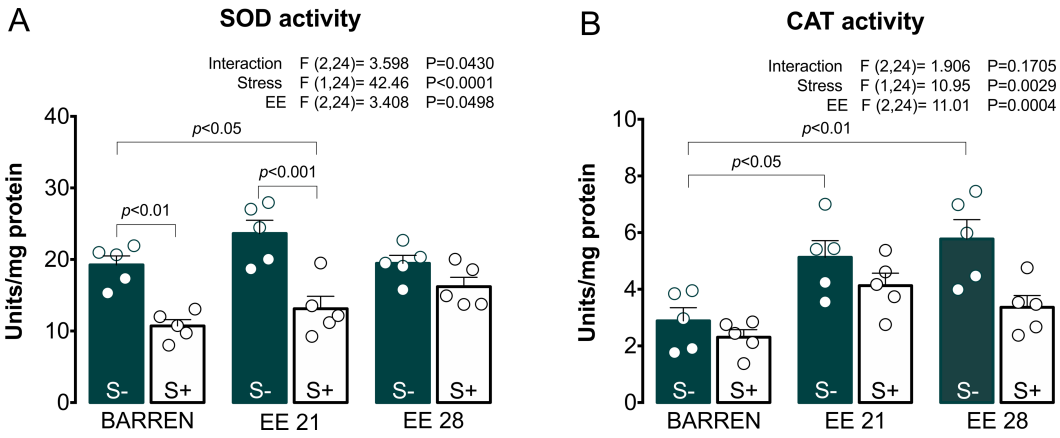

Figure 5 Effects of enriched environment for 21 (EE 21) or 28 days (EE 28) on antioxidant mechanisms in zebrafish brain submitted to unpredictable chronic stress (S+) or no (S−). Superoxide dismutase (SOD) and Catalase (CAT) activity. BARREN: barren tank. Data are expressed as a mean + S.E.M. $n = 5$. Two-way ANOVA/Tukey.

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DISCUSSION

In this study, we replicate a previous result and expand the characterization of the effects of EE on mechanisms associated with antioxidant defenses and oxidative stress in zebrafish submitted to UCS. We demonstrated for the first time that UCS protocol induced several changes in redox homeostasis in the zebrafish brain and revealed that EE has a protective effect against the oxidative stress induced by the UCS protocol.

UCS protocol induces several biochemical changes in the zebrafish brain and through sustained activation of the neuroendocrine axis leads to increased cortisol levels (*Piato et al., 2011*; *Manuel et al., 2014*; *Marcon et al., 2016*; *Rambo et al., 2017*; *Song et al., 2017*). This was confirmed by the results recently published in our previous study, which showed that the UCS protocol increased cortisol levels while EE for 21 or 28 days prevented this increase (*Marcon et al., 2018*). In this way, the response to sustained stress leads to great energy expenditure and for this reason some cellular metabolic processes are accelerated (*Otte et al., 2016*), such as oxidative phosphorylation (*Zorov, Juhaszova & Sollott, 2014*) and β-oxidation of fatty acids (*Carracedo, Cantley & Pandolfi, 2013*). As a consequence, the excessive production of ROS can reach levels above the antioxidant defense capacity of the organism and consequently oxidize cellular structures leading to oxidative stress (*Sies, Berndt & Jones, 2017*; *Poprac et al., 2017*).

Particular features of nervous tissue, such as neurotransmitters metabolism, high iron content, low antioxidant capacity, neuronal membrane rich in polyunsaturated fatty acids and high oxygen consumption, make the brain an organ extremely susceptible to oxidative stress (*Clarke & Sokoloff, 1999*; *Halliwell, 2006*). Therefore, sustained antioxidant mechanisms are necessary for the maintenance of cerebral homeostasis (*Finkel & Holbrook, 2000*).

Glutathione (GSH) is the main antioxidant component in brain tissue. It is essential for maintenance of redox homeostasis, serving as the cofactor of the enzymes glutathione peroxidase (GPx) and glutathione-S-transferase (GST) and a direct neutralizer of ROS

(*Dringen, 2000*; *Dringen & Hirrlinger, 2003*). Here we showed that the UCS protocol decreased NPSH levels, a measure that reflects the levels of GSH, while it did not alter the SH levels (thiols groups associated with cysteine residues), suggesting that chronic stress promoted the depletion of cerebral GSH in zebrafish.

At the same time, the UCS protocol decreased SOD but did not alter CAT activities. Physiologically the SOD enzyme plays a key role in neutralizing the superoxide anion ($O_2^{\bullet -}$) to hydrogen peroxide (H_2O_2), which is synergistically converted to water (H_2O) and oxygen (O_2) by CAT (*Fukai & Ushio-Fukai, 2011*). Therefore, we hypothesized the decrease in the SOD activity induced by UCS may led to an excessive accumulation of $O_2^{\bullet -}$. $O_2^{\bullet -}$ in high concentrations may contribute to oxidative stress through direct or indirect damage, for example, by the formation of other reactive species, such as peroxynitrite ($ONOO^-$), H_2O_2 or hydroxyl radical ($OH^{\bullet}$) (Fenton reaction) (*Pacher, Beckman & Liaudet, 2007*). Additionally, we revealed here that the UCS protocol increases ROS production and therefore we suggest that the high levels of ROS associated with the decrease of the antioxidant mechanisms (GSH level and decreased SOD activity) led to an imbalance between its production and detoxification and consequently increased lipid peroxidation in stressed animals leading to oxidative stress. This is according to a previous study that showed a decrease in the values of total antioxidant status, SOD activity and the increase of lipid peroxidation in mice submitted to chronic unpredictable mild stress (*Biala et al., 2017*).

Oxidative stress is related to the development of mental disorders (*Ng et al., 2008*) and it was demonstrated to contribute to the pathophysiology of neurodegenerative diseases (*Christen, 2000*). Therefore, it is remarkable the need for studies that bring new discoveries in this line. Interestingly, here, we have shown for the first time that EE promoted protection against oxidative stress induced by UCS. We report that EE for 28 days prevented all changes induced by UCS in the oxidative status while EE for 21 days prevented the decreased of NPSH levels and the increased of the lipid peroxidation and ROS levels. Besides, both EE for 21 or 28 days increased the CAT activity.

In this study, we suggest that EE prevented the oxidative by preventing the decrease of antioxidant defenses (GSH level and SOD enzyme activity), as well as the increase of ROS levels. Furthermore, EE increases the expression of glucocorticoid receptors (*Shilpa et al., 2017*), which is associated with downregulation of neuroendocrine axis activity; this occurs by negative feedback at the cortisol receptor and reduces the response to sustained stress.

CONCLUSION

Our findings are in agreement with our previous study and together with the literature findings reinforce the idea that EE exerts neuromodulatory effects. Here, we revealed that EE promoted positive effects in the maintenance of redox homeostasis, which may reduce the susceptibility to stress and its oxidative impact. However, our data are still preliminary and require further investigation to establish and clarify the exact neurobiological mechanisms by which EE prevents changes in oxidative status. Also, we reinforce and suggest that zebrafish is a suitable animal model to investigate the neurobiology of stress and the effects of EE.

ADDITIONAL INFORMATION AND DECLARATIONS

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Competing Interests

Angelo Piato is an Academic Editor for PeerJ.

Author Contributions

- Matheus Marcon conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Ricieri Mocelin and Adrieli Sachett performed the experiments, approved the final draft.
- Anna M. Siebel contributed reagents/materials/analysis tools, approved the final draft.
- Ana P. Herrmann conceived and designed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Angelo Piato conceived and designed the experiments, analyzed the data, contributed reagents/materials/analysis tools, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.

Animal Ethics

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

All experiments were approved by the Ethics Committee of Universidade Federal do Rio Grande do Sul (#30992/2015).

Data Availability

The following information was supplied regarding data availability:

The raw data are provided in Data S1.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.5136#supplemental-information.

REFERENCES

Aebi H. 1984. Catalase *in vitro*. *Methods in Enzymology* **105**:121–126 DOI 10.1016/S0076-6879(84)05016-3.

Ali SF, LeBel CP, Bondy SC. 1992. Reactive oxygen species formation as a biomarker of methylmercury and trimethyltin neurotoxicity. *Neurotoxicology* **13**:637–648.

Arunachalam M, Raja M, Vijayakumar C, Malaiammal P, Mayden RL. 2013. Natural history of zebrafish (Danio rerio) in India. *Zebrafish* **10**:1–14 DOI 10.1089/zeb.2012.0803.

Bennett JC, McRae PA, Levy LJ, Frick KM. 2006. Long-term continuous, but not daily, environmental enrichment reduces spatial memory decline in aged male mice. *Neurobiology of Learning and Memory* **85**:139–152 DOI 10.1016/j.nlm.2005.09.003.

Biala G, Pekala K, Boguszewska-Czubara A, Michalak A, Kruk-Slomka M, Budzynska B. 2017. Behavioral and biochemical interaction between nicotine and chronic unpredictable mild stress in mice. *Molecular Neurobiology* **54**:904–921 DOI 10.1007/s12035-016-9701-0.

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72**:248–254 DOI 10.1016/0003-2697(76)90527-3.

Brown J, Cooper-Kuhn CM, Kempermann G, Van Praag H, Winkler J, Gage FH, Kuhn HG. 2003. Enriched environment and physical activity stimulate hippocampal but not olfactory bulb neurogenesis. *The European Journal of Neuroscience* **17**:2042–2046 DOI 10.1046/j.1460-9568.2003.02647.x.

Carracedo A, Cantley LC, Pandolfi PP. 2013. Cancer metabolism: fatty acid oxidation in the limelight. *Nature Reviews. Cancer* **13**:227–232 DOI 10.1038/nrc3483.

Cechetti F, Worm PV, Lovatel G, Moysés F, Siqueira IR, Netto CA. 2012. Environmental enrichment prevents behavioral deficits and oxidative stress caused by chronic cerebral hypoperfusion in the rat. *Life Sciences* **91**:29–36 DOI 10.1016/j.lfs.2012.05.013.

Christen Y. 2000. Oxidative stress and Alzheimer disease. *The American Journal of Clinical Nutrition* **71**:621s–629s DOI 10.1093/ajcn/71.2.621s.

Clarke DD, Sokoloff L. 1999. Circulation and energy metabolism of the brain. In: Siegel GJ, Agranoff BW, Albers RW, Fisher SK, Uhler MD, eds. *Basic neurochemistry: molecular, cellular and medical aspects*. 6th edition. Philadelphia: Lippincott-Raven. Chapter 31.

Diamond MC, Krech D, Rosenzweig MR. 1964. The effects of an enriched environment on the histology of the rat cerebral cortex. *The Journal of Comparative Neurology* **123**:111–120 DOI 10.1002/cne.901230110.

Draper HH, Hadley M. 1990. Malondialdehyde determination as index of lipid peroxidation. *Methods in Enzymology* **186**:421–431 DOI 10.1016/0076-6879(90)86135-I.

Dringen R. 2000. Metabolism and functions of glutathione in brain. *Progress in Neurobiology* **62**:649–671 DOI 10.1016/S0301-0082(99)00060-X.

Dringen R, Hirrlinger J. 2003. Glutathione pathways in the brain. *Biological Chemistry* **384**:505–516 DOI 10.1515/BC.2003.059.

Ellman GL. 1959. Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics* **82**:70–77 DOI 10.1016/0003-9861(59)90090-6.

Griñan Ferré C, Puigoriol-Illamola D, Palomera-Ávalos V, Pérez-Cáceres D, Companys-Alemany J, Camins A, Ortuño Sahagún D, Rodrigo MT, Pallàs M. 2016. Environmental enrichment modified epigenetic mechanisms in SAMP8 mouse hippocampus by reducing oxidative stress and inflammaging and achieving neuroprotection. *Frontiers in Aging Neuroscience* **8**:241 DOI 10.3389/fnagi.2016.00241.

Finkel T, Holbrook NJ. 2000. Oxidants, oxidative stress and the biology of ageing. *Nature* **408**:239–247 DOI 10.1038/35041687.

Fukai T, Ushio-Fukai M. 2011. Superoxide dismutases: role in redox signaling, vascular function, and diseases. *Antioxidants & Redox Signaling* **15**:1583–1606 DOI 10.1089/ars.2011.3999.

Gapp K, Bohacek J, Grossmann J, Brunner AM, Manuella F, Nanni P, Mansuy IM. 2016. Potential of environmental enrichment to prevent transgenerational effects of paternal trauma. *Neuropsychopharmacology* **41**:2749–2758 DOI 10.1038/npp.2016.87.

Halliwell B. 2006. Oxidative stress and neurodegeneration: where are we now? *Journal of Neurochemistry* **97**:1634–1658 DOI 10.1111/j.1471-4159.2006.03907.x.

Herring A, Blome M, Ambrée O, Sachser N, Paulus W, Keyvani K. 2008. Reduction of cerebral oxidative stress following environmental enrichment in mice with Alzheimer-like pathology. *Brain Pathology* **20**:166–175 DOI 10.1111/j.1750-3639.2008.00257.x.

Kang H, Choi D-H, Kim S-K, Lee J, Kim Y-J. 2016. Alteration of energy metabolism and antioxidative processing in the hippocampus of rats reared in long-term environmental enrichment. *Developmental Neuroscience* **38**:186–194 DOI 10.1159/000446772.

Kempermann G, Kuhn HG, Gage FH. 1997. More hippocampal neurons in adult mice living in an enriched environment. *Nature* **386**:493–495 DOI 10.1038/386493a0.

Kim WY, Cho BR, Kwak MJ, Kim J-H. 2017. Interaction between trait and housing condition produces differential decision-making toward risk choice in a rat gambling task. *Scientific Reports* **7**:5718 DOI 10.1038/s41598-017-06408-4.

Lawrence C, Mason T. 2012. Zebrafish housing systems: a review of basic operating principles and considerations for design and functionality. *ILAR Journal/National Research Council, Institute of Laboratory Animal Resources* **53**:179–191 DOI 10.1093/ilar.53.2.179.

Lazarov O, Robinson J, Tang Y-P, Hairston IS, Korade-Mirnics Z, Lee VM-Y, Hersh LB, Sapolsky RM, Mirnics K, Sisodia SS. 2005. Environmental enrichment reduces Abeta levels and amyloid deposition in transgenic mice. *Cell* **120**:701–713 DOI 10.1016/j.cell.2005.01.015.

LeBel CP, Ali SF, McKee M, Bondy SC. 1990. Organometal-induced increases in oxygen reactive species: the potential of 2',7'-dichlorofluorescin diacetate as an index of neurotoxic damage. *Toxicology and Applied Pharmacology* **104**:17–24 DOI 10.1016/0041-008X(90)90278-3.

Manuel R, Gorissen M, Zethof J, Ebbesson LOE, Van de Vis H, Flik G, Van den Bos R. 2014. Unpredictable chronic stress decreases inhibitory avoidance learning in Tuebingen long-fin zebrafish: stronger effects in the resting phase than in the active phase. *The Journal of Experimental Biology* **217**:3919–3928 DOI 10.1242/jeb.109736.

Marcon M, Herrmann AP, Mocelin R, Rambo CL, Koakoski G, Abreu MS, Conterato GMM, Kist LW, Bogo MR, Zanatta L, Barcellos LJG, Piato AL. 2016. Prevention of unpredictable chronic stress-related phenomena in zebrafish exposed to bromazepam, fluoxetine and nortriptyline. *Psychopharmacology* **233(21–22)**:3815–3824 DOI 10.1007/s00213-016-4408-5.

Marcon M, Mocelin R, Benvenutti R, Costa T, Herrmann AP, De Oliveira DL, Koakoski G, Barcellos LJG, Piato A. 2018. Environmental enrichment modulates the response to chronic stress in zebrafish. *The Journal of Experimental Biology* **221(Pt 4)**:1–7 DOI 10.1242/jeb.176735.

Mármol F, Rodríguez CA, Sánchez J, Chamizo VD. 2015. Anti-oxidative effects produced by environmental enrichment in the hippocampus and cerebral cortex of male and female rats. *Brain Research* **1613**:120–129 DOI 10.1016/j.brainres.2015.04.007.

Meshi D, Drew MR, Saxe M, Ansorge MS, David D, Santarelli L, Malapani C, Moore H, Hen R. 2006. Hippocampal neurogenesis is not required for behavioral effects of environmental enrichment. *Nature Neuroscience* **9**:729–731 DOI 10.1038/nn1696.

Misra HP, Fridovich I. 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *The Journal of Biological Chemistry* **247**:3170–3175.

Muhammad MS, Magaji RA, Mohammed A, Isa A-S, Magaji MG. 2017. Effect of resveratrol and environmental enrichment on biomarkers of oxidative stress in young healthy mice. *Metabolic Brain Disease* **32**:163–170 DOI 10.1007/s11011-016-9891-1.

National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. 2011. Environment, housing, and management. In: *Guide for the care and use of laboratory animals*. 8th edition. Washington, D.C.: National Academies Press.

Ng F, Berk M, Dean O, Bush AI. 2008. Oxidative stress in psychiatric disorders: evidence base and therapeutic implications. *The International Journal of Neuropsychopharmacology* **11**:851–876 DOI 10.1017/S1461145707008401.

Nilsson M, Perfilieva E, Johansson U, Orwar O, Eriksson PS. 1999. Enriched environment increases neurogenesis in the adult rat dentate gyrus and improves spatial memory. *Journal of Neurobiology* **39**:569–578 DOI 10.1002/(SICI)1097-4695(19990615)39:4<569::AID-NEU10>3.0.CO;2-F.

Nithianantharajah J, Hannan AJ. 2006. Enriched environments, experience-dependent plasticity and disorders of the nervous system. *Nature Reviews Neuroscience* **7**:697–709 DOI 10.1038/nrn1970.

Otte C, Gold SM, Penninx BW, Pariante CM, Etkin A, Fava M, Mohr DC, Schatzberg AF. 2016. Major depressive disorder. *Nature Reviews Disease Primers* **2**:16065 DOI 10.1038/nrdp.2016.65.

Pacher P, Beckman JS, Liaudet L. 2007. Nitric Oxide and Peroxynitrite in Health and Disease. *Physiological Reviews* **87**:315–424 DOI 10.1152/physrev.00029.2006.

Piato ÂL, Capiotti KM, Tamborski AR, Oses JP, Barcellos LJG, Bogo MR, Lara DR, Vianna MR, Bonan CD. 2011. Unpredictable chronic stress model in zebrafish (Danio rerio): behavioral and physiological responses. *Progress in Neuro-Psychopharmacology & Biological Psychiatry* **35**:561–567 DOI 10.1016/j.pnpbp.2010.12.018.

Poprac P, Jomova K, Simunkova M, Kollar V, Rhodes CJ, Valko M. 2017. Targeting free radicals in oxidative stress-related human diseases. *Trends in Pharmacological Sciences* **38**:592–607 DOI 10.1016/j.tips.2017.04.005.

Rambo CL, Mocelin R, Marcon M, Villanova D, Koakoski G, De Abreu MS, Oliveira TA, Barcellos LJG, Piato AL, Bonan CD. 2017. Gender differences in aggression and cortisol levels in zebrafish subjected to unpredictable chronic stress. *Physiology & Behavior* **171**:50–54 DOI 10.1016/j.physbeh.2016.12.032.

Sale A, Maya Vetencourt JF, Medini P, Cenni MC, Baroncelli L, De Pasquale R, Maffei L. 2007. Environmental enrichment in adulthood promotes amblyopia recovery through a reduction of intracortical inhibition. *Nature Neuroscience* **10**:679–681 DOI 10.1038/nn1899.

Sherwin CM. 2004. The influences of standard laboratory cages on rodents and the validity of research data. *Animal Welfare* **13**:9–15.

Shilpa BM, Bhagya V, Harish G, Srinivas Bharath MM, Shankaranarayana Rao BS. 2017. Environmental enrichment ameliorates chronic immobilisation stress-induced spatial learning deficits and restores the expression of BDNF, VEGF, GFAP and glucocorticoid receptors. *Progress in Neuro-Psychopharmacology & Biological Psychiatry* **76**:88–100 DOI 10.1016/j.pnpbp.2017.02.025.

Sies H, Berndt C, Jones DP. 2017. Oxidative stress. *Annual Review of Biochemistry* **86**:715–748 DOI 10.1146/annurev-biochem-061516-045037.

Song C, Liu B-P, Zhang Y-P, Peng Z, Wang J, Collier AD, Echevarria DJ, Savelieva KV, Lawrence RF, Rex CS, Meshalkina DA, Kalueff AV. 2017. Modeling consequences of prolonged strong unpredictable stress in zebrafish: complex effects on behavior and physiology. *Progress in Neuro-Psychopharmacology & Biological Psychiatry* **81**:384–394 DOI 10.1016/j.pnpbp.2017.08.021.

Sun XR, Zhang H, Zhao HT, Ji MH, Li HH, Wu J, Li KY, Yang JJ. 2016. Amelioration of oxidative stress-induced phenotype loss of parvalbumin interneurons might contribute to the beneficial effects of environmental enrichment in a rat model of post-traumatic stress disorder. *Behavioural Brain Research* **312**:84–92 DOI 10.1016/j.bbr.2016.06.016.

Sztainberg Y, Chen A. 2010. An environmental enrichment model for mice. *Nature Protocols* **5**:1535–1539 DOI 10.1038/nprot.2010.114.

Tanti A, Westphal W-P, Girault V, Brizard B, Devers S, Leguisquet A-M, Surget A, Belzung C. 2013. Region-dependent and stage-specific effects of stress, environmental enrichment, and antidepressant treatment on hippocampal neurogenesis. *Hippocampus* **23**:797–811 DOI 10.1002/hipo.22134.

Toth LA, Kregel K, Leon L, Musch TI. 2011. Environmental enrichment of laboratory rodents: the answer depends on the question. *Comparative Medicine* **61**:314–321.

Van Praag H, Kempermann G, Gage FH. 2000. Neural consequences of environmental enrichment. *Nature Reviews. Neuroscience* **1**:191–198 DOI 10.1038/35044558.

Westerfield M. 2000. *The zebrafish book. A guide for the laboratory use of zebrafish (Danio rerio)*. 4th edition. Eugene: University of Oregon Press.

Young D, Lawlor PA, Leone P, Dragunow M, During MJ. 1999. Environmental enrichment inhibits spontaneous apoptosis, prevents seizures and is neuroprotective. *Nature Medicine* **5**:448–453 DOI 10.1038/7449.

Zorov DB, Juhaszova M, Sollott SJ. 2014. Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. *Physiological Reviews* **94**:909–950 DOI 10.1152/physrev.00026.2013.