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Oxidative Parameters in the Rat Brain of Chronic Mild Stress Model for Depression: Relation to Anhedonia-Like Responses

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Abstract The chronic mild stress (CMS) protocol is widely used to evoke depression-like behaviors in the laboratory. Some animals exposed to CMS are resistant to the development of anhedonia, whereas the remaining are responsive, CMS-resilient and CMS-sensitive, respectively. The aim of this study was to examine the effects of chronic stress on oxidative parameters in the rat brain. The consumption of sweet food, protein and lipid oxidation levels and superoxide dismutase and catalase activities in the rat hippocampus, cortex and cerebellum were assessed. We found a significant increase in protein peroxidation (hippocampus and cortex), a significant increase in catalase activity (cortex, hippocampus and cerebellum) and a decrease in superoxide dismutase activity (cortex, hippocampus and cerebellum) in the CMS-sensitive group compared to the CMS-resilient group and normal controls as well as an increase in lipid peroxidation (cerebellum) in the CMS-sensitive and CMS-resilient groups compared to normal controls. However, there was no significant difference in protein peroxidation (cerebellum) and lipid peroxidation (cortex and hippocampus) among the three groups. In conclusion, our results indicate that the segregation into CMS-sensitive and -resilient groups based on sucrose intake is paralleled by significant differences in oxidative parameters. CMS induces oxidative damage and alterations in the activity of antioxidants which may lead to increased oxidative damage, irrespective of the anhedonia-like status of the stressed animals.

Keywords Anhedonia · Oxidative parameter · Stress · Anhedonia-like response

Introduction

Chronic stress is an etiological factor in anxiety disorder and depression, and therefore, based on this observation, the chronic mild stress (CMS) animal model has been developed to mimic the development and progress of clinical depression. In the CMS model, one of the main symptoms of major depression, anhedonia, is mimicked (Willner et al. 1992). Anhedonia has been widely measured as decreased consumption of and preference for palatable sweet solutions, indicating decreased responsiveness to rewarding stimuli as a consequence of sequential exposure to a variety of mild stressors (Henningsen et al. 2009; Moreau et al. 1995; Papp et al. 1991; Willner 1997). The decrease in sweet consumption does not occur in all animals exposed to CMS; i.e., a group of rats are stress-resistant or -resilient (Bergstrom et al. 2007; Bisgaard et al. 2007; Jayatissa et al. 2006; Strekalova et al. 2004). Previous studies have shown that rats subjected to CMS segregate into two subgroups: a group that develops anhedonia-like symptoms (CMS-sensitive) and a group that appears to be resilient to the influence of chronic stress on hedonic status (CMS-resilient) as assessed by sucrose-intake profiles (Bergstrom et al. 2008). This segregation was confirmed by a place preference conditioning test and on the molecular level by global gene and protein expression analysis (Bisgaard et al. 2007). Additionally, studies have implied that the HPA axis is activated in both

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CMS-sensitive and CMS-resilient animals, hence indicating other processes as responsible for the development of, and resistance to, anhedonia. In particular, this would argue for some protective mechanism only in CMS-resilient animals (Bergstrom et al. 2008).

The brain metabolizes 20 % of total-body oxygen and has a limited amount of antioxidant capacity, so it is very vulnerable to reactive oxygen species (ROS) production. In the CMS model, the generation of free radicals can exceed the capacity of antioxidant defense in the brain. And oxidative stress, which results from increased production of ROS, decreased antioxidant defense or failure to repair oxidative damage, may lead to membrane degradation, cellular dysfunction and apoptosis. Recent studies have consistently reported increased ROS in plasma of patients with major depression, especially with melancholia associated (Bilici et al. 2001). Recent studies also have shown the effects of the CMS paradigm on lipid and protein oxidation levels (markers of oxidative stress) and on superoxide dismutase (SOD) and catalase (CAT) activities (the major antioxidant enzymes) in the rat brain, and it is believed that stress produces oxidants and an imbalance between SOD and CAT activities that contributes to stress-related diseases such as depression (Lucca et al. 2009a). But the relationship between oxidative parameters and an anhedonia-like state remains unknown.

In the present study, we analyzed possible differences in the oxidative parameters among CMS-resilient, CMS-sensitive and normal control rats in the cortex, hippocampus and cerebellum to investigate if any alteration of oxidative stress was specific for the anhedonia-like state and the relationship between them.

Materials and Methods

Subjects

We used adequate measures to minimize pain or discomfort of the rats. The research was conducted in accordance with the guidelines published in the NIH *Guide for the Care and Use of Laboratory Animals* and the principles presented in the "Guidelines for the Use of Animals in Neuroscience Research" by the Society for Neuroscience. All experimental protocols were approved by the Review Committee for the Use of Human or Animal subjects of the Fourth Military Medical University.

Male Wistar rats were purchased from the animal center of the Fourth Military Medical University. Animal weight was approximately 220 g when adaptation for sucrose consumption was initiated and approximately 340 g at the start of the stress regime. Animals were singly housed, except when grouping was applied as a stress parameter. Food and water were available ad libitum except when food and/or water deprivation was applied as a stress parameter. The standard 12-h light/dark cycle, with lights on from 6:00 a.m. to 6:00 p.m., was changed only in the course of the stress regime.

Sucrose-Consumption Test

Animals were first trained to consume a palatable sucrose solution (1.5 %). Training lasted 5 weeks. In this period, the sucrose test was made twice a week during the first 3 weeks and once a week during the last 2 weeks. Animals were food- and water-deprived 14 h before the test. The test consisted of 1-h exposure to a bottle with sucrose solution. During the stress period the sucrose-consumption test was performed once a week.

CMS Protocol and Study Design

On the basis of sucrose intake in the three final baseline tests, animals were divided into two matched groups and placed in separate rooms. One group was exposed to an initial 2 weeks of chronic mild stressors and the other was left undisturbed. The unchallenged group was food- and water-deprived 14 h before the sucrose-consumption test; otherwise, food and water were freely available. The stress procedure is a slight modification of the protocol developed by Papp (Garcia et al. 2008; Sanchez et al. 2003). It consisted of seven different stressors: one period of intermittent illumination, stroboscopic light, grouping and food or water deprivation; two periods of soiled cage and no stress; and three periods of 45° cage tilting. All stressors lasted from 10 to 14 h. Stress was continued during the entire period of treatment. Based on end-point sucrose intake, animals were finally subdivided in three groups: normal controls ($n = 7$), CMS-sensitive ($n = 7$) and CMS-resilient ($n = 10$). At the end of the experiment, all rats were handled and accustomed to the environment where the killing was to be performed. All subjects were removed from their home cages and killed by decapitation. The hippocampus, cortex and cerebellum were immediately isolated and stored at −80 °C for posterior analyses for oxidative parameters.

Oxidative Stress Parameters

In order to assess oxidative damage, the formation of thiobarbituric acid-reactive species (TBARS) was measured during an acid-heating reaction (Esterbauer and Cheeseman 1990; Lucca et al. 2009a). Samples were mixed with 1 ml of trichloroacetic acid (TCA) 10 % and 1 ml of thiobarbituric acid 0.67 % and then heated in a boiling water bath for 15 min. TBARS were determined by

absorbance at 535 nm. Oxidative damage to proteins was measured by quantification of carbonyl groups based on the reaction with dinitrophenylhydrazine (DNPH), as previously described (Levine et al. 1994). Proteins were precipitated by the addition of 20 % TCA and redissolved in DNPH; absorbance was read at 370 nm. To determine CAT activity, brain tissue was sonicated in 50 mmol/l phosphate buffer (pH 7.0), and the resulting suspension was centrifuged at 3,000×g for 10 min. The supernatant was used for enzyme assay. CAT activity was measured by the rate of decrease in hydrogen peroxide absorbance at 240 nm (Aebi 1984). SOD activity was assayed by measuring the inhibition of adrenaline auto-oxidation, as previously described (Bannister and Calabrese 1987). All biochemical measures were normalized to the protein content, with bovine albumin as standard (Lowry et al. 1951).

Statistical Analysis

Results were presented as mean ± SEM. Statistical analysis of data was performed with SPSS 11.0 (SPSS, Inc., Chicago, IL). Data were analyzed by one-way ANOVA followed by a pairwise multiple comparison test (Student–Newman–Keul). The significance level was set at $p < 0.05$.

Results

CMS: Sucrose Intake as a Measure of Anhedonia

Sucrose consumption was monitored once a week throughout the experiments, and the results are shown in Table 1. Based on end-point sucrose intake, animals were subdivided into three groups: normal controls ($n = 7$), CMS-sensitive ($n = 7$) and CMS-resilient ($n = 10$). This segregation lasted throughout the experiment. An anhedonia-like state (CMS-sensitive) was defined as a minimum of 40 % reduction in sucrose intake in response to stress. CMS resilience was defined as remaining on a sucrose-intake level corresponding to the baseline level. Normal control animals did not decrease sucrose intake. No significant difference among the three groups was present at baseline. The segregation was significant through the entire period ($p < 0.05$). The normal control group was significantly different from the CMS-sensitive ($p < 0.05$), but not from the CMS-resilient, animals ($p > 0.05$). The segregation was evident already after 7 days of exposure to CMS and persistent after 14 days.

Effects of CMS on Oxidative Stress Variables in Rat Brain

As shown in Fig. 1, protein peroxidation and carbonyl in the cortex and hippocampus were significantly higher in the CMS-sensitive group compared to the CMS-resilient group (cortex $F_{2,21} = 179.244$, $p = 0.000$; hippocampus $F_{2,21} = 234.557$, $p = 0.000$) and to normal controls (cortex $F_{2,21} = 179.244$, $p = 0.000$; hippocampus $F_{2,21} = 234.557$, $p = 0.000$). Compared with normal controls, protein peroxidation and carbonyl in the cortex and hippocampus were also significantly higher in the CMS-resilient group (cortex $F_{2,21} = 179.244$, $p = 0.000$; hippocampus $F_{2,21} = 234.557$, $p = 0.000$). In the cerebellum, there was no significant main effect of groups for protein peroxidation and carbonyl ($F_{2,21} = 4.588$, $p = 0.055$).

We also measured the lipid peroxidation–TBARS variation (Fig. 2). No significant differences were found for lipid peroxidation–TBARS in the cortex ($F_{2,21} = 0.966$, $p = 0.403$) and hippocampus ($F_{2,21} = 0.966$, $p = 0.095$). In the cerebellum, lipid peroxidation–TBARS was significantly increased in CMS-sensitive ($F_{2,21} = 50.867$, $p = 0.000$) and CMS-resilient ($F_{2,21} = 50.867$, $p = 0.000$) compared to normal controls. No differences were found between CMS-sensitive and CMS-resilient animals ($F_{2,21} = 50.867$, $p = 0.127$).

Effects of CMS on Antioxidant Variables in Rat Brain

As shown in Fig. 3, SOD activity in the cortex and hippocampus was significantly higher in normal controls compared to the CMS-sensitive group (cortex $F_{2,21} = 47.175$, $p = 0.001$; hippocampus $F_{2,21} = 39.527$, $p = 0.001$) and to the CMS-resilient group (cortex $F_{2,21} = 47.175$, $p = 0.007$;

Table 1 Sucrose consumption (mean ± SD) in the chronic mild stress model in three groups: CMS-sensitive ($n = 7$), CMS-resilient ($n = 10$) and normal control ($n = 7$)

	Baseline	1 week	2 weeks	3 weeks	4 weeks	5 weeks
Normal control	10.53 ± 1.29	12.38 ± 2.03	13.78 ± 2.95	14.45 ± 2.38	14.92 ± 3.41	14.73 ± 2.55
CMS-resilient	10.12 ± 1.37	11.47 ± 2.56	12.41 ± 2.12	13.87 ± 2.54	13.68 ± 2.49	14.21 ± 2.37
CMS-sensitive	10.92 ± 1.45	9.14 ± 1.12[a,b]	7.34 ± 0.86[a,b]	6.02 ± 0.93[a,b]	5.32 ± 0.74[a,b]	5.45 ± 0.83[a,b]

[a] Significant difference between normal control and CMS-sensitive ($p < 0.05$)

[b] Significant difference between CMS-sensitive and CMS-resilient ($p < 0.05$)

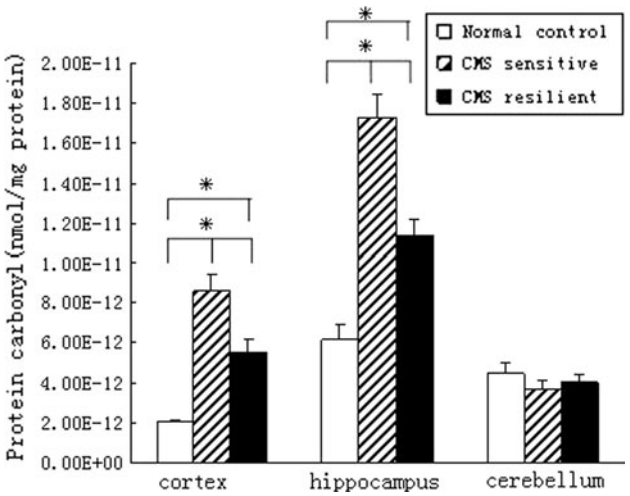

Fig. 1 Effect of the CMS paradigm on protein peroxidation in brain of normal control, CMS-sensitive and CMS-resilient rats. *Bars* represent mean ± SEM. *Vertical lines above bars* indicate standard deviation. $^*p < 0.05$

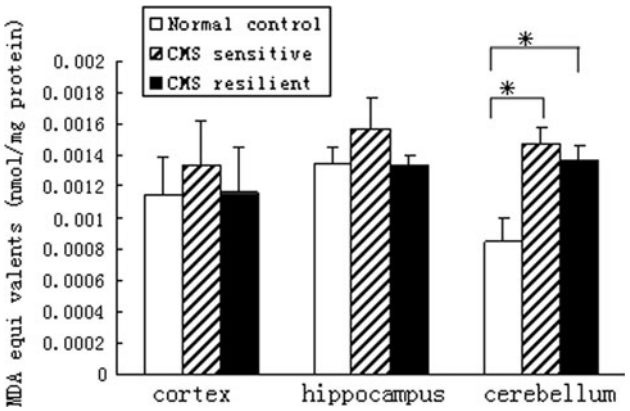

Fig. 2 Effect of the CMS paradigm on lipid peroxidation in brain of normal control, CMS-sensitive and CMS-resilient rats. *Bars* represent mean ± SEM. *Vertical lines above bars* indicate standard deviation. $^*p < 0.05$

hippocampus $F_{2,21} = 39.527$, $p = 0.007$). Compared with the CMS-sensitive group, protein SOD activity in the cortex and hippocampus was also significantly higher in the CMS-resilient group (cortex $F_{2,21} = 47.175$, $p = 0.000$; hippocampus $F_{2,21} = 39.527$, $p = 0.002$). Compared with the CMS-sensitive group, SOD activity in the cerebellum was significantly higher in normal controls ($F_{2,21} = 12.776$, $p = 0.005$) and the CMS-resilient group ($F_{2,21} = 12.776$, $p = 0.032$). No differences were found between normal controls and the CMS-resilient group ($F_{2,21} = 12.776$, $p = 0.110$).

In Fig. 4, CAT activity in the hippocampus and cerebellum was significantly higher in the CMS-sensitive group compared to the CMS-resilient group (hippocampus

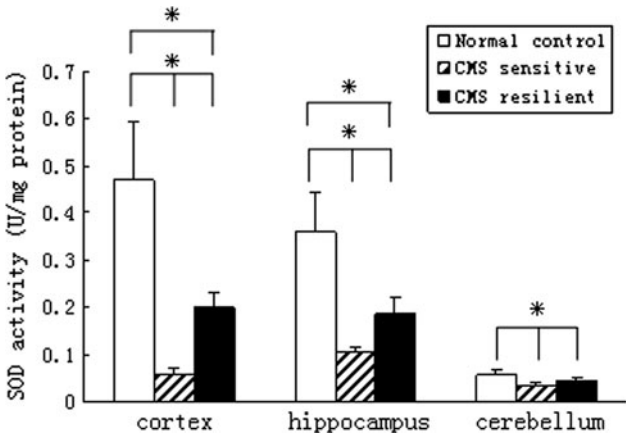

Fig. 3 Effect of the CMS paradigm on superoxide dismutase activity in brain of normal control, CMS-sensitive and CMS-resilient rats. *Bars* represent mean ± SEM. *Vertical lines above bars* indicate standard deviation. $^*p < 0.05$

$F_{2,21} = 50.967$, $p = 0.001$; cerebellum $F_{2,21} = 55.264$, $p = 0.001$) and to normal controls (hippocampus $F_{2,21} = 50.967$, $p = 0.000$; cerebellum $F_{2,21} = 55.264$, $p = 0.000$). Compared with normal controls, CAT activity in the hippocampus and cerebellum was also significantly higher in the CMS-resilient group (hippocampus $F_{2,21} = 50.967$, $p = 0.000$; cerebellum $F_{2,21} = 55.264$, $p = 0.000$). In the cortex, CAT activity was significantly increased in the CMS-sensitive group compared to the CMS-resilient group ($F_{2,21} = 20.841$, $p = 0.000$) and compared to normal controls ($F_{2,21} = 20.841$, $p = 0.001$). No differences were found between the CMS-resilient group and normal controls ($F_{2,21} = 20.841$, $p = 0.499$).

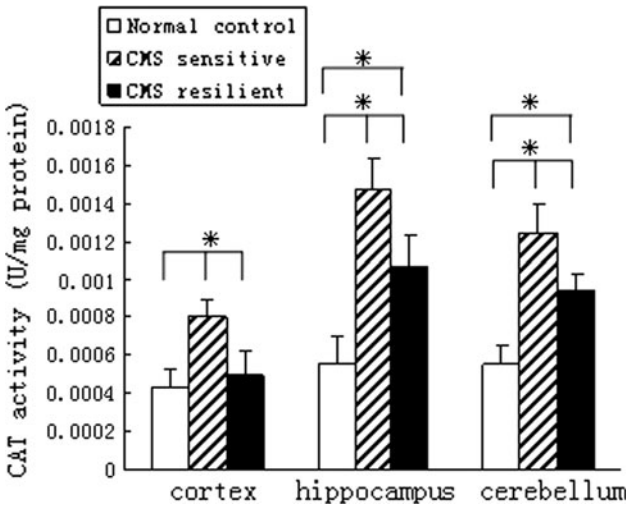

Fig. 4 Effect of the CMS paradigm on catalase activity in brain of normal control, CMS-sensitive and CMS-resilient rats. *Bars* represent mean ± SEM. *Vertical lines above bars* indicate standard deviation. $^*p < 0.05$

Discussion

The CMS model, originally described by Willner et al. (1992), is a model of depression that employs chronic unpredictable mild stressors. Most of the symptoms of depression have been modeled and mimicked in the CMS rats. In this model reduced consumption of sucrose solution as well as decreased intracranial self-stimulation behavior serve as markers of a decrease in reward sensitivity and may simulate anhedonia (Bekris et al. 2005; Bergstrom et al. 2008; Gamaro et al. 2003).

This is the first study to demonstrate a clear difference in distinct oxidative parameters between CMS-resilient and CMS-sensitive animals, which could be involved in the pathogenesis of depression. We segregated animals exposed to CMS into the above two groups, decreasing and not decreasing sucrose intake, respectively. This was paralleled by significant differences in oxidative parameters. We found a significant increase of protein peroxidation in the hippocampus and cortex in the CMS-sensitive group compared to the CMS-resilient group and normal controls. Increased lipid peroxidation in the cerebellum was also observed in the CMS-sensitive and CMS-resilient groups compared to normal controls. Moreover, we found a significant increase of CAT activity and a decrease of SOD activity in the cortex, hippocampus and cerebellum in the CMS-sensitive group compared to the CMS-resilient group and normal controls. However, there was no difference in protein peroxidation in the cerebellum and lipid peroxidation in the cortex and hippocampus among the three groups.

In a previous study an animal model of repeated restraint stress showed that this kind of model induced an increase in TBARS levels in the hippocampus (Fontella et al. 2005). In another study it was demonstrated that, compared to unstressed controls, an animal model of immobilization stress caused significant increases in lipid peroxidation in the cerebral cortex, cerebellum and hippocampus and significant increases in levels of protein oxidation in the cortex, hypothalamus and striatum (Liu et al. 1996). In humans, it was demonstrated that ROS was elevated in the plasma of patients with major depression, especially in those with melancholic type (Bilici et al. 2001). Moreover, in a previous study it was demonstrated that CMS induced an increase in protein peroxidation (prefrontal, hippocampus, striatum and cortex) and lipid peroxidation (cerebellum and striatum) and an increase in CAT (cerebellum, hippocampus, striatum and cortex) and a decrease in SOD (prefrontal, hippocampus, striatum and cortex) activities in stressed rats compared with normal controls (Lucca et al. 2009a). Therefore, our findings in this study are consistent with previous results suggesting that oxidative stress is crucially involved in the pathophysiology of depression.

There are four major sources of ROS: (1) oxidative burst, (2) oxidative processes, (3) lipid peroxidation and (4) oxidative stress. Studies have reported numerous oxidative disturbance parameters in patients with major depression, including oxidative damage in erythrocytic membranes (Peet et al. 1998) as well we elevated superoxide anion generation and lipid peroxidation products (Sarandol et al. 2007). And it is believed that stress in adult male rats which were immobilized for 6 h per day over 21 days inhibits the activities of the first complexes of the mitochondrial respiratory chain, which may potentiate the formation of peroxynitrite, leading to depletion of antioxidant defenses and increased lipid peroxidation (Darley-Usmar et al. 1992). Lipid peroxidation can cause structural damage to membranes, including those which form the mitochondria, further potentiating their dysfunction (Darley-Usmar et al. 1992; Lucca et al. 2009b). Our results also demonstrated increased protein peroxidation in the cortex and hippocampus, but not in the cerebellum, of CMS rats compared to controls. An increase in lipid peroxidation was also detected in the cerebellum, but not in the hippocampus and cortex, of CMS rats. These discrepancies may reflect the fact that basal activities of diverse antioxidant enzymes, such as SOD, CAT, and glutathione reductase, are highly variable across brain regions (Carvalho et al. 2001). However, since repeated restraint stress showed an increase in TBARS levels in the hippocampus and immobilization stress caused significant increases in lipid peroxidation in the cerebral cortex and hippocampus (Fontella et al. 2005; Liu et al. 1996), this may reflect that the stressors in our setting were too mild to cause changes in lipid peroxidation in the cortex and hippocampus. There was also significantly higher lipid peroxidation (cortex and hippocampus) in CMS-sensitive than in CMS-resilient rats. This demonstrated that the CMS-resilient group experienced lower oxidative stress than the CMS-sensitive group. The CMS-resilient group may be prone to neuroprotective support of the neurons in the brain and the CMS-sensitive group may be more prone to brain damage.

SOD is an enzyme that uses the superoxide anion as substrate and produces hydrogen peroxide. This molecule is a substrate to the peroxidases, such as CAT, which is one of the most important peroxidases in the organs. In situations of SOD overactivation without a compensatory increase in the peroxidases, the excess of hydrogen peroxide could react with metal ions and generate hydroxyl radicals, which are thought to be the most dangerous radicals. CAT metabolizes the excess of H_2O_2, producing $O_2 + H_2O$ and then decreasing the intracellular redox status. The brain is particularly prone to oxidative damage due to its relatively high content of peroxidizable fatty acids and limited antioxidant capacity (Floyd 1999).

Recently, studies have demonstrated in patients that major depression, especially with melancholia, is associated

with elevated CAT and SOD (antioxidative enzyme) activities in the plasma. And there was a significant positive correlation between SOD activity and Hamilton Depression Rating Scale score (Bilici et al. 2001). In this study, CMS had a marked effect on CAT and SOD activities, with both CMS-sensitive and resilient rats showing significant increases of CAT activity in the cortex and hippocampus and decreased SOD activity in the hippocampus and cerebellum compared with normal controls. In situations where SOD levels are increased without a concomitant CAT increase, the intermediate product hydrogen peroxide may accumulate and generate hydroxyl radicals, which may lead to lipid and protein oxidation (oxidative damage). This effect was evident for both CMS-sensitive and CMS-resilient rats, suggesting that chronic unpredictable stress has a detrimental effect on generating dangerous radicals, regardless of whether the stressed animals developed anhedonia-like responses. And the differences are also paralleled with the segregation of CMS. Hence, the CMS-resilient group may in fact be stressed, as the presence of different oxidative parameters would argue, which may be induced by some protective responses in the brain. But this kind of protective response does not occur in the CMS-sensitive group.

In conclusion, this study has shown that segregation of animals exposed to CMS into sensitive and resilient groups is paralleled by significant differences in oxidative parameters in the hippocampus, cortex and cerebellum. CMS induces oxidative damage and alterations in the activity of antioxidants, which may lead to increased oxidative damage, irrespective of the anhedonia-like status of the stressed animals. Further investigation of essential mechanisms underlying the development of oxidative damage during CMS administration will be necessary for a better understanding of the relationship between oxidative stress and anhedonia-like.

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