Chronic melatonin administration reduced oxidative damage and cellular senescence in the hippocampus of a mouse model of Down syndrome

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Abstract

Previous studies have demonstrated that melatonin administration improves spatial learning and memory and hippocampal long-term potentiation in the adult Ts65Dn (TS) mouse, a model of Down syndrome (DS). This functional benefit of melatonin was accompanied by protection from cholinergic neurodegeneration and the attenuation of several hippocampal neuromorphological alterations in TS mice. Because oxidative stress contributes to the progression of cognitive deficits and neurodegeneration in DS, this study evaluates the antioxidant effects of melatonin in the brains of TS mice. Melatonin was administered to TS and control mice from 6 to 12 months of age and its effects on the oxidative state and levels of cellular senescence were evaluated. Melatonin treatment induced antioxidant and antiaging effects in the hippocampus of adult TS mice. Although melatonin administration did not regulate the activities of the main antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and glutathioneS-transferase) in the cortex or hippocampus, melatonin decreased protein and lipid oxidative damage by reducing the thiobarbituric acid reactive substances (TBARS) and protein carbonyls (PC) levels in the TS hippocampus due to its ability to act as a free radical scavenger. Consistent with this reduction in oxidative stress, melatonin also decreased hippocampal senescence in TS animals by normalizing the density of senescence-associated β-galactosidase positive cells in the hippocampus. These results showed that this treatment attenuated the oxidative damage and cellular senescence in the brain of TS mice and support the use of melatonin as a potential therapeutic agent for age-related cognitive deficits and neurodegeneration in adults with DS.

Keywords: Down syndrome; melatonin; Ts65Dn mice; cellular senescence; oxidative stress.

Introduction

Down syndrome (DS) is characterized by a triplication of a complete or partial copy of chromosome 21 (Hsa21). The cognitive impairment of DS individuals is partially due to developmental alterations, although in later life stages, this impairment is progressively aggravated due to accelerated aging and the development of Alzheimer's disease (AD) neuropathology [1, 2].

Among the different mouse models of DS, the Ts65Dn (TS) mouse, that contains three copies of 92 genes orthologous to Hsa21 genes [3], recapitulate numerous phenotypic characteristics of DS, including cognitive deficits and alterations in brain morphology and function [1, 4], that become more pronounced as the animals age [5].

One of the mechanisms that contributes to the accelerated aging, cognitive and neuronal dysfunction in DS is increased oxidative stress that is present from early life stages affecting neurogenesis and differentiation, connection and survival [6-8]. During later life stages, oxidative stress is aggravated in DS and in the TS mouse [9-12] contributing to their progression of cognitive and neuronal degeneration [4, 9,13].

The enhanced oxidative stress found in DS individuals and in the TS mouse is caused by the triplication of several Hsa21 genes [9] including the *SOD1/Sod1*, the gene responsible for the formation of superoxide dismutase (SOD), which catalyzes the conversion of superoxide anions into hydrogen peroxide (H_2O_2). The increase in SOD activity results in the formation of disproportionate levels of H_2O_2, leading to the overproduction of highly reactive oxygen species (ROS). In addition, oxidative stress induces cell senescence [14, 15], a process that is characterized by permanent arrest of cell proliferation [16].

Melatonin is an indoleamine mainly synthesized and secreted by the pineal gland. Its production progressively decreases as animals age [17], and its exogenous administration has been demonstrated to induce neuroprotective effects [18-20]. Melatonin protects against oxidative

stress regulating anti- and pro-oxidant enzymes, acting as a potent ROS scavenger [21] and repairing molecules damaged by ROS overgeneration. Thus, melatonin has been proposed to be a powerful tool in the treatment of neuropathologies in which oxidative stress is enhanced.

Our previous studies [10, 22], showed that melatonin treatment during adulthood improved spatial memory and hippocampal long-term potentiation (LTP) in TS mice. This functional benefit was associated with protection against cholinergic neurodegeneration and the normalization or attenuation of several hippocampal neuromorphological alterations of TS mice. The aim of this study was to evaluate the oxidative status and the density of senescent cells in the brain of adult TS mice and to evaluate the effect of chronic melatonin treatment on these processes.

Materials and methods

Animals and housing

This study was approved by the Cantabria University Institutional Laboratory Animal Care and Use Committee and was carried out in accordance with the Declaration of Helsinki and the European Communities Council Directive (86/609/EEC). Mice were generated, karyotyped, housed and maintained as previously described Corrales et al. [22].

Melatonin treatment and experimental groups

TS and euploid littermates (CO) mice were orally treated with melatonin (Mel: 100 mg/L; Sigma-Aldrich, Madrid, Spain) or its diluent from 6 to 12 months of age as previously described Corrales et al. [10] and assigned to one of the following experimental groups: TS-Mel, CO-Mel, TS-vehicle or CO-vehicle. Six animals per group were used to assess the effects of melatonin administration on the oxidative stress assessments, while 7 extra animals per group were used to perform the senescence study.

Oxidative stress assays

Sample preparation. Cortex and hippocampal samples were homogenized in cold buffer containing 20 mM sodium phosphate, pH 7.4; 0.1 % Triton; and 150 mM NaCl (1:20 w/v) and centrifuged at 5000 *g* for 5 min. The supernatant was used to perform all the biochemical determinations that were carried out in triplicates.

Antioxidant enzyme assays. Enzymatic activities were measured as described by Parisotto et al. [23]. Briefly, CAT activity was analyzed at 240 nm quantifying the decrease in the level of H_2O_2 (expressed in mmol/min/g) in a 10 mM H_2O_2 solution. In order to determine SOD activity, the oxidation of epinephrine (pH 2.0 to pH 10.2), which produces superoxide anion and a pink chromophore (expressed in USOD/g), was quantified at 480 nm. GPx activity was determined

measuring the oxidation of NADPH at 340 nm (expressed in µmol/min/g). Glutathione reductase (GR) activity (expressed in µmol/min/g) was analyzed quantifying the oxidation of NADPH at 340 nm due to the formation of GSH from GSSG via the GR that is present in the assay solution. Glutathione S-transferase (GST) activity (expressed in µmol/min/g) was measured at 340 nm using 1-chloro-2,4-dinitrobenzene as the substrate and 0.1 M GSH concentration.

Lipid peroxidation assessment: Lipid oxidation was determined spectrophotometrically at 535 nm via the quantification of thiobarbituric acid-reactive substances (TBARS, expressed in nmol/g) as described by Parisotto et al. [23].

Protein carbonyls (PC): Oxidative damage caused by protein carbonylation was determined measuring carbonyl absorbance at 360 nm as previously described [23]. The PC concentration was expressed in mmol/mg.

Histological procedures

Tissue preparation: The animals were anesthetized and perfused and the hippocampi were removed and processed for histology and cell counting as previously described [10].

Nissl staining. To calculate the area of the subgranular zone (SGZ) of each mouse, a randomly chosen series was used to perform Nissl staining. The SGZ area was measured via the standard Cavalieri method as described previously Llorens-Martín et al. [24]. Briefly, the total SGZ extension was measured using a semiautomatic system (ImageJ v.1.33, NIH, USA, http://rsb.info.nih.gov/ij/) with the series of images from toluidine blue-stained sections. We then drew the SGZ below the internal side of the granular cell layer on the computer screen and measured the length of the resulting lines. The SGZ area of a series was calculated by multiplying the total SGZ extension by the thickness of the sections (50µm).

Histochemical detection of senescence-associated β-galactosidase. To estimate the density of senescent cells in the SGZ of the dentate gyrus (DG) in the different groups of mice we used the SA-β-gal assay (senescence-associated β-galactosidase) method described by He et al. [25]. The hippocampal sections were washed twice with PBS and fixed for 15 min at room temperature with a 0.5 % glutaraldehyde solution. Next, the sections were washed and incubated with a staining solution containing 5-bromo-4chloro-3-indolyl-β-D-galactopyranoside (X-gal, Thermofisher Scientific, MA, USA) for 24 h at 37°C, mounted on Superfrost plus glass slides, dehydrated, cleared, and coverslipped with mounting medium. The density of SA-β-gal- positive cells (showing a blue reaction product over the cell soma) was determined by counting all blue cells in the SGZ of the DG of each animal using a Zeiss Axioskop 2 plus microscope with a 40X objective and dividing this number by the SGZ area.

Statistical analysis

Data were analyzed using MANOVA ('genotype' x 'treatment') followed by *post-hoc* group comparisons after Bonferroni corrections when all groups were compared and Student's t-test when two individual groups were compared. All of the analyses were performed in SPSS (version 22.0, Chicago, IL, USA) for Windows.

Results

To determine the brain oxidative stress status of adult TS mice and the possible beneficial effects of chronic administration of melatonin, we evaluated the levels of brain oxidative damage and the state of the antioxidant enzymatic system in the hippocampus and cortex of vehicle- and melatonin-treated TS and CO mice. Table 1 shows the results of the multivariance analysis on each variable assessed.

Table 1. *F* values of MANOVA (genotype x treatment) for oxidative stress markers and antioxidant enzymatic activities in the cortex and hippocampus of the four groups of animals.

	MANOVA $F_{(1,21)}$					
	CORTEX			***HIPPOCAMPUS***		
	Genotype	***Treatment***	***Genotype x Treatment***	***Genotype***	***Treatment***	***Genotype x Treatment***
PC level	0.21, p=0.65	1.07, p=0.32	1.18, p=0.29	4.20, p=0.063	**22.81, p<0.001**	2.60, p=0.13
TBARS level	1.30, p=0.27	2.92, p=0.10	0.18, p=0.67	0.01, p=0.89	**9.52, p=0.008**	1.42, p=0.25
SOD activity	**33.71, p<0.001**	0.001, p=0.97	1.33, p=0.26	0.33, p=0.571	0.046, p=0.83	1.18, p=0.29
CAT activity	**9.46, p=0.08**	0.002, p=0.96	0.46, p=0.50	0.75, p=0.39	0.008, p=0.92	**5.97, p=0.02**
GPx activity	**4.85, p=0.04**	0.11, p=0.74	1.09, p=0.31	**12.13, p=0.003**	1.02, p=0.32	0.40, p=0.53
GR activity	0.009, p=0.92	0.13, p=0.71	0.12, p=0.72	0.021, p=0.88	0.78, p=0.38	0.50, p=0.48
GST activity	1.57, p=0.22	0.008, p=0.93	3.33, p=0.08	**8.51, p=0.01**	1.02, p=0.32	1.40, p=0.25

To evaluated the effects of melatonin treatment on the levels of brain oxidative damage, we measured the levels of PC (a marker of protein damage induced by ROS) and of TBARS (a marker of lipid damage induced by ROS or lipid peroxidation).

Although MANOVA revealed no significant differences due to the genotype, *post hoc* analyses showed a significant increase in the level of oxidized proteins (PC, ~10%; t=2.48; p=0.038; Fig. 1A) and of lipid peroxidative damage (TBARS, ~16%; t=2.33; p=0.042; Fig. 1B) in the

hippocampus of adult vehicle-treated TS mice compared to CO mice under the same treatment. However, no differences in the cortical levels of both markers were observed between the different groups of animals (Fig. 1A and B). Melatonin significantly decreased the levels of protein damage (~12%; t=5.86; p=0.001) and lipid peroxidation (~29%; t=5.14; p<0.001) in the hippocampus of TS mice (Fig. 1A and B, Table 1) and produced a significant decrease in the levels of PC in the hippocampus of CO mice (~10%; t=2.68; p=0.028; Fig 1A, Table 1).

Next, we examined whether melatonin might also exert its antioxidant effects by regulating the activity of the most important antioxidant enzymes in the cortex and hippocampus of the animals. As expected, SOD activity was increased (~143%) in the cortex of TS mice treated with vehicle compared to their CO littermates (Fig. 2A, Table 1). In the hippocampus, although MANOVA revealed the absence of significant differences due to genotype, *post hoc* comparisons showed higher SOD activity in vehicle-treated TS mice than in CO mice (~33%) under the same treatment (t=2.31; p=0.046). Melatonin administration did not modify SOD activity in either brain region (Table 1).

We then analyzed the activity of CAT and GPx because the activity of SOD must be coordinated with the activity of these two antioxidant enzymes to metabolize the H_2O_2 that is produced by SOD into H_2O and O_2. As shown in Fig. 2B, CAT activity was increased in the cortex (~64%) but not in the hippocampus of TS animals. In addition, GPx activity was increased in both brain structures (cortex: ~34%; hippocampus ~27%, Fig. 2C) in vehicle-treated TS mice when compared to CO animals (Table 1). Chronic melatonin administration did not modify the activity of CAT or GPx in the cortex or hippocampus of TS or CO mice (Figs. 2B and C, Table 1).

To detoxify hydroperoxides, GPx requires the participation of glutathione (GSH) as a co-factor to counteract the continuous formation of its oxidized form (GSSG), which is very toxic to cells. Thus, we also measured the activity of GR, a flavoprotein that allows the conversion of GSSG

back into GSH. GR activity did not differ in the cortex or hippocampus of TS or CO mice that were treated with melatonin or vehicle during adulthood (Fig. 2D, Table 1).

Finally, we measured the activity of GST, an enzyme that participates in the detoxification of the endogenous hydroperoxides continuously generated through cellular lipoperoxidation processes. TS mice presented less hippocampal GST activity (~12%) than CO mice that received the same treatment. However, GST activity was similar in the cortex of the different groups of mice (Fig. 2E, Table 1).

Because oxidative stress induces premature cellular senescence, we assessed the density of SA-β-gal-positive cells in the hippocampus of the four groups of mice. TS mice treated with vehicle presented a higher density of β-gal-positive cells than CO mice, indicating greater hippocampal senescence (MANOVA `genotype´: $F_{(1,25)}$=6.35, p=0.019; Fig. 3). Interestingly, melatonin treatment significantly reduced the density of cells with a senescent phenotype in the DG of TS mice (MANOVA `treatment´: $F_{(1,25)}$=9.07, p=0.005; Fig. 3B).

Discussion

We have previously demonstrated that melatonin exerts cognitive-enhancing effects in the adult TS mouse [10, 22]. These beneficial effects of melatonin in TS animals were partially due to the prevention of cholinergic degeneration and to the normalization of the function and/or morphology of the hippocampus. In this study, we evaluated other mechanisms that could be involved in the altered cognition of these mice, i.e., the oxidative stress status and the density of senescent cells in the brain, as well as the effect of melatonin treatment on these processes.

We first evaluated the status of the antioxidant defense system in the brain of TS and CO mice. Consistent with the triplication of the *Sod1* gene and the increased activity of SOD observed in different tissues in DS individuals and TS mice [15, 23, 26, 27], we found that SOD activity was increased in the cortex and hippocampus of TS mice. Although SOD catalyzes the dismutation of superoxide, a free radical with high toxicity, to non-radical molecules such as oxygen and H_2O_2, the accumulation of H_2O_2 or its inefficient removal, leads to the formation of the most deleterious hydroxyl radical (HO^-) that damage membrane lipids, proteins and other biomolecules [28]. Thus, the increase activity of SOD found in the brain of TS mice may lead to an excess of OH^- production resulting in high oxidative damage.

To prevent the accumulation of H_2O_2, SOD activity must be balanced with GPx and CAT activities. In DS neurons, higher SOD activity without the concomitant increase of complementary antioxidant enzymes activities, CAT and GPx, create a redox imbalance that may not efficiently neutralize the excess of H_2O_2 [29]. In this study, while GPx activity was increased in the cortex and hippocampus, CAT activity was only increased in the cortex of TS mice. The increase in GPx activity found in the brains of TS mice may be due to induction of the enzyme by excess H_2O_2 and lipid peroxides as an adaptative response to oxidative stress [30]. However, the fact that CAT activity was not increased in the hippocampus of TS mice to compensate the enhanced SOD

activity could result in insufficient removal of H_2O_2, which would favor the generation of HO^-; this, in turn, could produce persistent oxidative stress.

Among the anti-oxidative stress-related enzymes, GST acts in xenobiotic detoxification by catalyzing the conjugation of GSH to chemical toxins [31] and contributing to the detoxification of the endogenous hydroperoxides that are continuously generated through cellular lipoperoxidation processes [32]. In agreement with previous observations in the blood of DS subjects [27, 31], in this study, the activity of the GST enzyme in the hippocampus of TS mice was lower than that in CO mice. Considering the antioxidant effects of this enzyme [31], this may be an additional factor contributing to the oxidative damage in this structure in this model of DS.

The levels of GSH, another major neuronal endogenous antioxidant, are reduced in DS subjects [27, 31]. Consistent with previous studies [33], we found no differences in the brain activity of GR (a central player in the conversion of GSSH to GSH) between CO and TS mice. However, the levels of GSH also depend on the levels of γ-glutamylcysteine synthase and glucose-6-phosphate dehydrogenase [34] that were not measured in our study.

Melatonin has been shown to reduce oxidative stress through different mechanisms [21, 34, 35]: indirectly, by modulating the activity of the enzymes that are involved in controlling oxidative processes (up-regulating antioxidant and down-regulating pro-oxidant enzymes); and directly, by acting as a free radical scavenger and interacting with oxidative radicals.

In this study, chronic melatonin treatment did not modify the activity of SOD, CAT, GPx, GR or GST enzymes, suggesting that melatonin does not exert its antioxidant effects in the brains of adult TS mice by regulating the antioxidant defense system. This lack of effect of melatonin to modulate the indirect mechanism to reduce oxidative stress in TS mice might be due to the fact that the ability of this indoleamine to detoxify free radicals is highly variable and depends on the tissues and species involved [35, 36]. For example, Olcese et al. [37] found that long-term

melatonin administration to a mouse model of AD diminish the mRNA expression levels of the antioxidant enzymes SOD, CAT and GPx. However, a previous study in another model of AD reported opposite effects of melatonin treatment on antioxidant enzymes [38]. In TS mice, the genetically predetermined increase in SOD activity induces a dysregulation of the other two enzymes and other triplicated genes also influence the redox-metabolism [39]. These factors are likely to be contributing to the divergent results found after melatonin administration in the expression levels of these enzymes in TS mice and in murine models of AD. Furthermore, the regulation of antioxidant enzymes by melatonin can be also different depending upon under basal or elevated oxidative stress conditions [34] as occur in the brain of TS mice.

Melatonin can also contribute to neuroprotection exerting other indirect antioxidative and pro-survival effects by regulating different anti- or pro- apoptotic proteins and enzyme cofactors [40, 41]. In addition, melatonin also modulates a broad set of ROS- and survival-related signaling pathways that implicate transcriptional factors, such as NF-κB or Akt [42- 44], that activate different pro-inflammatory genes involved in age-related processes under increased oxidative stress. Future studies should explore the effects of melatonin administration in these signaling pathways in TS mice.

Lipids and proteins are the molecules that are most prone to undergo major oxidative injury. Therefore, we measured the levels of oxidative damage in proteins and lipids in the brains of TS mice. Consistent with previous studies in DS individuals and TS mice in which other markers of oxidative stress were analyzed [10, 12], the levels of TBARS and PC were increased in the hippocampus, but not in the cortex, of vehicle-treated TS mice. These findings suggest that in adult TS mice, cells in the cortex may exhibit greater tolerance or better regulation against oxidative stress than in the hippocampus. Further support for this hypothesis comes from the finding that the activity of CAT and GPx was also increased in the cortex of TS mice. These effects may compensate for the enhanced SOD1 activity, leading to a reduction in the production

of ROS and of oxidative injury in the cortex. Conversely, an imbalance of these enzymes in the hippocampus would lead to increased oxidative stress in this structure, which could play a role in the cognitive deficits and neurogenesis alterations that have previously been described in this model of DS [4]. Therefore, further studies are needed to determine whether brain structure-specific manipulations of the levels of the SOD and catalase enzymes, may account for the higher vulnerability of hippocampus to suffer oxidative stress damage.

The second mechanism by which melatonin reduces oxidative damage is its direct ROS scavenging properties [21], detoxifying a variety of free radicals and reactive oxygen intermediates, including OH^-, peroxynitrite anion, singlet oxygen and nitric oxide [45] to avoid oxidative damage. In this study we showed that melatonin treatment rescued the levels of protein damage and lipid peroxidation in the hippocampus of TS mice because they did not differ from those of CO mice, indicating that the main antioxidant action of this indoleamine in this mouse model of DS may be due to its action as a free radical scavenger. This protective effect of melatonin against lipid and protein damage is consistent with previous reports in other mouse models with different neuropathologies [20, 38] or with brain damage induced by radiation or by toxin exposure [46, 47] in which oxidative stress plays an important role.

In addition, oxidative stress is an important factor that causes cell senescence [14, 15, 25]. Because the hippocampus of TS mice seems to be exposed to greater amounts of oxidative stress, we analyzed the density of senescent cells in this structure. We found that vehicle-treated TS mice showed a higher density of SA-β-gal-positive cells in the DG of the hippocampus than CO mice. Consistent with these findings, it was recently demonstrated that fibroblasts with trisomy 21 present signs of premature cell senescence secondary to increased oxidative damage [15].

Interestingly, melatonin normalized the density of SA-β-gal-positive cells in TS mice to a level comparable to the one found in vehicle-treated CO mice, indicating a potential antiaging protective effect in the hippocampus of this model. These results are consistent with previous studies that showed that melatonin treatment effectively reverses H_2O_2-induced senescent phenotypes in mesenchymal stem cells [14]. Thus, it is likely that the melatonin-induced antioxidant effects in the hippocampus of TS mice may be involved in the reduction of senescent cells in this structure.

Although administration of the antioxidant analogue of the nootropic piracetam SGS-111 from conception or during adulthood failed to improve cognition in the TS mouse [48], other antioxidants, such as vitamin E, normalize oxidative stress and delay impairments in cognitive performance [11]. It has been demonstrated that, due to a variety of physiological and metabolic advantages [35], the protective effects of melatonin against oxidative damage are more potent than the ones induced by vitamins C or E [21], partially because the metabolites formed by free radical scavenging also present antioxidant activity [21, 35]. Because the pro-cognitive effects of melatonin are related to its multiple antioxidant actions [20, 37, 46, 47] and the learning and memory deficits in TS mice are associated with increased brain oxidative stress, our results suggests that the beneficial effects found in TS mice cognition after melatonin treatment [22] could be partially due to its antioxidant action as a ROS scavenger.

However, because melatonin has been also demonstrated to exert other neuroprotective effects, its administration could improve cognition by other mechanisms such as recovering LTP or promoting neurogenesis [19, 20, 49-51]. In our previous work [10], melatonin also decreased cholinergic degeneration, increased the density of proliferating cells, of differentiated neuroblast and of mature granular cells and improved the impaired LTP in the hippocampus of the TS mice. And, in the present study, melatonin also reduced the density of cells with a senescent phenotype in TS mice. These results support the idea that the pro-cognitive effects of this indoleamine could

also be due to other neuroprotective actions. Therefore, melatonin could be a more effective pharmacological tool to reduce several DS-altered phenotypes than other antioxidants due to its multiple effects.

Conclusions

In summary (see Fig. 4), the results of the present study revealed an enhanced pro-oxidant status in the hippocampus of adult TS mice. This persistent oxidative state may account for the higher density of cells with a senescent phenotype that was demonstrated for the first time in the hippocampus of TS mice. Melatonin administration exerted antioxidant effects in the hippocampus of TS animals that were apparently not mediated by regulation of the activity of the antioxidant defense system but by the effects of melatonin as a ROS scavenger, which led to attenuation of the levels of oxidative damage. In addition, melatonin exerted an antiaging effect, as demonstrated by a reduction in the density of senescent cells in the hippocampus of TS mice. The present results provide further support for the neuroprotective effects of melatonin administration to adult TS animals and suggest that melatonin could be a potential beneficial supplement for the treatment of the age-related cognitive decline in DS individuals.

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Author contributions

E.B.P. and S.G. performed the experiments. V.V. and S.L. developed the colony and karyotyped the animals. D.W.F. and E.S.-B. discussed the experiments. C.M.-C. and N.R. conceived the project; designed, supervised and interpreted the experiments; analyzed the data; and wrote the manuscript.

Conflicts of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper

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Figure legends

Fig. 1. Mean ± S.E.M. of the levels of PC (A) and TBARS (B) in the cortex and hippocampus of TS and CO mice treated with melatonin or vehicle. *p<0.05, TS vs. CO; #p<0.05; ##p<0.01; ###p<0.001, vehicle-treated vs. melatonin-treated mice. *p* values reflect the outcomes of *post hoc* tests following a Bonferroni correction performed after MANOVAs.

Fig. 2. Mean ± S.E.M. of the activity levels of different antioxidant enzymes in the hippocampus and cortex of TS and CO mice treated with melatonin or vehicle. Superoxide dismutase (SOD, A), catalase (CAT, B), glutathione peroxidase (GPx, C), glutathione reductase (GR, D), and glutathione S-transferase (GST, E). Mean ± SEM. *p<0.05; **p<0.01; ***p<0.001 TS vs. CO. *p* values reflect the outcomes of *post hoc* tests following a Bonferroni correction performed after MANOVAs.

Fig. 3. Representative photomicrographs of SA-β-gal-stained sections (A) and mean ± S.E.M. of the density of β-gal-positive cells (B) of the SGZ of the hippocampus of TS and CO mice treated with melatonin or vehicle. *p<0.05; TS vs. CO; #p<0.05; melatonin vs. vehicle. *p* values reflect the outcomes of *post hoc* tests following a Bonferroni correction performed after MANOVAs.

Fig. 4. Schematic diagram summarizing the differences in the brain oxidative status between TS and CO mice and the effects produced by melatonin treatment in the cortex and hippocampus of TS mice.

Fig. 1

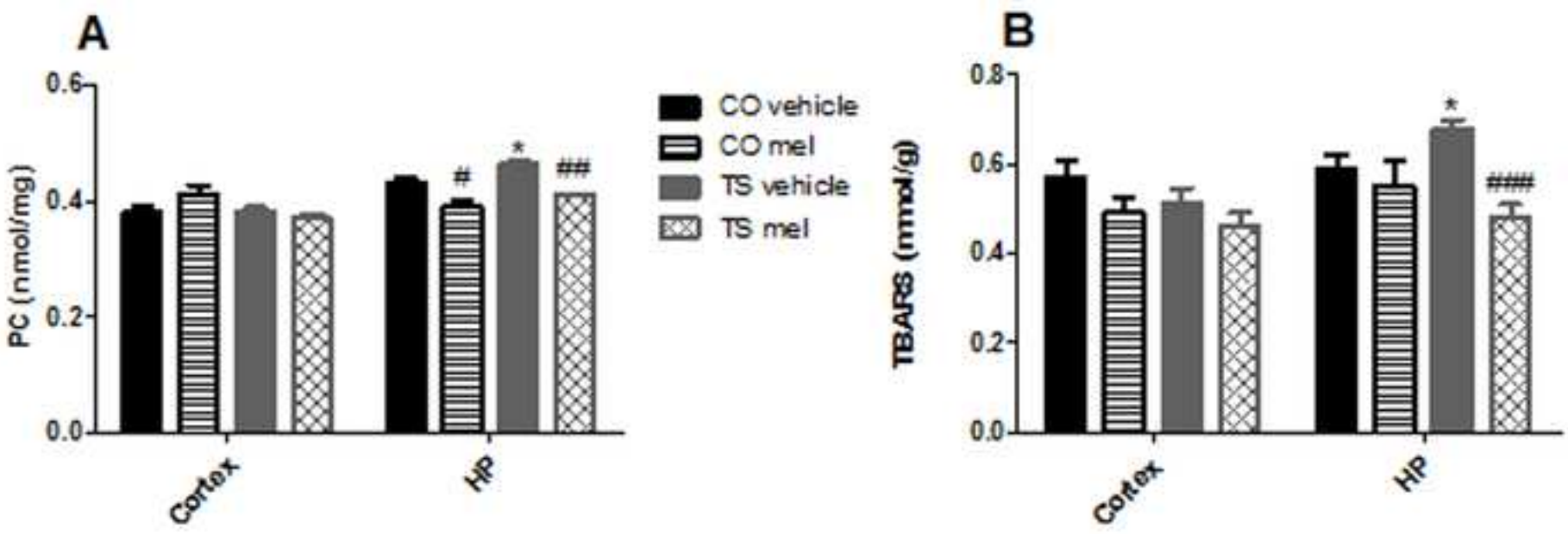

Figure2

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Fig. 2

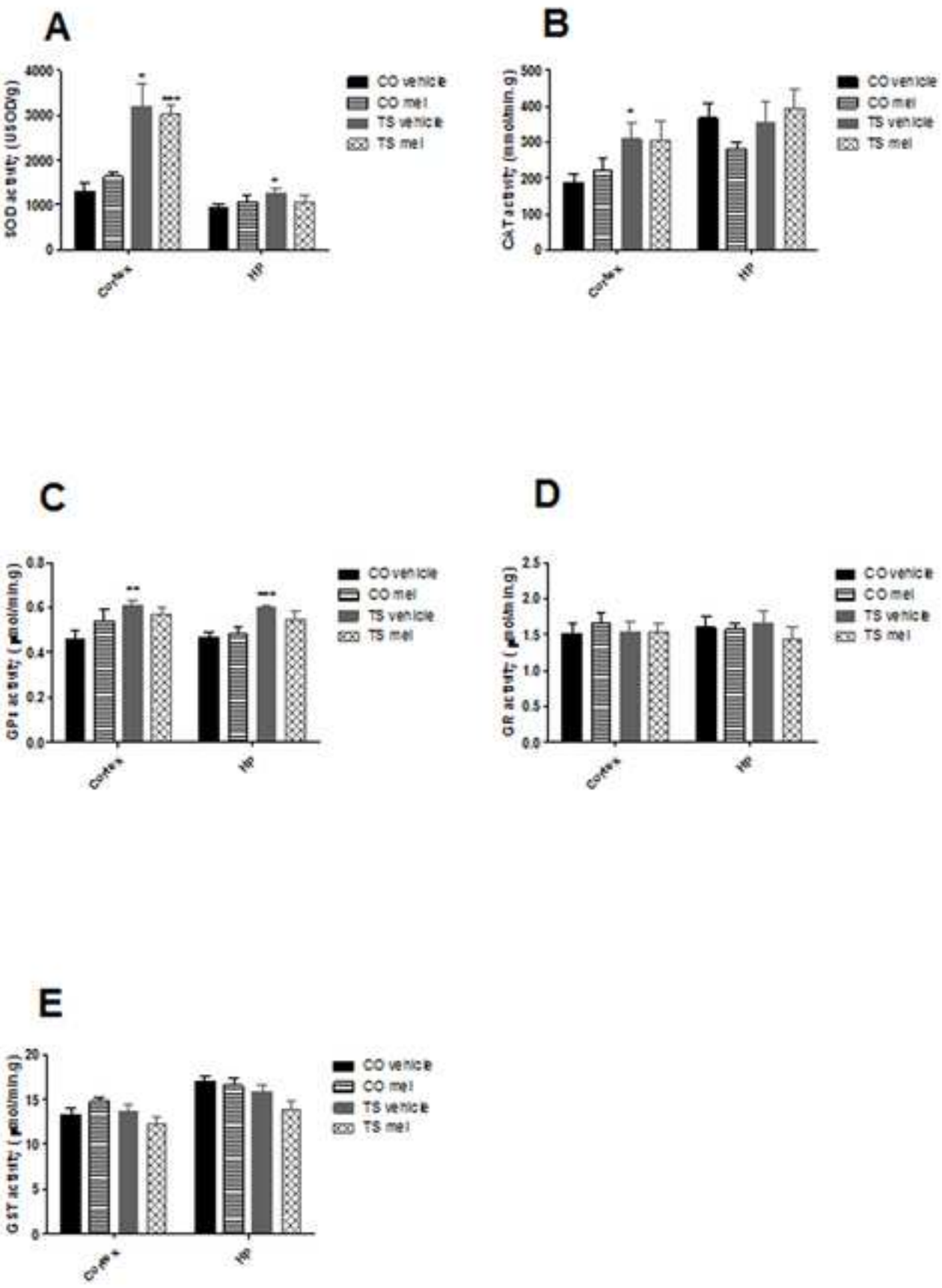
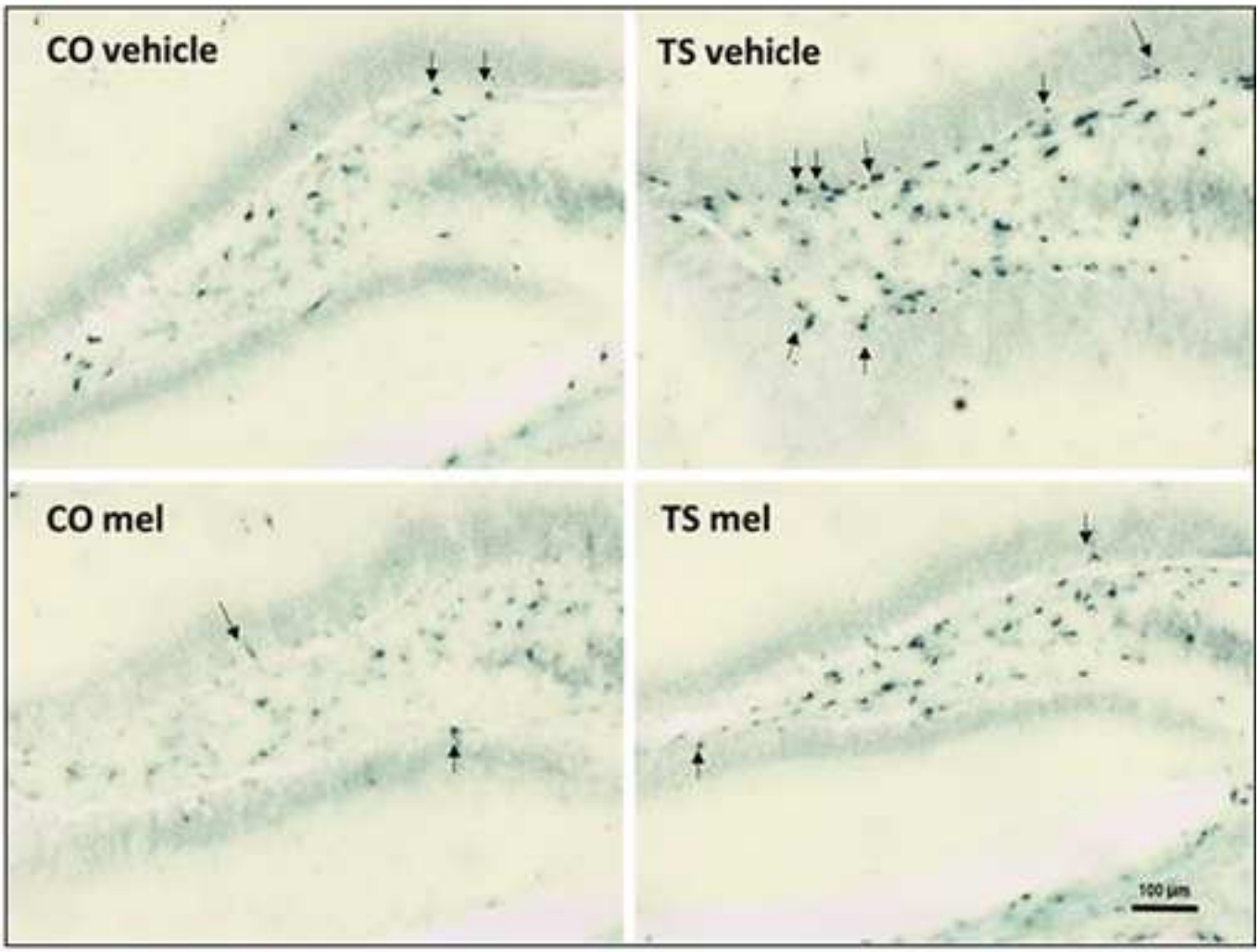


Fig. 3

A



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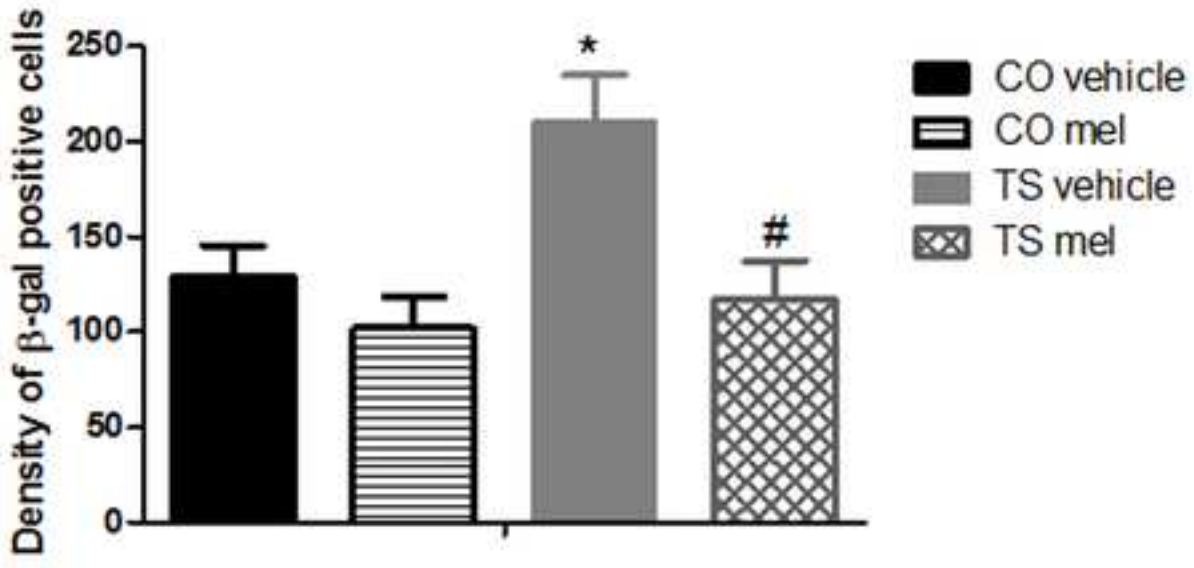

Figure4

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