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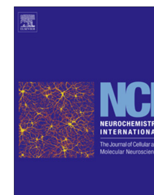

Effects of green tea and physical exercise on memory impairments associated with aging

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ABSTRACT

We investigated the effects of physical exercise and green tea supplementation (associated or not) on biochemical and behavioral parameters in the time course of normal aging. Male Wistar rats aged 9 months were divided into groups: control, physical exercise (treadmill running), and supplemented with green tea while either performing physical exercise or not. A young control group was also studied. Physical exercise and green tea supplementation lasted 3 months. Afterwards, behavioral and biochemical tests were performed. Biochemical measurements revealed differences in antioxidant and oxidant responses in hippocampus, prefrontal cortex and striatum. Behavioral testing showed age-related memory impairments reversed by physical exercise. The association of green tea supplementation and physical exercise did not provide aged rats with additional improvements in memory or brain oxidative markers. Green tea *per se* significantly decreased reactive oxygen species levels and improved antioxidant defenses although it did not reverse memory deficits associated with normal aging.

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1. Introduction

Oxidative stress is a biological phenomenon associated with a range of degenerative diseases observed during aging, including those resulting in learning and memory deficits (Fukui et al., 2001; Serrano and Klann, 2004). Age-related deficits may be associated with structural and functional changes in macromolecules and cell membranes, which, at least in part, result from direct or indirect effect of free radicals and reactive oxygen species (ROS) (Cui et al., 2012). Regular physical exercise is an efficient strategy to protect the brain functions against deficits associated with the aging process (Asl et al., 2008).

ROS generated during sessions of aerobic exercise contribute to the long term adaptation to aerobic training, which improves both enzymatic and non-enzymatic antioxidants (Andrews, 1965). However, low adherence to physical exercise programs in the general population increases interest in behavioral or pharmacological interventions aiming to minimize ROS, especially among aged subjects not able to exercise regularly. In this regard, nutritional interventions are a frequent strategy to improve ingestion of antioxidant substances (Morillas-Ruiz et al., 2006).

A natural compound often included in the daily diet of the general population is the green tea (*Camellia sinensis*). Green tea (GT) contains a large amount of catechins (30–40% of dry weight), a polyphenol with potential antioxidant activity (Berube-Parent et al., 2005). Among elderly population, the regular ingestion of GT has been described as frequent, especially in the Mediterranean and Asian diets (Stefani and Rigacci, 2014). Specifically in the Asian countries, such as China (Tseng and Hernandez, 2005) and Japan (Hayasaka et al., 2013), which is the major tea producing country. Catechins are the major components of GT, and its intake has been associated with a variety of beneficial health effects, including neuroprotective effects in Alzheimer and Parkinson disease (Bastianetto et al., 2006; Hu et al., 2007; Kim et al., 2010; Mandel et al., 2008; Rezai-Zadeh et al., 2008, 2005; Singh et al., 2008). This effect is associated with the activity of epigallocatechin gallate (EGCG), a major constituent of GT, which has been investigated for its preventive and therapeutic potential role in cerebral aging, as well as in the progression of neurodegenerative diseases in the aging (Assuncao et al., 2011; Unno et al., 2004; Unno et al.,

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2007). A former epidemiological study (Feng et al., 2010) suggested that both black and green tea consumption were associated with better cognitive performance. Despite of this, more intervention trials and large prospective studies are needed to further assess the relation of GT ingestion and the development, for example, of Alzheimer's disease.

Considering combined interventions, regular physical exercise has been associated with ingestion of GT to reduce exercise-induced muscle damage (Haramizu et al., 2013). This was described to be dependent on the antioxidant potential of GT (Haramizu et al., 2011) after long term treatments (Haramizu et al., 2011) but not after single doses (Jowko et al., 2012). However, the biochemical and behavioral effects of GT supplementation associated with regular physical exercise on cognition have been briefly addressed (Gibbons et al., 2014; Schimidt et al., 2014). It has been suggested that effects of exercise on brain can be enhanced by concurrent consumption of natural products such as omega fatty acids or plant polyphenols (van Praag, 2009). These interventions could involve similar cellular pathways important for neurogenesis, cell survival, synaptic plasticity and vascular function. Therefore, any positive association of long term GT intake and physical exercise could have important implications as both constitute low cost interventions. Here we studied the specific effect of exercise and GT, either combined or not, on the prevention of memory impairment and brain oxidative damage related to aging in rats.

2. Material and methods

2.1. Animals and experimental design

Male Wistar rats were purchased from Central Vivarium of Federal University of Santa Maria (RS/Brazil). During all the experimental period, they were housed three per cage (dimensions 50 × 60 × 22 cm) and maintained under controlled light and environmental conditions (12 h light/12 h dark cycle; temperature of 23 ± 2 °C; humidity 50 ± 10%) with food and water *ad libitum*. All experiments were conducted in accordance with the "*Principles of laboratory animal care*" (NIH publication n° 80–23, revised 1996) and in agreement with the guidelines established by the Institutional Animal Care and Use Committee of the Local Institution (IRB #0422012) to ensure that number of rats and their suffering were kept to a minimum. During the experiment, the weight of each rat and liquid consumption for each house cage were measured once a week. When rats were 9 months of age, they were divided into 4 groups: aged group ($n = 16$), exercise aged group ($n = 18$), green tea supplementation aged group ($n = 18$) and exercise and green tea supplementation aged group ($n = 21$). Moreover, at the time of memory and biochemical testing, a group of rats 2 month-old rats control group not submitted to any intervention was also studied ($n = 10$).

After memory testing, rats from all groups were euthanized using a rodent guillotine. Anesthesia was not used due to its effects on central nervous system (Tan et al., 2012). The brain was removed and the areas of interest were quickly dissected out on an inverted Petri dish, and homogenized in buffer solution for posterior brain tissue preparation and biochemical analyses. Biochemical analyses permitted to quantify the concentration of glutathione (GSH), reactive oxygen species levels (ROS), thiobarbituric acid reactive substances (TBARS) and glutathione peroxidase (GPx) activity.

NADPH, 2′,7′-dichlorofluorescein diacetate (DCFH-DA) and GSH reagents were purchased from Sigma (St. Louis, MO, USA). Other reagents used in this study were of analytical grades and obtained from standard commercial supplier. Experiments followed the experimental designed illustrated in Fig. 1.

During the behavioral tests, which were necessary to perform before biochemical analyses, interventions were sustained.

2.2. Experimental groups and conditions

2.2.1. Aged control group

Rats in the aged control group had access to food and water *ad libitum*. They were free to move and manipulated daily in parallel to rats from other groups.

2.2.2. Exercise aged group

Rats in this group had access to food and water *ad libitum*. They were submitted to the physical exercise intervention. Physical exercise was individual treadmill running performed at an intensity of 60–70% maximal oxygen uptake (VO_2) (belt velocity between 9 and 13 m/min), in sessions lasting 30 min, 5 times a week, always in the same period of day, in light time period (Andrews, 1965). In the week previous to the onset of intervention, rats performed daily treadmill running for 10 min to habituate before performing the first VO_2 test. To determine the individual intensity of exercise, we conducted an indirect VO_2 test, consisting in a treadmill running, starting with low velocity increased in 5 m/min steps every 3 min, until the rat was unable to keep running. The time to fatigue (min) and work volume (m/min) were considered as an indirect measure of maximum oxygen consumption, as described elsewhere (Cechetti et al., 2012; Costa et al., 2012). In the middle of exercise intervention (week 6), an additional indirect VO_2 test was conducted to adjust the exercise intensity for each rat. At the end of the exercise intervention (week 12), a last indirect VO_2 test was performed. The last indirect VO_2 test was performed to ensure that there was an improvement in aerobic condition of the rats after the exercise intervention.

2.2.3. Green tea supplementation aged group

Rats in this group received food and GT. They received GT mixed with drinking water (13.33 g/L), as described elsewhere (Mustata et al., 2005). Food and GT were *ad libitum* and rats were free to move and manipulated daily. GT was prepared daily in the early morning, and administered at room temperature. For tea preparation, 750 ml of water were boiled and then rest until temperature was 90 °C. Afterwards, an infusion of GT (10 g) was muffled for 3 min and allowed to rest. Finally, tea was filtered using filter paper in a container immersed in ice. Once tea was cold, it was distributed in bottles protected from light at environment temperature.

GT samples (Madrugada Co.) used in this study were purchased from standard markets and analyzed by spectrophotometry using the Folin–Ciocalteu method modified (Chandra and De Mejia Gonzalez, 2004), which measured the total polyphenols content (at a concentration of 819.5 μEq GAE/mL), and by high-performance liquid chromatography, which measured the presence of epicatechin (concentration of 83.35 μg/mL), epigallocatechin gallate (299.56 μg/mL) and epicatechin gallate (86.05 μg/mL).

2.2.4. Exercise and green tea supplementation aged group

Rats from this group exercised as described for the exercise aged group, and received green tea as described for the green tea supplementation aged group.

2.2.5. Young control group

Rats from this group were 2 months old and served as a control to verify effects of aging. They were housed as the others groups with food and water *ad libitum*. They were free to movement and daily manipulated in the same form of rats from other groups.

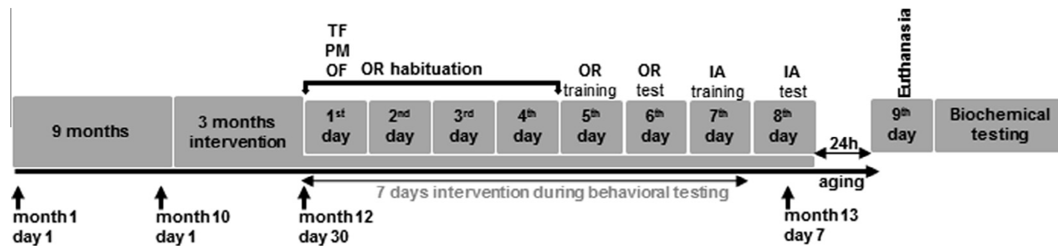

Fig. 1. Experimental design considering the 9 months normal aging period, interventions during 3 months and until the 7th day of behavioral testing, behavioral testing, and the biochemical testing of the animals. TF: tail flick; PM: plus maze; OF: open field; OR: object recognition; IA: inhibitory avoidance.

2.3. Behavioral testing

2.3.1. Object recognition memory test (OR)

Training and testing in the object recognition (OR) task were carried out in an open-field arena (50 × 50 × 50 cm) built with polyvinyl chloride plastic, plywood and transparent acrylic (Ennaceur and Delacour, 1988). Rats were first habituated individually in the apparatus and left to freely explore it for 20 min during 4 consecutive days before the training. In the training session, two different objects (A and B) were placed in the apparatus and rats were allowed to explore them freely during 5 min. The objects were made of metal, glass, or glazed ceramic. Exploration was defined as sniffing or touching the objects with the nose and/or forepaws. Sitting on or turning around the objects was not considered an exploratory behavior. A video camera was positioned over the arena, and the rats' behavior was recorded using a video tracking and analysis system for offline evaluation. After 24 h, in the test session, one of the objects was randomly exchanged for a novel object (C) and the rats were reintroduced into the apparatus during 5 min. To avoid confounds by lingering olfactory stimuli and preferences, the object and the arena were cleaned after testing each animal with 70% ethanol. The time spent exploring the familiar and the novel object was recorded. Afterwards, the discrimination index was calculated taking into account the difference of time spent exploring the new (Tnovel) and the familiar (Tfamiliar) objects ([(Tnovel − Tfamiliar)/(Tnovel + Tfamiliar)] × 100 (%)), and used as a memory parameter (Damgaard et al., 2010; Mello-Carpes and Izquierdo, 2013; Nava-Mesa et al., 2013).

2.3.2. Inhibitory avoidance/aversive memory test (IA)

Rats were trained in a one-trial step-down inhibitory avoidance task. The training apparatus was a 50 × 25 × 25 cm plexiglass box with a 5 cm-high, 8 cm-wide, and 25 cm-long platform on the left end of a series of bronze bars which made up the floor of the box. For training, animals were placed on the platform facing the left rear corner of the training box. When they stepped down and placed their four paws on the grid, received a 2 s, 0.5 mA scrambled footshock. Memory retention was evaluated in a no reinforced test session carried out 24 h after training. Latency testing data were compared between groups to verify memory retention. In addition, differences between testing and training latencies were evaluated (Rosa et al., 2013; Vargas et al., 2014).

2.3.3. Open field, plus maze and tail flick

To analyze exploratory and locomotor activities and ensure that any procedure impaired such behaviors, altering the memory tests results, after 3 months of intervention each rat was placed on the left quadrant of a 50 × 50 × 39 cm open field made with wooden pained white, with a frontal glass wall. Black lines were drawn on the floor to divide it into 12 equal quadrants. Crossing and rearing, as measures for locomotor activity and exploration, respectively, were measured over 5 min (Bonini et al., 2006). To evaluate anxiety state rats were exposed to an elevated plus maze (Pellow et al., 1985). The time spent into the open arms was recorded over a 5 min session. To ensure the inhibitory avoidance testing efficacy, nociception was measure using tail flick test (Tjolsen et al., 1989). In the tail flick test, pain was induced by giving infrared light on the tail of the rat 5 cm away from the tip of the tail. Reaction time (tail-flick latency) was noted by observing the interval between placing the tail on the infrared light source and the withdrawal of the tail. Data from these tests were compared between the groups to ensure all rats presented any impairment in behavior that could affect the variables of interest in our study.

2.4. Biochemical testing

Response of some antioxidant markers (GSH and GPx) to exercise may differ between brain regions (Somani et al., 1995). Therefore, we analyzed biochemical markers in three different regions of the brain that are related to memory (striatum, prefrontal cortex and hippocampus). The hippocampus is one of the most important structures involved on all types of memory processes, especially in the declarative ones (Izquierdo and Medina, 1995). In this sense, the recognition and aversive memories share some characteristics, both depends on hippocampus, but object recognition memory depends more on prefrontal cortex activity (Barker and Warburton, 2013; Feld et al., 2014; Lopez-Ramos et al., 2012), whereas inhibitory avoidance learning, even though involving prefrontal cortex, also requires significant activity of other structures such as the amygdale and striatum (Chau and Galvez, 2012; Ehrlich et al., 2009).

2.4.1. Tissue preparation

For the preparation of brain tissues, the rats were euthanized 24 h after the behavioral experiments were finished. The brain was removed and the hippocampus, prefrontal cortex and striatum were quickly dissected out on an inverted Petri dish and homogenized in 50 mM Tris HCl, pH 7.4, (1/10, w/v). Afterwards, samples were centrifuged at 2400*g* for 20 min at 4 °C, and supernatants (S1) obtained were used for assay.

2.4.2. Detection of TBARS level

Lipoperoxidation was evaluated by the thiobarbituric acid reactive substance (TBARS) test (Ohkawa et al., 1979). One aliquot of S1 was incubated with a 0.8% thiobarbituric acid solution, acetic acid buffer (pH 3.2) and sodium dodecyl sulfate solution (8%) at 95 °C for 2 h, and the color reaction was measured at 532 nm. Results were expressed as nmol of malondialdehyde (MDA) per mg protein.

2.4.3. Glutathione peroxidase

Glutathione peroxidase (GPx) activity was measured spectrophotometrically (Wendel, 1981) in a system containing GSH/NADPH/GR by dismutation of H_2O_2 at 340 nm. S1 was added in GSH/NADPH/glutathione reductase system and the enzymatic reaction was initiated by adding H_2O_2. In this assay, the enzyme

activity is indirectly measured by means of NADPH decay. H_2O_2 is decomposed generating GSSG from GSH. GSSG is regenerated back to GSH by glutathione reductase presents in the assay media at the expenses of NADPH. The enzymatic activity was expressed by the consumption of NADPH expressed as nmol/min/mg of protein.

2.4.4. Glutathione (GSH)

GSH levels were determined fluorometrically (Hissin and Hilf, 1976). An aliquot of S1 was mixed (1:1) with perchloric acid ($HClO_4$) and centrifuged at 3000*g* for 10 min at 4 °C. After the centrifugation, the protein pellet was discarded and free-SH groups were determined in the clear supernatant. An aliquot of supernatant was incubated with ortho-phthalaldehyde, and fluorescence was measured at excitation of 350 nm and emission of 420 nm. Results were expressed as nmol g^{-1} of tissue.

2.4.5. Reactive oxygen species (ROS) levels

ROS content was assessed by a spectrofluorimetric method using 2′,7′-dichlorofluorescein diacetate (DCFH-DA) as a probe. The sample (S1) was incubated in darkness with 5 μL DCFH-DA (1 mM). The oxidation of DCHF-DA to fluorescent dichlorofluorescein (DCF) was measured for the detection of intracellular reactive species.

The formation of the oxidized fluorescent derivative (DCF), measured by DCF fluorescence intensity, was recorded at 520 nm (480 nm excitation) 1 h after the addition of DCFH-DA to the medium. Results were expressed as AU (arbitrary units).

2.5. Statistical analysis

Data were first checked for normality of distribution. Daily intake of water and animals' weight were compared between days for the experiment duration using one-way ANOVA. Mean weight was compared between the start and at the end of interventions using a *t*-test. The discrimination index of the OR task and IA results were compared by Kruskal–Wallis test followed by individual Mann–Whitney tests for comparisons between specific groups. In OF, PM and TF tests the data were analyzed using ANOVA with Duncan post hoc when necessary. Biochemical results were compared between the aged control and the intervention groups by *t*-test. The differences were considered statistically significant when $P < 0.05$.

3. Results

3.1. Animal weight and fluid intake

The individual weight of the rats at the start and end of interventions was similar between the groups ($P > 0.05$, one-way ANOVA). Water or green tea intakes were similar between the groups ($P > 0.05$; *t*-test).

3.2. Behavioral results

3.2.1. Object recognition memory

In the control aged group a significant decrease of discrimination index for novel object was observed when compared to young group ($P = 0.040$, Fig. 2). Importantly the aged exercise group presented a significantly higher discrimination index than control aged group ($P = 0.048$, Fig. 2). Also, the aged group submitted to exercise and GT supplementation presented a significant higher discrimination index than the control aged group ($P = 0.044$, Fig. 2).

As shown by the similar total exploration time in the test session between all groups (Table 1, exploration time in OR test), all rats dedicated time to the new object, and young rats and rats from

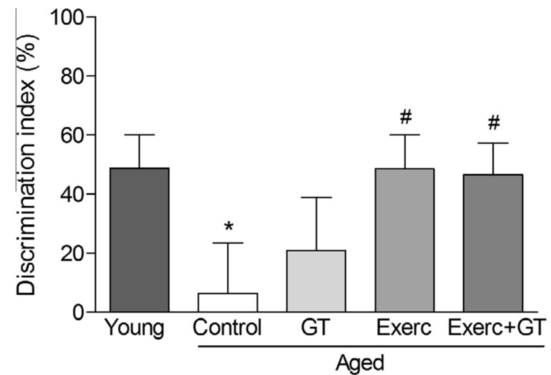

Fig. 2. Discrimination index for novel object in the object recognition task. * Indicates difference when compared to young group ($P < 0.05$), and # indicates difference when compared to control aged group ($P < 0.05$). Data presented as mean and SEM. Statistical analysis was performed using Kruskal–Wallis test followed by Mann–Whitney test between specific groups.

some interventions groups spent a longer time exploring the novel object.

3.2.2. Inhibitory avoidance

In the inhibitory avoidance task, rats from all groups presented similar step down latency in the training session ($P = 0.850$; Kruskal–Wallis test, Fig. 1A training).

In the test session, aged rats presented significantly lower step down latencies than young ($P = 0.042$; Mann–Whitney test, Fig. 3A). Among the aged groups, only the exercise aged group showed enhanced latencies ($P = 0.044$; Mann–Whitney test, Fig. 3A) reaching values similar to those found in the young control group ($P = 0.940$; Mann–Whitney test).

When we compared the differences between test and training latencies, used as a memory measurement, we verified that aged groups presented significantly lower differences than young group ($P = 0.029$; Mann–Whitney test, Fig. 3B). However, exercise aged groups showed significant higher latency difference than control aged group ($P = 0.044$ for control aged *vs.* exercise aged group and $P = 0.026$ for control aged *vs.* exercised and GT group; Mann–Whitney test; Fig. 2).

3.2.3. Open field, elevated plus maze and tail flick

Normal aging and the various interventions did not affect the number of crossings and rearing during the 5 min free exploration session at the open field (Table 1 – open field). Similarly, no effect of aging nor of the various interventions were found on the total number of entries or in the time spent at open arms during the plus maze session (Table 1 – plus maze) neither in latency time to reaction in the tail flick (Table 1 – tail flick).

3.3. Biochemical results

Biochemical data for the different brain regions are shown in Table 2. Rats from the aged control group presented lower antioxidant defenses (expressed by GSH levels and GPx activity) compared to the rats in the young control group ($P = 0.002$, $P = 0.008$ and $P = 0.002$, in hippocampus, striatum and prefrontal cortex, respectively, for GSH; $P = 0.010$ in hippocampus for GPx).

When comparing aged control group with the aged groups submitted to the different interventions, increased GSH levels were found in the exercise aged group in prefrontal cortex ($P = 0.0043$), and increased GPx activity was found in hippocampus ($P = 0.0043$) and in striatum ($P = 0.041$). Similar results were observed in the prefrontal cortex GSH ($P < 0.0022$) and

Table 1
Results of behavioral tasks. Aging and the diverse interventions had no effect on total time of exploration in the object recognition task (OR), or on locomotor and exploratory activities in the open field, onanxiety measured in the elevated plus maze or on pain threshold measured in the tail flick test. Data are expressed as mean ± SD of the total time of exploration for OR, number of crossings (open field), the time spent in the open arms (plus maze), and latency (tail flick). There were no differences between the groups (one-way ANOVA; n = 6–10 per group in all tests).

Behavioral tasks		Groups				
		Young group	Aged control group	Exercise aged group	GT aged group	Exercise + GT aged group
Exploration time in OR	Total exploration time in test (s)	29.89 ± 21.22	19.62 ± 15.85	9.90 ± 8.78	16.25 ± 11.66	20.43 ± 14.07
Open field	Crossings (n°)	31.89 ± 15.90	22.38 ± 17.86	22.17 ± 14.94	14.56 ± 11.36	23.43 ± 14.71
Plus maze	Time in open arms (s)	13.73 ± 14.40	13.37 ± 13.66	13.43 ± 14.70	9.40 ± 12.61	6.57 ± 15.98
Tail flick	Times (s)	5.79 ± 1.64	5.40 ± 1.47	5.83 ± 1.18	5.38 ± 1.16	5.50 ± 1.09

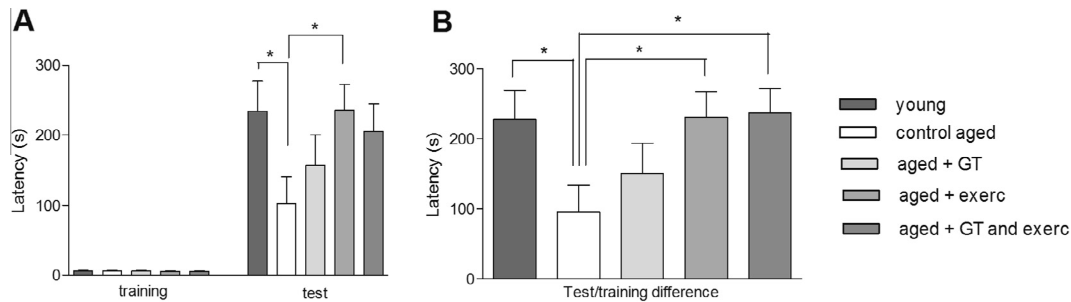

Fig. 3. (A) Step down latency in the inhibitory avoidance task in the training and test sessions. (B) Differences between test and training step down latencies in IA. Data were present as mean ± SEM. *P < 0.05 in Mann–Whitney test.

Table 2
Antioxidant defenses and oxidant markers in different brain regions in the different experimental groups (mean ± SD). Group numbers represent: 1: young control group; 2: aged control group; 3: exercise aged group; 4: green tea supplementation aged group; 5: exercise and green tea supplementation aged group.

Brain region	Group	Antioxidant		Pro-oxidant	
		GSH	GPx	ROS	TBARS
Hippocampus	1	524.50* ± 52.70	9.25* ± 2.49	272.70 ± 23.51	107.20 ± 8.54
	2	285.90 ± 66.14	4.96 ± 1.93	250.30 ± 19.11	114.90 ± 12.60
	3	372.80 ± 68.95	10.17* ± 3.16	187.90* ± 27.11	130.60 ± 12.06
	4	269.20 ± 47.84	9.03 ± 3.34	167.80* ± 25.02	103.10 ± 23.64
	5	343.30 ± 27.61	7.34 ± 4.10	169.60* ± 47.29	120.60 ± 17.09
Striatum	1	218.00* ± 18.96	6.32 ± 1.67	160.50 ± 32.12	43.99 ± 11.32
	2	179.30 ± 18.86	3.79 ± 2.13	172.70 ± 38.44	46.32 ± 11.22
	3	201.90 ± 24.11	6.67* ± 1.68	75.17* ± 32.15	42.56 ± 6.31
	4	198.00 ± 20.06	7.32* ± 2.25	109.10* ± 35.38	35.72 ± 5.99
	5	195.00 ± 20.05	8.64* ± 5.53	104.10* ± 32.33	38.86 ± 8.64
Prefrontal cortex	1	244.50* ± 45.02	3.04 ± 1.60	137.30 ± 23.16	139.60 ± 8.91
	2	154.20 ± 16.77	2.68 ± 0.75	130.00 ± 22.79	131.90 ± 11.51
	3	250.10* ± 46.60	2.11 ± 1.14	125.70 ± 16.66	155.60* ± 7.45
	4	223.10* ± 44.13	3.88 ± 1.08	123.90 ± 16.06	112.60 ± 21.25
	5	227.20* ± 29.32	3.93 ± 1.81	124.20 ± 14.32	116.70 ± 11.21

GSH was higher in young control group (P < 0.01) than aged control group. All interventions enhanced GSH in the prefrontal cortex when compared to the aged control (P < 0.01). Gpx values were higher in the young control group (P < 0.01) in the hippocampus, and in all interventions groups (3, 4 and 5; P < 0.05) in striatum. Hippocampal (P < 0.01) and striatal ROS (P < 0.01) decreased in response to all interventions (groups 3, 4 and 5). TBARS values were higher in the prefrontal cortex of exercise aged group (3; P < 0.01).

* Statistical significant difference when compared to the aged control group (P < 0.05; Mann–Whitney test).

striatum GPx (P = 0.041) when aged control group was compared to the exercise and GT supplementation aged group, in striatum GPx (P = 0.041) and cortex GSH (P = 0.015) when aged control group was compared to the GT supplementation aged group.

Considering the oxidant markers, all interventions decreased ROS levels in the hippocampus and striatum compared to those observed in the aged control group (P < 0.01), but no change was observed in prefrontal cortex.

The lipid peroxidation (TBARS) was not altered in the intervention groups, which presented values similar to the aged control group, except for prefrontal cortex (P < 0.01) in the exercise aged group.

4. Discussion

Considering the importance of the activity of antioxidants along the aging process, here we show the influence of interventions based on physical exercise and GT, either by separate or associated, on memory and brain oxidative parameters of normal aging in rats. After biochemical and behavioral testing we observed memory deficits in the aged rats. Although such deficits were not reduced by GT supplementation *per se*, long term GT intake was associated with lower ROS levels and improvement in antioxidant defenses in rats.

Aged rats submitted to physical exercise (treadmill running) presented no memory deficits. In addition, these rats presented

improved antioxidant status as well as reduced ROS levels. However, when combining physical exercise and GT supplementation we did not observe any additional improvement on biochemical and behavioral parameters monitored.

The memory deficits in the aged control rats were evident in the object recognition (recognition long term memory) and inhibitory avoidance (aversive long term memory) tasks. These deficits were reverted in the aged control rats submitted to physical exercise, but not in the rats supplemented with GT. Cognitive performance after physical training is related to improved hippocampus activity (Berchtold et al., 2010; O'Callaghan et al., 2007; Radak et al., 2006), and such improvements are most likely resulting from neuroplasticity in different brain regions related to learning and memory (Lin et al., 2012), which may explain our result.

The effects of GT supplementation can be influenced by factors such as the age of the animal (Haque et al., 2008, 2006), the duration of intervention (Haque et al., 2008; Rodrigues et al., 2013), the dose concentration and animal species (Haque et al., 2008) and the bioavailability of the bioactive constitutes. The bioavailability of a compound indicates the fraction of the bioactive constituents that have been absorbed by the body and can mediate their biological actions at specific target tissue sites. We did not measure the bioavailability of GT in our study, but, it is know from literature that GT catechins are brain permeable (Nakagawa and Miyazawa, 1997; Suganuma et al., 1998). Despite of this, the bioavailability of tea catechins is considered poor (Visioli, 2011). In this sense, recent studies have addressed strategies to improve bioavailability of GT (Haratifar et al., 2014; Wang et al., 2014; Zou et al., 2014).

It is important to consider that the GT presents other biocompounds that can contribute to GT neuroprotective effects, such as caffeine (Unno et al., 2009) and theanine (Tamano et al., 2013). Additionally, GT contains large amounts of brain-accessible polyphenols; many of these compounds are monomeric catechins, which have been shown to exert antioxidant effects (Andrade and Assuncao, 2012). In our study we assess the antioxidant and pro-oxidant markers in brain. We found that aged rats had lower brain antioxidant defenses when compared with young group, which may help to explain the worst performance in memory tasks in aged control rats. Additionally, our results suggest the onset of GT effects on oxidative status and antioxidants defenses early than observed for memory. While behavioral tasks were not improved in GT supplemented groups, we found higher GSH levels and GPx activity, both related with antioxidant defenses, in the prefrontal cortex and striatum, respectively, in both exercise aged group and exercise and GT supplementation aged group. It suggests the possibility that GT may eventually potentiate the effect of exercise exists. Previous investigations reported similar effects in the striatum after physical training (Somani et al., 1995). In the hippocampus, only exercise promoted changes in the antioxidant defenses, which agreed with the concept that antioxidant enzymes activity may vary within brain regions and the effects of exercise may also depend on the brain region (Radak et al., 2013).

Previous investigations eliciting favorable changes in glutathione levels and antioxidant enzymes activity after long term GT intake (Assuncao et al., 2011; Xu et al., 2010) had led us to expect effects of GT on cognitive parameters presumably dependent on those biochemical parameters. Others authors also report the effects of GT on neural plasticity. A period of 6-month of GT catechins administration increased levels of cAMP-response element binding protein (CREB) phosphorylation in the hippocampus and the expressions of brain-derived neurotrophic factor (BDNF), a target gene of CREB (Li et al., 2009). The same authors verified that long term GT catechins administration prevents reductions of three representative proteins of synaptic function and synaptic structure, including brain-derived neurotrophic factor (BDNF), post-synaptic density protein-95 (PSD95) and Ca(2+)/calmodulin-dependent protein kinase II (CaMKII). Additionally, EGCG from GT can potentiates neuritogenic action of BDNF, and this effect requires only submicromolar concentrations of EGCG (Gundimeda et al., 2014). These effects of GT on BDNF can also be related with GT effects on learning and memory and highlight that GT polyphenols can have a promising role in the reversal of age-related loss of neuronal plasticity and recovery after neuronal lesions associated with aging (Andrade and Assuncao, 2012).

Here, we measure the effects of GT on biochemical markers. The reduction in ROS most likely results of the activity of GT catechins as radical scavenger (Assuncao et al., 2011). Haque et al. (2008) reported reduced hippocampal lipoperoxides and ROS levels after long-term GT administration in rats. We highlight this result since the memory impairment observed with aging has been suggested as a result of increase in the ROS levels. Although significant in our experiments, the magnitude of these effects may also rely on the doses administrated. Haque et al. (2006) observed benefits of 5 g/L intake during 26 weeks with ROS levels measured in the hippocampus but not in prefrontal cortex. Here we found effects in hippocampus and striatum, but not in the prefrontal cortex after 3 months of supplementation with GT. Similar effects were observed for physical exercise group. In the prefrontal cortex GT had any detectable effect. However, we observed increase in TBARS, suggesting increased lipid peroxidation (Coskun et al., 2005). ROS levels were lower in both physical exercise groups compared to control, which denote the protective effect of exercise against oxidative stress and inflammation in the brain (Gerecke et al., 2013).

5. Conclusion

GT *per se* was not effective to avoid memory deficits associated with aging, but significantly decreased ROS levels and improved antioxidant defenses.

Physical exercise improved all biochemical and memory parameters tested. Associating GT supplementation and physical exercise was not related to additional improvements in memory or biochemical markers in aged rats.

Author disclosure statement

Authors declare no competing financial interests exist concerning the content of this document.

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References

Andrade, J.P., Assuncao, M., 2012. Protective effects of chronic green tea consumption on age-related neurodegeneration. Curr. Pharm. Des. 18, 4–14.
Andrews, R.J., 1965. Treadmill for small laboratory animals. J. Appl. Physiol. 20, 572–574.
Asl, N.A., Sheikhzade, F., Torchi, M., Roshangar, L., Khamnei, S., 2008. Long-term regular exercise promotes memory and learning in young but not in older rats. Pathophysiology 15, 9–12.
Assuncao, M., Santos-Marques, M.J., Carvalho, F., Lukoyanov, N.V., Andrade, J.P., 2011. Chronic green tea consumption prevents age-related changes in rat hippocampal formation. Neurobiol. Aging 32, 707–717.
Barker, G.R., Warburton, E.C., 2013. Object-in-place associative recognition memory depends on glutamate receptor neurotransmission within two defined hippocampal-cortical circuits: a critical role for AMPA and NMDA receptors in the hippocampus, perirhinal, and prefrontal cortices. Cereb. Cortex. http://dx.doi.org/10.1093/cercor/bht1245 (Epub ahead of print).

Bastianetto, S., Yao, Z.X., Papadopoulos, V., Quirion, R., 2006. Neuroprotective effects of green and black teas and their catechin gallate esters against beta-amyloid-induced toxicity. Eur. J. Neurosci. 23, 55–64.

Berchtold, N.C., Castello, N., Cotman, C.W., 2010. Exercise and time-dependent benefits to learning and memory. Neuroscience 167, 588–597.

Berube-Parent, S., Pelletier, C., Dore, J., Tremblay, A., 2005. Effects of encapsulated green tea and Guarana extracts containing a mixture of epigallocatechin-3-gallate and caffeine on 24 h energy expenditure and fat oxidation in men. Br. J. Nutr. 94, 432–436.

Bonini, J.S., Bevilaqua, L.R., Zinn, C.G., Kerr, D.S., Medina, J.H., Izquierdo, I., Cammarota, M., 2006. Angiotensin II disrupts inhibitory avoidance memory retrieval. Horm. Behav. 50, 308–313.

Cechetti, F., Worm, P.V., Elsner, V.R., Bertoldi, K., Sanches, E., Ben, J., Siqueira, I.R., Netto, C.A., 2012. Forced treadmill exercise prevents oxidative stress and memory deficits following chronic cerebral hypoperfusion in the rat. Neurobiol. Learn. Mem. 97, 90–96.

Chandra, S., De Mejia Gonzalez, E., 2004. Polyphenolic compounds, antioxidant capacity, and quinone reductase activity of an aqueous extract of *Ardisia compressa* in comparison to mate (*Ilex paraguariensis*) and green (*Camellia sinensis*) teas. J. Agric. Food Chem. 52, 3583–3589.

Chau, L.S., Galvez, R., 2012. Amygdala's involvement in facilitating associative learning-induced plasticity: a promiscuous role for the amygdala in memory acquisition. Front. Integr. Neurosci. 6, 92.

Coskun, S., Gonul, B., Guzel, N.A., Balabanli, B., 2005. The effects of vitamin C supplementation on oxidative stress and antioxidant content in the brains of chronically exercised rats. Mol. Cell. Biochem. 280, 135–138.

Costa, M.S., Ardais, A.P., Fioreze, G.T., Mioranzza, S., Botton, P.H., Souza, D.O., Rocha, J.B., Porciuncula, L.O., 2012. The impact of the frequency of moderate exercise on memory and brain-derived neurotrophic factor signaling in young adult and middle-aged rats. Neuroscience 222, 100–109.

Cui, H., Kong, Y., Zhang, H., 2012. Oxidative stress, mitochondrial dysfunction, and aging. J. Signal Transduct. 2012, 646354.

Damgaard, T., Larsen, D.B., Hansen, S.L., Grayson, B., Neill, J.C., Plath, N., 2010. Positive modulation of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors reverses sub-chronic PCP-induced deficits in the novel object recognition task in rats. Behav. Brain Res. 207, 144–150.

Ehrlich, I., Humeau, Y., Grenier, F., Ciocchi, S., Herry, C., Luthi, A., 2009. Amygdala inhibitory circuits and the control of fear memory. Neuron 62, 757–771.

Ennaceur, A., Delacour, J., 1988. A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. Behav. Brain Res. 31, 47–59.

Feld, M., Krawczyk, M.C., Sol Fustinana, M., Blake, M.G., Baratti, C.M., Romano, A., Boccia, M.M., 2014. Decrease of ERK/MAPK overactivation in prefrontal cortex reverses early memory deficit in a mouse model of Alzheimer's disease. J. Alzheimer's Dis. 40 (1), 69–82.

Feng, L., Gwee, X., Kua, E.H., Ng, T.P., 2010. Cognitive function and tea consumption in community dwelling older Chinese in Singapore. J. Nutr. Health Aging 14, 433–438.

Fukui, K., Onodera, K., Shinkai, T., Suzuki, S., Urano, S., 2001. Impairment of learning and memory in rats caused by oxidative stress and aging, and changes in antioxidative defense systems. Ann. N. Y. Acad. Sci. 928, 168–175.

Gerecke, K.M., Kolobova, A., Allen, S., Fawer, J.L., 2013. Exercise protects against chronic restraint stress-induced oxidative stress in the cortex and hippocampus. Brain Res. 1509, 66–78.

Gibbons, T.E., Pence, B.D., Petr, G., Ossyra, J.M., Mach, H.C., Bhattacharya, T.K., Perez, S., Martin, S.A., McCusker, R.H., Kelley, K.W., Rhodes, J.S., Johnson, R.W., Woods, J.A., 2014. Voluntary wheel running, but not a diet containing (−)-epigallocatechin-3-gallate and beta-alanine, improves learning, memory and hippocampal neurogenesis in aged mice. Behav. Brain Res. 272C, 131–140.

Gundimeda, U., McNeill, T.H., Fan, T.K., Deng, R., Rayudu, D., Chen, Z., Cadenas, E., Gopalakrishna, R., 2014. Green tea catechins potentiate the neuritogenic action of brain-derived neurotrophic factor: role of 67-kDa laminin receptor and hydrogen peroxide. Biochem. Biophys. Res. Commun. 445, 218–224.

Haque, A.M., Hashimoto, M., Katakura, M., Hara, Y., Shido, O., 2008. Green tea catechins prevent cognitive deficits caused by Abeta1-40 in rats. J. Nutr. Biochem. 19, 619–626.

Haque, A.M., Hashimoto, M., Katakura, M., Tanabe, Y., Hara, Y., Shido, O., 2006. Long-term administration of green tea catechins improves spatial cognition learning ability in rats. J. Nutr. 136, 1043–1047.

Haramizu, S., Ota, N., Hase, T., Murase, T., 2011. Catechins attenuate eccentric exercise-induced inflammation and loss of force production in muscle in senescence-accelerated mice. J. Appl. Physiol. 111, 1654–1663.

Haramizu, S., Ota, N., Hase, T., Murase, T., 2013. Catechins suppress muscle inflammation and hasten performance recovery after exercise. Med. Sci. Sports Exerc. 45, 1694–1702.

Haratifar, S., Meckling, K.A., Corredig, M., 2014. Antiproliferative activity of tea catechins associated with casein micelles, using HT29 colon cancer cells. J. Dairy Sci. 97, 672–678.

Hayasaka, S., Goto, Y., Maeda-Yamamoto, M., 2013. The effects of bathing in hot springs on the absorption of green tea catechin: a pilot study. Compl. Ther. Clin. Pract. 19, 243–245.

Hissin, P.J., Hilf, R., 1976. A fluorometric method for determination of oxidized and reduced glutathione in tissues. Anal. Biochem. 74, 214–226.

Hu, G., Bidel, S., Jousilahti, P., Antikainen, R., Tuomilehto, J., 2007. Coffee and tea consumption and the risk of Parkinson's disease. Mov. Disord. 22, 2242–2248.

Izquierdo, I., Medina, J.H., 1995. Correlation between the pharmacology of long-term potentiation and the pharmacology of memory. Neurobiol. Learn. Mem. 63, 19–32.

Jowko, E., Sacharuk, J., Balasinska, B., Wilczak, J., Charmas, M., Ostaszewski, P., Charmas, R., 2012. Effect of a single dose of green tea polyphenols on the blood markers of exercise-induced oxidative stress in soccer players. Int. J. Sport Nutr. Exerc. Metab. 22, 486–496.

Kim, J., Lee, H.J., Lee, K.W., 2010. Naturally occurring phytochemicals for the prevention of Alzheimer's disease. J. Neurochem. 112, 1415–1430.

Li, Q., Zhao, H.F., Zhang, Z.F., Liu, Z.G., Pei, X.R., Wang, J.B., Cai, M.Y., Li, Y., 2009. Long-term administration of green tea catechins prevents age-related spatial learning and memory decline in C57BL/6 J mice by regulating hippocampal cyclic amp-response element binding protein signaling cascade. Neuroscience 159, 1208–1215.

Lin, T.W., Chen, S.J., Huang, T.Y., Chang, C.Y., Chuang, J.I., Wu, F.S., Kuo, Y.M., Jen, C.J., 2012. Different types of exercise induce differential effects on neuronal adaptations and memory performance. Neurobiol. Learn. Mem. 97, 140–147.

Lopez-Ramos, J.C., Jurado-Parras, M.T., Sanfeliu, C., Acuna-Castroviejo, D., Delgado-Garcia, J.M., 2012. Learning capabilities and CA1-prefrontal synaptic plasticity in a mice model of accelerated senescence. Neurobiol. Aging 33 (627), e613–e626.

Mandel, S.A., Amit, T., Kalfon, L., Reznichenko, L., Weinreb, O., Youdim, M.B., 2008. Cell signaling pathways and iron chelation in the neurorestorative activity of green tea polyphenols: special reference to epigallocatechin gallate (EGCG). J. Alzheimer's Dis. 15, 211–222.

Mello-Carpes, P.B., Izquierdo, I., 2013. The Nucleus of the Solitary Tract → Nucleus Paragigantocellularis → Locus Coeruleus → CA1 region of dorsal hippocampus pathway is important for consolidation of object recognition memory. Neurobiol. Learn. Mem. 100, 56–63.

Morillas-Ruiz, J.M., Villegas Garcia, J.A., Lopez, F.J., Vidal-Guevara, M.L., Zafrilla, P., 2006. Effects of polyphenolic antioxidants on exercise-induced oxidative stress. Clin. Nutr. 25, 444–453.

Mustata, G.T., Rosca, M., Biemel, K.M., Reihl, O., Smith, M.A., Viswanathan, A., Strauch, C., Du, Y., Tang, J., Kern, T.S., Lederer, M.O., Brownlee, M., Weiss, M.F., Monnier, V.M., 2005. Paradoxical effects of green tea (*Camellia sinensis*) and antioxidant vitamins in diabetic rats: improved retinopathy and renal mitochondrial defects but deterioration of collagen matrix glycoxidation and cross-linking. Diabetes 54, 517–526.

Nakagawa, K., Miyazawa, T., 1997. Absorption and distribution of tea catechin, (−)-epigallocatechin-3-gallate, in the rat. J. Nutr. Sci. Vitaminol. (Tokyo) 43, 679–684.

Nava-Mesa, M.O., Lamprea, M.R., Munera, A., 2013. Divergent short- and long-term effects of acute stress in object recognition memory are mediated by endogenous opioid system activation. Neurobiol. Learn. Mem. 106, 185–192.

O'Callaghan, R.M., Ohle, R., Kelly, A.M., 2007. The effects of forced exercise on hippocampal plasticity in the rat: a comparison of LTP, spatial- and non-spatial learning. Behav. Brain Res. 176, 362–366.

Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95, 351–358.

Pellow, S., Chopin, P., File, S.E., Briley, M., 1985. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J. Neurosci. Methods 14, 149–167.

Radak, Z., Marton, O., Nagy, E., Koltai, E., Goto, S., 2013. The complex role of physical exercise and reactive oxygen species on brain. J. Sport Health Sci. 2, 87–93.

Radak, Z., Toldy, A., Szabo, Z., Siamilis, S., Nyakas, C., Silye, G., Jakus, J., Goto, S., 2006. The effects of training and detraining on memory, neurotrophins and oxidative stress markers in rat brain. Neurochem. Int. 49, 387–392.

Rezai-Zadeh, K., Arendash, G.W., Hou, H., Fernandez, F., Jensen, M., Runfeldt, M., Shytle, R.D., Tan, J., 2008. Green tea epigallocatechin-3-gallate (EGCG) reduces beta-amyloid mediated cognitive impairment and modulates tau pathology in Alzheimer transgenic mice. Brain Res. 1214, 177–187.

Rezai-Zadeh, K., Shytle, D., Sun, N., Mori, T., Hou, H., Jeanniton, D., Ehrhart, J., Townsend, K., Zeng, J., Morgan, D., Hardy, J., Town, T., Tan, J., 2005. Green tea epigallocatechin-3-gallate (EGCG) modulates amyloid precursor protein cleavage and reduces cerebral amyloidosis in Alzheimer transgenic mice. J. Neurosci. 25, 8807–8814.

Rodrigues, J., Assuncao, M., Lukoyanov, N., Cardoso, A., Carvalho, F., Andrade, J.P., 2013. Protective effects of a catechin-rich extract on the hippocampal formation and spatial memory in aging rats. Behav. Brain Res. 246, 94–102.

Rosa, J., Myskiw, J.C., Furini, C.R., Sapiras, G.G., Izquierdo, I., 2013. Fear extinction can be made state-dependent on peripheral epinephrine: role of norepinephrine in the nucleus tractus solitarius. Neurobiol. Learn. Mem. 113, 55–61.

Schimidt, H.L., Vieira, A., Altermann, C., Martins, A., Sosa, P., Santos, F.W., Mello-Carpes, P.B., Izquierdo, I., Carpes, F.P., 2014. Memory deficits and oxidative stress in cerebral ischemia-reperfusion: neuroprotective role of physical exercise and green tea supplementation. Neurobiol. Learn. Mem. 114, 242–250.

Serrano, F., Klann, E., 2004. Reactive oxygen species and synaptic plasticity in the aging hippocampus. Ageing Res. Rev. 3, 431–443.

Singh, M., Arseneault, M., Sanderson, T., Murthy, V., Ramassamy, C., 2008. Challenges for research on polyphenols from foods in Alzheimer's disease: bioavailability, metabolism, and cellular and molecular mechanisms. J. Agric. Food Chem. 56, 4855–4873.

Somani, S.M., Ravi, R., Rybak, L.P., 1995. Effect of exercise training on antioxidant system in brain regions of rat. Pharmacol. Biochem. Behav. 50, 635–639.

Stefani, M., Rigacci, S., 2014. Beneficial properties of natural phenols: highlight on protection against pathological conditions associated with amyloid aggregation. BioFactors. http://dx.doi.org/10.1002/biof.1171.
Suganuma, M., Okabe, S., Oniyama, M., Tada, Y., Ito, H., Fujiki, H., 1998. Wide distribution of [3H](−)-epigallocatechin gallate, a cancer preventive tea polyphenol, in mouse tissue. Carcinogenesis 19, 1771–1776.
Tamano, H., Fukura, K., Suzuki, M., Sakamoto, K., Yokogoshi, H., Takeda, A., 2013. Preventive effect of theanine intake on stress-induced impairments of hippocamapal long-term potentiation and recognition memory. Brain Res. Bull. 95, 1–6.
Tan, S., Lam, W.P., Wai, M.S., Yu, W.H., Yew, D.T., 2012. Chronic ketamine administration modulates midbrain dopamine system in mice. PLoS One 7, e43947.
Tjolsen, A., Lund, A., Berge, O.G., Hole, K., 1989. An improved method for tail-flick testing with adjustment for tail-skin temperature. J. Neurosci. Methods 26, 259–265.
Tseng, M., Hernandez, T., 2005. Comparison of intakes of US Chinese women based on food frequency and 24-hour recall data. J. Am. Diet. Assoc. 105, 1145–1148.
Unno, K., Takabayashi, F., Kishido, T., Oku, N., 2004. Suppressive effect of green tea catechins on morphologic and functional regression of the brain in aged mice with accelerated senescence (SAMP10). Exp. Gerontol. 39, 1027–1034.
Unno, K., Takabayashi, F., Yoshida, H., Choba, D., Fukutomi, R., Kikunaga, N., Kishido, T., Oku, N., Hoshino, M., 2007. Daily consumption of green tea catechin delays memory regression in aged mice. Biogerontology 8, 89–95.
Unno, K., Yamamoto, H., Maeda, K., Takabayashi, F., Yoshida, H., Kikunaga, N., Takamori, N., Asahina, S., Iguchi, K., Sayama, K., Hoshino, M., 2009. Protection of brain and pancreas from high-fat diet: effects of catechin and caffeine. Physiol. Behav. 96, 262–269.
van Praag, H., 2009. Exercise and the brain: something to chew on. Trends Neurosci. 32, 283–290.
Vargas, L.S., Lara, M.V., Goncalves, R., Mandredini, V., Ponce-Soto, L.A., Marangoni, S., Dal Belo, C.A., Mello-Carpes, P.B., 2014. The intrahippocampal infusion of crotamine from *Crotalus durissus terrificus* venom enhances memory persistence in rats. Toxicon 85C, 52–58.
Visioli, F., 2011. Polyphenol studies: time for a physiological tea party? Br. J. Nutr. 106, 1321–1322.
Wang, P., Vadgama, J.V., Said, J.W., Magyar, C.E., Doan, N., Heber, D., Henning, S.M., 2014. Enhanced inhibition of prostate cancer xenograft tumor growth by combining quercetin and green tea. J. Nutr. Biochem. 25, 73–80.
Wendel, A., 1981. Glutathione peroxidase. Methods Enzymol. 77, 325–333.
Xu, Y., Zhang, J.J., Xiong, L., Zhang, L., Sun, D., Liu, H., 2010. Green tea polyphenols inhibit cognitive impairment induced by chronic cerebral hypoperfusion via modulating oxidative stress. J. Nutr. Biochem. 21, 741–748.
Zou, L.Q., Liu, W., Liu, W.L., Liang, R.H., Li, T., Liu, C.M., Cao, Y.L., Niu, J., Liu, Z., 2014. Characterization and bioavailability of tea polyphenol nanoliposome prepared by combining an ethanol injection method with dynamic high-pressure microfluidization. J. Agric. Food Chem. 62, 934–941.