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Differential impairment of spatial and nonspatial cognition in a mouse model of brain aging

Wei Wang [a,2], Song Li [a,b,1,2], Hui-ping Dong [a], Shen Lv [c], Yi-yuan Tang [a,*]

[a] Laboratory of Brain and Mind, Institute of Neuroinformatics, Dalian University of Technology, 116024, Dalian, China
[b] Department of Pharmacology and Toxicology, School of Life Sciences and Biopharmacy, Shenyang Pharmaceutical University, 110016, Shenyang, China
[c] Laboratory of Molecular Biology, Second Affiliated Hospital, Dalian Medical University, 116011, Dalian, China

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ABSTRACT

Aims: Chronic exposure to d-galactose (D-Gal), which causes acceleration in aging and simulated symptoms of natural senescence, has been used as a reliable animal model of aging. However, the different influences of D-Gal on spatial and nonspatial cognition are as yet unclear.
Main methods: In the present study, the object recognition test (ORT), object location test (OLT) and Y-maze test were carried out to assess the cognitive performance of mice after 8 weeks of chronic D-Gal exposure. The expression of oxidative-stress biomarkers in the prefrontal cortex (PFC) and caspase-3 in the hippocampus (HIP) were also determined.
Key findings: The results of the behavioral tests indicated that after chronic D-Gal exposure, the spatial memory of mice was seriously impaired, whereas nonspatial cognition remained intact. D-Gal exposure also induced more significant changes in malondialdehyde (MDA) levels, superoxide dismutase (SOD) and catalase (CAT) activities in the HIP than in the PFC. Furthermore, chronic D-Gal exposure triggered more substantial caspase-3 overexpression in the HIP than in the PFC.
Significance: Together, these findings suggest the impairment of spatial, but not nonspatial, cognitive ability after chronic D-Gal exposure. The differential nature of this impairment might be due to the more substantial reduction of antioxidant enzyme activities and more severe neuronal apoptosis mediated by caspase-3 in the HIP. The present results also indicate that the HIP and HIP-dependent spatial cognition might be more susceptible to oxidative stress during senescence or other pathological processes.

Introduction

Chronic exposure to d-galactose (D-Gal) causes an acceleration of senescence in different animal species and has been used as a reliable animal model for gerontological research (Xu 1985; Cui et al. 2004, 2006; Chen et al. 2006; Ho et al. 2003). D-Gal-treated animals have a shortened life span and exhibit symptoms similar to those of natural aging, especially the decline in cognitive functions (Xu and Zhao 2002; Holden et al. 2003; Wei et al. 2005; Cui et al. 2006; Lu et al. 2006; Hua et al. 2007; Sun et al. 2007; Zhang et al. 2007). Previous studies have also demonstrated that such cognitive impairment may be due to the excessive formation of reactive oxygen species (ROS), to a significant reduction of antioxidant activities and to over-expression of caspase-3 in the brain of chronic D-Gal-treated animals (Lu et al. 2006, 2007). There is neurochemical evidence that distinct brain structures have different levels of vulnerability to oxidative stress. Therefore, we hypothesize that hippocampus (HIP)-dependent spatial cognition and HIP-independent nonspatial cognition may be affected to a different extent in this animal model of brain aging.

Spatial and nonspatial cognition are two important aspects of cognitive function. Previous studies have confirmed that D-Gal-treated animals appear to be cognitively impaired (Xu and Zhao 2002; Wei et al. 2005; Zhang et al. 2007). However, most of these studies tested nonspatial or spatial cognition using conventional memory tasks that incorporate various aversive stressors (such as electroshock in the passive avoidance test, hunger in the radial maze, water immersion in the T water maze or Morris water maze). In contrast, human cognitive performances are not usually tested with strong stressors, such as intense electroshock or hunger. Therefore, it may not be possible to directly extrapolate to the human population the results of animal studies that use such conventional stressors. Cognitive tests without stressors may be more appropriate to study the neuronal mechanisms underlying cognitive performance (Murai et al. 2007). Furthermore, most of the previous studies assessed only one type of cognitive function (such as spatial cognition in the water maze) or both spatial and nonspatial cognition but using different tasks with different contexts or different difficulties (for example

* Corresponding author. Tel.: +86 411 84706039; fax: +86 411 84706046.
E-mail address: yy2100@126.com (Y. Tang).
[1] At the time of the behavioral tests, Dr. Song Li was a member of the Department of Pharmacology and Toxicology, Shenyang Pharmaceutical University, China. Now he is a member of the Institute of Neuroinformatics, Dalian University of Technology, China.
[2] These authors contribute equally to this work.

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spatial cognition in the Morris water maze and nonspatial cognition in an object recognition test).

In the present study, to confirm our hypothesis and to compare the differences between nonspatial and spatial cognitive performance following D-Gal exposure, non-stressed behavioral tests, the object recognition test (ORT), the object location test (OLT) and the Y-maze test, were performed. In addition, to explore the possible mechanisms underlying the differential alteration of distinct cognitive functions, the expression of oxidative stress biomarkers and caspase-3 was measured in the prefrontal cortex (PFC) and HIP.

Materials and methods

Animals and treatment

Three-month-old male Swiss albino mice (28±2 g) were obtained from Experimental Animal Center of Dalian Medical University and housed under standard conditions (12 h light/dark cycle; lights on from 0730 to 1930; 22±2 °C ambient temperature; 55±10% relative humidity; food and water ad libitum). After one week's acclimation, the mice were randomized into the following three groups: 100 mg/kg D-Gal group (n=15), 150 mg/kg D-Gal group (n=15) and control group (n=15). Each animal was subcutaneously injected with D-Gal (100 or 150 mg/kg, 10 ml/kg) or vehicle (0.9% saline, 10 ml/kg) once daily for 8 weeks. D-Gal was purchased from Sigma (St. Louis, MO, U.S) and dissolved in sterilized 0.9% saline at concentrations of either 10 mg/ml or 15 mg/ml. The procedures for both breeding and experiments complied with the Provisions and General Recommendations of Chinese Experimental Animal Administration Legislation.

Experimental schedule

The present study was performed according to the experimental schedule shown in Fig. 1. After 8 weeks of D-Gal exposure, mice were subjected to behavioral tests to determine their nonspatial or spatial cognitive performance. Twenty-four hours after performance in the Y-maze, animals were sacrificed. After brain removal, the two subregions of interest, the PFC and HIP – were dissected to allow application of the western blotting technique and determination of the levels of antioxidant enzymes.

Behavioral tests

Object recognition test

In the present study, ORT was performed by slightly modifying the method described previously (Zhao et al. 2007; De Rosa et al. 2005). The test apparatus was a white, open-field plastic box (60 cm × 50 cm × 40 cm) placed in a sound-proof testing room, which was lit by constant illumination (about 40 lx) to eliminate shadows. The discriminated objects (A, B and C) were identically-sized (5 cm × 5 cm × 5 cm), cuboid, woody blocks. Objects A and B were black, whereas object C had a black-and-white pattern. Each of the three objects was sufficiently heavy to prevent the mice from moving it.

The ORT consisted of two phases: the sample trial and the test trial (Fig. 2A). In order to acclimate the mice, each was first placed into an empty box for 5 min. Subsequently, during the sample trial the two identical objects (A and B) were placed on opposite sides (at a distance of 8 cm from the walls and 34 cm from each other). To start a 5-minute period of exploration, each animal was placed in the middle of the two objects. After this period, they were returned to their home cages for an interval of 4 h. In the test trial, object B was replaced by object C, which

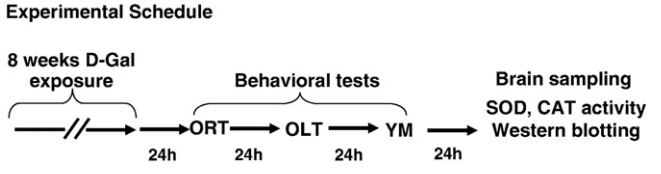

Fig. 1. Experimental schedule. After 8 weeks of D-Gal exposure, mice were subjected to behavioral tests to determine their nonspatial or spatial cognitive performance. Then, animals were sacrificed. The brains were removed and subregions, frontal cortex and hippocampus, were dissected for antioxidant enzyme determination and western blotting.

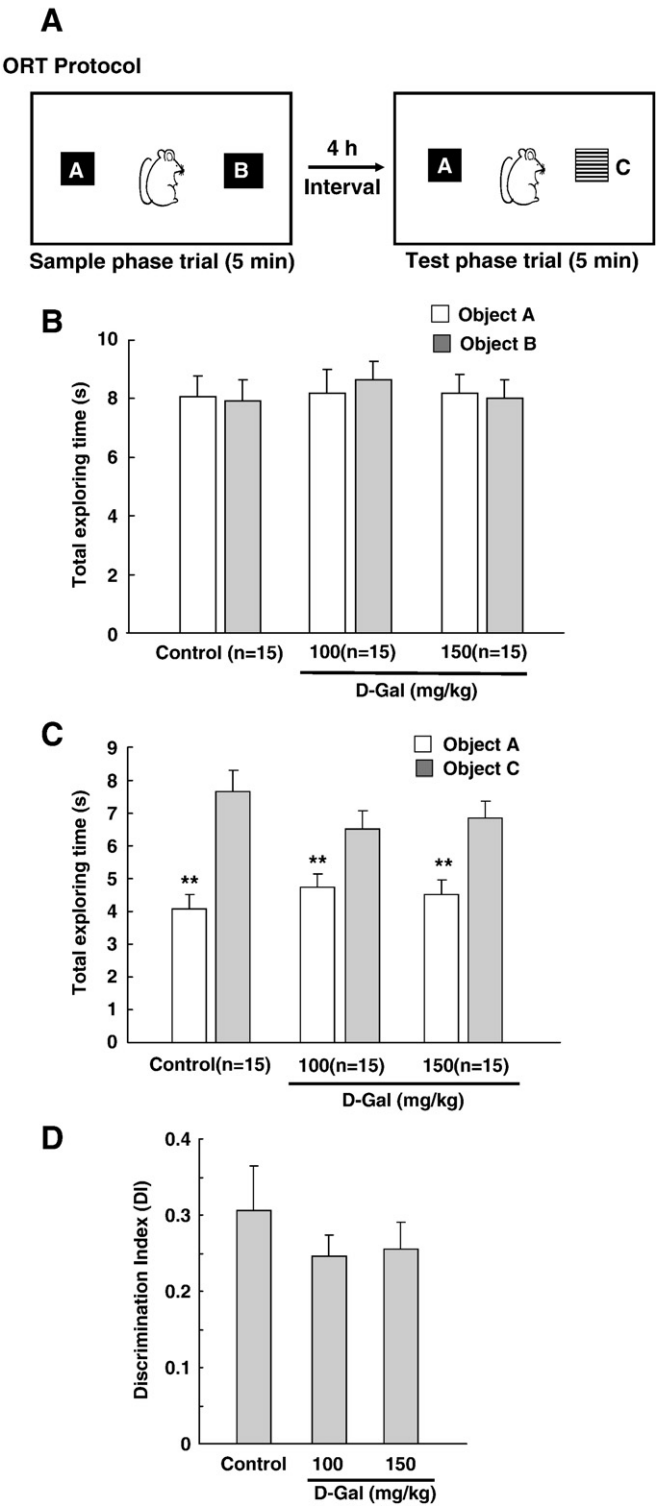

Fig. 2. Effects of 8 weeks D-Gal exposure on ORT performances of mice. (A) Experimental schedule and schematic representation for the sample and test phase trials in ORT. (B) The performances of mice in the sample phase trial; (C) The performances of mice in the test phase trial. **P<0.01 vs. exploration time of new object C in each group; (D) The discrimination indices obtained from the test phase trial using the equation mentioned in the text.

was novel to the mice and different from either object A or B. The mice were then left to explore objects A and C for 5 min. The animals' behavioral performances during the tests were video-taped and the exploration time of the sample and test phases for each of the three objects was recorded by a trained observer.

Object location test

The apparatus used for this test was the same box that was used in the ORT. The discriminated objects, D and E, were two identically-sized (5 cm diameter and 10 cm height), cylindrical vitreous bottles (Fig. 3A). The period of acclimation was performed as in the ORT. In the sample trial, objects D and E were placed in the apparatus as described in the ORT. After 5 min object exploration, the mice were returned to their home cage for a 4-hour interval. Subsequently, in the test trial, object E was moved to a location that was diagonally opposite to object D, and the mouse was left in the box for 5 min exploration. The exploration time of both the sample and test phases was recorded.

The criterion used to assess exploration in both the ORT and the OLT was that the nose of the mouse was oriented towards the objects at a distance of less than 2 cm. Periods in which the rat moved around, climbed over or sat on the objects were not recorded. To prevent olfactory cues, the objects and the test box were cleaned with 75% ethanol after each trial. A discrimination index (DI) was calculated according to the following equation (Zhao et al. 2007; De Rosa et al. 2005):

$$DI = (Tn - Tf) / (Tf + Tn).$$

Here, Tn and Tf represented the time spent (during a 5-min observation period) exploring new and familiar objects (or locations) respectively.

Y-maze tests

Two versions of the Y-maze were used (Fig. 4A and B). The Y-maze with extra-maze cues (YMe) was similar to that originally described (Dellu et al. 1992); this has been validated as a test of spatial memory (Conrad et al. 1996). The second version of the Y-maze was created by including salient intra-maze cues (YMi). In this cued version, one large woody painted object (different from those used in the ORT and heavy enough to prevent the mice from moving them) was placed at the end of each arm. These objects could be distinguished by texture as well as by their paint scheme. The objects were present during both training and testing.

During the training phase, a mouse was placed in the Start arm and allowed to explore the Start and Other arms for 15 min while the Novel arm was blocked with black Plexiglass. Before each mouse was trained and tested, the corncob bedding on the maze floor was mixed. To prevent the use of odor cues in maze navigation, the maze was cleaned and rotated between training and testing periods. The terms Novel, Start and Other refer to the spatial locations of the arms with respect to extra-maze cues. In addition, objects at the ends of the arms remained in the same spatial locations with respect to the extra-maze cues for training and testing. After a 4-hour interval, the mouse was placed into the Start location for testing and was allowed to explore all three arms for 5 min. The location of the Start, Novel and Other arms was varied among mice, and the observers were never present while the mice explored the maze. All trials were videotaped and subsequently analyzed by a trained observer who was blind to the treatment condition. If memory and novelty-seeking behavior remained intact, mice were expected to enter the Novel arm more than the Other arm, due to the innate tendency of mice to explore novelty (Granon et al. 1996).

An entry was scored when a mouse's front paws crossed into an arm. The Start arm was not included in the analysis because the mice were placed there at the beginning of the testing trial, meaning that it was inherently biased and not orthogonal to the Novel and Other arms.

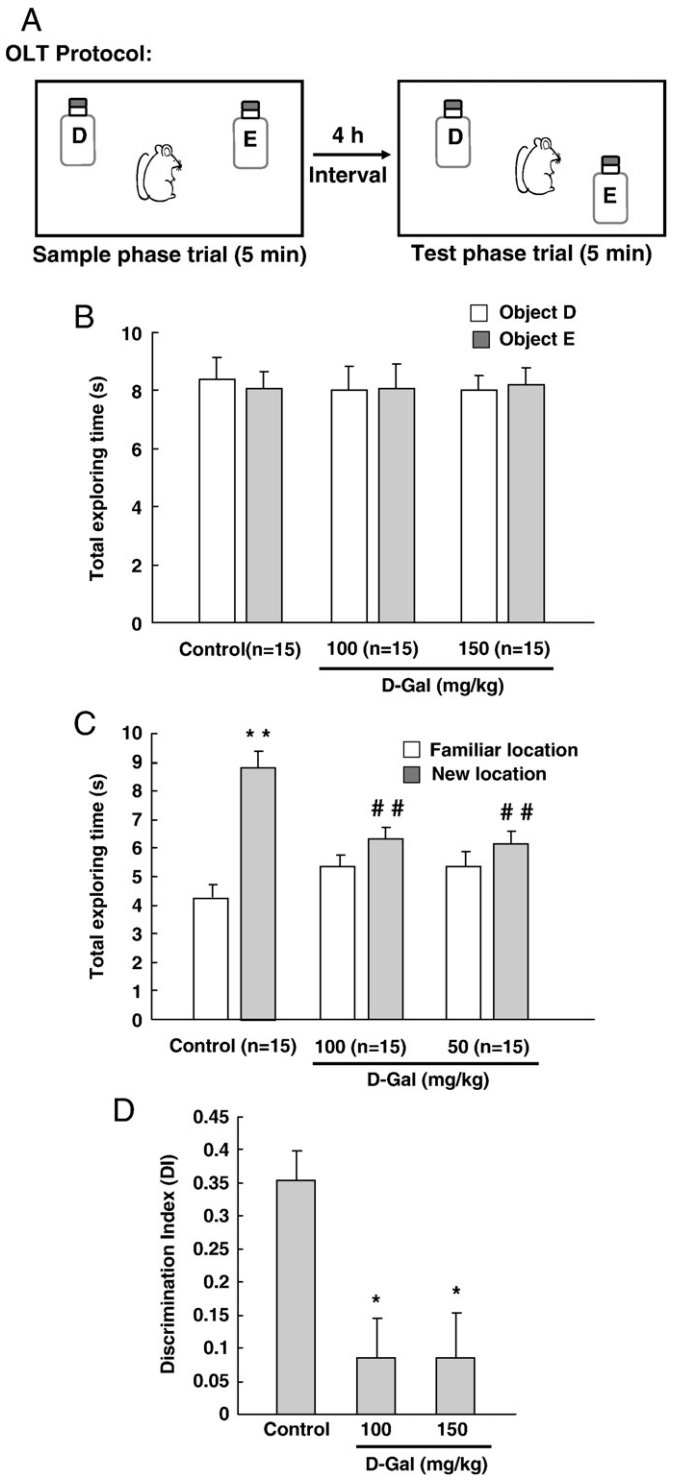

Fig. 3. Effects of 8 weeks of D-Gal exposure on OLT performances of mice. (A) Experimental schedule and schematic representation for the sample and test phase trials in OLT. (B) The performances of mice in the sample phase trial; (C) The performances of mice in the test phase trial. $^{**}P<0.01$ vs. exploration time of object in new location, $\#\#P<0.01$ vs. exploration time of object in new location in control group; (D) The discrimination indices obtained from the test phase trial using the equation mentioned in the text. $^{*}P<0.05$ vs. control group.

Brain sampling

The mice were anesthetized with 10% chloral hydrate and sacrificed. Brains were removed carefully and quickly to 0.9% cold saline. The prefrontal cortices and hippocampi from each hemisphere were immediately dissected out on a cold plate (within 5 min) and frozen in dry ice. All tissues were homogenized and properly prepared for the

detection of biomarkers or signal transduction molecules. All samples were kept frozen at −80 °C until required.

SOD and CAT activities and MDA levels

On the day of the assays (24 h after the Y-maze test), the PFC and HIP from the left hemispheres were homogenized and then centrifuged at 4000 ×*g* for 10 min at 4 °C. The supernatant was then extracted to allow for detection of SOD, CAT and MDA levels. These procedures were performed according to the manuals of the commercial assay kits. Spectrophotometric determinations of SOD, and CAT activities and MDA concentrations were performed at 550, 405 and 532 nm respectively.

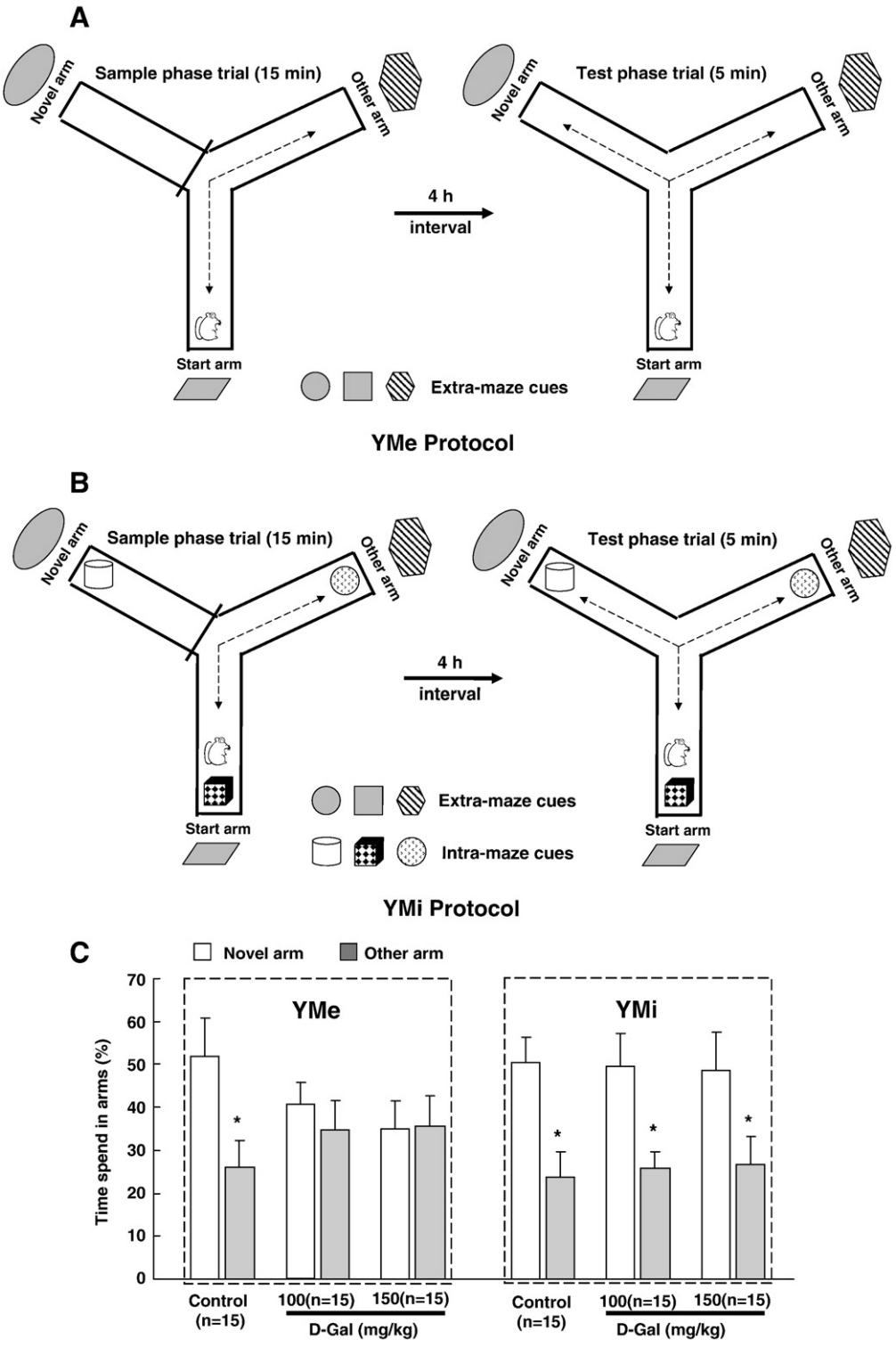

Fig. 4. Effect of Chronic D-Gal exposure on Memory and Exploration. (A) Experimental schedule and schematic representation for the sample and test phase trials in YMe; (B) Experimental schedule and schematic representation for the sample and test phase trials in YMi; (C) Y-maze performance based upon time spent in arms; (D) Y-maze performance based upon entries made into arms; (E) Total number of arm entries. Data are represented as means ± SEM. *$P<0.05$, Other arm compared to Novel arm.

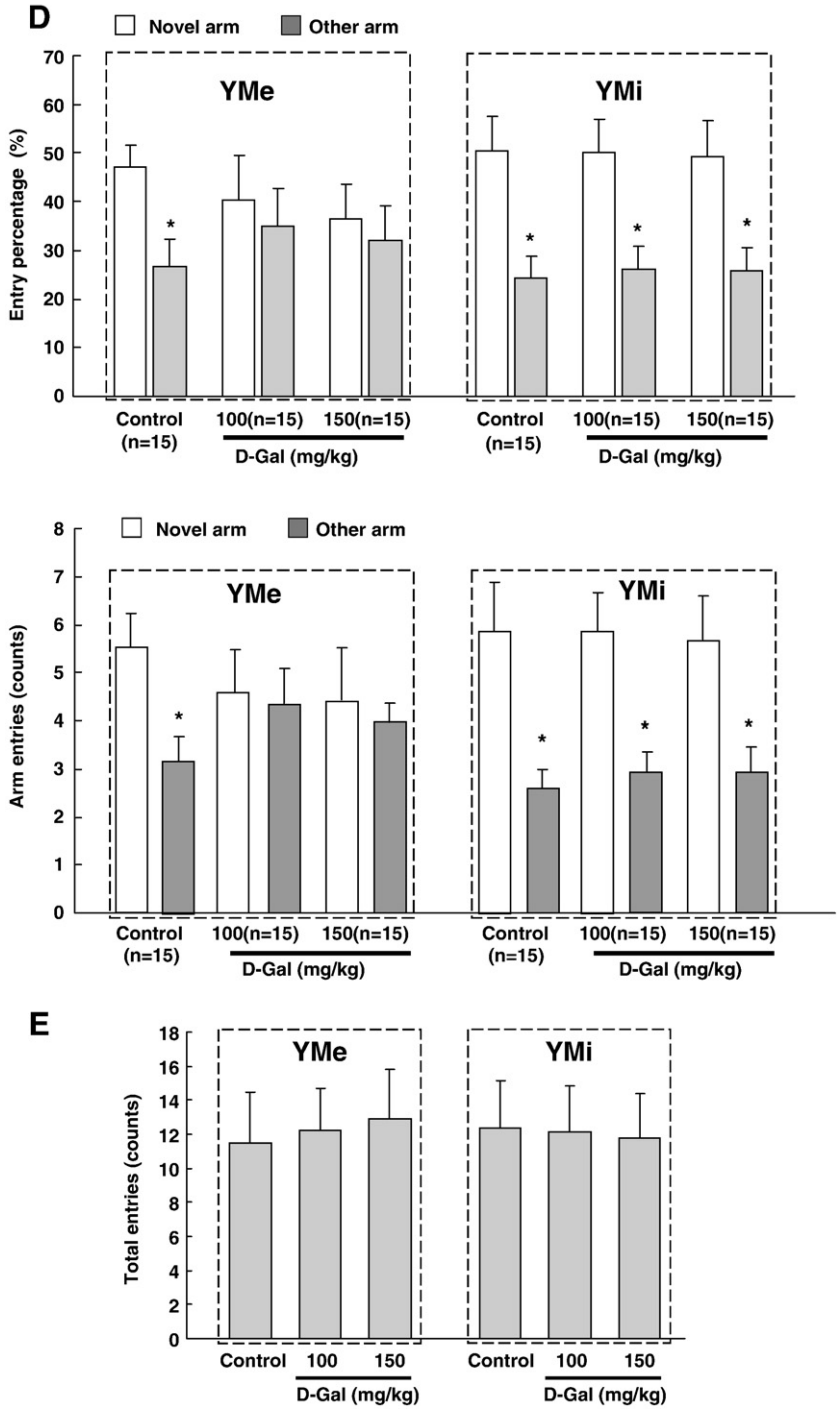

Fig. 4 (*continued*).

Caspase-3 western blot

Caspase-3 analysis was done as previously described but with some modifications (Ieraci and Herrera 2006; Pei et al. 2006). Brain subregions were divided in half and lysed by homogenizing in 400 ml SDS buffer (250 mM Tris–HCl [pH 6.8], 2.5% SDS) followed by brief sonication and further centrifugation at 14,000 ×*g* for 20 min. The supernatant was collected and stored at −80 °C until further analysis.

Protein samples (40 mg per lane) were diluted in SDS-PAGE sample buffer (50 mM Tris–HCl, pH 6.8, 100 mM DTT, 2% SDS, 0.1% bromophenol blue, and 10% glycerol), electrophoresed on a 15% SDS-PAGE, and transferred to PVDF membrane (Immobilon P; Millipore, Burlington, MA, USA). The membrane was subsequently incubated with anti-caspase-3 antibody (Cell Signaling Technology, Beverly, MA, USA). Thereafter, it was exposed to the appropriate horseradish peroxidase-conjugated secondary antibodies, and visualized with enhanced chemiluminescence (Amersham, Piscataway, NJ, USA). For densitometric analysis, immunoreactive bands were scanned and intensity-quantitated using NIH Image software (Scion, Frederick, MD, United States).

Statistical analysis

All data were expressed as mean ± S.E.M. Either one-way or two-way analysis of variance (ANOVA) with post-hoc tests or paired Student's *t* tests was used to determine significance. One-way ANOVAs were used to test

for differences among groups in exploration time during the sample trials (ORT, OLT or Y-maze). For the test trial, two-way ANOVAs were performed: group (control or D-Gal) × object/place/arm (old or new). If significant F values were found ($P<0.05$), then post hoc tests were applied. To test whether time spent with the new object (location) or in the novel arm was greater than time spent with the old one, paired t tests were performed for each group. Levels of caspase-3, and SOD and CAT activities were analyzed using one-way ANOVA followed by the Tukey HSD post hoc test for group differences.

Results

Behavioral tests

Animal conditions after D-Gal exposure

After 8-weeks exposure to the lower dose of D-Gal (100–150 mg/kg), none of the mice subjected to behavioral tests demonstrated obvious impairments, such as significant weight loss, visual deficit or symptoms reflecting the effects of toxicity. However, when animals were treated with D-Gal at doses of 300, 500 and 1000 mg/kg in a preliminary test for dose determination, significant impairments in health (including weight loss, hypolocomotion, or greater than 20% mortality) were observed.

Object recognition test

In the sample phase, there were no differences between the cognitive performances of control and D-Gal-treated mice, as indicated by the total exploring time (Fig. 2B). In the test phase, the mice in all three groups spent more time exploring the novel object C than the familiar object A ($P<0.01$, Fig. 2C). When the data were used to calculate the discrimination index (DI), there was no significant difference between the ability of mice in the D-Gal-treated group and those in the control group to discriminate between familiar and new objects (Fig. 2D); however, the values of the DI for D-Gal groups declined.

Object location test

In the sample phase, statistical analyses revealed no differences in the total exploring time between control and D-Gal-treated groups (Fig. 3B). During the test phase, the time spent exploring the displaced object by the control group was longer than the exploring time for the non-displaced object ($P<0.01$, Fig. 3C). This can be contrasted with the D-Gal-treated groups, in which there were no statistical differences between the new and familiar location exploring times. Moreover, there was a significant difference in the exploring time for the displaced object between mice of the control and D-Gal-treated groups ($P<0.01$, Fig. 3C). The DI of the D-Gal treated groups was also significantly reduced when compared with the control group ($P<0.05$, Fig. 3D).

Y-maze test

After chronic D-Gal exposure, mice demonstrated normal novelty-seeking behavior but impaired spatial memory. Statistical analysis revealed that mice chronically exposed to D-Gal spent more time in the Novel arm than the Other arm of the YMi ($P<0.05$) but spent similar amounts of time in the Novel and Other arms when tested on the YMe. Control mice tested on either version of the Y-maze spent more time in the Novel arm than the Other arm ($P<0.05$, Fig. 4C). The entry data are consistent with these findings (Fig. 4D).

To determine whether the motivation to explore the Y-maze differed between groups during the testing phase, the total entries (the sum of entries into the Novel, Start and Other arms) and the latency of leaving the Start arm were analyzed with ANOVA. Neither the total entries nor the latency to leave the Start arm were affected by chronic treatment with D-Gal. Analysis of the total entries revealed that D-Gal exposure had no significant effects (Fig. 4E). The analysis for latency of leaving the Start arm revealed a similar lack of effect of D-Gal exposure. Means ± SEM were as follows: Control (YMe) 22.3 ± 8.6 s; Control (YMi) 17.9 ± 10.8 s; 100 mg/kg D-Gal (YMe) 24.3 ± 12.1 s; 100 mg/kg D-Gal (YMi) 19.4.3 ± 8.1 s; 150 mg/kg D-Gal (YMe) 21.9 ± 10.4 s; 150 mg/kg D-Gal (YMi) 19.6 ± 7.8 s.

SOD, CAT and MDA assays

After 8 weeks of chronic exposure to D-Gal, the SOD and CAT activities in the HIP were significantly decreased ($P<0.05$, Fig. 5A and B), whereas the concentration of hippocampal MDA increased ($P<0.05$, Fig. 5C) compared to those of controls. However, in the PFC of mice exposed to D-Gal, the SOD activity was significantly enhanced only with higher doses (150 mg/kg). There were no significant changes in the CAT activity and MDA content in the PFC compared to the control group.

Caspase-3 expression in HIP and PFC

The increase in the intensity of bands obtained from western blots indicated a significant increase in the levels of pro-caspase-3 in the HIP and PFC of mice after 8 weeks treatment with 100 ($P<0.05$, Fig. 6B) or 150 mg/kg of D-Gal ($P<0.01$, Fig. 6A,B). Furthermore, the increase in caspase-3 expression was significantly higher in the HIP than in the PFC ($P<0.05$ in 100 mg/kg group, $P<0.01$ in 150 mg/kg group, Fig. 6B). There

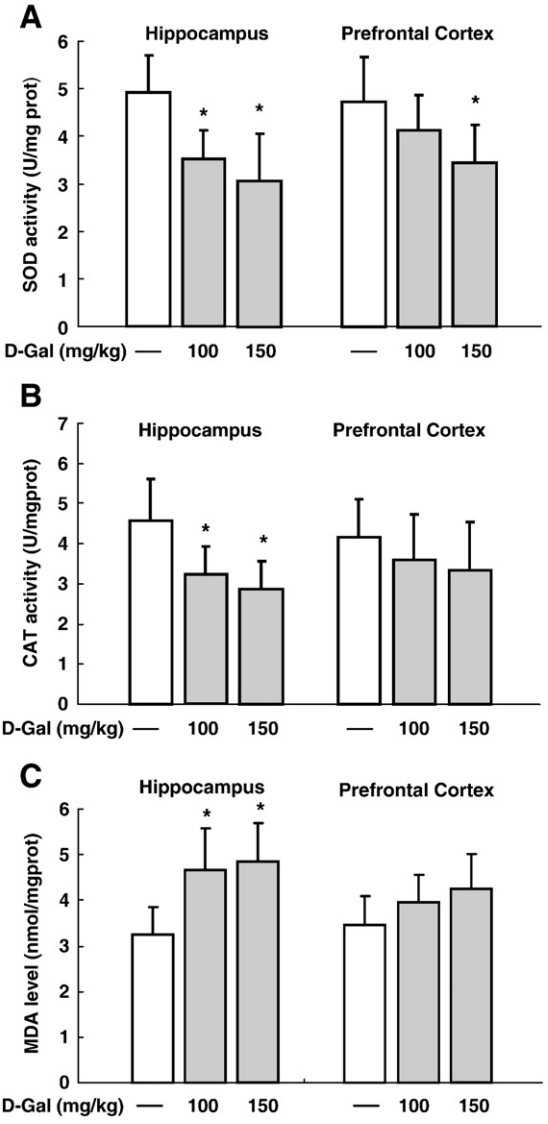

Fig. 5. Effects of D-Gal on SOD, CAT activity and MDA after 8 weeks exposure. *$P<0.05$ and **$P<0.01$ vs. control group.

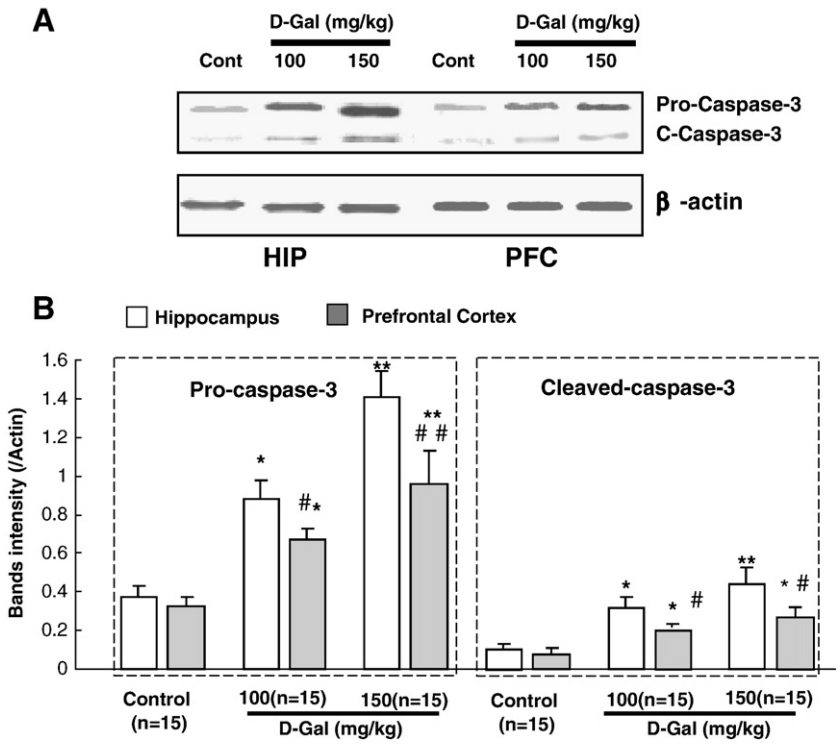

Fig. 6. Effect of D-Gal on the expression of caspase-3-related signal transduction pathway components in the frontal cortex and hippocampus of mice. (A) Representative western blots. (B) Effect of D-Gal on caspase-3 level. Data are expressed as mean ± S.E.M. (n = 15). *P<0.05 and **P<0.01 vs. control group. #P<0.05 and ##P<0.01 protein expressions in PFC vs. HIP.

was a comparable change in the expression of cleaved-caspase-3 in HIP and PFC (Fig. 6B).

Discussion

Using natural-aged and cerebral ischemic animal models, it has previously been reported that ORT and OLT are related to non-spatial and spatial cognitive function, respectively (Hodges et al. 1997; Benice et al. 2006; Bevins and Besheer 2006; Murai et al. 2007; Tong et al. 2007; Cao et al. 2007; Zhao et al. 2007). In the present study, we performed both ORT and OLT to test the hypothesis that chronic exposure to D-Gal could induce a differential impairment of non-spatial and spatial cognitive function.

In the sample phases of ORT and OLT, there were no statistical differences between the three groups in exploring objects A, B, D, and E. This result suggests that the mice do not have objective preferences or visual recognition dissimilarity (Zhao et al. 2007). In the test phase, mice treated with D-Gal spent less time exploring novel or displaced objects than control group mice. This means that treatment with D-Gal induces cognitive impairment in mice. However, the degree of impairment of novelty-recognition and location-recognition was dissimilar. In ORT, mice treated with D-Gal still spent more time exploring the new object than the familiar one. This suggested that these animals maintained recognition memory obtained during the sample phase and therefore discriminated the new object from the familiar one. In the test phase of OLT, the exploring times of D-Gal-treated mice for non-displaced and displaced objects were not statistically different. The DIs of the ORT and the OLT reflected these variations. According to these data, the different performances in the ORT and OLT proved our hypothesis that nonspatial and spatial cognition were unequally impaired after chronic D-Gal exposure.

The behavioral performance of mice in the Y-maze test also supported our hypothesis that chronic D-Gal treatment leaves novelty-recognition intact while impairing spatial memory. If the D-Gal-treated mice exhibited reduced novelty-seeking compared to controls, they would either have avoided the Novel arm or explored the Novel and Other arms to a similar extent in both versions of the Y-maze. However, D-Gal-treated mice explored the Novel arm more than the Other arm in the Ymi (a version of Y-maze with intra-cues), but explored the Novel and Other arms to a similar extent in the YMe. Thus, chronic D-Gal exposure left novelty-recognition (non-spatial cognition) intact.

To explore the possible mechanisms underlying D-Gal-induced cognitive impairment, levels of antioxidant system biomarkers and caspase-3 expression were evaluated. SOD and CAT, two critical antioxidant enzymes working in a cooperative way, are necessary for the elimination of oxidative stress and the survival of neurons. CAT can clear up hydrogen peroxide generated in the course of SOD eliminating the superoxide anion (Sohal et al. 1994; Serrano and Klann 2004). Previous studies have demonstrated that cognitive ability is impaired by oxidative stress and that antioxidants can ameliorate and prevent these cognitive deficits (Lu et al. 2006; Sun et al. 2007; Cui et al. 2006; Yasui et al. 2002; Sharma and Gupta 2001). Increases, decreases or a lack of change in the activities of SOD and CAT have been reported in various studies that used D-Gal-treated or normal aging models (Lu et al. 2006; Sohal et al. 1994). The inconsistency of these studies could be due to variations in the species, age, and periods and dosage of D-Gal administration. Furthermore, most of these studies determined biomarker activities using whole brain tissue but did not study individual subregions that are closely related to specific cognitive functions. In our study, the activity of both of these antioxidant enzymes was measured in the PFC and HIP. Our data demonstrated that the HIP might have a higher sensitivity to chronic D-Gal-induced impairment of the antioxidant system. Furthermore, such weakening of the antioxidant system might induce neuronal toxicity through specific molecular signal transduction pathways which could lead to cognitive impairment.

Proteolytic activation of caspase-3 is a vital event in the execution of apoptosis, resulting from a variety of challenges such as oxidative stress or metabolic inhibition of neurons (Wang 2000; Zhang et al. 2004; Annunziato et al. 2003; Hutton et al. 2007; Tanaka et al. 2006), in central nervous system (Yamashima 2000). Therefore, the increased caspase-3

expression in the brains of D-Gal-treated mice implied that neurons were committed to apoptosis. Previous studies have indicated that the HIP might be more sensitive to oxidative stress during various pathological states and also that the underlying signal transduction pathways may be more sensitive in the HIP than in other brain regions (Naimark et al. 2007; Rall et al. 2003; Wu et al. 2006; Maiti et al. 2006; Behl et al. 1997; Herrera et al. 2003). The multiplicity of apoptosis-related pathways in the HIP might explain why the HIP is more vulnerable to oxidative stress than other brain subregions.

Intact functioning of the HIP and/or its projection sites, such as the PFC, is vital for the ability to form memories (Floresco et al. 1997). In particular, the HIP is thought to convey spatial information and/or information concerning the appropriate behavioral plan relevant to the performance of navigational tasks subserved by the medial PFC. The PFC, in turn, organizes and provides multiple motor plans to drive goal-directed behavior (Floresco et al. 1997; Kolb 1984; Izaki et al. 2000; Ruit and Neafsey 1988; Vertes 2002; Verwer et al. 1997; Broadbent et al. 2004). Previous studies have also demonstrated that the HIP is critical for object-place associations (Gilberta and Kesner 2004; Mumby et al. 2002; Lee et al. 2005; Murai et al. 2007; De Rosa et al. 2005). The increased expression of caspase-3 in the HIP suggests that spatial memory and its information transfer were damaged. This was responsible for the impaired performance in behavioral tasks such as the OLT and YMe. Nonspatial memory is thought to depend not only on the frontal cortex but also on the perirhinal cortex (Bussey et al. 2000), the parahippocampal gyrus (Murai et al. 2007; Murray and Richmond 2001; Zhang et al. 2004), and the entorhinal cortex (Capsoni et al. 2002). Broadbent et al. suggested that hippocampal damage might not be sufficient to completely abolish recognition memory, and also, that spatial memory performance might require more hippocampal tissue than dose recognition memory (Broadbent et al. 2004). Previous studies have reported that damage to the HIP impaired contextual or spatial memory, whereas cholinergic substances were important in object recognition (Bartolini et al. 1996; Whinters and Bussey 2005; Murray and Richmond 2001), and recognition performance was largely spared by hippocampal lesions (Holdstock et al. 2002; Murray and Mishkin 1998; Mumby 2001; Winters et al. 2004). Thus, increased caspase-3-mediated apoptosis in the PFC or HIP might lead to impairment of spatial cognition but does not result in the serious or complete behavioral deficiency of nonspatial recognition memory.

In conclusion, the differences between the control group and D-Gal groups in our study confirm that chronic D-Gal-treated mice can be used as a reliable aging animal model. Moreover, the current data demonstrate that chronic D-Gal exposure induced differential impairment of spatial and non-spatial cognitive functions. These differences might be attributed both to the greater sensitivity of the HIP to reductions in the activity of antioxidant enzymes and to enhanced caspase-3-mediated neuronal apoptosis.

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