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Effect of long-term treatment with rasagiline on cognitive deficits and related molecular cascades in aged mice

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ABSTRACT

The present study aimed to investigate the protective effects of prolonged treatment with the selective, irreversible monoamine oxidase-B inhibitor, novel anti-parkinsonian drug, rasagiline (Azilect) in aged animals. Our findings from behavioral experiments demonstrated that long-term treatment of aged mice with rasagiline (0.2 mg/kg) exerted significant beneficial effects on mood-related dysfunction and spatial learning and memory functions. At this dose of rasagiline, chronic drug administration significantly inhibited monoamine oxidase-B activity and caused an increase in striatal dopamine and serotonin levels, while decreasing their metabolism. In addition, rasagiline treatment elevated striatal mRNA expression levels of dopamine receptors D1 and D2. Furthermore, we found that rasagiline upregulated expression levels of the synaptic plasticity markers brain-derived neurotrophic factor, tyrosine kinase-B receptor, and synapsin-1, increased Bcl-2 to Bax antiapoptotic ratio and the activity of the antioxidant enzyme, catalase in brain of aged mice. The present study demonstrated that long-term treatment with rasagiline could affect behavioral deficits in aged mice and upregulate various neuroprotective parameters in the aging brain, indicating that the drug may have therapeutic potential for treatment of age-associated neurodegenerative disorders.

1. Introduction

Aging in human subjects and experimental animals is often characterized by changes in brain volume, accompanied by a decline in motor and cognitive performance (Schulze et al., 2011). In particular, the functional integrity of the hippocampus region in the brain, which plays an important role in memory formation and spatial navigation, is vulnerable to the aging process, impacting learning and memory (Driscoll and Sutherland, 2005). Brain aging is associated with a progressive imbalance between antioxidant defenses and intracellular accumulation of reactive oxygen species and free radicals (Harman, 1992; Poon et al., 2006), as well as downregulation of neurotrophic factors (Halbach, 2010), which may contribute to synaptic and cellular loss and memory deficits (Tapia-Arancibia et al., 2008).

Rasagiline (N-propargyl-1(R)-aminoindan) is an aromatic propargylamine and highly potent selective irreversible monoamine oxidase (MAO)-B inhibitor (Youdim et al., 2001), indicated for the treatment of motor symptoms in both early- and moderate-to-late stage of Parkinson's disease (PD) (Olanow et al., 2008, 2009). Several preclinical studies have demonstrated that rasagiline exerted neuroprotective effects against various neurotoxins in both cell cultures and animal PD models and in a variety of non-PD-related models (see reviews, Finberg and Gillman, 2011; Maruyama and Naoi, 2013; Naoi and Maruyama, 2010; Weinreb et al., 2010, 2011). Various molecular mechanisms appear to be associated with the neuroprotective effects of rasagiline, including upregulation of cellular antioxidant activity; induction of neurotrophic factors and neuroactive ligand receptors (Maruyama et al., 2004; Weinreb et al., 2009); prevention of the decline in mitochondrial membrane potential and nuclear translation of glyceraldehyde 3-phosphate dehydrogenase, activation of prosurvival antiapoptotic molecules (e.g., Bcl-2 and Bcl-xL), and suppression of cell death cascades initiated by proapoptotic Bcl-2 family molecules (e.g., Bax and Bad) and caspase-3 (Akao et al., 2002b; Blandini, 2005; Maruyama et al., 2001a, Weinreb et al., 2004, 2007). Moreover, it was demonstrated that rasagiline may be involved in the regulation of the molecular composition of the excitatory postsynaptic density (Gardoni et al., 2011).

Structure-activity experiments have indicated that the propargyl moiety is essential for the neuroprotective activity of rasagiline, since N-propargylamine itself was shown to promote neuronal survival via similar neuroprotective and/or neurorescue pathways

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(Bar-Am et al., 2005). Previous studies have shown that N-propargylamine induced antiapoptotic Bcl-2 family proteins reduced the expression of proapoptotic Bax and Bad and increased the availability of neurotrophic factors (Bar-Am et al., 2005; Maruyama et al., 2000b; Yogev-Falach et al., 2003). The neuroprotective activity of rasagiline was also demonstrated at concentrations below the MAO inhibition threshold (Sagi et al., 2007), as well as in cell cultures that do not contain MAO-B (Akao et al., 2002a), suggesting that the neuroprotective activity of rasagiline is not completely attributable to MAO-B inhibition and that multiple mechanisms are involved. In this regards, comparable protective effects have been also obtained with the S-enantiomer of rasagiline, which lacks MAO-B inhibitory activity (Maruyama et al., 2000a, Maruyama et al., 2001b). Additionally, the major metabolite of rasagiline, 1-(R)-aminoindan has been reported to possess antioxidant activity and neuroprotective capabilities of its own, and thus could also contribute to the overall neuroprotective activities of the parent molecule (see review, Bar-Am et al., 2010).

Previous proteomics and genomics studies in aged rats have shown that rasagiline affected various hippocampal mitochondrial genes involved in neurodegeneration, cell survival, synaptogenesis, oxidation, and metabolism (Weinreb et al., 2007). In the present study, we have further investigated the neuroprotective effects of prolonged rasagiline treatment on depressive-like behavior and spatial learning and memory impairments in normal aged mice. Additionally, the regulatory effect of rasagiline on expression of neurotrophic factors, Bcl-2 to Bax ratio and the antioxidant enzyme catalase in the hippocampus and striatum of aged mice were evaluated.

2. Materials and methods

2.1. Materials

Chemicals and reagents were of the highest analytical grade and were purchased from local commercial sources. Primers were purchased from QIAGEN Ltd (Germany). Rasagiline mesylate (N-propargyl-1R-aminoindan) was purchased from Sigma-Aldrich Co LLC (USA).

2.2. Animal treatment procedures

All procedures were carried out in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by the Animal Ethics Committee of the Technion, Haifa, Israel. Animals were kept 3–4 per cage on a 12-hour light-dark cycle with food and water available ad libitum. The dose of rasagiline chosen on the basis of previous studies (Lamensdorf et al., 1996; Youdim et al., 2001). Aged (24-month-old) male C57Bl/6J mice were obtained from Harlan Laboratories, Inc (Israel). Rasagiline (0.2 mg/kg, per day) or vehicles (water) were orally administered daily to aged mice (7–10 mice per each experimental group) for 3 months. In parallel, young (6-month-old) mice were administered with vehicles (water, orally). In all experimental protocols, animals were weighted once a week, and no significant change in body weight was observed during treatment period. At the end of the experiment, the mice were sacrificed and brains were dissected and stored and −80 °C for further biochemical analyses.

2.3. Behavioral analyses

With the aim of assessing the effects of rasagiline on depressive-like behavioral and cognitive impairments in aged mice, behavioral studies were performed 2–3 weeks before the end of drug treatment, as followed:

2.3.1. Depressive-like behavior tests

Forced swimming test (FST) in mice, previously established as behavioral despair test (Borsini and Meli, 1988; Porsolt et al., 1977). The animals were placed individually in glass cylinders (40 cm height, 20 cm diameter) containing 20 cm depth of water at 24–26 °C. After the initial 2-minute acclimatization period, the total duration of immobility was measured for 4 minutes.

Tail suspension test (TST) in mice was performed according to the method described previously (Cryan et al., 2005). The mice were individually suspended in the hook of the TST box, 40 cm above the surface of table with an adhesive tape placed 3 quarters of the distance from the base of the tail. After 2 minutes acclimatization, immobility duration was recorded for 4 minutes.

2.3.2. Morris water maze test

Spatial learning and memory was assessed in mice, using the Morris water maze (MWM) test as previously described (Bromley-Brits et al., 2011; Morris, 1984). We used a circular tank (120 cm diameter × 50 cm height) filled to a depth of 25 cm with tepid water and a white escape platform (10 cm diameter), divided into 4 equal quadrants. The water (23 °C–26 °C) was made opaque by addition of milk. Mice were released into the water, always facing the tank wall, and given 60 seconds to find the platform. On reaching the platform, the mice were allowed to remain on it for 20 seconds. The training schedule consisted of 7 consecutive days of testing. During the 2 first days of testing, the mice were training with visible platform for three 60-second trials per day. During the 4 following days of testing, the mice were trained with hidden platform for three 60-second trials per day. Each subsequent trial was starting at a different direction for each trial. To assess memory consolidation, a probe trial is performed after the platform training trials. In this trial, the platform was removed from the tank, and mice were allowed to swim freely. For these tests, time spent in the target quadrant within 60 seconds was recorded. All trials were monitored by a video camera positioned above the pool and the behavior of each mouse is acquired by a computerized video-tracking system (Smart JUNIOR, Panlab, Spain).

2.3.3. Open field performance

The open field was a 40 cm × 45 cm arena surrounded by 50-cm high walls. The floor of the arena was divided into 12 equal squares by black lines. Mice were placed in the near left corner and left to explore the field freely for 5 minutes. Latency to start locomotion, line crossing, rearing, and the number of fecal pellets produced were counted (Markowska et al., 1998).

2.3.4. Object recognition test

Mice were trained and tested in the novel object recognition task, as previously described (de Lima et al., 2005, 2008). Training in the object recognition task took place in the same arena used for the open field. The object recognition test required that the mice recalled which of the 2 objects they had been previously familiarized with. Twenty-four hours after arena exploration, training was conducted by placing individual mouse into the field, in which 2 identical objects (objects A1 and A2) were positioned in 2 adjacent corners. Mice were left to explore the objects until they had accumulated 30 seconds of total object exploration time or for a maximum of 20 minutes. In a short-term memory test, given 1.5 hours after training, the mouse explored the open field for 5 minutes in the presence of 1 familiar and 1 novel object. In a long-term memory test, given 24 hours after training, the same mouse

Table 1
Inhibition of MAO by chronic treatment of rasagiline in aged mice

Treatment	Enzyme inhibition (% of control)	
	MAO-A	MAO-B
Rasagiline (0.2 mg/kg)	16.13 ± 3.89	97.46 ± 0.50[a]

Data are expressed as percent of MAO inhibition (% of control) in the cerebellum of aged mice after chronic administration of rasagiline (0.2 mg/kg). Values represent mean ± SEM (n = 7–10).
Key: MAO, monoamine oxidase; SEM, standard error of the mean.
[a] $p < 0.05$ versus vehicle-treated aged mice.

explored the field for 5 minutes in the presence of familiar object and a novel object. All objects presented similar textures, colors, and sizes, but distinctive shapes. A discrimination index was calculated as the difference between the time spent exploring new (TB) and old (TA) object divided by the total time spent exploring the objects (TB − TA)/(TB + TA) (Wieschollek et al., 2014). A recognition index was calculated for each mouse and expressed by the ratio TB/(TA + TB).

2.4. MAO activity assay

The effect of rasagiline on MAO-A and B activities was measured as previously described (Gal et al., 2005). Briefly, tissues of cerebellum were dissected and incubated with [C^{14}]serotonin (5-HT) for 30 minutes (final concentration 100 μM) as a substrate for MAO-A, or with [C^{14}]phenylethylamine (PEA) for 20 minutes (final concentration 100 μM) as a substrate for MAO-B, respectively. The radioactivity was determined by liquid-scintillation counter. Protein determination for standardization of MAO activity was performed by the method of Bradford (Sigma-Aldrich Co, USA). Enzyme activity in drug-treated tissues was expressed as a percentage of that in vehicle-treated control tissues.

2.5. Analysis of catecholamine levels

Striatal tissues were homogenized for 1 minute in 400 μL ice cold 0.1-M perchloric acid with mini-homogenizer (Pellet Pestel Motor, Kontes, USA). After centrifugation at 12,000*g* for 10 minutes, the supernatant fraction was recentrifuged at 12,000*g* for 5 minutes, and then injected into a high performance liquid chromatography-electrochemical detector (ECD) (ESA, Inc USA), using an autosampler (Jasco, USA). The levels of dopamine (DA) and its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 3-methoxytyramine (3-MT); 5-HT and its metabolite and 5-hydroxyindoleacetic acid (5-HIAA) were determined by using a hypersil column H30DS-H3427 (Hichrom, Theale, Berkshire, UK), as previously reported (Gal et al., 2005). The content is calculated by comparison to monoamines standards in known concentrations and normalized relatively to tissue weight. Results were analyzed using CoulArray for Windows Application Software.

2.6. Total RNA extraction, reverse transcription, and quantitative real-time reverse transcription polymerase chain reaction

Isolation of total RNA was performed using PerfectPure RNA Cultured Cell Kit (5′PRIME Inc, MD, USA), as recommended by the manufacturer, and reverse transcribed by using PrimeScript RT reagent kit (Takara Bio Inc, Korea), as previously described (Kupershmidt et al., 2011). Real-time polymerase chain reaction was performed with specific primers, for the genes in search: dopamine receptor D1 (DRD1), dopamine receptor D2 (DRD2), brain-derived neurotrophic factor (BDNF), glial cell-derived neurotrophic factor (GDNF), tyrosine kinase-B (Trk-B) receptor, growth-associated protein-43 (GAP-43), synapsin ([Trk-B] receptor)-1, Bcl-2, Bax, and catalase, purchased from QIAGEN Ltd (Germany) on the provided program of 7500 Real time polymerase chain reaction system and SYBR premix Ex Taq ll (Hot Start; Takara Bio Inc). The relative expression level of a given messenger RNA (mRNA) was assessed by normalizing to the housekeeping genes β-actin and γ-tubulin, compared with control values.

2.7. Catalase enzyme activity

Catalase activity was measured according to the assay previously described (Bar-Am et al., 2009). The measurement of catalase activity is based on monitoring of H_2O_2 breakdown using spectrophotometer at 240 nm. The reaction mixture contained 0.033-M H_2O_2 in 0.05-M phosphate buffer pH 7.0. One unit of enzyme was defined as 1 μM of H_2O_2 cleaved per minute at 25 °C and defined as units per mg protein.

2.8. Statistical analysis

Differences among means were analyzed using 1-way analysis of variance and results were expressed as the means ± standard error of the mean; for the statistical analysis, either 1- or 2-way analysis of variance with Dunnett's test for 1-way and Bonferroni

Table 2
Effect of rasagiline on striatal DA and 5-HT and their metabolites in aged mice

A.

Treatment	Striatal levels (pmol/mg)				DOPAC/DA	HVA/DA	3-MT/DA	3-MT/DOPAC
	DA	DOPAC	HVA	3-MT				
Aged/vehicle	51.24 ± 5.68	7.43 ± 0.75	3.82 ± 0.42	1.76 ± 0.10	0.15 ± 0.02	0.07 ± 0.006	0.03 ± 0.001	0.25 ± 0.03
Aged/rasagiline (0.2 mg/kg)	73.97 ± 5.77[a]	3.86 ± 0.30[a]	4.71 ± 0.33	3.81 ± 0.32[a]	0.05 ± 0.002[a]	0.06 ± 0.002	0.05 ± 0.002[a]	0.98 ± 0.04[a]

B.

Treatment	Striatal levels (pmol/mg)		5-HIAA/5-HT
	5-HT	5-HIAA	
Aged/vehicle	2.17 ± 0.23	1.13 ± 0.08	0.54 ± 0.02
Aged/rasagiline (0.2 mg/kg)	2.93 ± 0.18[a]	1.24 ± 0.09	0.42 ± 0.01[a]

(A) Striatal levels of DA and its metabolites, DOPAC, HVA, and 3-MT were determined by using high performance liquid chromatography analysis. Striatal DA metabolism was expressed as the following ratios of: DOPAC to DA, HVA to DA, and 3-MT to DA. Inhibition of DA reuptake was expressed as the ratio of 3-MT to DOPAC. (B) Striatal levels of 5-HT and its major metabolite 5-HIAA were determined by using high performance liquid chromatography analysis. 5-HT metabolism was expressed as the ration of 5-HIAA to 5-HT. Results represent mean ± standard error of the mean (n = 7–10).
Key: DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin; HVA, homovanillic acid; 3-MT, 3-methoxytyramine.
[a] $p < 0.05$ versus vehicle-treated aged mice.

Table 3
Effect of rasagiline on striatal DRD1 and DRD2 mRNA expression levels in aged mice

Treatment	mRNA (arbitrary units)	
	DRD1	DRD2
Aged/vehicle	0.21 ± 0.02	0.25 ± 0.02
Aged/rasagiline (0.2 mg/kg)	0.32 ± 0.03[a]	0.35 ±0.03[a]

The mRNA expression levels of DRD1 and DRD2 were measured in the striatum by quantitative real time reverse transcription polymerase chain reaction. The amount of each product was normalized to the housekeeping genes, β-actin and γ-tubulin. Data are expressed as arbitrary units and represent mean ± standard error of the mean (n = 7–10).
Key: DRD1, dopamine receptor D1; DRD2, dopamine receptor D2; mRNA, messenger RNA.
[a] $p < 0.05$ versus vehicle-treated aged mice.

for 2-way post hoc tests were used. p-Values < 0.05 were considered significant.

3. Results

3.1. Regulatory effects of rasagiline on cerebral MAO, content of amines and their metabolites, and mRNA expression of DRD1/DRD2 in aged mice

Initially, we determined the inhibitory effect of rasagiline on MAO-A and -B activities in the cerebellum of aged mice. The activity of cerebral MAO-B was significantly higher in aged mice (130.7 ± 5.8%; $p < 0.05$), compared with young mice, whereas no significant change was demonstrated in MAO-A activity in aged mice (115.6 ± 6.7%), compared with young mice. As shown in Table 1, chronic drug administration resulted in a marked and significant inhibition of brain MAO-B activity and nonsignificant low inhibition of MAO-A activity, compared with vehicle-aged mice.

Additionally, the effect of chronic rasagiline treatment on the levels of striatal amines (DA, DOPAC, HVA, 3-MT, 5-HT, and 5-HIAA) in aged mice was determined. As shown in Table 2, rasagiline treatment increased striatal levels of DA, decreased the levels of the intraneuronal metabolite DOPAC, and elevated the levels of the extraneuronal metabolite 3-MT, compared with vehicle-treated aged mice. DOPAC to DA ratio (MAO-associated oxidative pathway), was significantly decreased by administration of rasagiline, thus indicating a decreased intraneuronal DA metabolism in the striatum. Rasagiline treatment also significantly increased 3-MT/DA (catechol-O-methyltransferase-associated methylation pathway) and 3-MT to DOPAC (DA reuptake index) ratios. In addition, rasagiline treatment increased 5-HT levels and reduced 5-HT turnover, as assessed by 5-HIAA to 5-HT ratio, compared with vehicle-treated aged mice (Table 2).

As the dopaminergic system was found to regulate the expression of DA receptor genes (see review, Jaber et al., 1996), we further analyzed the effect of rasagiline administration on mRNA expression of DRD1 and DRD2 in the striatum and hippocampus of aged mice. Table 3 shows that administration of rasagiline significantly increased mRNA expression levels of DRD1 and DRD2 in the striatum, indicating that the drug possesses the ability to modulate gene expression of DRD1 and DRD2 in aged animals.

3.2. Rasagiline attenuates depressive-like behavior and cognitive deficits in aged mice

The possible beneficial effect of rasagiline on depressive-like activity in aged mice was analyzed by 2 most commonly used models, the TST (Cryan et al., 2005) and FST (Borsini and Meli, 1988; Pirondi et al., 2005). Results for the TST demonstrated a significant elevation of time spent in immobility in vehicle-treated aged mice, compared with vehicle-treated young mice (Fig. 1). Treating aged animals with rasagiline (0.2 mg/kg) for 3 months significantly reduced the duration of immobility in both depressive-like behavior tests, the TST (Fig. 1A) and the FST (Fig. 1B) in aged mice, compared with vehicle-treated aged animals. Results for open field exploration behavior in aged mice treated with rasagiline did not affect the number of crossing and rearings, as compared with vehicle-treated aged mice, indicating that the locomotor activity was not affected by the treatment (data not shown).

In the next behavioral experiments, we assessed the effect of rasagiline administration on cognitive deficits in aged mice with MWM test, one of the most accepted behavioral tests of hippocampal function that evaluates hippocampal dependent spatial

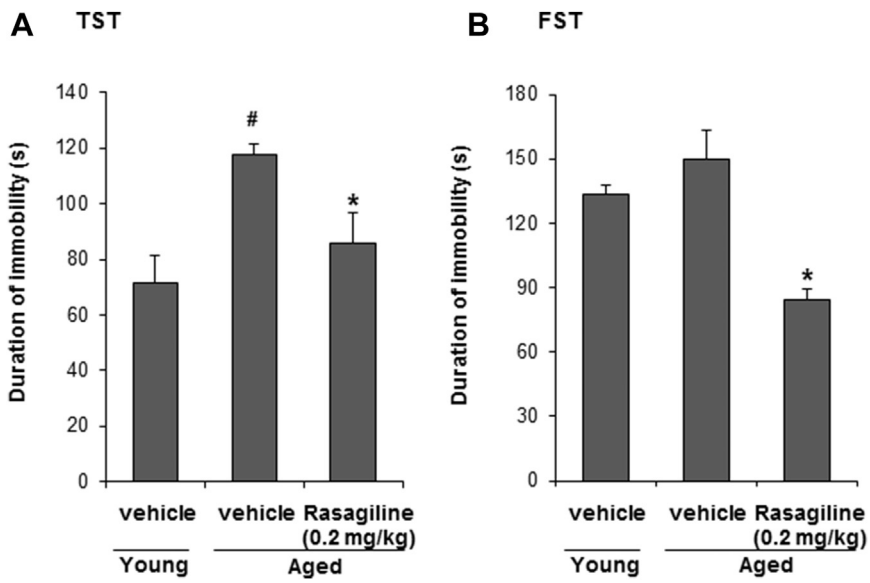

Fig. 1. Effect of rasagiline on the duration of immobility in the TST and FST. The immobility time (seconds) of aged mice treated with either vehicle (water) or rasagiline (0.2 mg/kg; orally, daily for 3 months) was analyzed in TST (A) and the FST (B). Values are expressed as the mean ± standard error of the mean (n = 7–10). #$p < 0.05$ versus vehicle-treated young mice; *$p < 0.05$ versus vehicle-treated aged mice. Abbreviations: FST, forced swimming test; TST, tail suspension test.

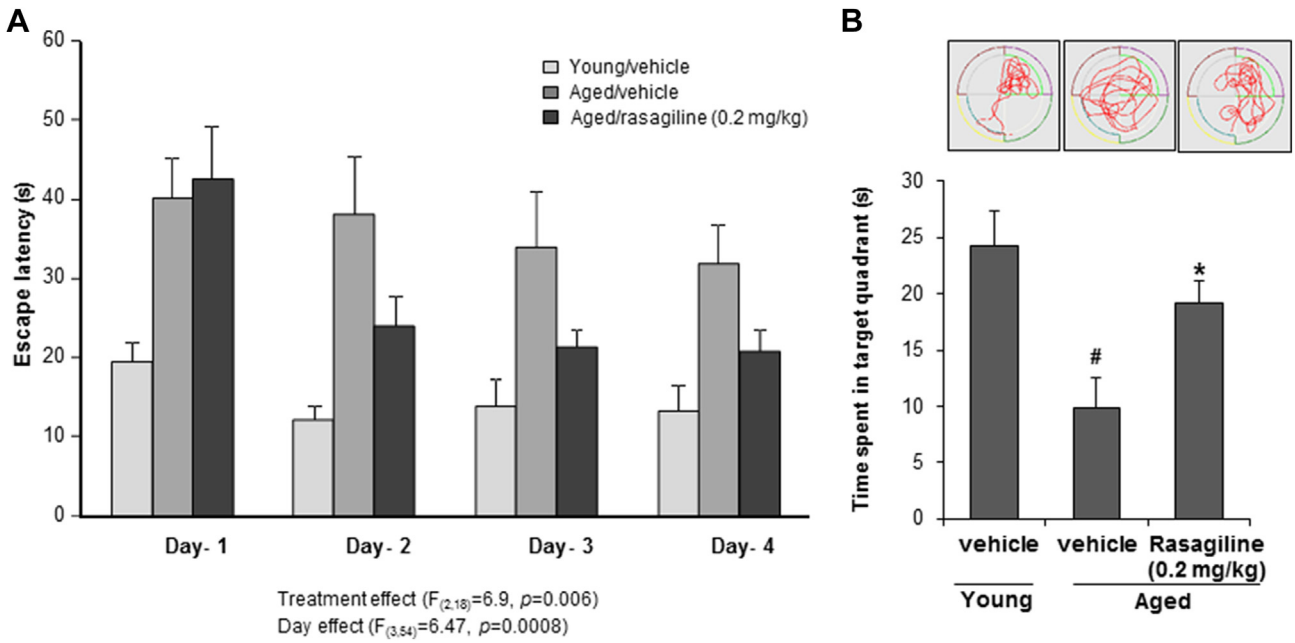

Fig. 2. Effect of rasagiline on spatial learning and memory of aged mice in Morris water maze (MWM) test. Mice were treated as described in Fig. 1 and subjected to MWM test. The analyses of mice performance were consisted of escape latency (A) and time spending in the target quadrant (B) in the probe trial. Values represent mean ± standard error of the mean of 4 trials (n = 7–10). #$p < 0.05$ versus vehicle-treated young mice; *$p < 0.05$ versus vehicle-treated aged mice.

learning and memory (Morris, 1984). Spatial learning was assessed by the time required to find the hidden platform (escape latency). When comparing aged versus young mice, the results showed impaired acquisition of spatial learning in vehicle-treated aged mice, compared with vehicle-treated young mice, as indicated by longer duration to locate the hidden platform and much slower improvements in the escape latency across consecutive trials (Fig. 2A). On the fourth day of training, the escape latency of aged mice was longer than that of the young mice; however, rasagiline-treated aged mice, despite the marked initial spatial learning impairment exhibited on the first day, the animals were able to proficiently locate the hidden platform by the fourth day of the session (Fig. 2A). All groups demonstrated a significant decrease in the distance to locate the hidden platform by the fourth day of training (data not shown). Probe trials, in which the platform was removed to assess spatial bias, are shown in Fig. 2B. The time spent in the target quadrant was significantly decreased in vehicle-treated aged mice ($p < 0.05$), as compared with vehicle-treated

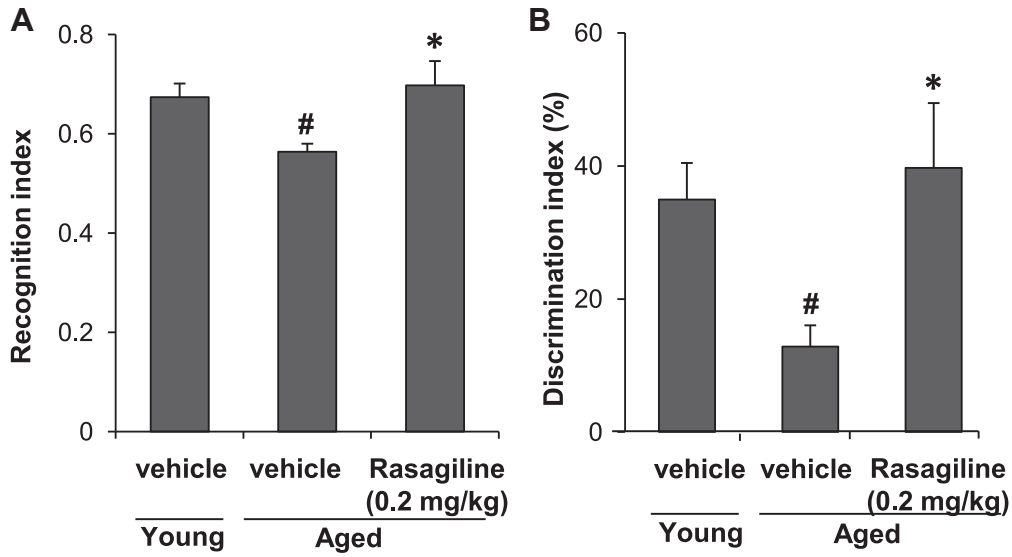

Fig. 3. Effect of rasagiline on memory acquisition of aged mice in the novel object recognition test. Mice were treated as described in Fig. 1 and subjected to the novel object recognition test in a long-term retention (24 hours after training). The proportion of the total exploration time that the animal spent investigating the novel object was expressed as the recognition index, calculated for each animal by the ratio of time spent exploring the novel object to the time spent exploring both objects (A) and the discrimination index, calculated as the difference in time spent exploring the new object minus the time spent exploring the familiar object (B). Each bar represents the mean ± standard error of the mean of 4 trials (n = 7–10). #$p < 0.05$ versus vehicle-treated young mice; *$p < 0.05$ versus vehicle-treated aged mice.

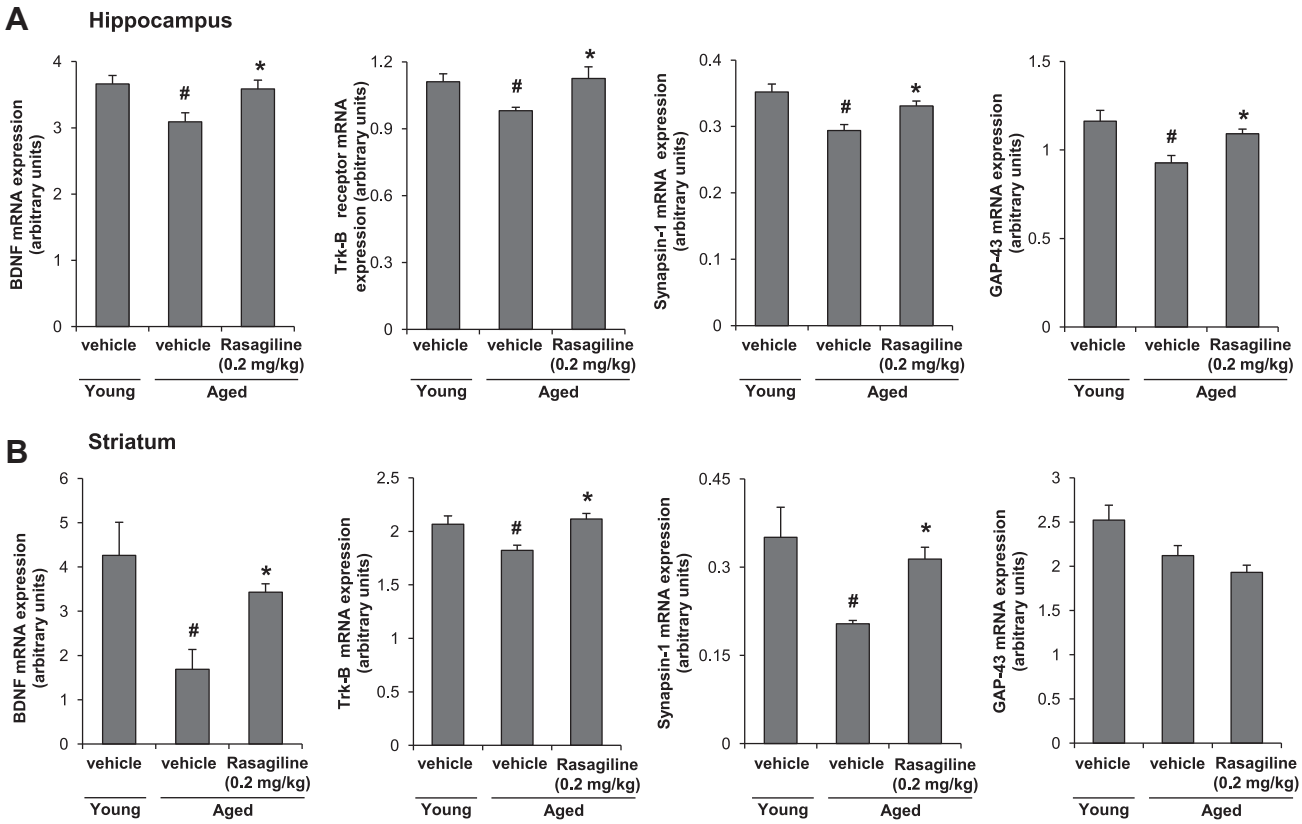

Fig. 4. Effect of rasagiline on mRNA expression levels of BDNF, Trk-B receptor, and synaptic factors in the hippocampus and striatum of aged mice. Mice were treated as described in Fig. 1. The expression levels of BDNF, Trk-B receptor, synapsin-1, and GAP-43 mRNAs were measured in the hippocampus (A) and striatum (B) of aged mice by quantitative real time RT-PCR. The amount of each product was normalized to the housekeeping genes, β-actin and γ-tubulin. Data are expressed as arbitrary units and represent mean ± standard error of the mean (n = 7–10); #$p < 0.05$ versus vehicle-treated young mice; *$p < 0.05$ versus vehicle-treated aged mice. Abbreviations: BDNF, brain-derived neurotrophic factor; GAP-43, growth-associated protein-43; mRNA, messenger RNA; Trk-B, tyrosine kinase-B.

young mice. Chronic rasagiline treatment attenuated the decrease in acquisition phase of place learning and improved memory retention during the probe trial (Fig. 2B).

In the novel object recognition test, vehicle-treated aged mice showed significantly lower preference toward the novel object, as indicated in their recognition (Fig. 3A) and discrimination (Fig. 3B) indexes, compared with vehicle-treated young mice, in the long-term memory retention trial. However, rasagiline-treated animals exhibited a significantly higher preference in exploring the novel object during the long-term memory retention trial than vehicle-treated aged mice, as their recognition (Fig. 3A) and discrimination (Fig. 3B) indexes were significantly higher, compared to the vehicle-treated aged group. Taken together, these data, obtained from MWM and novel object recognition tests, indicate that rasagiline treatment significantly improved spatial learning-memory deficits in aged mice.

3.3. Effect of rasagiline on hippocampal and striatal mRNA expression levels of BDNF and/or Trk-B receptor, synaptic factors, and Bcl-2 family members in aged mice

To further investigate rasagiline-induced changes in the brain and their relationship to the improved cognition observed in the aged mice, we compared the gene expression of various neuroprotective parameters in the hippocampus and striatum of rasagiline-treated aged mice, with their corresponding controls. As shown in Fig. 4, the levels of the transcripts of BDNF, its receptor Trk-B, synapsin-1, and GAP-43 were decreased in the hippocampus and striatum of aged mice, compared with young mice. However, in rasagiline-treated aged mice, the mRNA levels of BDNF, Trk-B receptor, and synapsin-1 were significantly upregulated, both in the hippocampus (Fig. 4A) and striatum (Fig. 4B), as compared with vehicle-treated aged mice. Rasagiline administration upregulated mRNA expression levels of GAP-43 in the hippocampus of aged mice, as compared with vehicle-treated aged mice (Fig. 4A).

In addition, rasagiline upregulated mRNA expression levels of the antiapoptotic Bcl-2 in the hippocampus (Fig. 5A) and down-regulated mRNA expression levels of the proapoptotic Bax (Fig. 5B) in the striatum in aged mice, as compared with respective levels in vehicle-treated aged mice. The ratio of Bcl-2 to Bax, which correlates with cellular apoptosis, was significantly increased in the hippocampus and striatum of rasagiline-treated versus vehicle-treated aged mice (Fig. 5).

3.4. Effect of rasagiline on mRNA expression levels and activity of catalase in the brain of aged mice

Prevention of oxidative stress and maintenance of oxidative status at the cellular level is critical to normal physiological function and inhibition of age-associated disorders (Venkateshappa et al., 2012). The results from the present study demonstrated that catalase mRNA levels were increased in the hippocampus (Fig. 6A) and striatum (Fig. 6B) of rasagiline-treated, as compared with vehicle-treated aged mice. Moreover, rasagiline administration to aged mice antagonized the age-dependent decrease of catalase enzyme activity in the frontal cortex (Fig. 6C).

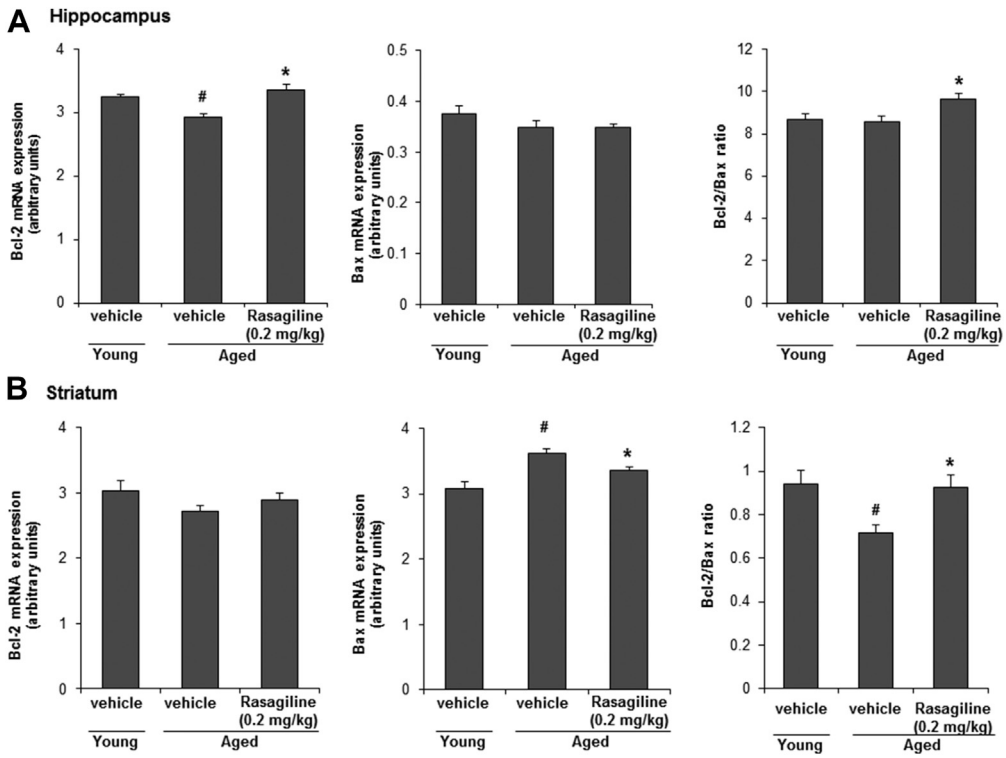

Fig. 5. Effect of rasagiline on Bcl-2 and Bax mRNA expression levels in the hippocampus and striatum of aged mice. Mice were treated as described in Fig. 1. The expression levels of Bcl-2 and Bax mRNAs and Bcl-2 to Bax apoptotic ratio were determined in the hippocampus (A) and striatum (B) of aged mice by quantitative real time reverse transcription polymerase chain reaction. The amount of each product was normalized to the housekeeping genes, β-actin and γ-tubulin. Data are expressed as arbitrary units and represent mean ± standard error of the mean (n = 7–10); #$p < 0.05$ versus vehicle-treated young mice; *$p < 0.05$ versus vehicle-treated aged mice. Abbreviation: mRNA, messenger RNA.

3.5. *Effect of rasagiline on mRNA expression levels of GDNF, synapsin-1, and Bcl-2 and enzyme activity of catalase in the brain of aged rats*

We further evaluated the effect of chronic rasagiline administration on mRNA expression levels of neurotrophic factors and Bcl-2 in the hippocampus and catalase activity in the frontal cortex in aged rats. Supplementary Fig. 1A shows that the levels of the transcripts of GDNF, synapsin-1, and Bcl-2 were decreased in the hippocampus of aged rats, compared with young rats. However, in rasagiline-treated aged rats, the mRNA levels of GDNF, synapsin-1, and Bcl-2 were significantly increased in the hippocampus, as compared with vehicle-treated aged rats (Supplementary Fig. 1A). Moreover, rasagiline administration to aged rats significantly contracted the age-dependent decrease of catalase activity in the frontal cortex (Supplementary Fig. 1B).

4. Discussion

The present study provides evidence for the protective potential of rasagiline on depressive-like behavior and cognitive impairment in aged mice. Our findings from the behavioral experiments demonstrate that chronic treatment of aged mice with rasagiline (0.2 mg/kg) exerted a significant beneficial effect on mood-related dysfunction, tested by the TST and FST, as well as on cognitive behavior deficits, as assessed by MWM and object recognition tests. We observed that chronic treatment with rasagiline, at low dose, selectively inhibited MAO-B activity in the cerebellum (without a

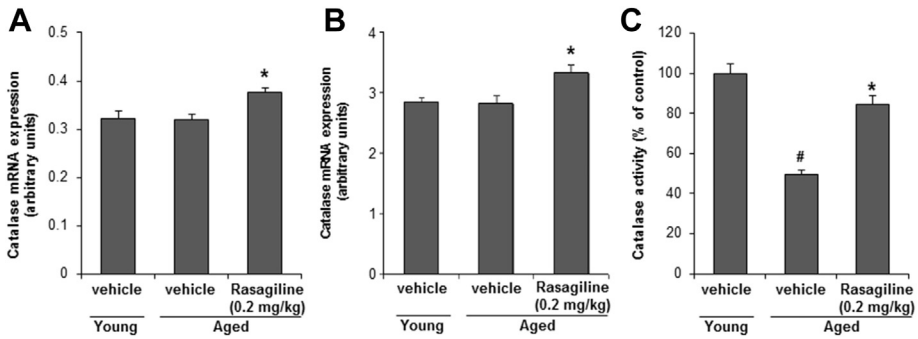

Fig. 6. Effect of rasagiline on mRNA expression levels and enzyme activity of catalase in the brain of aged mice. Catalase mRNA expression was analyzed in the hippocampus (A) and striatum (B) by quantitative real time reverse transcription polymerase chain reaction. The amount of each product was normalized to the housekeeping genes, β-actin and γ-tubulin. Data are expressed as arbitrary units. Catalase enzyme activity (C) was measured in the frontal cortex and expressed as relative activities (% of control, vehicle-treated young mice). The values represent mean ± standard error of the mean (n = 7–10); #$p < 0.05$ versus vehicle-treated young mice; *$p < 0.05$ versus vehicle-treated aged mice. Abbreviation: mRNA, messenger RNA.

significant inhibition of MAO-A); increased mRNA expression levels of DRD1 and DRD2 in the striatum and hippocampus and enhanced striatal DA levels. This may be related to the involvement of the amphetamine-like trace amine beta-PEA levels that are markedly increased in the brain by selective inhibition of MAO-B (Lauber and Waldmeier, 1984). In accordance, a previous study demonstrated that chronic treatment with selective MAO-B inhibitors (e.g., deprenyl and rasagiline) increased extracellular striatal DA, suggesting that this effect may be mediated by an increase in endogenous PEA levels or inhibition of DA reuptake (Lamensdorf et al., 1996). In addition, we showed that rasagiline treatment decreased striatal levels of the intraneuronal metabolite, DOPAC, and elevated those of the extraneuronal metabolite 3-MT. Concomitantly, DA oxidation levels, expressed as DOPAC to DA ratio, were decreased and the marker of DA reuptake inhibition, expressed as the 3-MT to DOPAC ratio was significantly increased. The levels of 5-HT in the striatum were only slightly increased, after rasagiline administration to aged mice and are in accordance with nonsignificant low inhibition of MAO-A and the selective inhibition of MAO-B by the drug. Indeed, selective inhibition of MAO-B does not cause significant changes in central nervous system steady-state levels of 5-HT (Green and Youdim, 1975; Twist et al., 1991; Waldmeier, 1987). In addition, several studies have reported that striatal DA may play a key role in motivated behavior and has a direct impact on memory formation in the hippocampus, suggesting hippocampal-striatal DA network interaction that affected learning and memory capabilities [see reviews, (Shohamy and Adcock, 2010; Baudonnat et al., 2013)].

The findings from behavioral experiments with rasagiline in aged mice were confirmed by neurochemical studies, conducted in the hippocampus and striatum, two brain regions that have been shown to mediate various aspects of depression, spatial cognition, and memory (Berton and Nestler, 2006). In the present study, rasagiline treatment was shown to upregulate mRNA expression levels of BDNF and TrK-B receptor and the synaptic plasticity marker synapsin-1 in the hippocampus and striatum in aged mice. Furthermore, rasagiline treatment resulted in increasing the ratio of the antiapoptotic molecule Bcl-2 and the proapoptotic molecule Bax. Our results also show that chronic treatment of aged rats with rasagiline significantly upregulated mRNA expression levels of GDNF, synapsin-1, and Bcl-2 in the hippocampus (Supplementary Data). In agreement, we have previously reported that rasagiline treatment could partially reverse age-related mitochondrial and key regulator genes that are involved in the neurodegenerative process, synaptogenesis, cell survival, oxidation, and metabolism in the hippocampus (Weinreb et al., 2007). These results are also in accordance with studies, demonstrating that the neuroprotective activity exerted by rasagiline against various neurotoxins, both in cell culture and in animal models of PD is dependent on the intervention of mitochondrial apoptotic cascade and on regulation of various neurotrophic factors [e.g., BDNF, nerve growth factor, and GDNF] and Bcl-2 family proteins (e.g., Bcl-2, Bcl-xL, Bcl-w, Bad, and Bax) (Akao et al., 2002a; Bar-Am et al., 2005; Maruyama et al., 2004; Weinreb et al., 2004, 2009; Yogev-Falach et al., 2006).

Considering the oxidative stress as a major factor in the aging brain and age-related neurodegenerative diseases, we observed in the present study that rasagiline increased mRNA levels of the antioxidant enzyme, catalase in the hippocampus and striatum, compared with vehicle-treated aged animals. Rasagiline also enhanced the enzyme activity of catalase, further indicating the significant antioxidative action of the drug. These data are consistent with previous reports that demonstrated that a series of propargylamines, including rasagiline, deprenyl, and lasdostigil, all increased catalase activity in several brain regions (e.g., hippocampus, striatum, and frontal cortex) and in systemic organs, such as heart and kidney (Bar-Am et al., 2009; Carrillo et al., 2000; Kitani et al., 2001). Moreover, given that products of MAO-catalyzed reaction, for example, H_2O_2 and toxic aldehydes are compelling inductors of lipid peroxidation, it can be assumed that the inhibition of MAO-B by rasagiline may reduce oxidation and free radical accumulation and also increase the content of monoamines in the brain, by inhibiting their catabolism (Khaldy et al., 2000).

Taken together, the results of the present study demonstrated that long-term treatment with rasagiline could attenuate depressive-like behavior and age-related cognitive impairments in aged mice and upregulate neurotrophic parameters in the aging brain, indicating that the drug might be of therapeutic benefits in aging.

Disclosure statement

Moussa B. H. Youdim developed with Teva pharmaceutical company (Israel) the anti-Parkinson's drug rasagiline and received royalty.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neurobiolaging.2015.05.009.

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