Rasagiline improves learning and memory in young healthy rats

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The effect of rasagiline on learning and memory in Lister-Hooded rats was investigated in this study. Two cognitive tests were used: a 24-h temporal deficit novel object recognition test and a modified water maze task. Rasagiline (0.3 and 1 mg/kg) was administered subcutaneously 15 min before the cognitive tests. In a novel object recognition test, rasagiline treatment enhanced object recognition memory. A small effect was observed with 0.3 mg/kg rasagiline; at 1 mg/kg, rasagiline-treated animals spent twice as much time exploring the novel object. On the water maze test, the use of an on-demand platform allowed adjustment of the difficulty of this spatial learning task. This enabled the detection of a small positive effect of rasagiline (1 mg/kg) on spatial learning, which was not observed in earlier reports. For the first time, our study has showed the procognitive effect of rasagiline in young healthy rats. On the basis of these findings, a monoamine oxidase-B inhibitor would seem to be a potential symptomatic treatment for cognitive impairments affecting patients with neurodegenerative disorders. *Behavioural Pharmacology* 00:000–000 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.

Introduction

Rasagiline is a potent selective inhibitor of type B monoamine oxidase (MAO-B). It has been approved for the symptomatic treatment of mild-to-advanced Parkinson's disease (PD) in the USA, Canada, Israel, and the European Union (for review, see Guay, 2006). The inhibition of MAO-B reduces the breakdown of various monoamine neurotransmitters that are important in learning and memory processes (Bushnell and Levin, 1993; Arnsten et al., 1998; Lidow et al., 1998). Earlier studies have shown that central dopaminergic and cholinergic functions were significantly enhanced after rasagiline treatment (Lamensdorf et al., 1996; Speiser et al., 1998b). In various animal models of neurodegeneration and behavioral deficit, rasagiline has been shown to exhibit neuroprotective and cognition-enhancing properties. Acute rasagiline treatment has been shown to reduce infarct volume in an ischemic stroke model (Speiser et al., 2007) and decrease cerebral damage after closed head injury (Huang et al., 1999). Chronic rasagiline treatment has also been shown to increase survival and reduce the incidence of stroke in spontaneously hypertensive rats (Eliash et al., 2001), and improved learning and memory in a rat model of anoxia (Speiser et al., 1998a, 1998b).

However, the effects of rasagiline are not limited to its MAO-B inhibitory action, especially when it is administered for a prolonged period of time. Chronic rasagiline induced significant changes in the brain as compared with an acute administration. In a recent study, 4 weeks of rasagiline treatment resulted in changes in mRNA level and proteins that are mainly involved in cell survival and death pathways, metabolic/oxidative stress, and signaling systems (Weinreb et al., 2009). Furthermore, the levels of monoamine neurotransmitters were also affected (Finberg and Youdim, 2002). In addition, the drug has been shown to induce neuroprotective effects through its propargyl moiety (Abassi et al., 2004; Bar-Am et al., 2004).

As chronic rasagiline treatment can lead to a mixture of complex events that are independent of MAO-B inhibition, this study investigated the direct effect of MAO-B inhibition by acute rasagiline treatment on learning and memory in healthy rats. Two cognitive tests were used: a novel object recognition (NOR) test and a modified water maze task. These two tests have been used to investigate the cognitive-enhancing properties of various drugs, which were small and difficult to detect in most of the commonly used cognitive tests in young healthy animals (Atcha et al., 2009; Goh et al., 2009). Our findings have shown a positive effect of rasagiline on learning and memory, and support the potential use of rasagiline in the symptomatic treatment of cognitive impairments.

Methods
Subjects
Male Lister-Hooded rats were obtained from Harlan, UK. Animals were housed four in each cage in a temperature ($20 \pm 1^{\circ}$C) and humidity ($40 \pm 2\%$)-controlled environment

for a 12-h light/dark cycle (lights on 07:30 h). Food and water were freely available. All experiments were carried out in accordance with the Singapore National Advisory Committee for Laboratory Animal Research guidelines for the use and care of animals for scientific purposes and GlaxoSmithKline animal research ethical standards.

Novel object recognition

Animals were handled before and after a daily 1-h habituation session to the test cages (Tecniplast, Buguggiate, Italy) for 2 days before the initial presentation of the objects (T1 trial). Objects used in this study were custom-made black acrylic cubes and cylinders (Labman Design, Singapore). A small magnet was embedded at the bottom of each object to prevent the animals from moving the objects during trials. In the T1 trial, the animals were habituated to the test cage without objects for 3 min. The animals were then briefly moved to an adjacent cage for approximately 10 s, whereas two identical objects were placed into the test cage. The animals were then placed back to the test cage for a further 3 min habituation period and the time spent exploring each object was recorded by an experienced observer. For the T2 trial, animals were placed back into the test cage for a further 3 min habituation period, 24 h after the T1 trial. They were then presented with one familiar and one novel object for a total of 3 min and object exploration was recorded. Objects were randomly assigned to ensure that treatment groups were fully balanced for both the novel object and its position within the test cage (either left or right).

Water maze (on-demand platform)

The water maze apparatus was a white fiberglass pool (diameter, 1.7 m; height, 0.65 m). Surrounding the pool were a variety of spatial cues (SCs) (posters and halogen light sources), which remained constant throughout the entire study. The water maze was filled with clean water warmed to $26 \pm 1°C$ every morning and made opaque by adding 1 l of opacifier (Syntran 5905; Interpolymer, Canton, USA). The pool was divided into four imaginary quadrants and an on-demand platform (diameter, 20 cm) was placed in the center of one of the four quadrants. When the platform was fully raised, it was covered by 2 cm of water and therefore invisible to the rat. A video camera was positioned directly above the tank to record the rat's swim trajectory, and this was connected to a personal computer in which escape latency and swim speed were acquired using Watermaze software (Actimetrics Inc., Wilmette, Illinois, USA).

During the visual cue (VC) training session run on the first study day, a curtain was completely drawn around the water maze shielding the SCs. The platform was set in the raised position and a black acrylic cylindrical object was suspended 40 cm directly above the platform. During each of the four VC trials, the animals were trained to locate the platform using the black acrylic object as a VC. When the platform was located, the trial was stopped and the rat was left on the platform for 30 s.

During the SC training sessions (Tuesday–Friday, six trials each day), the black acrylic cylindrical object (VC) was removed and the curtain was fully retracted so that the animals could use the SCs surrounding the pool. The on-demand platform enables the task difficulty to be gradually increased over the 4 training days. This is achieved by gradually extending the time that the animal has to dwell within the trigger zone across the 4 days (day 1, 0.8 s; day 2, 1.5 s; day 3, 2.3 s; and day 4, 3.0 s). In any particular trial, if the animal failed to locate and/or activate the platform, it was automatically raised after 90 s, and after 2 min, the animal was led to the platform using a pole. Once the animal had located the platform, the trial was stopped and the animal was left on the platform for 30 s.

Locomotor activity monitoring

The LABORAS is a validated behavior registration system for the automatic registration of different behavioral elements of mice and rats (Van de Weerd et al., 2001). Locomotor activity was monitored based on the vibrations because of the movements detected by force transducers. The data were then analyzed using the LABORAS software (Metris B.V., Hoofddorp, North Holland, The Netherlands). Before the test sessions, all the cages were calibrated to ensure that the settings were specific for each animal. The animals were placed into the cages and monitored for 1 h. Food and water were freely available. After the completion of the experiment, the animals were returned to their home cages.

Drug administration and pretreatment time

Rasagiline mesylate (J. Inc., Ahmedabad, Gujarat, India) was dissolved in sterile 0.9% sodium chloride solution and administered subcutaneously at an injection volume of 2 ml/kg, 15 min before T1 and T2 trials of NOR, water maze training trials, and LABORAS monitoring. All the control animals were administered sterile 0.9% sodium chloride solution 15 min before the tests.

Statistical analysis

All the graphs were prepared using GraphPad Prism (version 4, La Jolla, California, USA), and all data are expressed as mean ± SEM. Statistical analysis was made using StatSoft Statistica (version 6.0, Tulsa, Oklahoma, USA), and all data were checked for normality before the analysis. For the water maze and NOR T2 trial data, repeated-measures analysis of variance (ANOVA) followed by planned comparisons was used. For NOR T1 and T2 total exploration time data, and LABORAS data, a one-way ANOVA followed by planned comparisons was used to compare the treatment groups.

Results

Effect of rasagiline on novel object recognition performance

Vehicle-treated animals spent comparable amount of time exploring the novel and familiar objects during the T2 trial, indicating that they had forgotten the familiar object after 24 h. There was a significant effect of rasagiline treatment on novel versus familiar exploration [$F_{(1,33)} = 11.17$, $P < 0.002$] (Fig. 1a). Planned comparison analysis showed a significant effect of both the doses when compared with the vehicle group (0.3 mg/kg, $P < 0.02$; 1 mg/kg, $P < 0.001$). No significant difference in total exploration time was found in either the T1 trial [$F_{(2,33)} = 1.00$, NS] or the T2 trial [$F_{(2,33)} = 0.65$, NS] (Fig. 1b).

Effect of rasagiline on water maze performance

VC data were analyzed using repeated-measures ANOVA and this showed no differences in either latency [$F_{(2,18)} = 0.35$, NS] or swim speed [$F_{(2,18)} = 1.88$, NS] between the assigned treatment groups before dosing. Repeated-measures ANOVA of the SC latency data showed a significant effect of rasagiline treatment [day × treatment interaction, $F_{(6,54)} = 3.54$, $P < 0.005$] (Fig. 2a).

Planned comparison analysis showed that the 1 mg/kg rasagiline group performed significantly better than the vehicle group on SC day 4 ($P < 0.01$). Repeated-measures ANOVA of the SC swim speed data showed that there was no effect of rasagiline treatment [day × treatment interaction, $F_{(6,54)} = 1.28$, NS] (Fig. 2b).

Fig. 1
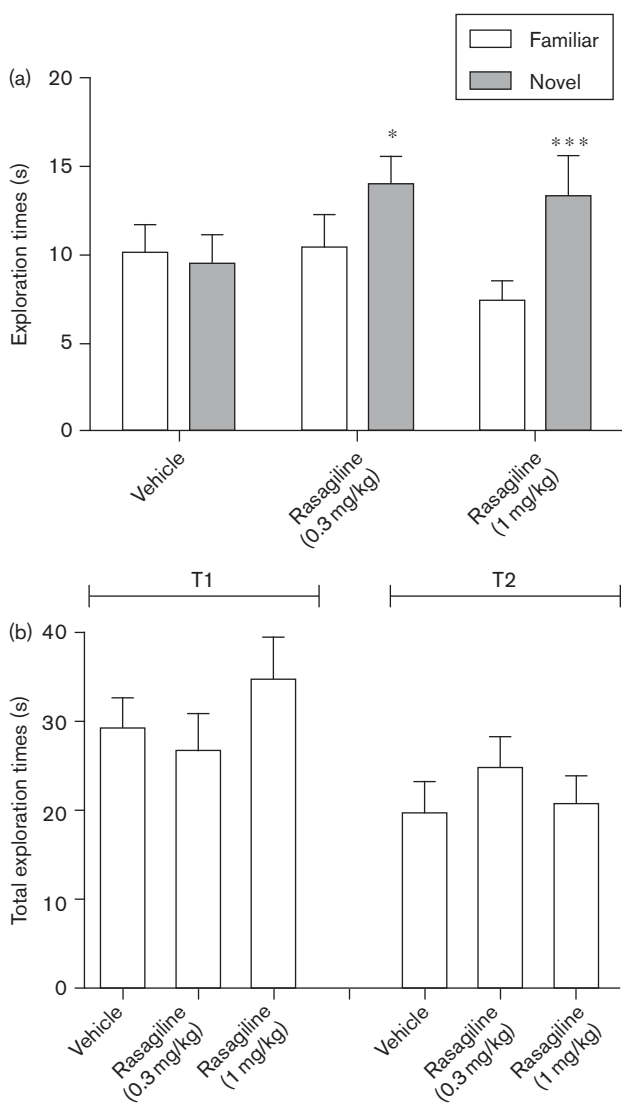
Effect of 0.3 and 1 mg/kg rasagiline on (a) NOR T2 trial novel and familiar objects exploration time. (*$P < 0.05$ and ***$P < 0.0001$), (b) T1 and T2 total exploration time. Rasagiline significantly enhanced object recognition memory in healthy rats. Data are expressed as mean ± SEM ($n = 12$).

Fig. 2
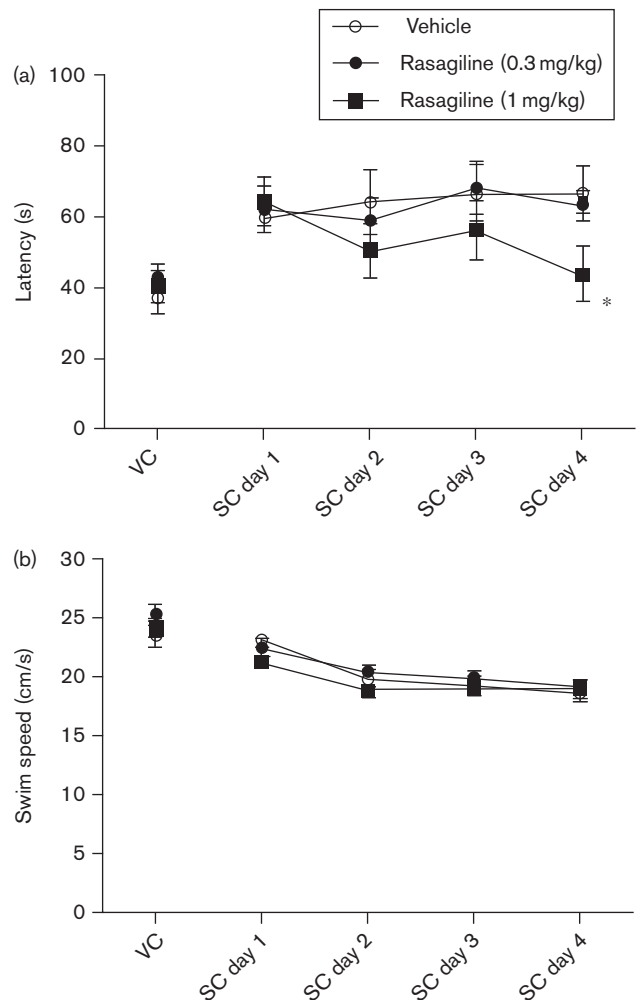
Effect of 0.3 and 1 mg/kg rasagiline on (a) latency to locate the hidden water maze platform and (b) swim speed in the water maze. Rasagiline (1 mg/kg) significantly enhanced spatial learning. Data are expressed as mean ± SEM ($n = 10$); *$P < 0.05$. SC, spatial cue; VC, visual cue.
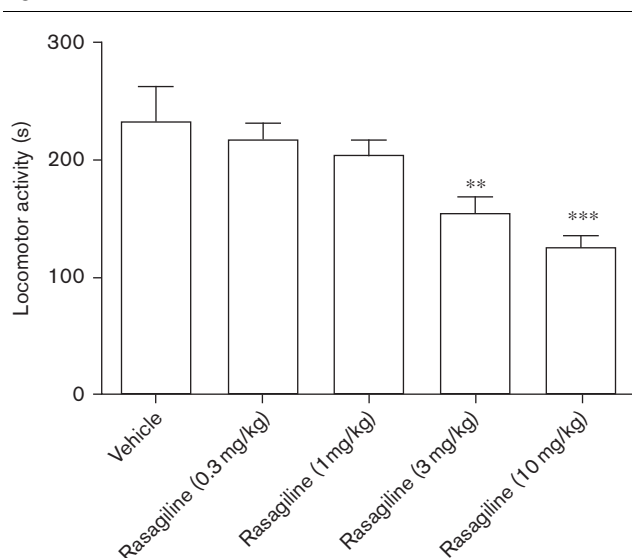
Effect of rasagiline on locomotor activity

A significant reduction in locomotor activity was observed in animals treated with rasagiline at doses of 3 and 10 mg/kg or more [$F(4,35) = 6.48$, $P < 0.001$]; Fisher's least significance difference (3 mg/kg, $P < 0.005$; 10 mg/kg, $P < 0.001$) (Fig. 3).

Discussion

NOR is a standard behavioral test designed to study object recognition memory that exploits the tendency of rodents to explore a novel rather than a familiar object in their environment (Ennaceur and Delacour, 1988; for review, see Dere et al., 2007). The current protocol is a temporal deficit model in which a healthy animal will forget the familiar object and explore both familiar and novel objects with the same level of interest, after a 24-h retention interval. The 24-h delay was chosen from the results obtained in our own validation study (data not shown), and it was also described in a number of earlier reports (Obinu et al., 2002; Bertaina-Anglade et al., 2006). The inclusion of the 2-day habituation to the test cages in our protocol greatly minimized the stress level and reduced the variability in the results obtained, allowing us to detect a smaller effect of drug treatment, as seen with 0.3 mg/kg rasagiline. Animals treated with 1 mg/kg rasagiline spent nearly twice as much time exploring the novel object compared with the familiar object. In addition, the total T2 exploration times of the rasagiline groups were not significantly different from the vehicle group (Fig. 1b), excluding the possibility of a drug-induced increase in exploratory drive.

Fig. 3

Effect of rasagiline (0.3–10 mg/kg) on locomotor activity monitored using the LABORAS. Rasagiline at doses equal or more than 3 mg/kg significantly reduced locomotor activity in healthy rats. Data are expressed as mean ± SEM ($n = 8$); **$P < 0.01$, ***$P < 0.001$.

Unlike the traditional Morris water maze, the water maze setup in this study included an on-demand platform that was raised near to the water surface only when the rat dwelled within a defined area above the platform for a programmed period of time, which could be adjusted to change the difficulty of the task. This approach also greatly reduced the likelihood of animals locating the platform by random chance. By gradually increasing the difficulty of the task over the four spatial training days, the vehicle group took longer time to locate the hidden platform. In contrast, 1 mg/kg rasagiline significantly enhanced spatial reference memory, as shown by the shorter escape latency required to find the hidden platform, particularly on SC day 4 when the maximum task difficulty was programmed. Earlier studies using rasagiline or another selective MAO-B inhibitor, selegiline, failed to improve the performance of healthy rats in water maze tasks (Barbelivien et al., 2001; Speiser et al., 2007). One possible reason is that the spatial learning and memory capability of healthy rats is more than sufficient to handle the difficulty of the normal water maze task (the only limitation is their physical swim speed). Treatment with any cognitive enhancer would not further improve the performance of a rat unless the task difficulty can be adjusted; one example is the use of the on-demand platform in this study.

Monoamine neurotransmitters are believed to play an important role in learning and memory. About 93% of PD patients were reported to suffer from various degrees of cognitive deficits (Dubois and Pillon, 1997). The administration of L-3,4-dihydroxyphenylalanine has been shown to improve the working memory and cognitive flexibility in PD patients (Savitz et al., 2006). In primates, dopamine agonists were shown to reverse the working memory deficits (Goldman-Rakic, 1995). In addition, an increase in dopaminergic activity in the dorsolateral prefrontal cortex of primates has been observed during the spatial working memory (Kodomo et al., 1997), suggesting a role for monoamines, such as dopamine, to modulate learning and memory. Rasagiline may enhance cognition in healthy rats by modulating circuitries that involve various monoamine neurotransmitters. It should be noted that, however, the enhancement of monoaminergic transmission is not always associated with a positive cognitive effect. In fact, in certain cases, enhancement of monoaminergic transmission was accompanied by a deficit in cognitive tasks. Kimberg et al. (1997) and Mattay et al. (2000) have showed that drugs that potentiate dopaminergic activity improved cognition in individuals with low-baseline levels of dopamine, but could also induce a negative effect once a certain threshold was reached. This could be mediated by the differential effects of D1 and D2 receptors activation (Savitz et al., 2006). A fine balance between the activation of these receptors is needed for achieving the cognitive-enhancing effect of drugs.

To date, the effect of rasagiline on cognition in humans has not been investigated. However, the effect of selegiline on learning and memory has been studied in a small number of patients with Alzheimer's disease. Selegiline, given at 10 mg/kg daily, significantly enhanced patients' performance on an episodic memory and learning task requiring complex information processing and sustained attention (Tariot *et al.*, 1987). In another study, patients with Alzheimer's disease treated with selegiline showed small but significant improvement on a number of cognitive tests (Schneider *et al.*, 1991). Nevertheless, clinical trials involving a larger number of patients and a longer treatment duration are required to confirm the cognitive enhancing action of MAO-B inhibitors in humans.

In summary, we have shown that acute rasagiline treatment enhanced object recognition and spatial learning in healthy rats. The doses of rasagiline used in our studies did not significantly affect locomotor activity, and were similar to the effective doses reported in studies using other neurodegenerative models (Eliash *et al.*, 2005; Speiser *et al.*, 2007). On the basis of current data, MAO-B inhibitors would seem to be a promising symptomatic treatment for cognitive impairments affecting patients with neurodegenerative disorders.

Acknowledgements

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