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Long-Term Treatment of Male F344 Rats with Deprenyl: Assessment of Effects on Longevity, Behavior, and Brain Function

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BICKFORD, P. C., C. E. ADAMS, S. J. BOYSON, P. CURELLA, G. A. GERHARDT, C. HERON, G. O. IVY, A. M. L. Y. LIN, M. P. MURPHY, K. POTH, D. R. WALLACE, D. A. YOUNG, N. R. ZAHNISER AND G. M. ROSE. *Long-term treatment of male F344 rats with deprenyl: Assessment of effects on longevity, behavior, and brain function.* NEUROBIOL AGING **18**(3) 309–318, 1997.—L-Deprenyl (selegiline) was chronically administered to male Fischer 344 rats via their drinking water beginning at 54 weeks of age (estimated daily dose: 0.5 mg/kg/day). Beginning at 84 weeks of age, the rats were behaviorally evaluated using a sensorimotor battery, a motor-learning task, and the Morris water maze. At 118 weeks of age, cerebellar noradrenergic function was evaluated in the surviving rats using in vivo electrochemistry. The rats were then sacrificed to measure brain monoamine oxidase activity and perform quantitative autoradiography to evaluate the effect of chronic deprenyl treatment on β-adrenergic receptors in the cerebellum, α_2-adrenergic receptors several brain regions, and D_1 and D_2 dopamine receptors in the striatum. Deprenyl treatment reduced brain monoamine oxidase B activity by 85%, but had no effect on brain monoamine oxidase A. A clear effect of chronic deprenyl treatment upon longevity was not observed. Several measures of CNS function were altered in the deprenyl-treated animals: 1) spatial learning in the Morris water maze was improved; 2) electrochemical signals recorded following local application of NE were reduced, and the responsiveness to the reuptake blocker nomifensine was enhanced, in the cerebellum; 3) β-adrenergic receptor binding affinity was increased in the cerebellum; 4) α_2-adrenergic receptor density was increased in the inferior colliculus; and 5) striatal D_1 dopamine receptor density was reduced but binding affinity was enhanced. In contrast, chronic deprenyl treatment did not cause changes in: 1) sensorimotor function, as evaluated by balance beam, inclined screen, or wire hang tasks; 2) motor learning; 3) α_2-adrenergic receptor density in any region examined except for the inferior colliculus, or binding affinity in any region examined; or 4) striatal D_2 dopamine receptor number or affinity. Thus, long-term oral administration of deprenyl extended the functional life span of rats with respect to cognitive, but not motor, performance. © 1997 Elsevier Science Inc.

Selegiline Aging Longevity Hippocampus Water maze Cerebellum Motor learning Dopamine receptors Noradrenergic receptors Electrochemistry

EXTENDING functional life span is an important goal of gerontological research. To date, progress in this area has been made primarily through the development of treatments for age-related diseases. An alternative approach is to investigate manipulations for prolonging the life of healthy individuals. While this problem has received considerable experimental attention, very few positive results have emerged [e.g., (32, 34)]. At present, essentially life-long caloric restriction is the only intervention that is generally recognized to enhance longevity in mammals (1, 33, 53).

L-Deprenyl (selegiline) is a selective and irreversible inhibitor of monoamine oxidase B (MAO-B) that has been used as an adjunct to the pharmacotherapy of Parkinson's disease. A decade ago it was reported that patients receiving deprenyl and *l*-dopa lived longer than patients receiving *l*-dopa alone (10). Subsequent studies in experimental animals have suggested that chronic treatment with deprenyl alone can enhance longevity [see Table 5; (24,25,29,30,35,47,55); but see (5,21,43)]. In previous studies in rats, deprenyl has routinely been administered by subcutaneous

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injection, several times per week, starting late in the expected life span of the animal. The purpose of this study was to evaluate the effect of chronic deprenyl treatment, administered orally, to male Fischer 344 rats starting at middle age.

Treatments that enhance longevity need to have beneficial effects above and beyond life extension. Ideally, these agents should also delay age-related declines in behavioral function. Thus, in the present study the effect of long-term deprenyl treatment on sensorimotor capacity and on two different types of learning tasks were studied prior to evaluating several measures of central nervous system function. Because of the known interaction of deprenyl with catecholamine systems, its effect upon noradrenergically mediated cerebellar motor learning (6,7) was made a particular focus of this work. In addition to a behavioral assessment of deprenyl's ability to affect the age-related decline in motor learning, in vivo electrochemistry and β-adrenergic receptor binding were used to evaluate noradrenergic function in the cerebellum.

METHOD

Animals and Treatment

Forty male Fischer 344 (F344) rats, 52 weeks (12 months) of age, were purchased from the NIA contract colonies maintained by Harlan Laboratories. The animals were housed in pairs in polyacrylate cages, which were placed in a laminar flow hood. A normal light cycle (12 h on/12 h off) was maintained, and food (Purina Lab Rodent Diet 5001) and tap water were available ad lib. Cage bedding (Sani-Chips, Murphy Forest Products, Montville, NJ) was renewed every 3 days.

After a 2-week acclimation period, the animals were divided randomly into two groups. Twenty rats had deprenyl (a gift from Somerset Pharmaceuticals, Tampa, FL) added to their drinking water (8 μg/ml), while the other 20 rats received no drug treatment. The concentration of deprenyl was calculated to provide an estimated dose of 0.5 mg/kg/day. Deprenyl solutions were made in tap water every 3 days and were stored in the dark at 4°C. Behavioral testing (described below) took place between 1000 and 1600 h.

Behavioral Testing

Sensorimotor Evaluation. At 84 weeks of age the rats were tested using a battery of tasks to evaluate sensorimotor skills [adapted from Wallace et al. (51)]. These tasks included walking on a balance beam, climbing an inclined screen, and hanging by the forepaws from a horizontally suspended wire. The balance beam apparatus was a 60 cm long, longitudinally grooved, wooden dowel rod (both 2.5 and 5 cm diameters were used) that was suspended between two safety platforms located 60 cm above a padded surface. The rat was placed in the center of the rod and the latency to fall was recorded. A rat that either remained on the rod for 2 min or reached a safety platform was assigned a score of 120 s. Each rat was given three trials (10-s intertrial interval); the best score of the three trials was used for subsequent analysis. Another task utilized a wire screen that was inclined at an angle of 60°. The rat was placed on the screen facing downward and allowed to stay for a maximum of 15 min. A single trial was given, and the latency to slip off the screen was recorded. In the final test, the rats were suspended by their forepaws from a 12-gauge copper wire (diameter: 2.03 mm), which was strung between two poles at a height of 60 cm above a padded surface. For a single trial, a rat was allowed to hang on to the wire for up to 120 s; otherwise, latency to fall was recorded. The data were analyzed using the Mann–Whitney *U*-test.

Motor Learning. Motor learning was assessed on 86-week-old rats using methods described previously (8). The apparatus for this task consisted of a straight runway (length = 127 cm, height = 25 cm, and width = 6 cm), at either end of which was a goal box (25 × 25 cm) in which a water spout was located. The floor of the runway consisted of an arrangement of 38 horizontally oriented aluminum rods. The rods (diameter = 4 mm, 2 to 4 cm long from the inner wall, and a minimum interrod distance of 2.5 cm) could be arranged in either a regular (REG) or irregular (IRR) configuration (see Fig. 3.) The goal of the rat was to traverse the runway to receive a water reward (0.3 ml) in the goal box. Water delivery was accompanied by a tone that served as a conditioned reinforcer. A number of photobeam detectors were positioned along the runway to allow the measurement of running time.

The rats were deprived of water for 12 h before training was begun. Initially, the rats were acclimated to the runway by covering the rods with a piece of Plexiglas. Shaping continued for 1 week, or until the animals consistently ran back and forth in the runway to drink at the goal boxes. The rats were given 3 min ad lib water access after each daily session, but were maintained on water restriction such that they averaged 90% (never less than 85%) of their original body weight during the entire 5-week training period. After shaping, training proceeded by gradually removing sections of the Plexiglas that covered the rods. Data collection began when the rat had performed two successful traverses of the entire length of the uncovered rods in less than 1 min. Average daily performance was calculated by determining the running time for 20 successive trials. After 5 days of training with the rods in the REG pattern, the rats were rested for a 2-week period. The animals were then trained in the IRR pattern until their performance was asymptotic. Comparisons between groups were made by simultaneous modeling of the data as has been previously described (8).

Spatial Learning. At 104 weeks of age, place learning in the Morris water maze was tested according to the protocol described in detail in Engstrom et al. (18). Briefly, the rats were required to use information provided by extra-apparatus cues to learn the location of a hidden escape platform located in a circular tank, 1.5 meters in diameter and 0.3 meters high, which was filled with water made opaque by adding 250 ml of white Createx, a nontoxic latex paint. Water temperature was maintained at 24–25°C. The tank was located in a room containing numerous sensory cues (e.g., a poster on one wall, a stand containing the cages of rats, an incandescent light) that were maintained in constant locations during the period of behavioral testing.

Prior to the first training trial, each rat was placed on the platform and allowed to remain there for 30 s. Then, with the platform in a consistent location, all animals were given four trials/day with an intertrial interval of approximately 10 min. A trial consisted of being placed into the water in each one of the four starting quadrants, as described above. The rat was required to swim for 60 s or until it located the platform and climbed onto it. If the platform was not located within 60 s, the rat was hand guided to it. In either case, the animal was allowed to remain on the platform for 15 s before being removed and returned to its home cage.

Training consisted of 20 trials given over 5 days. The behavioral measure recorded was the time to locate the platform (maximum 60 s). The data were analyzed by MANOVA, using trials as the repeated measure, with Tukey–Kramer post hoc comparisons.

In Vivo Electrochemistry

At 118 weeks of age, the surviving rats (four control, six deprenyl-treated) were anesthetized with urethane (1.5 g/kg) and

the clearance of norepinephrine (NE) from the cerebellar cortex was examined using in vivo electrochemistry as described in detail by Cass et al. (16). The electrochemical working electrodes were the multiple carbon fiber type, made with three carbon fibers (30 μm diameter) sealed by expoxylite in a glass capillary. The exposed surface area was a cylinder approximately 90 microns in diameter and 100–150 microns in length. The electrodes were coated with Nafion, which enhances their selectivity and sensitivity to monoamine neurotransmitters (19).

High-speed (5 Hz) chronoamperometric measurements were made using a microcomputer-controlled electrochemical instrument (IVEC-5, Medical Systems Corp., Greenvale, NY). A potential of +0.55 volt (vs. a Ag/AgCl reference located in the posterior neocortex) was applied to the working electrode for 100 ms. The resulting oxidation current was digitally integrated during the last 70 ms of the pulse. When the electrode was returned to resting potential (0.0 V), the reduction current produced by the oxidized electroactive species was integrated in the same manner. Before acquiring data in the brain, each recording electrode was calibrated to determine its sensitivity to NE and selectivity for NE against ascorbic acid as previously described (9).

The electrochemical electrode was fixed to a double-barrel micropipette (1.0 mm o.d.), with a tip separation of 280–320 μm, using sticky wax. One barrel of the micropipette was filled with 50 μM NE (Sigma Chemical Co., St. Louis, MO) and the other with 300 μM *d,l*-nomifensine maleate (Research Biochemicals International, Natick, MA). Both drugs were prepared in physiological saline and adjusted to pH 7.2–7.4. The electrode assembly was lowered into the cerebellum at the following coordinates: 13–14 mm posterior to bregma; 0.5 mm lateral to the midline. Drugs were ejected from pipettes in situ using a pneumatic pump (BH-2, Medical System Corp., Greenvale, NY). The pressure pulse for ejecting drugs were 2.5–40 psi for a duration of 5–10 s. The volume of drug applied was determined by measuring the volume of fluid ejected by the pressure pulse. A dissecting scope fitted with a reticule was used to observe the meniscus within a pipette barrel; volumes of drugs ejected were calculated based on the determination that 250 nl of solution was contained in a 1 mm segment of the pipette.

The characteristics of electrochemical signals produced by application of known volumes of NE were compared in control and deprenyl-treated rats. In addition, the effect of nomifensine on the clearance of exogenously applied NE was determined. Peak signal amplitudes represented the concentration of NE detected. The half-decay time of the signal from the peak value was used as an indicator of the dynamics of neurotransmitter uptake processes. These data were analyzed either by ANOVA or paired Student's *t*-test, as appropriate.

Measurement of Brain Monoamine Oxidase Activity

At the conclusion of the in vivo electrochemical experiments, the rats were overdosed with anesthetic. Their brains were rapidly removed and bisected along the midline. Samples of striatum, hippocampus, and cerebellum were dissected from one hemisphere for determination of MAO activity (40). The other hemisphere was further divided via coronal cuts at the level of the medial septum and the cerebellum for receptor autoradiography (described below). All brain pieces were frozen in powdered dry ice and stored at −70°C.

Quantitative Receptor Autoradiography

Tissue sections for receptor autoradiography were cut in the coronal (adrenergic receptors) or horizontal (dopamine receptors) plane at a thickness of 10 μm with a cryostat at −15°C, thaw-mounted onto gelatin-coated slides, and stored at −70°C.

Adrenergic Receptors. Beta-adrenergic receptors were examined in the cerebellum. For this, saturation curves were constructed using nine concentrations of the antagonist ^{125}I-iodopindolol [^{125}I-IPIN; (36)] ranging from 10 to 1000 pM. For competition curves, 100 pM ^{125}I-IPIN and 14 concentrations of the selective β_2-adrenergic receptor antagonist ICI 118,551 (a gift from Imperial Chemical Industries PLC, Cheshire, UK) ranging from 32 pM to 56 nM were used. Nonspecific binding was defined in the presence of 1 μM l-propranolol (Research Biochemicals International). Duplicate slide-mounted tissue sections were incubated in appropriate concentrations of ^{125}I-IPIN and drugs in buffer containing 20 mM Hepes and 154 mM NaCl (pH 7.5) for 30 min at 37°C. Following incubation, sections were washed in ice-cold buffer containing 10 mM Tris and 154 mM NaCl (pH 7.5) for 10 min, dipped in ice-cold deionized water and dried at 50°C. The slides were apposed to film along with ^{14}C-labeled standards (American Radiolabeled Chemicals, Inc., St. Louis, MO) for 7–11 days at room temperature.

α_2-Adrenergic receptors were measured in several brain regions using both the partial agonist p-^{125}I-iodoclonidine (^{125}I-PIC; DuPont/NEN, Boston, MA) and the antagonist ^{3}H-RX821002 (Amersham, Arlington Heights, IL). Saturation curves were constructed using seven to nine concentrations of radioligand ranging from 50 to 4500 pM. Nonspecific binding was defined in the presence of 10 μM phentolamine (gift from CIBA-Geigy, Summit, NJ). Incubations were carried out for 90 min at 21°C in buffer containing 50 mM Tris, 2 mM $MgCl_2$, and 0.6 mM EDTA (pH 7.4) for ^{125}I-PIC assays and in 25 mM glycylglycine buffer (pH 7.6) for ^{3}H-RX821002 assays. Washes were performed as above except that the appropriate assay buffers were used. The ^{125}I-PIC–labeled slides were apposed to film along with ^{14}C-labeled standards for 1–7 days at room temperature, whereas the ^{3}H-RX821002–labeled slides were apposed to film along with ^{3}H-labeled standards (Amersham) for 28–42 days.

The autoradiograms were analyzed using a computer-based image analysis system (Spatial Data Systems, Melbourne, FL). Saturation and competition curves were obtained using either GraphPad or LIGAND. The data were analyzed using either Student's *t*-test or repeated measures ANOVA with Tukey–Kramer post hoc comparisons, as appropriate.

Dopamine Receptors. D_1 and D_2 dopamine receptors were examined in the neostriatum. Slides for each animal were randomized to counteract the influence of any potential dorsoventral dopamine receptor gradient within the neostriatal tissue. Saturation curves for D_1 dopamine receptors were constructed using nine concentrations of the antagonist ^{3}H-SCH-23390 (Amersham) ranging from 0.1–10.4 nM. Nonspecific binding was defined with 2 μM (+)-butaclamol (RBI). Sections were incubated with radioligand in buffer containing 50 mM Tris, 154 nM NaCl, 10 mM $MgSO_4$, 2 mM EDTA, 10 mg/l bovine serum albumin and 300 nM ketanserin (a gift from Janssen Research Foundation, Beerse, Belgium) (pH 7.4) for 100 min at 37°C and then washed in this buffer (ice-cold) for 20 min, dipped in ice-cold water, and air dried [adapted from Boyson et al., 1986 (11)]. Saturation curves for D_2 dopamine receptors were constructed using nine concentrations of the antagonist ^{3}H-spiperone (Amersham) ranging from 0.06–6.03 nM. Assays were similar to those for the D_1 receptor with the exceptions that the assay buffer contained 20 mM Hepes, 154 mM NaCl, 5 mM EDTA, 10 mg/l bovine serum albumin, and 300 nM ketanserin (pH 7.5), and the wash was in this buffer for 80 min. Slides were apposed to film along with ^{3}H-labeled standards (ARC; Amersham) for 1 (D_1 receptors) or 2 (D_2 receptors) weeks. The autoradiograms were analyzed using the DUMAS Image-

Processing System (Drexel University, Philadelphia, PA), and the analysis was restricted to the rostral portion of the neostriatum to avoid previously reported rostrocaudal gradients in dopamine receptor levels (11). ANOVA was utilized for statistical analysis.

RESULTS

Effect of Deprenyl on Longevity and MAO Activity

Treatment of the rats with deprenyl (approximately 0.5 mg/kg/day, PO) began when the animals were 54 weeks of age. From this time point, the weights of the animals were regularly recorded during the remainder of the study. While food intake was not monitored, at no time was a significant difference in body weight between the deprenyl-treated and control rats observed. The survival curves for the two groups are shown in the upper portion of Fig. 1. A calculation based upon the longevity of the 16 control and 14 deprenyl-treated animals that died of natural causes prior to 118 weeks of age indicated that mean life span was significantly longer for the deprenyl-treated rats than for the controls (control: 103.5 ± 3.1 weeks; deprenyl: 111.0 ± 1.5 weeks; $p < 0.05$, two-tailed Student's *t*-test). However, an analysis that also considered the rats that were still living (48) indicated that there was no significant difference in longevity between the groups ($p = 0.21$). Closer examination of the survival data (Fig. 1, bottom) showed that deprenyl-treated rats exhibited higher survival rates at all time points after 62 weeks of age. The difference between the control and deprenyl-treated rats was maximum during the interval between approximately 100 and 108 weeks of age. Thereafter, the survival advantage of the deprenyl-treated rats rapidly declined, approaching no difference by 118 weeks of age.

The effect of deprenyl treatment upon the activities of monoamine oxidase (MAO) A and B was determined in samples taken from the striatum, hippocampus, and cerebellum of the four control and six experimental rats that survived to the age of 118 weeks (Fig. 2). There was no significant effect of deprenyl treatment on the activity of MAO-A in any brain region examined. In contrast, 85 to 88% inhibition of MAO-B activity was observed, depending upon the brain region sampled. Thus, administration of deprenyl via the drinking water was an effective method of drug delivery, and, at the dose employed, caused a selective inhibition of MAO-B activity.

Effect of Deprenyl on Behavioral Measures

Sensorimotor Tests. At 84 weeks of age (30 weeks after the start of deprenyl treatment), all surviving (18 control, 20 deprenyl-treated) rats were evaluated on a battery of sensorimotor performance tests, including a 2.5 and 5 cm round balance beam traverse, an inclined screen climb, and a wire hang test. The results of this work are summarized in Table 1. Overall, the performance of the two groups was not different on this battery of tests.

Motor Learning. At 86 weeks of age, 10 rats from each group were trained on a motor task that consisted of learning to cross a narrow runway by stepping on a series of aluminum pegs that protruded from the walls of the runway. This time point was chosen for evaluation because previous work has shown that age-related learning deficits in this task are apparent at this age (8), and based upon our experience that this task is beyond the ability of much older rats (greater than 100 weeks of age; unpublished observations). The rats were initially shaped to the task with the pegs placed in a regular pattern (Fig. 3, top). After all the animals had mastered this portion of the task, they were presented with a new runway in which the pegs were spaced in an irregular pattern (Fig. 3, top). The change in the amount of time necessary to negotiate the new runway with practice was taken as the measure of learning.

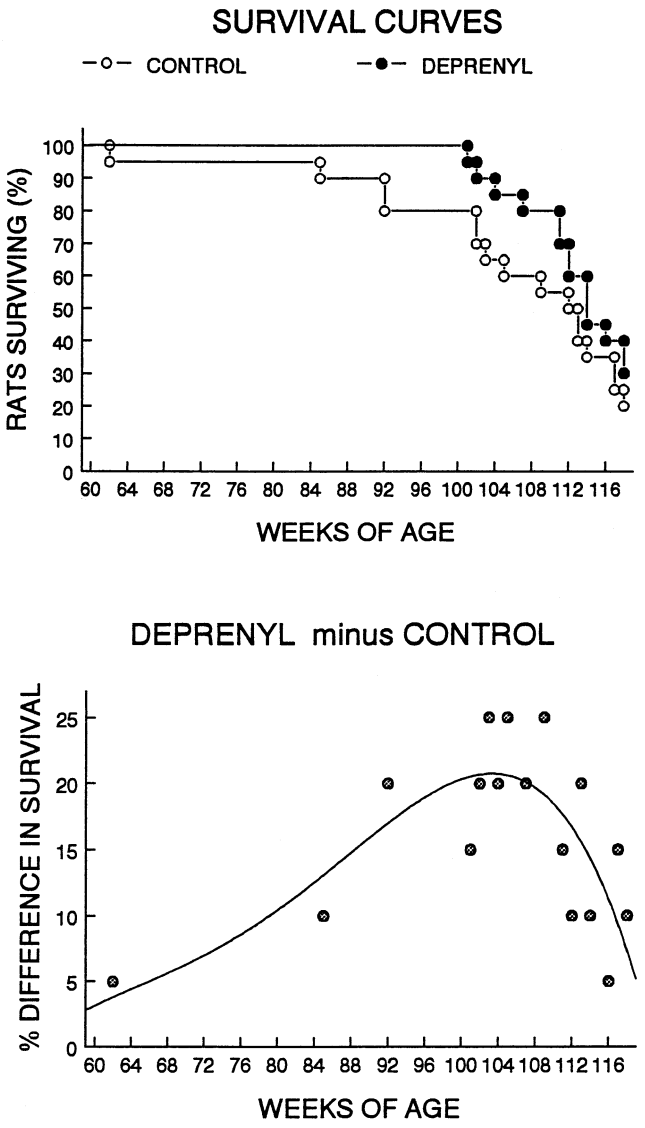

FIG. 1. Top: survival curves for male F344 rats that were either untreated or given deprenyl in the drinking water beginning at 54 weeks of age. Bottom: difference in percent survival between the populations of deprenyl-treated and control rats. The deprenyl-treated group had greater survival at all time points after 62 weeks of age. However, the difference between groups was maximal during the period between 100–108 weeks of age. Accelerated mortality in the deprenyl group resulted in a marked reduction in the group difference after 110 weeks of age.

The group of aged rats treated with deprenyl had slower running times than either the aged control group or young rats studied previously [day 1 runtimes: young—3.4 ± 0.3 s, $n = 9$ [from (8)]; aged control—4.3 ± 0.6 s, $n = 10$; aged deprenyl—6.1 ± 0.6 s, $n = 10$]. Thus, the data were normalized with respect to day 1 performance to determine the amount of learning in each group. Previous work has shown that young rats learn the new pattern of peg placements by the third day of training (8); in contrast, learning in the aged groups in the present experiment continued until training day 6 ($p < 0.05$ for both aged groups vs. young group). However, the normalized learning curves for the aged control and deprenyl-treated rats did not differ from each other (Fig. 3, bottom). In addition, it was found that the final

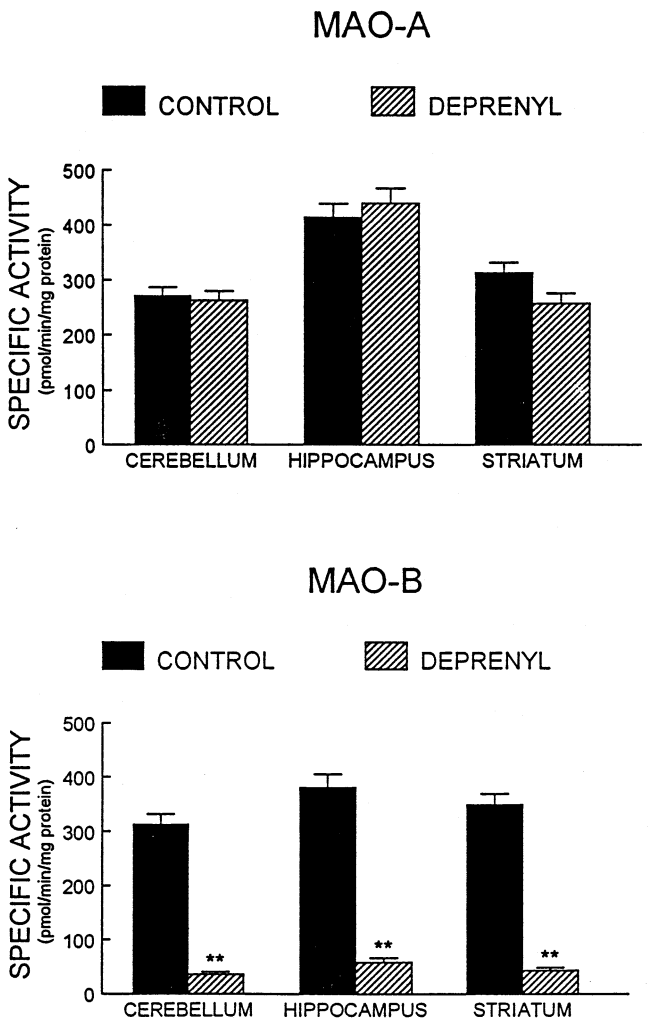

FIG. 2. Effect of chronic deprenyl on brain monoamine oxidase (MAO) activity. MAO-A (top) and MAO-B (bottom) were assayed in three brain regions after 64 weeks of deprenyl treatment. MAO-A activity was not affected; in contrast, MAO-B activity was significantly reduced. $^{**}p < 0.01$.

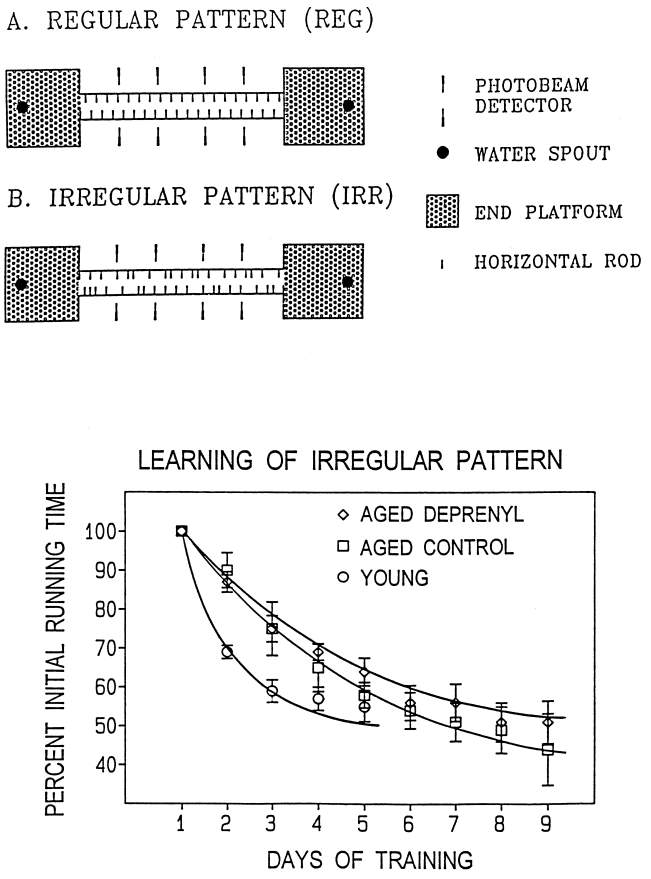

FIG. 3. Assessment of motor learning. Top: diagrams of the experimental apparatus. Rats are first trained to traverse a straight alley by stepping on a regularly patterned series of horizontal rods in order to receive water reward (A). After the animals are proficient in this task, the pattern of horizontal rod placements is changed to an irregular pattern (B), and the period required to regain maximum running speed is measured. Bottom: running times in the irregular pattern task, normalized to performance on the first training day. Aged (86-week-old) rats took longer to learn the task than did a young (12-week-old) control group, although the final level of performance was the same for all groups. There were no learning differences between the aged control and aged deprenyl-treated groups.

percent improvement in running time was equivalent for young and both groups of aged rats.

Spatial Learning. At 104 weeks of age, seven deprenyl-treated and six control rats were trained in the hidden platform version of the Morris water maze. Six 12-week-old rats were also trained. As is shown in Fig. 4, there were significant differences in learning between groups [group × day interaction: $F(8, 80) = 2.34$, $p = 0.026$]. Consistent with previous work (18), the aged control rats did not show any significant improvement over the 5-day training interval. By contrast, a significant improvement in swim time was seen in the aged deprenyl-treated rats, although learning in this group was not equivalent to the young animals. Thus, chronic deprenyl treatment lessened the reduction in spatial learning that is normally seen with aging.

TABLE 1

SENSORIMOTOR EVALUATION OF 84-WEEK-OLD RATS

Task	Group	Time to Fall (s)
Balance beam (2.5 cm)	Control	25.3 ± 4.6
	Deprenyl	21.9 ± 4.6
Balance beam (5.0 cm)	Control	22.6 ± 5.8
	Deprenyl	9.8 ± 2.2
Inclined screen	Control	44.2 ± 12.9
	Deprenyl	36.0 ± 7.2
Wire hang	Control	6.8 ± 1.2
	Deprenyl	9.1 ± 1.0

Data are presented as the mean ± SEM; $n = 18$ for control, 20 for Deprenyl. No differences were found between groups (Mann–Whitney *U*-tests).

Effect of Deprenyl on CNS Measures

In Vivo Electrochemistry. The rats that remained alive at 118 weeks (four control and six deprenyl-treated) were anesthetized with urethane and the clearance of NE from the extracellular space of the cerebellum was studied using in vivo electrochemistry. In addition, a group of eight 10–12-week-old rats was examined. To study clearance and uptake of NE, a fixed volume of 25 nl (3.75 pM) of NE was applied using pressure ejection and the signal

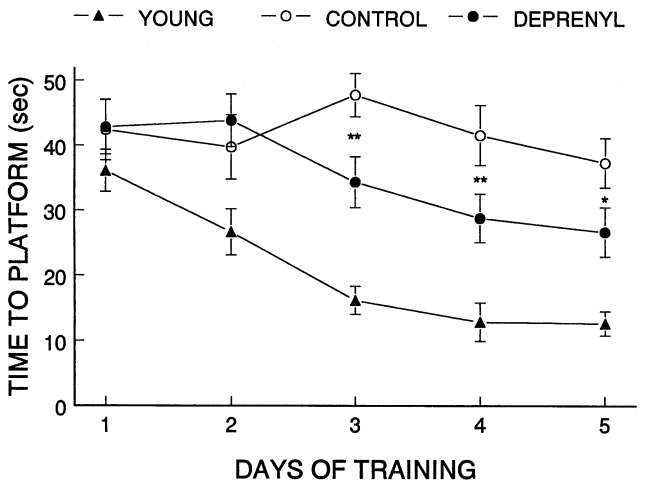

FIG. 4. Spatial learning in the Morris water maze. YOUNG (12-week-old) rats given four daily trials to find a hidden platform showed the greatest improvement in swim times over 5 days of training. No improvement over days was seen in aged (104-week-old) CONTROL rats. In contrast, learning was seen in aged DEPRENYL-treated rats. Asterisks indicate points that differ significantly between CONTROL and DEPRENYL groups. $^{**}p < 0.01$; $^{*}p < 0.05$. YOUNG rats were significantly better than both aged groups at all points except the first training day.

detected at the electrochemical electrode was recorded. The uptake blocker nomifensine was then applied prior to a second ejection of the same volume of NE.

The results of these experiments are presented in Fig. 5 and Table 2. The amplitude of signals following NE application was not different between young and aged control rats. However, the signals recorded in the deprenyl-treated group were significantly smaller in amplitude ($p < 0.05$). The time courses of the signals seen after NE application were significantly longer for both groups of aged rats compared to the young animals ($p < 0.05$), but were not different from each other. When nomifensine was applied prior to NE, the electrochemical signal in young rats was both larger in amplitude and had an extended time course as determined by the half-decay time ($p < 0.05$ for both measures; Fig. 5, top). By contrast, nomifensine application did not result in either a change in amplitude or time course in the aged control rats. In the deprenyl-treated rats, nomifensine also had no effect on signal amplitudes, but significantly prolonged the time course of the recorded signal in a manner similar to that observed in the young rats ($p < 0.05$; Fig. 5, bottom).

Adrenergic Receptors. Age-related impairments in motor learning have been associated with alterations in β-adrenergic receptors in the cerebellum (6). Thus, it was of interest to examine the effect of chronic deprenyl treatment upon β-adrenergic receptors in this brain region. The affinities and densities were determined from the nonselective antagonist ^{125}I-IPIN saturation curves, which were best fit to a single site. Deprenyl treatment resulted in a significant increase in the ^{125}I-IPIN binding affinity from 23 to 16 pM ($-\log K_d$ (M): Control 10.63 ± 0.02, $n = 4$; deprenyl 10.79 ± 0.04, $n = 4$; $p < 0.02$). However, receptor densities were unchanged (B_{max} (fmol/mg protein): control—10.9 ± 0.5, deprenyl—9.5 ± 0.6; $p = 0.08$). Competition curves with the selective β_2-adrenergic receptor antagonist ICI 118,551 were also best fit by a single site. This site had similar high affinities in both the control (970 pM) and deprenyl-treated (940 pM) groups ($-\log$ Ki value (M): control—9.01 ± 0.03, deprenyl—9.03 ± 0.04; $p = 0.81$). These data suggest that the β_2-receptor subtype accounts for greater than 90% of the β_2-adrenergic receptors in the cerebellum of aged F344 rats.

The effect of chronic deprenyl treatment upon β_2-adrenergic

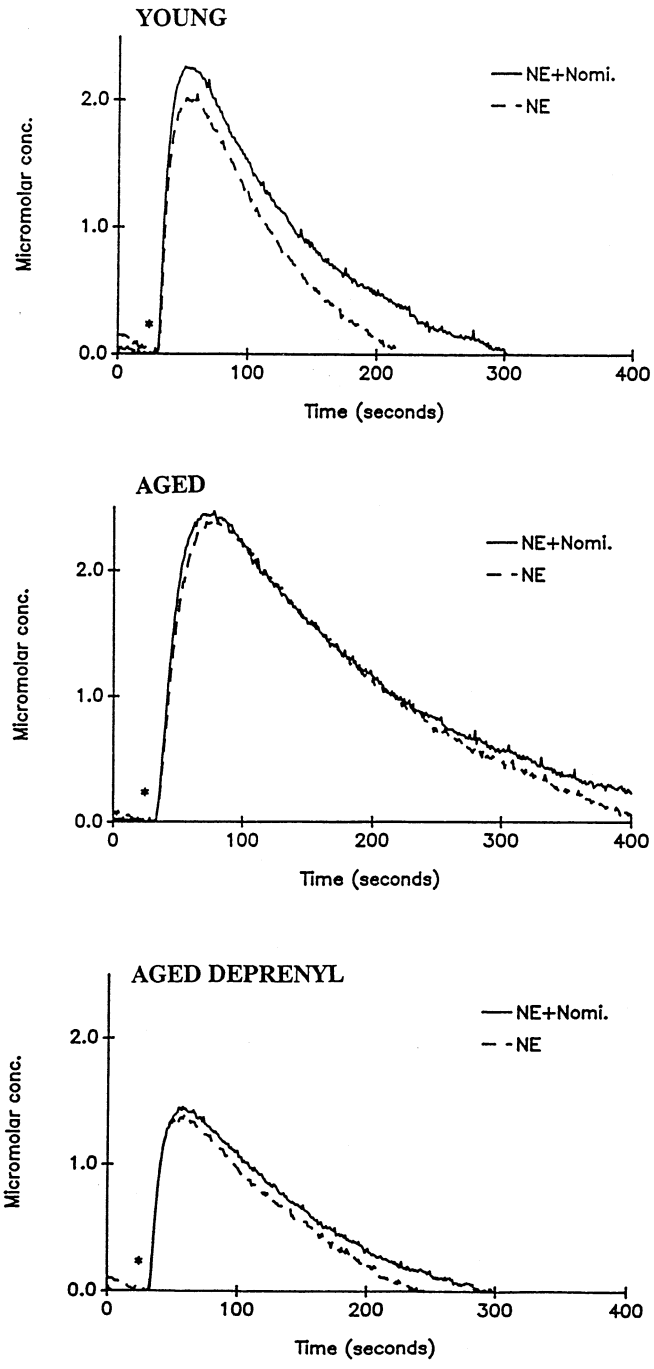

FIG. 5. Examples of electrochemical signals recorded in the cerebellum under urethane anesthesia. Top: in young (12-week-old) rats, application of nomifensine (Nomi.) augments both the amplitude and the time course of signals recorded following local application of norepinephrine (NE). Center: in aged (118-week-old) control rats, NE signals are not altered in the presence of nomifensine. Bottom: in aged deprenyl-treated rats, NE signals in the presence of nomifensine are not larger in amplitude, but have longer durations.

TABLE 2

CEREBELLAR ELECTROCHEMICAL SIGNALS RECORDED FOLLOWING LOCAL APPLICATION OF NOREPINEPHRINE: EFFECT OF REUPTAKE BLOCKADE BY NOMIFENSINE

Group	*n*	Signal Amplitude (μM)		Signal Half-Decay Time (s)	
		NE Alone	NE + Nomifensine	NE Alone	NE + Nomifensine
Young	8	1.80 ± 0.20	2.04 ± 0.20†	105 ± 4	124 ± 5§
Aged control	4	2.20 ± 0.30	2.20 ± 0.20	131 ± 7‡	137 ± 7
Aged Deprenyl	6	1.40 ± 0.20*	1.44 ± 0.18	142 ± 9‡	159 ± 9§

n = Number of rats per group. Data are presented as mean ± SEM for all measurements. The total number of measurements was: Young—*n* = 79; aged control—*n* = 46; aged Deprenyl—*n* = 31.

* Significantly different from other two groups ($p < 0.05$; ANOVA).

† Significantly different from NE alone ($p < 0.05$; paired *t*-test).

‡ Significantly different from the young group ($p < 0.05$; ANOVA).

§ Significantly different from NE alone ($p < 0.05$; paired *t*-test).

receptors was examined in the occipital/parietal cortex, hippocampus, piriform/entorhinal cortex, hypothalamus, inferior colliculus, and locus coeruleus using the agonist ^{125}I-PIC and the antagonist ^{3}H-RX821002 (Table 3). Binding of an agonist, as well as an antagonist, was measured because others have reported that 3 weeks of deprenyl treatment altered agonist, but not antagonist, interactions with hippocampal α_1-adrenergic receptors (23). In the present experiment, agonist binding was not different between the groups in any brain region examined. Similarly, no differences in antagonist binding affinity were observed between the groups in any brain region. However, chronic deprenyl treatment produced a significant 51% increase in α_2-adrenergic receptor density in inferior colliculus.

Dopamine Receptors. The effect of chronic deprenyl treatment upon D_1 and D_2 dopamine receptors in the dorsal striatum was examined using the antagonists ^{3}H-SCH-23390 and ^{3}H-spiperone, respectively (Table 4). Deprenyl treatment resulted in a significant increase in ^{3}H-SCH-23390 binding affinity from 708 to 447 pM, compared to the untreated control animals, as well as a 23% reduction in the density of D_1 receptors. No significant differences between groups were observed for D_2 receptor affinity or density.

DISCUSSION

Chronic oral self-administration of deprenyl at a dose of approximately 0.5 mg/kg/day caused essentially complete inhibition of brain MAO-B activity, but did not affect the activity of MAO-A. The effect of deprenyl on the lifespan of treated rats could not be unequivocally determined, because all the animals were not allowed to survive until natural death. When the surviving animals were excluded, statistical analysis suggested that the mean lifespan of deprenyl-treated rats was significantly greater than that of controls. However, a survival analysis that considered the animals still living indicated no difference between groups. This conflict arises because the largest effect of deprenyl was observed during the interval between 100–108 weeks of age, after which it rapidly declined. Thus, the population of deprenyl-treated rats lived longer to this point, but then began to die faster than the controls. However, similar findings have been observed in other studies examining the effect of chronic deprenyl treatment on survival (24,43).

A summary of previous experimental studies of deprenyl treatment on longevity is presented in Table 5. It is noteworthy that the effect of chronic deprenyl treatment in F344 rats, an inbred strain, appears to be much less pronounced than in other rat strains studied (Table 5). In addition, from the currently available data it appears that although chronic deprenyl treatment prolongs the life of rats, and perhaps hamsters, positive results are rarely reported for mice. While these findings may indicate species differences in the response to deprenyl, it is also possible that these results are another indication of differential effects between the strains of a particular species. We chose to initiate deprenyl treatment relatively early in the lifespan (approximately 12 months of age) of our F344 rats in an effort to enhance the effect on longevity to the level reported in studies using other rat strains. However, the observed increase in longevity was not significantly greater than was

TABLE 3

EFFECT OF CHRONIC DEPRENYL TREATMENT ON A_2-ADRENERGIC RECEPTORS

Region	Group	$-\log K_d$ (M)	B_{max} (fmol/mg protein)
A: Agonist Binding (p-^{125}I-Iodoclonidine)			
Occipital/parietal cortex	Control	9.22 ± 0.09	36.0 ± 5.7
	Deprenyl	9.12 ± 0.08	39.5 ± 4.9
Hippocampus	Control	9.35 ± 0.18	19.9 ± 3.0
	Deprenyl	9.40 ± 0.10	20.9 ± 2.7
Piriform/entorhinal cortex	Control	9.37 ± 0.09	83.8 ± 8.3
	Deprenyl	9.19 ± 0.11	94.5 ± 10.9
Hypothalamus	Control	9.15 ± 0.07	50.5 ± 3.7
	Deprenyl	9.18 ± 0.05	53.1 ± 9.5
Inferior colliculus	Control	9.29 ± 0.19	33.8 ± 8.5
	Deprenyl	9.12 ± 0.12	41.0 ± 5.7
Locus coeruleus	Control	9.05 ± 0.14	54.8 ± 12.1
	Deprenyl	9.10 ± 0.09	57.4 ± 2.4
B. Antagonist Binding (^{3}H-RX821002)			
Occipital/parietal cortex	Control	9.64 ± 0.02	54.6 ± 3.2
	Deprenyl	9.87 ± 0.10	63.8 ± 1.4
Hippocampus	Control	9.65 ± 0.02	36.5 ± 0.7
	Deprenyl	9.85 ± 0.10	40.8 ± 1.6
Piriform/entorhinal cortex	Control	9.61 ± 0.03	136.0 ± 7.3
	Deprenyl	9.76 ± 0.15	151.1 ± 7.8
Hypothalamus	Control	9.58 ± 0.03	104.4 ± 3.5
	Deprenyl	9.72 ± 0.10	112.2 ± 3.5
Inferior colliculus	Control	9.73 ± 0.04	61.1 ± 7.9
	Deprenyl	9.80 ± 0.02	92.0 ± 8.1*
Locus coerulus	Control	9.62 ± 0.04	98.6 ± 11.1
	Deprenyl	9.79 ± 0.02	112.2 ± 11.2

* $p < 0.05$ vs. the control group; $n = 4$ for each group.

TABLE 4

EFFECT OF CHRONIC DEPRENYL TREATMENT ON STRIATAL DOPAMINE RECEPTORS

	Receptor Subtype			
	D_1		D_2	
Group	$-\log K_d$ (M)	B_{max} (fmol/mg protein)	$-\log K_d$ (M)	B_{max} (fmol/mg protein)
Control	9.15 ± 0.07	469 ± 16	9.90 ± 0.08	103 ± 5
Deprenyl	9.35 ± 0.04*	359 ± 27*	9.94 ± 0.04	104 ± 4

* $p < 0.02$ vs. the control group.

recorded in experiments utilizing F344 rats in which treatment was begun much later [18–24 months of age; (24,35)]. This suggests that there is not a cumulative beneficial effect of deprenyl treatment. Finally, our study reinforces previous work indicating the effect of deprenyl is not the result of weight reduction, which would be the consequence of inadvertent caloric restriction (24,35,47,55).

The pattern of changes in behavioral performance with deprenyl treatment was complex. Sensorimotor function and motor learning were not improved. The former result replicates the findings of Ivy et al. (22), but was somewhat surprising in light of the known beneficial effect of deprenyl upon morphological and biochemical components of the extrapyramidal motor system (2,26). The motor-learning task employed in the present study requires intact cerebellar noradrenergic function (52) mediated through β-adrenergic receptors (7). Previous studies found no effect of 2 weeks of deprenyl administration on β-adrenergic receptors in rat cerebral cortex (37,39). However, long-term deprenyl treatment enhanced cerebellar β-adrenergic receptor binding affinity to a level equivalent to that seen in young rats (data not shown) and improved in vivo electrochemical measures of cerebellar noradrenergic function. In addition, other work has shown that chronic deprenyl treatment also reduces age-related morphological alterations in the cerebellum (2). The lack of improvement in the motor-learning task suggests either that the ameliorative effects of deprenyl were insufficient to overcome the negative effects of aging upon the cerebellum and noradrenergic system, or that changes in these systems are not the sole contributors to age-related dysfunction in the task.

In contrast to what was observed for motor learning, spatial learning was improved in deprenyl-treated rats. This result is consistent with previous work demonstrating enhanced performance in cognitive tasks (30,47,55), including the Morris water maze (22), after long-term deprenyl treatment. The effects of

TABLE 5

PREVIOUS EXPERIMENTAL STUDIES OF CHRONIC DEPRENYL TREATMENT ON PROLONGING LIFE SPAN IN RODENTS

Authors	Species (Strain)	Sex	Dose	Route	Age at Start of Treatment	Increase in Life Span (From Birth)	Behavioral Assessment
Knoll, 1988 (25)	Rat (CFY)	M	0.25 mg/kg 3 × per week	SC	24 months	+34.6%	+sexual activity
Knoll et al., 1989 (29)	Rat (Wistar–Logan F1 Hybrid)	M	0.25 mg/kg; 3 × per week	SC	24 months	+30.5%	+sexual activity
Milgram et al., 1990 (35)	Rat (F-344)	M	0.25 mg/kg every other day	SC	23–24 months	+ 2.3%*	n.d.
Kitani et al., 1993 (24)	Rat (F-344)	M	0.5 mg/kg 3 × per week	SC	18 months	+ 5.7%	n.d.
Knoll et al., 1994 (30)	Rat (Wistar–Logan)	M	as above	SC	8 months	+13.3% (low performers) +22.6% (high performers)	+sexual activity +learning of two-way shuttle box
Yen and Knoll, 1992 (55)	Mouse (OFA-1)	M	0.25 mg/kg; 3 × per week	SC	12 months	+23.9%	+passive avoidance retention
Barber et al., 1993 (5)	Mouse (Swiss White)	M	1 mg/kg/day	in water	12 months	None	no effect on locomotion or spatial learning
Ingram et al., 1993 (21)	Mouse (C57BL/6J)	M	0.5 or 1 mg/kg/day	in water	18 months	None	no effect on motor tasks
Piantanelli et al., 1994 (43)	Mouse (Balb/nu)	M	0.25 mg/kg 3 × per week	SC	22 months	None	n.d.
Stoll et al., 1994 (47)	Hamster (Syrian)	M	0.05 mg/kg/day	in food	12 months	+ (exact amount undetermined)	+spontaneous alternation

* Significant by one-tailed *t*-test. n.d. = not determined.

short-term treatment are less clear. Studies in aged rats (54) and mice (47) indicate that 2 weeks of treatment with a systemic dose of 0.25 mg/kg does not improve water-maze learning. However, improvements in aged rats have been observed when much higher oral doses (1.25–5.0 mg/kg, daily) were used (12). The effect of deprenyl in the latter study was of similar magnitude to the present results. The mechanism underlying the improvement is not clear. Hippocampal function is known to be critical for spatial learning (38), although no direct role for catecholamine function has been shown to date. It is possible that the antioxidant or neurotrophic actions of deprenyl (discussed further below) were responsible for the effect.

The biochemical evaluations performed in this experiment were targeted at evaluating the effects of chronic deprenyl treatment upon central catecholamine systems, because these systems would be expected to be most sensitive to alterations in MAO activity. Most of the differences observed were small. This could have been a consequence of making the measurements very late in the life of the rats, when the difference in survival between the control and deprenyl-treated group was not as great as at earlier time points. Changes in cerebellar noradrenergic function were discussed above. A change (increase in density) in α_2-adrenergic receptors was found only in the inferior colliculus. This finding is of interest because this is one of the few regions of rat brain in which a decrease in α_2-adrenergic receptors has been observed to occur during aging (Wallace and Zahniser, unpublished observations). It was unfortunate that the experimental protocol did not allow examination of α_2-adrenergic receptors in the prefrontal cortex, because adrenergic mechanisms in this area have been associated with cognitive decline in primates (4). In contrast with the present findings, short-term (3-week) treatment with deprenyl decreased binding of [^{3}H]idazoxan to α_2-adrenergic receptors in locus coeruleus and most of the regions innervated by the locus coeruleus (31).

Enhanced striatal D_1 dopamine receptor affinity was observed in the deprenyl treated group. However, whether this change was of sufficient magnitude to have functional consequences is not clear. The observed decrease in striatal D_1 dopamine receptor density following deprenyl treatment was likely an indirect effect. Striatal levels of β-phenylethylamine, a substrate of MAO-B, are increased following administration of deprenyl in both rats (42) and in postmortem brain samples taken from patients with Parkinson's disease (44). Beta-phenylethylamine, in turn, has been shown to decrease the density of striatal D_1, but not D_2, receptors (41). Therefore, our finding of a decreased striatal density of D_1 dopamine receptors is most likely due to an increase in β-phenylethylamine secondary to the blockade of MAO-B by deprenyl.

In addition to its well-known effects upon catecholamine metabolism (27), chronic deprenyl administration has been shown to upregulate the activity of the antioxidant enzymes catalase and superoxide dismutase in many, but not all, brain regions (13–15,25). It has also been shown that deprenyl has neurotrophic-like effects upon neurons [e.g., rescue following axotomy (3,45,56) and prevention of apoptosis (50)], and that deprenyl administration enhances expression of neurotrophins or their receptors (17,46). It appears that these effects of deprenyl are independent of its inhibitory action on MAO-B (3,20,28,49).

In summary, continuous oral administration of deprenyl to male Fischer 344 rats beginning at 54 weeks of age did have a clear effect upon longevity, but reduced the age-related impairment in a spatial learning task. In contrast, improvement in a motor task was not observed. Thus, deprenyl extended the functional life span with respect to cognitive performance. This work, taken together with other studies, underscores the possibility that the time course of biological aging may be altered by pharmacological manipulations, and that such manipulations could enhance both the quality and quantity of life.

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