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The Effects of an Extract of *Ginkgo biloba*, EGb 761, on Cognitive Behavior and Longevity in the Rat

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WINTER, J. C. *The effects of an extract of* Ginkgo biloba, *EGb 761, on cognitive behavior and longevity in the rat.* PHYSIOL BEHAV **63**(3) 425–433, 1998.—Extracts of the leaves of the *Ginkgo biloba* tree are widely used throughout the world for their purportedly beneficial effects on brain function. In the present investigation, a standardized extract, EGb 761, was self-administered orally by male Fischer 344 rats that were then tested in an eight-arm radial maze. The tasks employed were a) continuous learning and b) delayed nonmatching to position. Chronic postsession administration of EGb 761 at a dose of 50 mg/kg had no effect on continuous learning but the same dose given presession resulted in a trend toward fewer sessions to reach criterion performance as well as fewer errors. In addition, it was observed that rats chronically treated with EGb 761 lived significantly longer than vehicle-treated subjects. In a delayed nonmatching to position task using a 30-min delay in 20-month-old rats. EGb 761 administered presession produced a dose-related decrease in total, retroactive, and proactive errors; a repeated-measures design was used, with subjects serving as their own controls. Following the dose–response determination, the group, now 26 months of age, was divided in two with half receiving EGb 761 at a dose of 200 mg/kg presession and the other half vehicle (sweetened condensed milk). A statistically significant positive effect of treatment with EGb-761 was observed. The present data are consistent with the beneficial effects on cognitive performance which have been widely reported in human subjects. In addition, the data suggest that the methods employed, i.e., continuous learning and delayed nonmatching to position tasks in aged rats, are capable of detecting drugs of possible value in the treatment of human cognitive impairment. Finally, the present results encourage a search for the pharmacologically active principles of EGb 761 and for their mechanisms of action. © 1998 Elsevier Science Inc.

Ginkgo biloba EGb 761 Learning Memory Aging Rat Radial maze Delayed nonmatching to position

THE *Ginkgo biloba* tree has been a part of traditional Chinese medicine for several thousands of years (17). The subject of the present investigation, EGb 761, is a complex yet well-characterized mixture of chemicals obtained from ginkgo leaves via a patented extraction process (17,40). A leading prescription drug in the European Union, ginkgo extracts in the U.S.A. and elsewhere are sold directly to consumers as nutritional supplements. Ginkgo is presently most widely used for the treatment of ''cerebral insufficiency,'' i.e., nonspecific age-related deterioration of mental function. In their review of the subject, Kleijnen and Knipschild (38,39) identified forty trials in human subjects of which eight were judged to be adequate in terms of their design. Of the eight, seven reported positive results. In addition, recent double-blind placebo-controlled studies have provided evidence of the efficacy of EGb 761 in degenerative dementias of the Alzheimer and multi-infarct type (33,36). Plausible pharmacological mechanisms by which ginkgo might be beneficial include antioxidant effects (45,47), inhibition of platelet activating factor (63), alterations in membrane fluidity (67), and inhibition of glucocorticoid synthesis (2).

In contrast with the number of studies of EGb 761 either in human subjects or in isolated systems, directly relevant behavioral investigations in intact animals are few in number. EGb 761 facilitates acquisition and retention of a food-motivated two-lever response sequence task in mice given the extract for several weeks but the effects of age were not examined (73). Studies which have used aversively motivated avoidance tasks have variously concluded that gingko extracts can facilitate learning in both young and old rodents (50,53,67), differentially in old rats (13), or not at all (51). Complicating the issue is the questionable relevance to human cognition of drug-induced facilitation of avoidance in rodents (60,61,66).

A variety of devices and procedures have been employed to assess learning and memory in rodents [for reviews, see (30,34,60,61)]. Prominent among these are avoidance learning, both active and passive, and various maze-learning tasks. Most

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common of the latter types have been procedures in Olton's radial maze (46) and in the water maze described by Morris (24,44). Following its description by Olton and Samuelson in 1976, the radial maze has been widely employed in studies concerned with various aspects of learning and memory (71) and, most relevant to the present study, for the detection of age-related decrements in performance (10,26,74).

In assessing the effects of pharmacological interventions on performance in the radial maze, several experimental approaches may be taken. For example, numerous studies have found age-related deficits in the rate of acquisition by rats of food-reinforced tasks in the eight-arm radial maze [for example, (70,72)]. These deficits are usually manifest by an increase in the number of training sessions required to reach a predetermined performance criterion. However, once acquired, criterion performance in the radial maze appears to be relatively insensitive to the effects of age (1,4,23). For this reason, successive acquisition of a series of tasks in the radial maze, each requiring renewed learning (74), was assessed in the present investigation.

A second approach, complementary to the first, was the use in the present investigation of a delayed nonmatching to position (DNMTP) task. Typically, an animal is allowed to visit four arms of an eight-arm maze and then, after removal from the maze for a variable period of time, is given access to all eight arms. Beatty and Shavalia (5) reported that young adult rats are able to perform such tasks with minimal errors for periods of delay as long as 4 h. However, it has been observed both in cross-sectional (70) and in longitudinal studies (12) that, as rats age, there is a steady increase in errors. DNMTP and related delayed matching to sample tasks in animals have been the subject of recent critical reviews (9,16,20,61) and the consensus is that they represent the closest approximation to human declarative memory presently available.

METHOD

Animals

Male Fischer 344 rats were obtained from Charles River Breeding Laboratories, Inc. (Wilmington, MA) at an age of approximately 6 weeks. They were housed in pairs under a natural light–dark cycle and allowed free access to water in the home cage. All handling and testing occurred during daytime hours. Standard rat chow was provided immediately following training sessions. Caloric intake was controlled so as to maintain adult body weights of approximately 300 g. All animals used were maintained in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985). All experimental protocols were approved by the Laboratory Animal Care Committee of SUNY at Buffalo.

Apparatus

The radial maze consists of a central hub, 34 cm in diameter, with eight 86 cm $\times$ 9 cm arms radiating from it. The sides are 10 cm high at the center of the maze and slope to a height of 6 cm at the end of the arms. The maze is constructed of aluminum with the exception of the food wells, which are plastic cups, 1.5 cm deep with a diameter of 2 cm. The entire device is elevated 46 cm from the floor.

Procedure

Initial training. Rats were acclimated to the maze in 10-min sessions in which a piece of sweetened cereal (Post Fruity Pebbles; mean weight per piece approximately 40 mg) was placed at the entry, middle, and end of each arm with a fourth piece in the food cup. The number of reinforcers was gradually reduced until only the food cups were baited. Training in the initial eight-arm task was then begun. Criterion performance is reached when, in three consecutive sessions, no more than one reentry error is made per session. Arm entries are visually scored and entry or reentry into an arm is defined as movements at least three-quarters of the way to the food cup. The number of sessions required to reach criterion performance [sessions to criterion (STC)] and the number of errors committed in reaching criterion performance [errors to criterion (ETC)] are recorded for each subject. In addition, rate of responding is determined for each subject by dividing the total number of arms entered by time in minutes to complete the session. In Experiment I, assignment to treatment groups was made on the basis of STC in the initial eight-arm task.

Continuous learning. Upon acquisition of the eight-arm task, subjects were trained with only arms 1–4 baited, again with arms 1–8, and finally with only arms 5–8 baited; the series 1–4, 1–8, 5–8, 1–8 was then repeated throughout the life of the animals. Criterion performance in the 1–4 task consists of three consecutive sessions in which no errors in working memory, i.e., reentry into arms 1–4, are made and errors in reference memory, i.e., entry into arms 5–8, equal 1 or less. STC, ETC, and response rate were determined as described in the preceding paragraph.

The 30 subjects used to assess the effects of EGb 761 on continuous learning and longevity were divided between two groups on the basis of STC during the initial eight-arm task in the maze. The first two rats to reach criterion were assigned to the control group, the next rat to the EGb 761 group, and so on to yield a control group of $n = 20$ and an EGb 761 group of $n = 10$. All subjects were then trained in a series of tasks as already described. When a block of tasks consisting of 1–8, 1–4, 1–8, 5–8 was completed, a second block was begun and so on. Sessions were run five times per week. Following each session, EGb 761 rats drank 3 mL of a solution containing a dose of 50 mg/kg of EGb 761 in sweetened condensed milk diluted 2:1 with tap water. Control subjects drank 3 mL of sweetened condensed milk diluted 2:1 with tap water. At approximately 21 months of age, postsession ingestion of EGb 761 was stopped and the drug was given at a dose of 50 mg/kg immediately before the training sessions.

DNMPT. Each day, four arms of the eight-arm maze are chosen using a table of random numbers. Entry into these arms is prevented by wooden blocks inserted before subjects are introduced into the maze. Unblocked arms are baited in the normal fashion. A rat is then placed in the maze and, following entry into each of the four open arms, is removed for a delay period of 30 min. Upon reinsertion into the maze, all eight arms are open but only those previously blocked are baited. Errors made during the postdelay subsession are further segregated into retroactive errors, i.e., reentries into arms reinforced during the predelay session, and proactive errors, i.e., reentries into arms previously entered during the postdelay subsession. A maximum of four retroactive errors is possible, i.e., one for each of the arms baited in the predelay session (12). Response rate is calculated and total errors are recorded for both the predelay and the postdelay subsessions. The rats used in the delayed nonmatching to position task were approximately 20 months of age at the start of the experiments. A repeated-measures design was employed, with subjects serving as their own controls. After performance in the eight-arm task in the radial maze had stabilized, rats performed the DNMTP task in successive blocks of ten sessions each with five sessions per week. EGb 761 in sweetened condensed milk or vehicle control was ingested immediately before the predelay subsession. The order of treatment in the session blocks was control, 100 mg/kg EGb 761, control, 200 mg/kg EGb 761, and control.

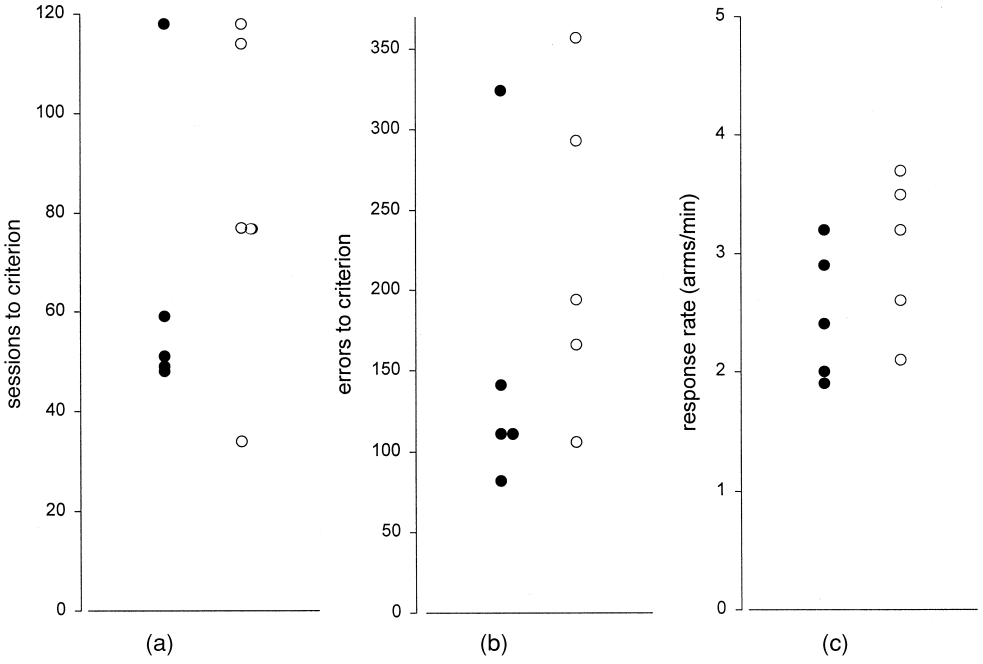

FIG. 1. Effects of chronic administration of EGb 761 on continuous learning (J. C. Winter, 1997) by 24–30-month-old Fischer 344 rats in a radial maze. Rats received either EGb 761 (50 mg/kg; per os; 30 min before testing; $n = 5$) or vehicle ($N = 5$). (a) Cumulative sessions to criterion for three successive tasks in individual subjects receiving either EGb 761 (closed circles) or vehicle (open circles); (b) cumulative errors to criterion; (c) response rate.

Drugs

EGb 761 is the most widely used form of *Ginkgo biloba* in clinical studies and is standardized in its content of ginkgo flavone glycosides and terpenoids. EGb 761 was obtained from Dr. S. S. Chatterjee, Department of Pharmacology, Dr. Willmar Schwabe GmbH & Co., Karlsruhe, Germany. Because EGb 761 is taken by humans by mouth, the oral route was used. The method described by Brailowsky et al. (7) for oral self-administration was employed using 3 mL of sweetened condensed milk as the vehicle.

Statistical Analysis

All analyses were conducted using SigmaStat for Windows™ (Jandel Scientific Software, San Rafael, CA).

RESULTS

Following assignment to treatment groups on the basis of their initial performance in the maze, subjects received either vehicle (3 mL of sweetened milk) or EGb 761 at a dose of 50 mg/kg. Both treatments were orally self-administered following training sessions. Chronic experiments in a radial maze were then begun using a series of tasks which previously has been shown to be sensitive to the effects of aging (74). Treatment and testing were continued for 24 months; no significant beneficial effects on acquisition of the tasks were apparent. Treatments were then shifted to 30 min before training sessions. As shown in Fig. 1, improved performance in the EGb 761 group is suggested by the fact that four of five EGb 761-treated subjects had lower values for both STC and ETC than did four of five control subjects. However, the differences in the mean values for the groups did not reach statistical significance. Examination of the individual data for sessions to criterion provides evidence that outliers in both groups may have accounted for the absence of statistical significance in that one subject in the EGb 761 group performed more poorly than all other subjects (1.98 standard deviations below the mean of the EGb 761 group) and one subject in the control group performed better than all other subjects (1.64 standard deviations above the mean of the control group). Although exclusion of these two subjects would yield a statistically significant difference between groups, exclusion of the possible outliers was not justified by generally accepted statistical criteria, i.e., deviation by two or more standard deviations from the group mean. The existence of these outliers is not surprising as age-related increases in the variability of cognitive performance in rodent populations have previously been noted (3). Following the maze experiments, treatments were continued until death intervened. A survival plot for the two groups is shown in Fig. 2. The EGb 761-treated rats lived significantly longer (Student's t-test, $p < 0.05$).

In a second series of experiments, a new group of 20-month-old rats ($n = 10$) was given varying doses of EGb 761 and tested in a delayed nonmatching to position task in which a 30-min delay was interposed between completion of the first four arms and completion of the maze. EGb 761 was given immediately before the predelay subsession and thus 30 min prior to the postdelay subsession. Subjects were used as their own controls and control sessions preceded each dose of EGb 761. The results are presented in Fig. 3 and indicate a dose-related reduction in postdelay errors as a result of EGb 761 treatment. To identify more precisely the nature of the effects of EGb 761, a more detailed analysis of the data at a dose of 200 mg/kg was done. Specifically, comparisons between control and EGb 761 sessions were made with respect to predelay errors as well as retroactive and proactive errors in the

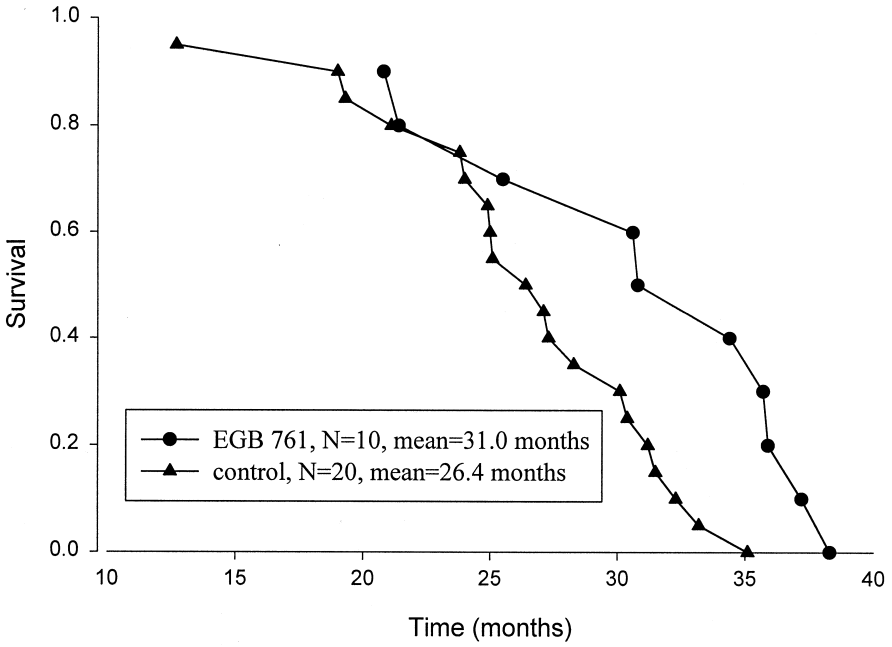

FIG. 2. Effects of chronic administration of EGb 761 on life span of Fischer 344 rats. At approximately 2 months of age, treatment with either EGb 761 ($n = 10$) or vehicle (sweetened condensed milk; $n = 20$) was begun and continued five times per week for the life of the subjects who were tested in a radial maze throughout this period. For the first 24 months, EGb 761 at a dose of 50 mg/kg was administered following test sessions; thereafter, EGb 761 was given 30 min before testing. Rats treated with EGb 761 lived significantly longer than control subjects (Student's $t = 2.06$; $p < 0.05$).

postdelay subsession, and response rates were made. In Table 1 it is seen that EGb 761 produced a significant decrease during the postdelay subsession both in retroactive and in proactive errors.

Following the completion of the DNMTP experiments illustrated in Fig. 3, the 10 rats, now 26 months of age, were assigned to two groups on the basis of their performance in their last series of control sessions. This yielded a control group ($n = 5$; mean errors = 3.4) which was thereafter to receive vehicle and a group ($N = 5$; mean errors = 4.0) to self-administer EGb 761 at a dose of 200 mg/kg. Other procedural details were unchanged, i.e., 30-min delay with treatments immediately before the predelay subsession. The performance of these two groups for 40 subsequent sessions is shown in Fig. 4 as Blocks 1–4. Application of a two-way repeated-measures ANOVA to Blocks 2–4 revealed a significant treatment effect ($F = 6.354$, $p < 0.04$).

DISCUSSION

The present data are consistent with the beneficial effects of EGb 761 on cognitive performance in human subjects (32,36,38,39). In addition, the data suggest that the methods employed are capable of detecting drugs of possible value in the treatment of human cognitive impairment. With respect to continuous learning, my use of a series of tasks in the radial maze has previously been shown to be sensitive to the effects of age (74) and this approach is akin to a number of other repeated acquisition tasks which have been described (48,49). The general goal of such methods is to permit the prolonged observation of deficits in learning and memory and thus to facilitate the discovery of agents able to prevent, ameliorate, or reverse the effects of age, toxins, or disease. However, the data of Fig. 1, while suggesting a reduction by EGb 761 in the number of sessions and errors to reach criterion performance, also provide evidence of a troubling degree of interanimal variation. Thus, one subject in each of the treatment groups differed by more than 1.5 standard deviations from the respective group means. Whereas it may be argued that these are random events easily overcome by the use of a larger sample size, a more general phenomenon may be operating in which variation between subjects increases with age. Whether this is viewed as an undesirable feature or an opportunity to examine subgroups within an aged population is a matter of opinion (25,42,56). As a practical matter, extreme interanimal variation may seriously detract from the utility of continuous learning as a means to detect cognitive enhancers in old rats. For example, in other studies of continuous learning (J. C. Winter, unpublished), variation over a period of 18 months was so great as to preclude completion of the experiments; at a time when some animals had completed the series, others were so far behind that they could not be expected to finish before death intervened.

An unexpected observation made during the continuous-learning experiments was that treatment with EGb 761 appeared to extend the life span (Fig. 2). Because an effect on longevity was not an original goal of the experiments and because several animals had died before the effect became apparent, formal assessment of the causes of death was not undertaken. Based on the well-established fact that caloric restriction extends the life span in rodents (37), it might be argued that EGb 761 somehow acted to alter food intake and weight gain. However, retrospective evaluation revealed no significant difference in weight between the EGb 761 and control groups at the time of death. Although the mechanism by which energy restriction extends life is unknown, oxidative stress and free radical production are thought to be important factors both in aging and in dementia (14,22). Thus, the known

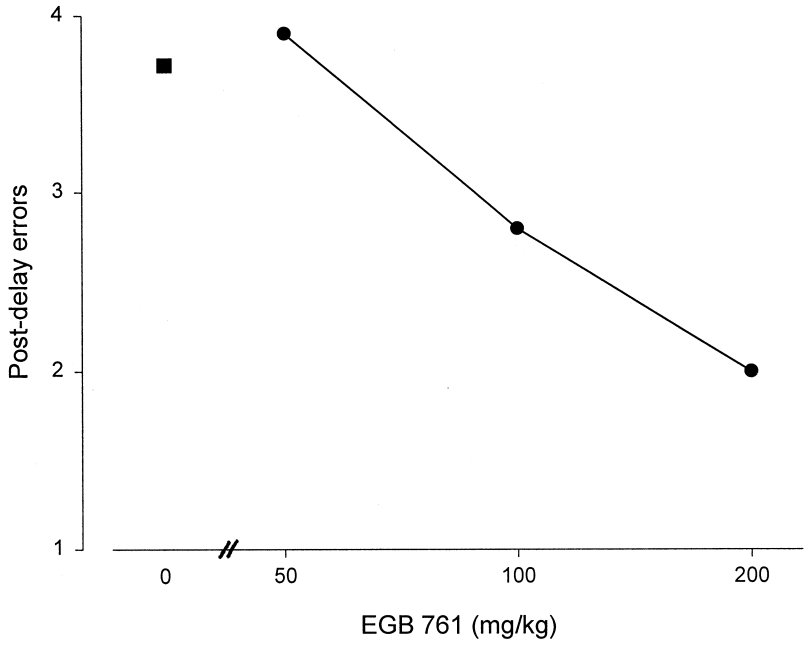

FIG. 3. Dose–response relationship for EGb 761 in 20–26-month-old Fischer 344 rats (n = 10) tested in a delayed nonmatching to position (DNMTP) task in an eight-arm radial maze. Ordinate: number of postdelay reentry errors. Abscissa: dose of EGb 761 (per os; immediately before the predelay subsession). Subjects were used as their own controls and the order of testing was 50, 100, and 200 mg. Vehicle (control) sessions preceded and followed each dose of EGb 761. Data shown are for 10-session blocks of testing and the point at zero dose is the mean of 40 control sessions. For statistical analysis; the effects of each dose of EGb 761 were compared with the mean of bracketing blocks of control sessions by means of Student's t-test for paired observations. EGb 761 produced a statistically significant reduction in errors at doses of 100 (t = 4.39; $p < 0.002$) and 200 mg/kg (t = 4.15; $p < 0.003$).

effects of *Ginkgo biloba* as an antioxidant (43,58) and free radical scavenger (18,19,21) provide alternative, though highly speculative, explanations of the data of Fig. 2. Because the data shown in Fig. 2 reflect lifetime administration of a relatively low dose of EGb 761, immediate questions arise as to whether initiation of treatment later in life would have had a comparable effect and as to whether higher doses would have been more effective. I am aware of no previous reports regarding possible effects of EGb 761 on longevity.

TABLE 1

EFFECTS OF EGB 761 (200 MG/KG) ON ERRORS AND RATES OF RESPONDING IN A DELAYED NONMATCHING TO POSITION TASK (30-MIN DELAY) IN THE RADIAL MAZE

	Control	EGb 761	p*
Errors			
predelay	0.9	0.7	NS
postdelay retroactive	2.2	1.4	<0.01
postdelay proactive	1.8	0.5	<0.01
Response rate (arms/min)			
predelay	4.3	3.7	<0.01
postdelay	4.3	4.6	NS

Subjects (n = 10) were tested in blocks of 10 sessions each in a repeated-measures design. Control values are the mean of two 10-session control blocks; one preceding and the other following the EGb 761 block.

* Signed ranks test; n = 10.

In Fig. 3 it is seen that an orderly dose-related reduction in errors is produced by EGb 761 in the DNMTP task. As was noted earlier, delayed matching and nonmatching to position or sample tasks have gained widespread acceptance in the study of the processes of learning and memory (9,16,20,61). Of direct relevance to the present results are previous DNMTP studies directed at the identification of cognitive enhancers. However, the majority of these have employed two-lever operant tasks in a Skinner box [e.g., (15,35,59)] and thus are not directly comparable. A notable exception is the work of Staubli and colleagues, who have examined in a radial maze a series of drugs modulating the AMPA-type glutamate receptor. These workers were able to demonstrate dose-related facilitation of performance using young adult rats, presession administration, and delays ranging from 2.5 to 8 h (28,64,65). Of particular interest was the demonstration by Granger et al. (27) of a restoration of performance by the glutaminergic drugs in age-impaired rats. Thus, the data of Fig. 3 are not without precedent. Significant procedural differences between the present investigation and those of Staubli et al. include my use of a) blocks of 10 sessions each and b) very old rats. The latter factor resulted in a higher baseline rate of errors even though a shorter period of delay was used.

In critical reviews of screening methods designed to detect cognitive enhancers for use in man, Sarter and colleagues (60,61) have emphasized the need, when animal performance is enhanced, to analyze the behavioral mechanisms which might be responsible.

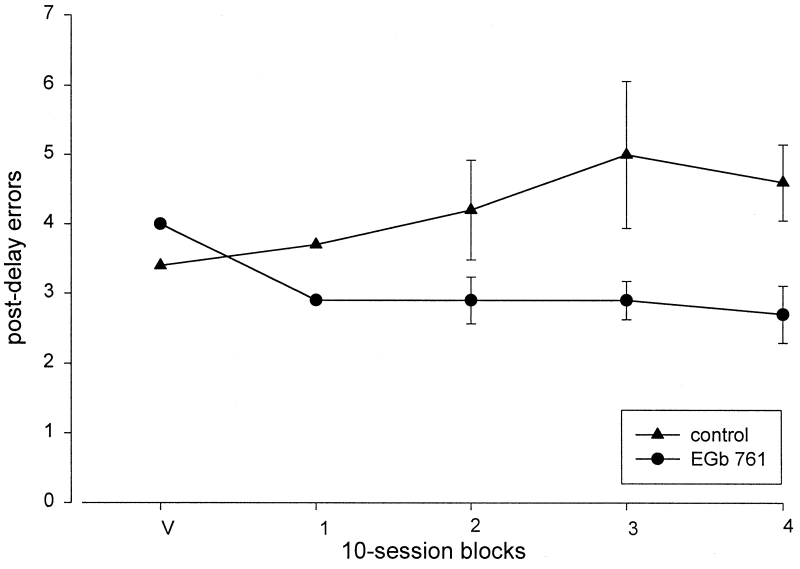

FIG. 4. Effects of chronic administration of EGb 761 in 26–29-month-old Fischer 344 rats ($n = 10$) tested in a delayed nonmatching to position (DNMTP) task. Assignment of the EGb 761 (200 mg/kg; $n = 5$) and control groups (vehicle; $n = 5$) was on the basis of performance in a block of 10 preceding sessions (session block ''V'') in which vehicle was administered to all. Ordinate: number of postdelay reentry errors (SEM indicated). Abscissa: blocks of 10 sessions each.

Thus, in addition to a direct effect upon learning and memory, drugs might act upon sensory, motor, attentional, or motivational factors. This is especially true when, as in the present investigation, drug treatment is administered prior to testing rather than postsession (61). In one approach to these issues, Chrobak and colleagues (12) have urged that errors in the postdelay subsession be further segregated into retroactive errors, i.e., reentries into arms reinforced during the predelay session, and proactive errors, i.e., reentries into arms previously entered during the postdelay subsession. Based on their comparison of young and old rats, it is their hypothesis that retroactive errors provide a true index of age-related impairment of working memory unconfounded by other factors. For the present investigation, Table 1 provides a more detailed analysis of the postdelay reentry errors shown in Fig. 3 at a dose of 200 mg/kg. It is seen that EGb 761 was associated with a significant decrease in both retroactive and proactive errors, which in turn may be interpreted as a beneficial effect upon both mnemonic and nonmnemonic factors. However, to the extent that rate of responding reflects motivation to perform, the absence of a significant effect of EGb 761 on response rate during the postdelay subsession suggests that motivation is not a significant factor.

Several studies have provided evidence that EGb 761 counters the effects of stress. Thus, chronic treatment with EGb 761 reduces the polydipsia induced by the stress of daily handling, anesthetization with ether, and oral intubation (57), blunts the elevation in plasma epinephrine, norepinephrine, and corticosterone following auditory stress (55), and prevents cold stress-induced desensitization of 5-HT_{1A} receptors (6). Effects on 5-HT_{1A} receptors are particularly interesting because of their suggested role in learning and memory (11,29,31,75) and the observation that EGb 761 prevents age-associated decreases in the density of this serotonin receptor subtype (33). In general terms, pretrial administration of an antistress or anxiolytic drug would be expected to interact with the anxiogenic or stressful aspects of aversively motivated learning tasks (61). Specifically, EGb 761 has been found to reduce learning deficits induced by exposure to inescapable shock in an active avoidance task (52) and to ameliorate the disruption of acquisition of a visual discrimination as a result of concurrent auditory stress (55). The degree to which stress reduction by EGb 761 might explain the beneficial effects seen in Figs. 1, 3, and 4 is uncertain but should be minimized by several features of the present experiments. First, performance in the maze was motivated by food, a positive reinforcer. Second, EGb 761 was orally self-administered by the subjects, thus avoiding the stress of injection or of oral intubation. Third, experiments were conducted in rats fully acclimated to the laboratory in general and to the radial maze task in particular.

Following completion of the dose–response curve seen in Fig. 3, I intended to ascertain the effects of EGb 761 upon lifespan when treatment is initiated late in life. These data would then be compared with those of Fig. 2 in which treatment was begun at an early age. Concurrently, effects upon performance in the DNMTP task were to be assessed. With respect to the latter, it is seen in Fig. 4 that the control and EGb 761 groups were well matched at the start of chronic treatment (Block 1) but that over the course of the next 40 sessions (Blocks 1–4), a performance-enhancing effect of the extract was maintained. Unfortunately, the remainder of the experiment could not be completed because of a suspected viral infection in the rat colony, later proven to be unfounded, which interrupted all behavioral studies.

The idea that EGb 761 acts directly upon a specific behavioral or biochemical aspect of learning and memory is attractive. However, it is at this time equally plausible that the extract works in a more subtle fashion. For example, it might enhance and preserve the functional activity of cells in general and neurons in particular. A nonspecific effect of this kind would explain not only improved cognition but also antiaging actions as reflected in an increased lifespan. In this regard the radical scavenging and antioxidant properties of EGb 761 have already been noted. In addition, there is evidence that EGb 761 may protect against chemically induced

neurotoxicity (54) and hasten recovery from brain lesions (8). If such a mechanism were active, it would be expected that chronic treatment would be more efficacious than acute treatment. Although many studies have employed chronic treatment, I am aware of none which have demonstrated a differential effect of chronicity on outcome. In the present investigation, blocks of 10 sessions each were used in an attempt to reduce variability but chronicity per se was not examined except at a dose of 50 mg/kg of EGb 761. At this dose, no statistically significant effect was seen following either acute (data not shown) or chronic treatment (Fig. 3). Clearly, additional experiments which explicitly address this hypothesis are needed.

Despite the urgent need to discover drugs able to prevent, delay, ameliorate, or cure age-related memory impairment and the degenerative dementias, progress has been slow. Two contributing factors are a) a lack of consensus regarding appropriate animal models and their predictive validity for man (16,30,60,61) and b) the absence of a drug of proven efficacy in humans with which to validate tests in animals (52,62,66). Thus the present demonstration of positive effects of EGb 761, a substance for which abundant evidence exists of at least a modestly beneficial clinical effect (32,36,38,39), in a delayed nonmatching to position task in animals is encouraging on both points. The immediate challenge is to provide convincing evidence that these effects on learning and memory are replicable, age-related, show a positive correlation with dose and with the duration of delay, and extend across a variety of tasks.

During the past decade the results of hundreds of studies of extracts of *Ginkgo biloba* in man and in animals, in vivo and in vitro, have been published. Despite this mass of data and despite the status of such extracts as prescription drugs in Germany, France, and elsewhere, the clinical efficacy of these preparations remains a matter of controversy (38,39). One of the major obstacles to establishing the mechanisms by which beneficial effects might be produced is the fact that these extracts are complex mixtures of chemicals which would be expected to have multiple pharmacological effects. Indeed, as a part of the lore of ancient Chinese medicine, it has been said that only in combination can these hundreds of constituents be effective (Dr. Won-Ki Kim, personal communication). As pharmacologically implausible as is that contention, it is only recently that attempts have been made to attribute specific effects to specific chemical constituents [e.g., (2,41,68,69)]. The present data urge a systematic evaluation of the chemical constituents of *Ginkgo biloba* extracts in tests of learning and memory and ultimately a determination of the pharmacological mechanisms by which they act. Once the active principles have been identified and characterized, work may proceed to explore their structure–activity relationships so that still more efficacious drugs may be synthesized. Such studies will as well be expected to contribute to an enhanced understanding of the molecular mechanisms of memory and the disturbances of that function so often imposed by disease and by the processes of aging.

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