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Facilitative Effects of EGb 761 on Olfactory Recognition in Young and Aged Rats

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WIRTH, S., J. STEMMELIN, B. WILL, Y. CHRISTEN AND G. DI SCALA. *Facilitative effects of EGb 761 on olfactory recognition in young and aged rats.* PHARMACOL BIOCHEM BEHAV **65**(2) 321–326, 2000.—The aim of the present study was to evaluate the effects of chronic and acute treatment by the Gingko biloba extract, EGb 761 (IPSEN, France) on olfactory short-term memory in rats, using a spontaneous recognition procedure. The effects of a daily EGb 761 treatment (30 or 60 mg/kg) over a period of 30 days (Experiment 1) were evaluated in young male rats. Those of a single injection of EGb 761 were assessed either in young male rats at 60 or 120 mg/kg (Experiment 2) or in aged female rats at 60 mg/kg (Experiment 3). Results showed that, at the highest dose (60 mg/kg), chronic EGb 761 treatment enhanced the recognition performances, allowing recognition at delays at which control animals did not show any recognition. Acute treatment enhanced recognition at both doses tested. The results of the third experiment showed that EGb 761 had an overall enhancement effect on the performances of aged rats. In summary, our results provide evidence for a short-term memory enhancement effect of EGb 761 in both young and aged rats. © 2000 Elsevier Science Inc.

Olfactory learning Recognition EGb 761 Gingko biloba Aging Memory

OLFACTORY function is markedly altered in "old age" and in age-related diseases such as dementia of the Alzheimer type (4,21). The alteration is known to combine a decline in olfactory perception and olfactory memory (5,13). Although less marked than in humans, similar deficits have been found in rodents: impairments in olfactory function have been reported in aged rats, as evidenced by deficits in their capacity to form odor reward associations (17), to perform olfactory delayed nonmatching-to-sample (29) and social olfactory recognition (24). Olfactory memory tasks may, therefore, be of interest for studying the neurobiological processes involved in age-related deficits and for testing potential therapeutic treatments.

EGb 761, a standardized (IPSEN, France) extract of Gingko biloba, has been widely used in Europe to alleviate symptoms associated with a large range of cognitive disorders [for review, see (3)]. For instance, several recent studies have found EGb 761 to stabilize the cognitive decline or to improve performances in patients with Alzheimer-type dementia (10, 11). In animal studies, promnesic effects of EGb 761 have been described in several tasks such as a radial maze (26); visual discrimination in a T-maze (15), and discriminative bar pressing in an operant chamber (25). Promnesic effects of the extract have also been reported in an olfactory delayed nonmatching-to-sample task (16). We have recently developed an olfactory recognition task (28), that may be of use to investigate the effect of potential treatments in short-term olfactory deficits related to aging, as it involves spontaneous recognition processes germane to those implicated in tests used in humans, particularly in aged people. The aim of the present study was, therefore, to test the putative effects of EGb 761 in young and aged rats using our olfactory recognition task. In brief, the test is based upon spontaneous exploratory behavior of rats towards odors. Rats display a decrease in exploratory behavior of one odor presented repeatedly over several trials. In contrast, they show a normal exploration of a novel odor presented on a subsequent trial. This difference of investigation between the new and the familiar odors reflects recognition (6). In our previous study (28), we showed that recognition depended on the pretest delay, as rats recognized the familiar odor only with brief delays (up to 40 min), but not with a longer one such as 120 min. Furthermore, the task has

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proven sensitive to promnesic effects of pharmacological compounds (FG 7142) and of cerebral lesions (entorhinal cortex lesion). Three experiments were carried out for the present study. The first experiment was aimed at testing whether a chronic treatment with EGb 761 would affect odor recognition in young adult rats. To this end, two doses (30 and 60 mg/kg) close to those commonly used in studies with EGb 761 [e.g., (8,16)] were daily administered over 30 days. The putative effects of the treatment were assessed with two conditions of pretest delays (5 and 120 min), to reveal either amnesic or promnesic effects. The second experiment tested the effects of an acute treatment with EGb 761 also in young rats. Most of the published studies concerning the extract have used chronic treatments, and the possible acute effects are still poorly investigated. This if of interest, as inference on the mechanisms of action of the compound may depend on the existence/absence of acute effects. In this regard, beneficial effects of EGb 761 have been interpreted as protective or restorative effects, possibly related to its free radical scavenger properties [see (3)]. It was hypothesized that acute promnesic effects may indicate additional properties of the compound. To this end, the effects of two doses were tested with a long pretest delay (120 min) only, to evaluated its putative promnesic effect. One dose corresponded to the one revealed to be efficient in Experiment 1 (60 mg/kg), and the second dose was chosen higher (120 mg/kg) as a putative dose-dependent effect might be observed. The third experiment assessed the odor recognition performances in aged animals, as it has logically been proposed that they may constitute good models for age-related cognitive deficits (29), and the effects of an acute treatment with EGb 761 were assessed in this population. Female rats were used in this experiment, as they are more suitable for long-term breeding than male rats. Thus, the test initially developed for young adult male rats was adapted to aged female animals, and the effects of only one dose of the extract (60 mg/kg) were tested but on two different pretest delays (45 and 90 min). Shorter delays were used than in previous experiments, as we expected that performances of aged rats would be lower than those of young rats.

METHOD

Animals and Housing

Ninety male Long–Evans rats obtained from the CERJ rat farm (Janvier, France) served as subjects for the first two experiments. They were housed two per cage with food and water available ad lib. They were maintained in a temperature controlled colony room on a 12:12-h light–dark cycle (lights at 0800 h). All testing took place during the light phase of the cycle. One week after their arrival to the laboratory, the rats were handled a few minutes per day for 5 days to familiarize them with the experimenter. Sixty aged (26 months) female Long–Evans rats were used for the third experiment. They were born and bred in the laboratory colony room. They were tested in a Morris water maze prior to this experiment. Except that they were housed three per cage, housing conditions were the same as for males. All procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national (council directive 87848, October 19, 1987, Ministère de l'Agriculture et de la Forêt, Service Vétérinaire de la Santé et de la protection animales; permission 5075 to G.D.S. and S. W. under the former's responsibility) and international (directive 86-609, November 24, 1986, European Community) laws and policies.

Olfactory Recognition: General Procedure

Behavioral testing took place in a dimly lit (50 lx) room adjacent to the colony room. A gray PVC octagonal arena (40 cm high, 55 cm width) with saw dust spread on the floor was used. Two sniff ports (2 cm wide, 3 cm above the floor) were centered on two walls 40 cm apart. Odor containers could be fixed to the outer wall of the arena, at the level of the sniff port. Each container consisted of a black tube in which a filter paper soaked with odor extract (5 μl/filter) could be inserted. Two odors, hazelnut and vanilla (Le Nez du Vin and Alchim Aromatiques), of equal attractiveness to the rats were used [for a detailed description of methods, see (27)] and designated as O1 and O2. The nature of these odors was counterbalanced, each single odor serving in a balanced manner as O1 and O2 for different rats. Rats were introduced separately in the arena, and their behavior was recorded by means of a video camera located above the arena, with the monitor and recorder located outside the room. Time spent sniffing the odor was quantified by means of a computer behavioral coding program, and was defined as the time the rat had its nose within 1 cm of the sniffing port. Blind coding was carried out by a second experimenter.

The experiment consisted of four sessions, each lasting 5 min. The three first sessions were designated as the "learning phase," and were separated by 5-min intervals. The last session was referred to as the "test." It started at different times after the last learning session: 5 or 120 min in Experiment 1; 120 min in Experiment 2; 45 or 90 min in Experiment 3. Between sessions, animals were returned to their home cages. During the first session (habituation), rats were familiarized to the test cage with the empty odor containers. On the two subsequent sessions, and odor (O1) was presented randomly in one of the two ports. The total amount of investigation of O1 and of the empty sniffing port was measured. After the chosen pretest delay, rats were placed in the arena for 5 min (test), in the presence of O1 and a novel odor O2. During this session, the position of the two odors was randomly assigned. The total amount of time investigating O1 and O2 was measured for each rat.

Experiment 1: Effects of a Chronic Treatment With EGb 761 in Young Adult Male Rats

Sixty young adult male rats (200–215 g) were submitted to a chronic treatment with EGb 761 or its vehicle for a period of 30 days. EGb 761 obtained from IPSEN was dissolved in saline with two drops of Tween 80 per ml. Treatment was delivered by daily intraperitoneal injection (2 ml/kg). Six groups were constituted: two groups received a 30-mg/kg treatment ($n = 10$ each); two groups received a 60-mg/kg treatment ($n = 10$ each), and two groups were daily injected with the vehicle ($n = 10$ each). For each treatment, one group was tested with a 5-min pretest delay, the other one with a 120-min pretest delay. The rats were tested between 1 to 6 h after their last injection.

Experiment 2: Effects of an Acute Treatment With EGb 761 in Young Adult Male Rats

Thirty young adult male rats (280–300 g) were used. The subjects were given an intraperitoneal injection of either EGb 761 or vehicle at equivalent volume 10 min before the start of the experiment. Three groups of rats were constituted: EGb 60 (60 mg/kg), EGb 120 (120 mg/kg), and control, with $n = 10$ in each group. All groups were tested following a 120 min pretest delay.

TABLE 1

MEAN (± SEM) SNIFFING TIME DURING THE SECOND AND THIRD SESSIONS OF THE LEARNING PHASE AFTER A CHRONIC TREATMENT WITH EGb 761 IN YOUNG ADULT MALE RATS

	Second Session		Third Session	
Treatment	O1	Empty Port	O1	Empty Port
EGb 30	5.47 ± 0.5	2.76 ± 0.27	1.33 ± 0.26	1.24 ± 0.24
EGb 60	5.78 ± 0.62	2.33 ± 0.41	2.34 ± 0.54	1.71 ± 0.46
Control	5.79 ± 0.65	2.27 ± 0.4	2.19 ± 0.43	1.94 ± 0.39

TABLE 2

MEAN (± SEM) SNIFFING TIME DURING THE SECOND AND THIRD SESSIONS OF THE LEARNING PHASE AFTER AN ACUTE TREATMENT WITH EGb 761 IN YOUNG MALE RATS

	Second Session		Third Session	
Treatment	O1	Empty Port	O1	Empty Port
EGb 60	6.17 ± 0.83	3.21 ± 0.67	1.24 ± 0.53	3.25 ± 0.88
EGb 120	4.57 ± 0.52	2.64 ± 0.48	1.27 ± 0.47	1.8 ± 0.63
Control	5.76 ± 0.74	2.42 ± 0.52	1.99 ± 0.51	4.3 ± 0.74

Experiment 3: Effect of an Acute Treatment With EGb 761 in Aged Female Rats

Sixty aged female rats were used. The subjects were given an intraperitoneal injection of either EGb 761 or vehicle at equivalent volume 10 min before the start of the experiment. The rats were randomly assigned to four groups: two groups received an intraperitoneal injection of EGb 761 at a dose of 60 mg/kg (n = 14 each) and the two other groups received an injection of vehicle (n = 14 each). Among each treatment group (aged-EGb and aged-vehicle), one subgroup was tested 45 min after the learning phase, the other at 90 min after this phase).

Data Analysis

For the learning phase, the time spent sniffing the odor was analyzed by a two-way ANOVA (drug factor and session factor). The difference in time spent sniffing O2 and O1 divided by the total amount of sniffing (i.e., O2−O1/O1+O2) is defined as the index of recognition. This variable was also analyzed by a two-way ANOVA for Experiment 1 (drug factor: EGb30, EGb60 and control, and delay factor: 5 min and 120 min), and Experiment 3 (drug factor: nontreated aged rats and treated rats and delay factor: 45 and 90 min). A one-way ANOVA was used for Experiment 2 (group factor: EGb60, EGb120, and control). Post hoc multiple comparison tests were carried out with the Newman–Keuls test.

RESULTS

Experiment 1: Effects of a Chronic Treatment with EGb 761 in Young Adult Male Rats

Two animals were discarded from the experiment because of an accidental interruption of the procedure; thus, the resulting number of rats was nine in each EGb60 group, and 10 for each other group. Time spent sniffing the odors during the learning phase is shown in Table 1. During this phase, animals spent more time sniffing the port containing O1 than the empty port. The three groups exhibited a similar duration of sniffing port investigation, and displayed a similar decrease in sniffing over successive learning sessions. The ANOVA confirmed this description, and showed no effect of drug factor, $F(5, 52) = 1.29$, NS; but a significant effect of session factor, $F(1, 52) = 86.74$, $p < 0.01$, and no interaction between these two factors, $F(2, 52) = 2.6$, NS.

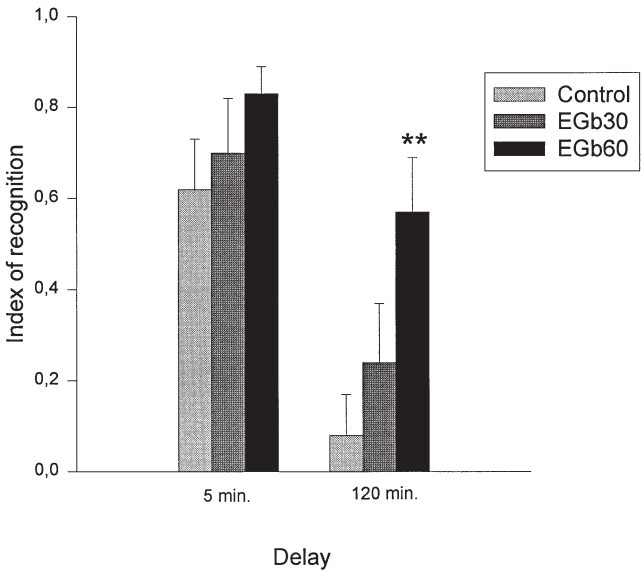

FIG. 1. Effect of a chronic treatment of EGb 761 on recognition in young adult male rats. The figure represents the ratios obtained during the test following either a 5- or 120-min delay for each group (control; EGb 30; EGb 60);**$p < 0.01$.

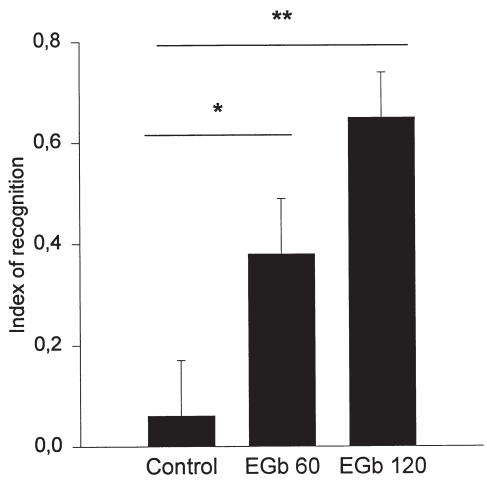

FIG. 2. Effect of an acute treatment of EGb 761 on recognition in young adult male rats. The figure represents the ratios obtained for each group (control, EGb 60, EGb 120) following a 120-min delay; *$p < 0.05$, **$p < 0.01$.

TABLE 3
MEAN (± SEM) SNIFFING TIME DURING THE LAST SESSIONS OF THE LEARNING PHASE AFTER AN ACUTE TREATMENT IN AGED FEMALE RATS

	Second Session		Third Session	
Treatment	O1	Empty Port	O1	Empty Port
Aged control	3.37 ± 0.21	0.9 ± 0.08	1.06 ± 0.1	0.83 ± 0.18
Aged + EGb 60	3.09 ± 0.31	0.48 ± 0.12	0.52 ± 0.19	0.56 ± 0.16

Figure 1 depicts the recognition ratios obtained during the test for all groups. When tested after a 5-min delay, animals from all groups exhibited a low sniffing duration of O1 and a normal investigation of the novel odor, as evidenced by a ratio nearing 1, indicating recognition of O1. When tested after a 120-min delay, control animals displayed equal investigation of each odor as indicated by a ratio nearing zero indicating an absence of recognition. Animals treated with 30 mg/kg of EGb 761 displayed a slightly higher exploration of O2 compared to O1, but this effect did not reach significance. In contrast, treatment with 60 mg/kg of EGb 761 tended to ameliorate recognition as animals from group EGb 60 displayed a low exploration of O1 and a normal exploration of O2 as indicated by a ratio close to 0.6. The two-way ANOVA confirmed this description, indicating an effect of the delay, $F(2, 52) = 5.13$; $p < 0.01$, an effect of the drug, $F(2, 52) = 21.42$, $p < 0.01$, and no interaction between these two factors, $F(2, 52) = 0.84$; NS. Two by two comparisons (Newman–Keuls) indicated a difference between the group EGb60, and the control group ($p < 0.01$) at the 120-min delay condition.

Experiment 2: Effect of an Acute Treatment With EGb 761 in Young Adult Male Rats

Table 2 depicts the time spent sniffing O1 during the learning phase. The three groups spent more time sniffing the port containing O1 than the empty port. The three groups exhibited similar investigation duration and decrease in time spent sniffing O1 over the two last sessions of the learning phase. The two-way ANOVA confirmed this description, and indicated an effect of the session factor, $F(2, 27) = 79.37$; $p < 0.01$, no effect of the drug factor, $F(2, 27) = 1.13$, NS, and no interaction, $F(2, 27) = 1.16$, NS.

The recognition index of the rats during the test is shown in Fig. 2. Control animals displayed a similar time of investigation of O1 and O2, as showed by the ratio close to zero. On the contrary, Egb-treated animals presented a low investigation of O1 and a higher investigation of O2 as expressed by ratios close to 0.4 (EGb 60) and 0.6 (EGb 120). This difference was significant and the ANOVA performed on the ratios indicated an effect of the drug factor, $F(1, 27) = 8.24$, $p < 0.01$. The Newman–Keuls test showed a significant difference between treated groups EGb 60 and EGb 120 when compared to the control group ($p < 0.05$ and $p < 0.01$, respectively).

Experiment 3: Effect of EGb 761 on Olfactory Recognition in Aged Female Rats

Six rats were discarded from the analysis because of their extremely low level of exploratory activity (lower than 1 s on ten first presentation of O1). The remaining "*n*" was 14 for each group of control rats and 13 for each group of EGb-treated rats. During the learning phase, the time spent sniffing O1 by the rats was lower than in young male animals (see Table 3 compared to Table 2), but rats still spent more time exploring the port containing O1 compared to the empty sniffing port. The two groups displayed a decrease in sniffing over the learning sessions as confirmed by the two-way ANOVA [drug factor, $F(1, 52) = 1.58$, NS; session factor, $F(1, 52) = 64.9$, $p < 0.01$; interaction, $F(1, 52) = 0.18$, NS].

Figure 3 represents the recognition indexes obtained after the two pretest delays. After a 45-min delay, control aged rats had a recognition index of 0.3, suggestive of a weak recognition. EGb-treated rats displayed a recognition index close to 0.6, suggesting that the treatment with EGb 761 facilitated recognition. At a 90-min delay, recognition indexes were lower in both groups, but the index of treated rats remained higher to nontreated rats. The two-way ANOVA indicated a significant effect of the drug factor, $F(1, 49) = 4.05$, $p < 0.05$; no effect of the delay factor, $F(1, 49) = 1.9$, NS, and no interaction between these two factors, $F(1, 49) = 0.16$, NS.

DISCUSSION

The results of the present study demonstrate that EGb 761, whether given chronically (Experiment 1) or acutely (Experiment 2), has facilitative effects on olfactory recognition in young adult rats. Furthermore, this study shows a similar fa-

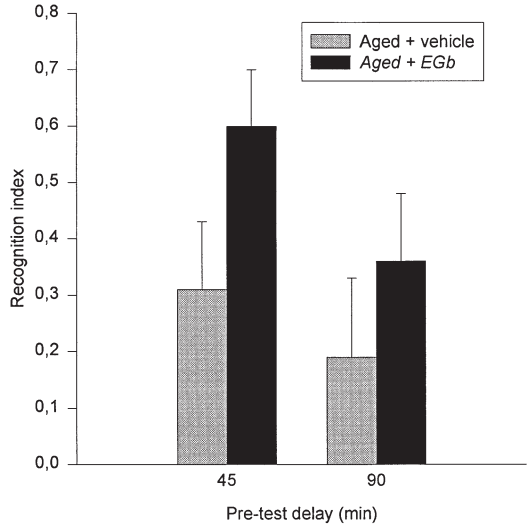

FIG. 3. Effect of an acute treatment of EGb 761 on recognition in aged female rats. The figure represents the ratios obtained during the test following either a 45- or 90-min delay for each group (aged nontreated; aged treated).

cilitative effect of an acute EGb 761 treatment in aged rats (Experiment 3). Taken together, these results show that EGb 761 has promnesic effects in an olfactory short-term memory test in both young adult and aged rats.

Experiment 1 demonstrates that a chronic treatment with EGb 761 induced a delay-dependent facilitative effect in young adult rats. In this experiment, control animals displayed a good recognition after a short pretest delay, but no recognition after a longer delay. This result confirms previous data (28), and indicates that our test reflects short-term memory as performance decreases with increasing delays. Probably due to a ceiling effect, EGb 761 did not modify performance at the short delay. In contrast, EGb 761 partially prevented the amnesic effect of the long delay; such a preventive effect may be interpreted as promnesic. These results confirm and extend previous studies showing promnesic effects of chronic EGb 761 treatment in various learning and memory tasks in young rodents (15,25), including an odor short-term memory (16).

Experiment 2 aimed at verifying whether an acute treatment would have similar effects, and showed that a single administration of EGb 761 also facilitated recognition. At a 120-min pretest delay, control rats did not display recognition, whereas EGb-treated rats showed a dose-dependent facilitative effect. More precisely, the dose of 60 mg/kg, which was the highest dose tested chronically, induced a moderate but significant effect, and the dose of 120 mg/kg induced a large effect, as the performances were close to ceiling. These results suggest that the facilitative effects observed with the chronic treatment may not depend only on a steady level of the compound in the organism, but may also at least partly be due to the effect of the last injection, though this injection was received between 1 and 6 h before the test. The Gingko biloba extract presents a very long-lasting availability in the organism, as shown by several studies on its pharmacokinetic [see (3)]. Thus, although the administration schedule is a repeated schedule, the pharmacokinetic properties of the drugs brings this schedule closer to a chronic one.

Experiment 3 was aimed at verifying whether the same test could be used for assessing memory performance in aged rats, and whether EGb 761 would have beneficial effects in such rats. The results show that although aged rats showed very little recognition at the longer delay (90 min), they showed some recognition at the shorter delay (45 min). This weak recognition performance observed in aged rats may be partly related to their weak investigatory activity during the learning phase, but may also reflect poor performances in short-term memory. In aged rats, acute treatment with EGb 761 had a general facilitative effect at both delays on recognition performances, but no effect on the exploration time of odors. This result indicates that in a population with low memory performances, EGb 761 can have promnesic effects that seem independent of sniffing during the learning phase. This suggests that these effects of EGb 761 are likely to involve learning or memory mechanisms as confounding factors, such as time spent sniffing or locomotor activity (unpublished data), were unaffected by the compound. These promnesic effects are germane to those described in humans [e.g., (7,10)] and they also confirm promnesic effects obtained in aged animals (1,15).

Hypotheses on the mode of action of EGb 761 are mainly based on experiments involving chronic treatment, and suggest the existence of a protective or restorative mechanism [e.g., (2,8,20,22)]. Our data obtained with an acute treatment, suggest that in addition to its protective and restorative effect, EGb 761 may have a fast and more direct action on a neurotransmission system. Several authors hypothesized that the effect of EGb 761 might be related to an improvement of cholinergic neurotransmission, as EGb 761 treatment was found to prevent the effects of scopolamine (16), and to enhance cortical muscarinic receptors density (23), and cholinergic transmission (14). It was also found recently that EGb 761 presents discriminative properties that generalize to serotonin agonists (27), raising the possibility that EGb 761 acts also on serotonin receptors. These hypotheses require further testing, especially in the context of acute treatment.

Processes involved in facilitative effects of a drug remain a matter of debate. When treatments are given prior to testing, the promnesic effect observed can result of several nonpurely mnemonic processes. In a study on the effect of EGb 761 on spatial learning (26), the author found that chronic administration of the drug at a low dose had no effect when administered after the learning session, whereas the same dose administered before that session had a significant effect. These results, together with ours, suggest that EGb 761 might act not only on purely mnemonic mechanisms but also on other mechanisms such as attentional processes. The link between acetylcholine and attentional/mnemonic function has been well documented [for a review, see (18,19)], and an increase in cortical acetylcholine has been found to be correlated with attentional demand. Recent research on aging-related mnemonic deficits have stressed out the role of attention in the deficits (9,12,18). As several results suggest that the cholinergic function is enhanced by EGb 761 treatment, a potential mechanism of action of the drug might be related to an increase in attentional function. This attentional mechanism of action might underlie the enhancement of recognition performances that we have observed in both young and aged rats.

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