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Effects of *Ginkgo biloba* administered after spatial learning on water maze and radial arm maze performance in young adult rats

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Abstract

Ginkgo biloba is reported to improve learning and memory in animals. However, many studies do not directly test the effects of *Ginkgo* on memory because the drug is administered during the learning phase of the experiments. In this study, we examined the effect of 10 mg/kg, 20 mg/kg, or 40 mg/kg *G. biloba* extract on spatial memory by administering the drug in the interval between training and testing. Rats were tested for long-term reference memory retention in the radial arm maze and in the Morris water maze during daily probe trials in which the hidden platform was removed. *G. biloba* had no effect on reference memory in either the water maze or radial arm maze. To test short-term working spatial memory using the radial arm maze, animals were removed after receiving the reward from 4 of the 8 arms and were returned to complete the maze 2 h later. While *Ginkgo* had no effect on working memory, over time animals exposed to *Ginkgo* learned task better than control animals. Thus, *Ginkgo* appears to enhance neither short-term working memory nor long-term reference memory, but it may promote learning of spatial information.

1. Background

A number of herbal medicines are routinely used for the treatment of neurological and psychological disorders (Beaubrun and Gray, 2000). Extracts from the leaves of Chinese maiden hair tree *Ginkgo biloba* have been used as an herbal medicine for thousands of years (Major, 1967). Recent claims have indicated that *G. biloba* has positive effects on learning, concentration, and memory (reviews by Kleijnen and Knipschild, 1992; O'Hara et al., 1998; Diamond et al., 2000). However, many of these studies are limited by small sample size and design. A larger randomized, double-blind, placebo controlled study indicated that *Ginkgo* did not have an effect of cognitive function (Solomon et al., 2002).

In human studies of young adults, *Ginkgo* promotes attention (Kennedy et al., 2000) but not learning and memory (Moulton et al., 2001). In older subjects with intact cognition, *Ginkgo* enhances working memory (Rigney et al., 1999; Wesnes et al., 2000) and long-term memory (Wesnes et al., 2000). In contrast, other studies have not demonstrated an effect of *Ginkgo* on learning, memory, concentration, or attention (Mix and Crews, 2000; Solomon et al., 2002). *Ginkgo* appears to have a more pronounced effect on brain activity (Itil et al., 1998) and short-term memory (Winther et al., 1998) in subjects with cognitive impairments. Despite these contradictory results, *G. biloba* remains one of the most commonly used herbal supplements (Itil et al., 1998).

Studies examining the effects of *G. biloba* on learning and memory in rodents have produced similarly mixed results. A mid-level dose of *Ginkgo* promoted inhibitory avoidance conditioning in rats, where a low- or high-dose was ineffective (Topic et al., 2002a). High levels of *Ginkgo* promoted short-term, but not long-term passive avoidance learning in senescent (20-month old) mice (Stoll et al., 1996). Similar high doses of *Ginkgo* promoted acquisition and long-term retention of operant conditioning in adult mice (Winter, 1991). Acute exposure to *Ginkgo* accelerates decision making time, but did not affect the accuracy of decisions (Wilson et al., 2000). *Ginkgo* did not promote acquisition or retention of memory in the water maze in

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senescent mice (Ward et al., 2002). While *Ginkgo* promoted spatial learning in aged rats (Topic et al., 2002b), it did not affect adult rats in the water maze (Hasenöhrl et al., 1998; Hoffman et al., 2004). However, *Ginkgo* has been shown to promote short-term, but not long-term spatial memory (Hoffman et al., 2004). *Ginkgo* also promoted acquisition of spatial learning using the radial arm maze in rats (Winter, 1998). *Ginkgo* exposure minimized β-amyloid induced spatial memory loss in the water maze in rats (Tang et al., 2002) and mice (Stackman et al., 2003), as well as decreasing the effects of memory impairment associated with aluminum-induced brain dysfunction (Gong et al., 2005). In addition to protection of motor functions following cerebral ischemic injury, *Ginkgo* also improved spatial memory on the radial arm maze (Lin et al., 2003). Evidence indicates that *Ginkgo* may have an anti-stress or anti-anxiety effect (Ward et al., 2002; Hasenöhrl et al., 1998). Pre-treatment of *Ginkgo* prior to bouts of restraint-stress or administration of corticosterone eliminates the normal stress-induced memory impairment in passive avoidance tasks (Walesiuk et al., 2005, in press).

The wide range of results using a variety of learning and memory paradigms may indicate that *Ginkgo* may have differential and task specific effects. In particular, it is important to differentiate possible effects on learning, short-term (working) memory and long-term (reference memory). For example, maze studies might focus on the effects of *Ginkgo* during the acquisition phase of the study, during working memory retrieval, or during long-term memory retrieval. Generally speaking, working memory refers to information that the subjects retains for minutes or hours and is used in the completion of a local trial or task. Such details (such as which arm of the maze was last visited) would not be expected to persist beyond the trial or session in which they were relevant (Olton et al., 1979). By contrast, long-term reference memory includes information involved in carrying out the procedures or "rules" of the task ("visit each arm of the maze no more than once"). Such information clearly persists for much longer periods of time (Izquierdo et al., 2000).

The current study was conducted utilizing the most commonly used maze designs in learning and memory retention studies, the Radial Arm Maze (RAM; Olton, 1978) and the Morris Water Maze (Morris, 1984). We used the RAM to examine both short-term working and long-term reference memory performance and the water maze to study long-term reference retention. The RAM consists of a central platform with eight arms radiating from the platform. In a short-term memory design, food is placed at the end of each of the arms and rats are allowed to freely explore the maze until they acquire all of the food. Learning is considered complete when animals visit each of the arms exactly once with no repeats. During short-term testing, rats are removed from the maze after their fourth choice and returned to the maze after a brief delay interval (minutes or hours). The number of correct choices made after this delay has been reported to provide a measure of short-term or working memory performance (Beatty and Shavalia, 1980). However, traditionally short-term memory has been used to describe the process involved in completing the current task such as remembering which arms had been visited previously in the on-going trial (Olton, 1978). An extended delay in the completion of the task on the order of a few minutes to a few hours may entail a different process involving intermediate-term memory (Sutton et al., 2004; Kesner and Hopkins, in press). For long-term reference memory testing using the RAM, animals are returned to complete the maze as trained after an extended non-testing interval. The number of errors made and time needed to complete the maze are examined to determine the extent of long-term memory retention of the original task. The Morris water maze was also used in this study to examine long-term reference memory. In this task, rats swim in a pool of water until they discover a hidden platform in a constant location. Once rats have learned the location of the platform, they are tested for retention of this knowledge after intervals of several days.

Previous studies with *G. biloba* have exposed animals to the drug during the acquisition phases of such maze studies. Thus, the effects of *Ginkgo* on memory retrieval are confounded with the effects of the drug on initial acquisition. The purpose of the present study was to examine the effects of *Ginkgo* on memory retrieval by delaying drug administration until after the initial learning phase of each study was complete.

2. Methods

2.1. Subjects

This study examined behavior in 123 young adult (60 day old) male Sprague Dawley rats (Charles River) utilizing two different mazes. Animals used in radial arm maze testing ($n=75$) were housed individually with water ad libitum. They were kept on a food restricted diet throughout the experiment. The rats were weighed daily to ensure that their body weight was at 82.5–87.5% of their ad libitum feeding weight. Animals used in water maze testing ($n=48$) were caged in pairs and were provided unrestricted amounts of food and water. Animals were trained to criteria in the respective tasks and assigned to groups based on performance as described in detail below. All procedures followed a protocol approved by the local Institutional Animal Care and Use Committee in accordance with institutional and federal guidelines.

2.2. Drug

A commercially available form of *Ginkgo*, Herbal Plus® Standardized *G. biloba* extract was obtained from a local health food store (General Nutrition Centers). The solution contained 24% *Ginkgo* Flavonglycosides along with 7.5% Stevia Extract (GNC product number 887224). The *Ginkgo* extract was prepared in 10% sucrose for a final delivery solution of 1 ml for each drug dosage. During the drug administration period, animals received daily one of three doses of *Ginkgo* (10, 20, or 40 mg/kg body weight) or an equal volume of the control vehicle. The drug was administered orally using a plastic syringe delivering the solution directly into the cheek pouch with no complications.

2.3. Behavioral testing

2.3.1. Morris water maze

Long-term reference memory was examined in 48 rats using a Morris water maze (Morris, 1984). The water maze (160 cm diameter pool) was surrounded by a black curtain raising 75 cm above the edge of the pool. Two extra maze cues were present on opposite ends of the curtain (a 30×50 cm white sheet; a 30×50 sheet with alternating vertical 2 cm black and white stripes). A hidden platform was submerged 5 cm below the surface of the water, and the surface of the water was made opaque through the presence of polypropylene beads. During acquisition training, rats were placed into the water maze for three trials per day for 10 days. All animals started from the same randomly assigned, but predetermined location on each trial. Rats were allowed to explore the maze until the platform was located or until 90 s had elapsed. Subjects that did not find the platform were physically placed on the platform for 1 min. The latency to find the hidden platform, distance traveled, and path of travel were recorded and analyzed using the Ethovision Behavioral Analysis System version 2.3 (Noldus Information Technology, The Netherlands). Animals were assigned to treatment groups based on their average performance (distance traveled to escape platform) over the last 3 days of acquisition training.

During the drug delivery phase, animals received the assigned amount of *Ginkgo* daily for 14 days during which time the animals also completed one acquisition trial per day to maintain performance (Fig. 1A1). At the completion of this 14 day period, animals underwent a 3 day retention interval during which time they received daily doses of *Ginkgo* but did not complete any water maze trials. The 3 day interval for reference memory testing was based on preliminary data indicating moderate memory of the task which could possibly be augmented by the effects of *Ginkgo* and was consistent with other reports (Ward et al., 2002). Over the next 6 days, spatial memory testing was conducted using single daily 90-second probe tests in which the hidden platform was removed. Distance traveled and travel path were recorded and analyzed. The latency to reach the zone that had previously contained the hidden platform, the number of platform zone crossings, and a search ratio calculated by the number zone crossings divided by the number of crossings into the comparable zones in each of the four quadrants.

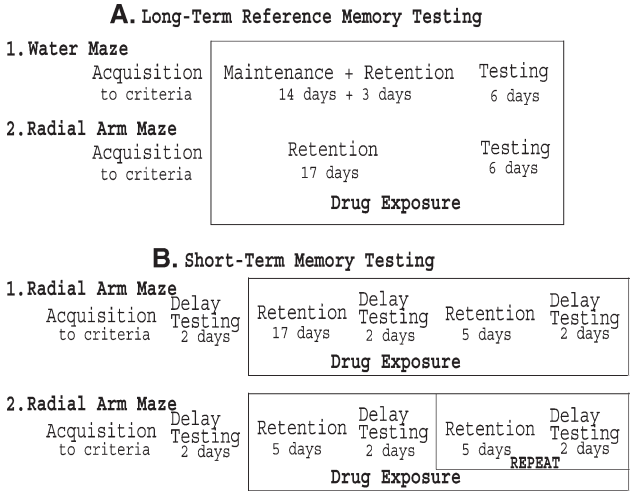

Fig. 1. The experimental design allowed for examination of long-term reference memory using the Morris water maze (A1) and the radial arm maze (A2), while short-term memory was examined using two paradigms using a 2-hour delay in the completion of the radial arm maze (B).

2.3.2. Radial arm maze

Short-term working or long-term reference memory was examined in 75 rats by using a radial arm maze with 8 arms (9 cm×63 cm) originating from a central platform (30 cm wide octagon; Olton, 1978) within a 9′×10′ testing room with additional equipment maintained in constant locations as potential extra maze cues. A reward cup containing a single sucrose pellet (Noyes Precision Food Pellets — 45 mg Sucrose) was located at the distal end of each arm. Each rat was individually placed on the RAM daily for 14–16 days, and allowed to explore the maze until all eight arms had been visited or until 6 min expired. At the completion of this acquisition training period, all animals completed the maze with less than 1 working error as indicated by a return to a previously visited arm on the same trial.

To examine the effects of *Ginkgo* on long-term reference memory using the radial arm maze, rats (n=21) were assigned to treatment groups based on performance during the last 2 days of acquisition trials (Fig. 1A2). Animals were assigned to groups so that there was no difference in performance in time needed to complete the 8-arm maze and number of working errors. These animals received low, medium, or high doses of *Ginkgo* or vehicle daily for 17 days. Animals were then tested to analyze their completion of the 8-arm radial arm maze with time to complete the maze, number of working errors (repeated arms visited), and number of arms entered prior to a repeat examined as dependent variables. In these tests, the animals completed the standard 8-arm task during a single trial with no delay interval.

The effects of *G. biloba* on short-term working memory was examined in 54 rats. Prior to assignment to treatment group, short-term spatial memory was examined by analyzing rat performance after a 2-hour delay interval. Animals were placed in the maze and removed after they had explored four of the eight arms. At the end of a 2-hour delay interval, the animals were returned to the maze which had not been altered during the interval (that is, food pellets were located only in the 4 arms which were unvisited prior to the delay interval). Performance of the rats following the delay interval testing was used to assign subjects to treatment groups so that there was no difference in total number of correct arms (i.e. arms that had not been entered prior to the delay interval) visited out of the first four choices post-delay interval between groups prior to *Ginkgo* exposure. Furthermore, during the post-delay testing the number of arms entered prior to repeated entry, the number of "pre-delay errors" associated with entering an arm that had been explored prior to the delay, and the number of "post-delay errors" associated with entering an arm that had been explored previously during the post-delay testing were also examined. Following 5 days of

drug administration, rats ($n=26$) were again given 2 days of delay testing in the RAM using the 2-hour delay interval (Fig. 2B). Drug administration continued during these 2 days. For the next 6 weeks, rats were exposed to daily drug administration. Delay testing occurred on the last 2 days of each week according to the procedure described above. A second series of animals ($n=28$) was used to control against possible learning effects of *Ginkgo* during the repeated weekly delay interval testing. In this series, animals were assigned to treatment groups based on performance in delay tests prior to drug exposure. The animals received *Ginkgo* for 17 days (the duration used in the long-term memory test) prior to the first drug-effect delay testing of short-term memory (Fig. 2B). To examine potential effects of learning under these circumstances, these animals were tested again 1 week later.

2.4. Statistical analysis

In the radial arm maze, analyses were conducted on the total number of correct arms entered out of the first four locations visited after the delay interval, number of arms entered prior to an error, and total number of errors. For water maze testing, time spent in the platform zone was recorded using the Noldus Ethovision Behavioral Analysis System. All data were analyzed statistically using the General Linear Module of SPSS for Windows 11.5. When appropriate, Tukey Post Hoc Tests were conducted. All data are expressed as mean±SEM.

3. Results

3.1. Morris water maze results

3.1.1. Long-term reference spatial memory retention in the Morris water maze

Following 17 days of exposure to the appropriate drug (the last 3 days of which involved no placement in the water maze), long-term spatial memory retention was analyzed over a six day period. The performance of rats on acquisition days 6–10 was examined using a standard ANOVA and the results indicated that there was no change in distance traveled to escape platform or trial latency indicating that the rats had learned the task prior to assignment to groups. Prior to drug exposure, rats were assigned to groups so that there was no difference in performance on the last 3 acquisition days as analyzed using a two-way repeated measures ANOVA with assigned group as a between factor and test day as a within factor. The performance of the rats during the fourteen maintenance days of drug exposure was analyzed through the use of a two-way repeated measures ANOVA with drug group as a between factor and test day as a within factor. There were no differences over time or between groups in distance traveled to the hidden platform or in the latency to reach the platform zone indicating no effect of *Ginkgo* on maze behaviors during the drug delivery phase of the experiment.

During the memory test phase, each test day consisted of a 90 second probe trial during which each subject was placed in the maze without a platform. Once again, results were analyzed using a two-way repeated measures ANOVA with drug group as a between factor and test day as a within factor. The amount of time spent in the area where the platform had been during the learning and drug phases (Fig. 2A), the latency to the first entry to the platform zone (Fig. 2B), and search ratio based on the number of platform zone crossings divided by the total number of crossings in the equivalent region in each of the four quadrants (Fig. 2C) were used as the dependent measure. Overall there was no group by time interaction in latency of the first entry to the zone or search ratio. However, there was a significant two-way interaction between dose of *Ginkgo* and memory test day ($F_{15,220}=1.97$, $p<0.05$). One-way ANOVA of the number of platform crossings over the memory test days conducted individually by group indicated that rats exposed to 20 mg *Ginkgo*/kg body weight did not exhibit the decrease in number of platform zone crossings that was seen in the other *Ginkgo* groups and vehicle-exposed animals ($p<0.05$). There was an overall effect of test days as subjects spent a decreasing percent of time in the trained zone with repeated trials in which

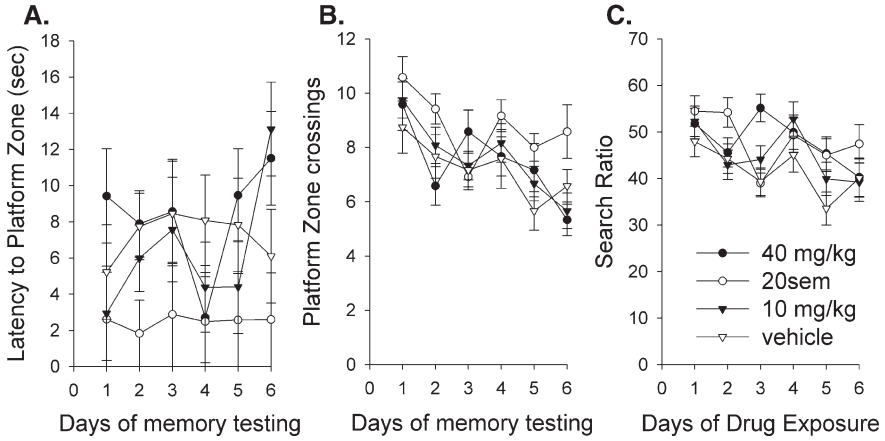

Fig. 2. *Ginkgo biloba* had no effect on long-term reference memory in the Morris water maze as measured by the latency to the platform zone (A), the number of platform zone crossings (B), or the search ratio based on the number of crossing into the zone that had previously contained the hidden platform divided by the total number of crossings into the equivalent region of each of the four quadrants (C). Over the 6 days of memory testing in the absence of an escape platform, the percent of time spent in the zone around the previous location of the platform (mean±SEM) and the search ratio decreased significantly.

the platform was missing ($p<0.001$). Of more interest, there was no main effect of drug group. These results indicate that *G. biloba* had no effect on long-term reference spatial memory during probe trials in the Morris water maze, or in the process of learning that the platform was no longer present.

3.2. Radial arm maze results

3.2.1. Long-term reference memory retention on the radial arm maze

Following 17 days of drug exposure, rats were returned to the radial arm maze to conduct long-term reference memory testing. Once again, two-way repeated measures ANOVAs revealed no differences over time or between groups in the time needed to complete the maze (Fig. 3A), the number of working errors as measured by arms repeated within the single trial (Fig. 3B), or the number of arms entered in a trial prior to making a working error (Fig. 3C). These results indicate that exposure to *Ginkgo* had no effect on improving long-term reference spatial memory using the radial arm maze.

3.2.2. Short-term spatial memory retention on the radial arm maze

For the 28 rats who received 17 days of drug prior to short-term memory testing, the effects of *Ginkgo* exposure were analyzed independently by day, as well as averaged between the two trial days using a two-factor ANOVA (drug group by time). The two-hour delay interval was chosen based on preliminary data so that the number of correct arms (approximately 2) provided the opportunity to measure an increase or decrease in post-delay performance over the duration of the experiment. The most common errors experienced during the delay testing were pre-delay errors where the rat entered an arm that had been entered during the pre-delay phase of the test. Only rarely did rats complete a post-delay error, which would be comparable to the traditional working memory error of reentering an arm during a single trial. The results indicate that there was no effect of *Ginkgo* on performance when comparing the pre-assignment delay results to the delay results on the first delay test day after 17 days of drug exposure, the second delay test day after drug exposure, or the average of the two delay test days (Fig. 4). Similarly, there was no difference in the number of arms entered prior to an error or number of pre-delay or post-delay errors during the delay testing.

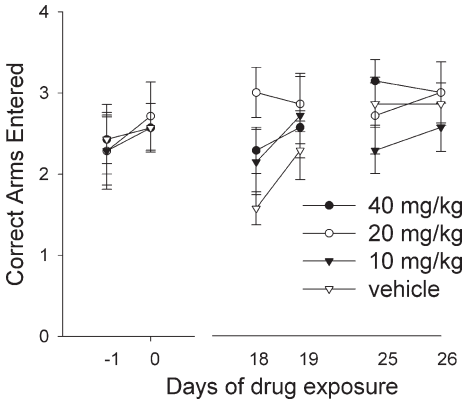

Fig. 4. *Ginkgo biloba* had no effect on the short-term working memory in the completion of the radial arm maze after a 2-hour delay interval (mean+SEM).

Following memory testing, drug exposure continued for an additional week prior to examining potential learning in two additional delay test days. There was no difference in the number of arms entered prior to an error, pre-delay, or post-delay errors during the memory tests. These results indicate that there was no effect of *Ginkgo* on learning of the memory retention task with this limited number of repeated trials, but there was a slight increase in correct arms entered post-delay interval during the second set of tests indicating that learning of the task may be occurring in all animals ($p=0.058$).

For the 26 rats who received 7 weeks of drug administration, a two-factor repeated measures ANOVA was employed with drug as a between factor (sugar, 10 mg/kg *Ginkgo*, 20 mg/kg *Ginkgo*, or 40 mg/kg *Ginkgo*) and test week as a within factor. The total number of correct arms entered after the two hour delay interval, the number of arms entered prior to an error, and the number of errors served as the dependent measures. These measures were averaged over the two test days for each week. In addition, equivalent two-factor repeated measures were

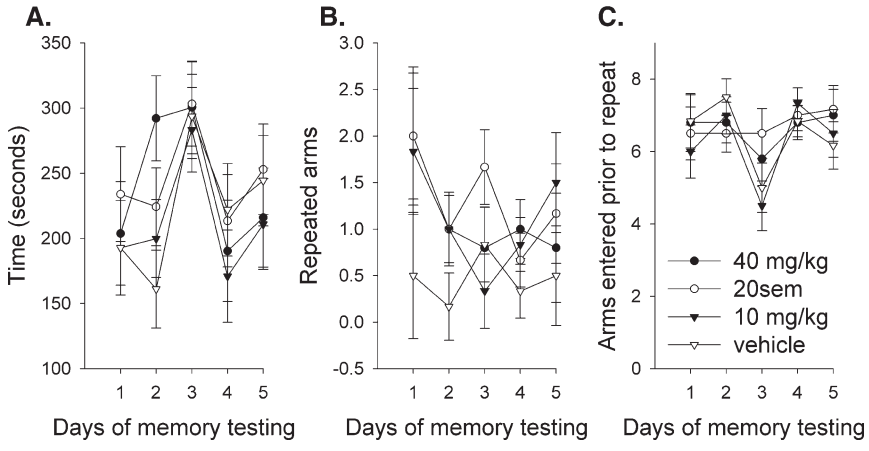

Fig. 3. *Ginkgo biloba* had no effect on long-term reference memory in the radial arm maze as measured by the latency to complete the task (A), the number of working errors (B), or the number of arms entered prior to conducting a working memory error (C). Results are represented as mean±SEM.

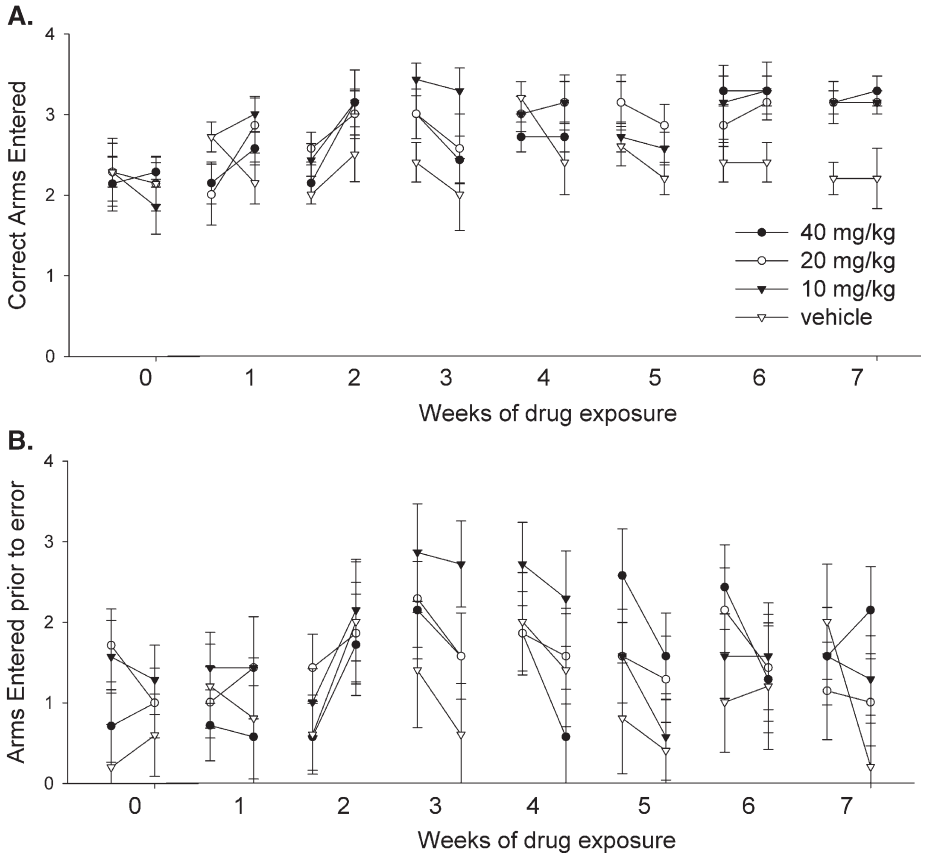

Fig. 5. *Ginkgo biloba* promoted the learning of the procedure for the completion of the radial arm maze after the 2-hour delay interval as indicated by improved performance in rats exposed to *Ginkgo* over the 7 weeks of repeated testing as measured by total number of correct arms entered (A; $p<0.001$) and arms entered prior to an error (B; $p<0.05$).

completed examining the effect of test day (first test day or second test day) across time.

There was no effect of *Ginkgo* on the initial memory test at 7 days, and there was no interaction between drug exposure and weeks of testing indicating no significant difference in performance over time based on exposure to different levels of *Ginkgo* (Fig. 5A). There was also an overall improvement in performance of animals in completing the delay task over weeks of testing as indicated by the significant main effect of test week ($p<0.001$) when the dependent variable was the results of the first day of testing each week ($p<0.001$), the results of the second day of testing ($p<0.001$), or the average results of both weekly test days ($p<0.001$). This is illustrated by the observation that rats had an average success rate of 53.8±2.7% correct arms entered on week 1 and improved to an average success rate of 73.3±2.3% on week 7. There did not appear to be a consistent difference between performance on the first and second delay tests each week. Similar results were observed for a gradual increase in the number of arms entered prior to an error (Fig. 5B) and an overall decrease in the total number of errors indicating that over time rats in all of the conditions learned to complete the delay memory task with greater success. During the repeated weeks of delay testing, there was a significant main effect of drug group on the second day of testing ($p<0.001$) that influenced the weekly average results ($p<0.05$). Tukey Post Hoc Tests of the results of the second day of weekly testing indicate that the number of correct arms entered after the 2-hour delay interval was significantly lower in the control animals when compared to animals receiving 10 mg/kg *Ginkgo* ($p<0.001$), 20 mg/kg ($p<0.01$), and 40 mg/kg *Ginkgo* ($p<0.01$). The overall poor performance by animals exposed to sugar water alone on weeks 3 ($p<0.05$) and 7 ($p<0.01$) is likely to account for the main effect of drug group, possibly indicating that these animals did not learn the procedure as well as animals exposed to *Ginkgo*.

4. Discussion

This experiment was designed to examine whether or not *G. biloba* extract had an effect on long-term reference and short-term working memory using the spatial RAM and Morris water maze testing techniques. Previous work in the laboratory had demonstrated an effect of *Ginkgo* on reference memory in the Morris water maze when tested at short, but not long intervals after training (Hoffman et al., 2004). However, analysis of previous results was often complicated by the presence of drug before or during the learning phase of the experiment (Hoffman et al., 2004; Winter, 1998). In this study, we separated the retention of spatial memory from initial acquisition by administering the *Ginkgo* supplement after the acquisition phase had been completed. This ruled out any

effects that *Ginkgo* might have had on the subjects' learning. Thus, our results reflect only differences in memory retrieval. Consistent with previous studies, *Ginkgo* had no effect on long-term reference memory using either the Morris water maze or the 8-arm radial maze. *Ginkgo* also did not promote short-term working memory in the completion of the radial arm maze after an interruption of 2 h. However, there appears to be an effect of *Ginkgo* in promoting the learning of the delay interval aspects of the test resulting from the repeated memory testing over a period of 7 weeks of *Ginkgo* exposure. This was somewhat surprising because previous work on our laboratory had indicated that *Ginkgo* had no effect during the acquisition (i.e. learning) phase in the Morris water maze (Hoffman et al., 2004). This dichotomy may reflect differential effects of *Ginkgo* on short-term, intermediate-term, and long-term memories of spatial tasks. This should not be sur-prising given the reported distinct anatomical, physiological, and molecular aspects of different memory types in animal and human models (Dudchenko, 2004; McDonald et al., 2004; Sutton et al., 2004; Kesner and Hopkins, in press).

The functional behaviors of different aspects of learning and memory have divergent neuroanatomical locations within the lateral amygdala, dorsal striatum, and hippocampus (McDonald and White, 1993). Lesions to the hippocampus impair spatial learning (Morris et al., 1982; White and McDonald, 2002), while injections of amphetamine into the hippocampus improve spatial memory (Packard et al., 1994). Research has traced short- and long-term spatial memories to specific regions of the hippocampus (Rosenzweig et al., 1999). Within the hippocampus, short-term memory is processed in the entorhinal cortex, and long-term memory is processed both in the entorhinal cor-tex and in the dorsal hippocampus (Izquierdo et al., 2000). These two sections of the hippocampus are affected by different inhibitors. It has also been suggested that memories of different lengths are controlled by different neural processes; short-term memory is a result of neurochemical changes at previously existing synapses, whereas long-term memory is a result of both neurochemical and structural changes in existing synapses and the formation of new synapses (Rosenzweig et al., 1999). Short-term and long-term memories are also affected differently by kinases; calcium kinases impair the formation of short-term me-mory, and protein kinases impair the formation of long-term memory (Rosenzweig et al., 1999; Izquierdo et al., 2000). Short-term memory was found to be inhibited by a protein ki-nase G (PKG) inhibitor and a mitogen-activated protein kinase kinase (MAPKK) inhibitor, when given into the CA1 area of the dorsal hippocampus or the entorhinal cortex. Long-term memory was shown to be influenced by the inhibitor staurosporin (STAU), calcium/calmodulin protein kinase II (KN62) inhibitor, two separate protein kinase A (PKA) and lavendustin A (LAV) inhibitors (Izquierdo et al., 2000). These long-term memory inhibitors were placed into the CA1 area of the dorsal hip-pocampus. Furthermore, pharmacological treatments can result in impairment of short-term memory without affecting long-term memory (Izquierdo et al., 1998), indicating that different aspects of memory may have separate mechanisms. Consequently, it is not surprising that *Ginkgo* might have differential effects on short- and long-term memory systems.

While a number of molecules have been isolated, two major fractions from *Ginkgo* extracts have been identified as having biological effects. The terpenoids, including ginkgolides are platelet-activating factor antagonists and may improve blood circulation (Smith et al., 1996). These molecules may also have anti-inflammatory effects (Oberpichler et al., 1990). The flavonoids, such as ginkgo-flavone glycosides may have antioxidant effects through free radical scavenging (Smith and Luo, 2003) and inhibition of monoamine oxidase (White et al., 1996). In previous studies *Ginkgo* has been shown to enhance neuronal plasticity (DeFeudis and Drieu, 2000; Gohil and Packer, 2002). *Ginkgo* may also play a more direct role in neurotransmitter signaling throughout the brain by increasing the rate of acetylcholine turnover (Itil and Martorano, 1995; DeFeudis and Drieu, 2000), increasing muscarinic acetylcholine receptor density (Racagni et al., 1988; DeFeudis and Drieu, 2000), decreasing the β-amyloid induced loss of choline acetyltransferase activity in the hippocampus (Tang et al., 2002), and stimulating binding to muscaranic acetylcholine receptors (Itil and Martorano, 1995). Similarly *Ginkgo* increases serotonin receptor density (Racagni et al., 1988; DeFeudis and Drieu, 2000) while the flavonoids from the extract mediate serotonin activity (Ramassamy et al., 1992). Ginkgo can also increase norephinepherine turnover (Brunello et al., 1985), while increasing alpha 1-adrenergic (DeFeudis and Drieu, 2000) and alpha 2-adrenergic (Huguet and Tarrade, 1992) receptor densities but decreasing beta-adrenoreceptor density (Racagni et al., 1988). This wide range of pharmacological actions of *Ginkgo* could provide for the beneficial effects of *Ginkgo* reported in studies with cognitively-impaired subjects, and may contribute to the differential effect on different memory types.

Consistent with the previous literature, the current study indicates mixed effects of *G. biloba* on learning and memory. This is one of the first studies that has examined the effects of *Ginkgo* during memory testing that is not confounded by possible differences in learning resulting from the presence of the drug during acquisition trials. Our results indicate the *Ginkgo* does not have a significant effect on long-term reference memory or short-term working memory. However, with prolonged testing, exposure to *Ginkgo* appears to contribute to learning of the delay task on the radial arm maze.

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