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# Effects of Ginkgo Biloba on Memory in Rats for Maze Tasks

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*Previous studies have investigated the effects of an extract of ginkgo biloba on acquisition, performance and retention in rats. It has been suggested that ginkgo increases mental precision by increasing blood flow to the brain. To determine whether ginkgo is a memory enhancer two female Sprague Dawley rats were tested for maze performance during four stages. Stages included four days of maze performance and four days of no maze performance. Subjects were tested in each stage with or without ginkgo in their water supply. It was predicted that subjects would show improved maze performance as measured by a decrease in the number of wrong turns in reaching the goal box when ginkgo was mixed with their water supply. A one-way ANOVA was used to calculate the total number of wrong turns for all four stages. Results of the analysis were not statistically significant. The present data were not consistent with results of previous studies with rats. Continued research is encouraged to investigate the external validity of those studies that proved ginkgo's effects to be beneficial to enhancing memory.*

Heise (1984) defined memory as the retention of learned memory over a period of time. Memory ". . .is a network of thoughts that's created as chemicals called neurotransmitters— acetylcholine, in particular—and electrical impulses trace pathways through the brain" (Business Week, 1 999, p. 1 1 6). The number of synapses within brain cells increase with the addition of new information.

The deterioration of memory over time remains a mystery. Memory loss has been attributed to a decreased number of brain cells or damage to a particular area in the brain. Dementia also represents a deterioration of memory and many factors contribute to dementia. 'Dementia is a pathology that is not part of normal aging,' says Andrew Morgan, chief of the neuropsychology of aging at the National Institute of Aging" (Business Week, 1 999, p. 1 16). Alzheimer's, the major cause of dementia in old age, results from amyloid plaques in the hippocampus, which damage brain cells that produce acetylcholine. Alzheimer's can also trigger inflammation of the brain and cause free radicals to develop harmful oxygen molecules within the brain. With free radicals, " ' . . . fatty acids in the membranes of brain cells become oxidized, and the brain stops functioning properly,' says Jerry Cott, head of adult psychopharmacology research at the National Institute of Mental Health" (Business Week, 1 999, p. 1 16).

Many "remedies" to Alzheimer's and other forms of dementia have been tested. Some of the so-called remedies that Cowley and Springen (1997) have suggested involve acetyl L carnitine, DMAE (a nutrient that stimulates the manufacture of acetylcholine), ginkgo biloba, ibuprofen, phosphatidylserine, and vitamin E. Ginkgo is the most popular remedy of choice.

Kleijnen and Knipschild (1992) used the term cerebral insufficiency to label twelve symptoms that were signs of dementia in elderly people. The twelve symptoms that could be relieved by ginkgo treatment were difficulties of concentration and of memory, absent mindedness, confusion, lack of energy, tiredness, decreased physical performance, depressive mood, anxiety, dizziness, tinnitus and headache. Many western countries such as France and Germany used standard dosages of ginkgo in prescription medicine. "It has recently been approved in Germany for the treatment of dementia" (Le Bars, Katz, Berman, Itil, Freedman, and Schatzberg, 1 997, p. 1327). In the United States there is no standard dosa-ge for ginkgo. Recommended dosages have ranged from 60 to 80 mg twice a day to 40 mg tablets three times a day. However, no specific dosage has been recommended as effective due to the lack of research.

Extracts of ginkgo are obtained from leaves of the ginkgo tree. The most popular extract of ginkgo biloba in Europe is EGb 761 which is standardized with " . . . ginkgo-flavone glycosides (24%) and terpenoids (6%)" (Kleijnen and Knipschild, 1992, p. 136). The ginkgo-flavone glycosides are used to offset free radicals while the terpenoids reduce inflammation and prevent blood clotting. As with other herbs, ginkgo ". . .has not been generally accepted by the federal drug administration for general distribution" (Kolakowsky and Parente', 1997, p. 12) and its potency cannot be predicted.

Despite the effects of ginkgo on memory it should not be considered as a treatment for conditions that cause dementia. "'Ginkgo biloba is not a smart pill,' says Varro Tyler, PhD, professor emeritus at the Purdue University School of Pharmacy and a leading expert on herbal supplements, and 'it won't work for everyone' "(Tufts University Health & Nutrition Letter, 1997, p.8). Ginkgo seems to benefit people who have trouble with blood flow to the brain due to advanced age and clogged arteries. The lack of blood flow results in loss of short-term memory and difficulties in concentration. Ginkgo acts as a blood thinner, which helps circulation of blood.

Experiments on the effects of ginkgo started in the late eighties. Two experiments demonstrated that ginkgo increased blood flow. Kleijnen and Knipschild (1 992) reported on a study by Koltringer, Eber, and Lind (1989) that investigated the effects of ginkgo on blood flow. In a randomized double blind experiment, 60 subjects were given 200-mg dosages of ginkgo for four consecutive days, which led to an increase in skin perfusion and a decrease in blood viscosity and elasticity.

In another experiment studying blood flow Jung, Mrowietz, Kiesewetter, and Wenzel (1990) (cited in Kleijnen and Knipschild, 1 992) performed a randomized single blind study using 10 subjects. Each subject received 1 12 milligram dosages of LI 13 70, which was a standardized preparation of ginkgo. Upon completion of the experiment, the subjects showed increased blood flow in capillaries and decreased erythrocyte aggregation.

Le Bars, et al. (1997) gained worldwide attention for their research at the New York Institute for Medical Research. These investigators conducted a randomized double blind study with 309 subjects. They were randomly assigned to experimental and control groups. The experimental group was given 120 milligrams of ginkgo per dose while the control group received a placebo for 52 weeks. At the end of this time the Alzheimer' s Disease Assessment Scale, which is a standardized test to measure cognitive function, was administered to subjects. The results indicated an improvement in cognitive function for 27% of subjects who received ginkgo compared to 14% who were given the placebo. The researchers concluded that ginkgo was safe and capable of delaying the process of dementia for six months to a year. However, the long-term benefits of ginkgo are still unknown.

Ginkgo experimentation has not been limited to human subjects. Experimentation with rats has also been conducted. J.C. Winter

(1997) conducted research on the effects of EGb 761 on cognitive behavior of rats in a radial maze. In this experiment 20-month-old male Fisher 344 rats were assigned a diet that included either ginkgo biloba extract or no extract. Over several weeks, the animals performed tasks in the radial maze that required them to master new challenges and retain learned information over time. Results showed that rats receiving ginkgo biloba extract learned quicker and made fewer errors than the control group, and unexpectedly, lived an average of five months longer. Winter found that a significant positive relationship between the amount of active ingredient and degree of learning. The standard dose during most of the study was 50 mg/kg. However, one sub-group of animals was assigned to receive EGb 761 in doses of 1 00 mg/kg followed by 200 mg/kg, interspersed with periods when they performed tasks while receiving no extract. Results showed that at the highest dose, errors declined by 50 percent.

A year after his work with cognitive behavior in rats, Winter and Timineri (1998) conducted a study with rats to investigate the stimulus properties of EGb 761 . The investigators hypothesized that ginkgo administered via intraperitoneal injections could trigger stimulus control. The subjects were nine male Fisher-344 rats. Subjects were tested on performance in an animal test chamber " . . . containing two levers mounted at opposite ends of one wall" (Winter, 1998, p. 544). Upon pressing the appropriate lever, ". . .a dipper. . .delivered 0.1 ml of sweetened condensed milk diluted 2:1 with tap water" (Winter, 1998, p. 544). Prior to testing, subjects were given a dosage of 10 mg/kg of ginkgo in order to establish "stimulus control" (Winter, 1998, p. 544). A fixed-ratio 10 schedule was used for liquid reinforcement during testing sessions. Every tenth response to the appropriate lever was reinforced. "Drug-induced stimulus control was assumed to be present when, in five consecutive sessions, 83% or more of all responses prior to the delivery of the first reinforcer were on the appropriate lever" (Winter, 1998, p. 544). The results indicated that rats required an average of 24 sessions to reach the desired criterion. These findings supported the hypothesis.

Eva Winter (1 989) conducted an experiment to study the effects of EGb 761 on learning and memory in rats. Performance, acquisition, and retention in mice were measured with an operant box. During the conditioning stage, subjects were trained for 30 days in order to learn a two-lever response that resulted in food reward. Subjects were randomly assigned either to an experimental or a control group. Experimental groups received a 1 00 mg/kg dosage of ginkgo. Control groups received a placebo. "Drug treatment started four and eight weeks before the training and was maintained until a retention test 1 0 weeks after it" (Winter, 1 989, p. 109). Performance involved lever pressing in a sequence (correct response) that led to the availability of food reward. Acquisition was determined by the ratio of correct to incorrect responses. Retention was measured 1 0 weeks after the conditioning stage. The results revealed that the experimental groups made more correct responses than the control groups. Experimental groups also showed a faster acquisition rate than control groups. Retention testing proved "EGb 761- treated mice performed more correct responses than the controls. . . " (Winter, 1 989, p. 1 1 3). The researcher concluded that ginkgo quickened acquisition, increased performance, and improved retention of learned responses.

Gajewski and Hensch (1999) conducted a study on the effects of ginkgo on rat performance in a maze. Five rats were tested with a within-subjects design. Subjects ran through four mazes. Maze performance was based on the amount of wrong turns before reaching the goal box. Ginkgo availability was alternated with the use of each maze, i.e.; a mixture of ginkgo and water was available for mazes two and four while regular water was available for mazes one and three. Results indicated a decrease in wrong turns by 8% and 60% when ginkgo was added to the drinking water. The decrease was not as significant after the reintroduction of ginkgo. "The decrease in the number of wrong turns. . .when ginkgo was reintroduced into the diet was less dramatic (5% to 26%)" (Gajewski and Hensch, 1 999, p. 483). The researchers concluded that rats demonstrated improved maze performance (fewer wrong turns) whenever the ginkgo

water mixture was available, implying that ginkgo improved memory.

Although still in the early stages, research on ginkgo biloba seems promising with respect to memory improvement. Thus additional research is necessary and important to understand how ginkgo affects the nervous system and what those effects are.

In the present study effects of ginkgo biloba on memory in rats was tested on maze performance during four stages. Maze performance was measured by the number of turns required to reach the goal box. Stages included four days of maze performance and four days of no maze running. Subjects were tested in each stage with or without ginkgo in their water supply. The researcher hypothesized that the availability of ginkgo would result in a decrease in the number of wrong turns required to reach the goal box.

## METHOD

*Design*

This experiment involved a within subject design with an A-B-A-B reversal.

Participants

Five white female Sprague Dawley rats were purchased from Charles River Breeding Laboratories, Inc. (Wilmington, MA) at the age of twenty-one days. At the beginning of this experiment they were approximately sixteen months old, with an average weight of 250.6 g prior to being food deprived to 90% of their body weight. During the food deprivation period three rats died. The researcher continued the study with two subjects. Animals were housed one rat per cage and maintained with constant temperature conditions. Illumination was provided by a fifty-five watt light bulb that operated on a twelve-hour cycle. Access to water was unlimited and access to food was unlimited prior to deprivation.

Apparatus

Animals were housed in standard plexiglass cages measuring approximately 23 .5 x 1 5 x 9 in. Four separate mazes were constructed for use in this study. Each maze consisted of four 11.5 x 5 x 5 in connecting arms, four 12 x 5 x 5 in dead ends and two 7.5 x 6 x 5 in start and goal boxes. Pictures of the maze pieces appear in Appendix A. All mazes were placed on the floor. Pictures of the actual mazes appear in Appendix B. A Denver Instrument Company digital scale (TR-402) was used to measure weight in grams. An Ohaus digital scale (CS-200) was used to measure water in ounces. A Sony Handycam Vision camcorder (CCD-TRV67) was used with a Sony P6-120MPD videocassette to record all rat activity in the mazes.

*Materials*

Herbal Authority Ginkgo Leaf manufactured by United Vitamin Manufacturing Corp. (Bohemia, NY) was used in this study. This liquid form of the herbal extract was mixed with water to be ingested orally by the subjects. A calibrated dropper was purchased with the herbal extract for ease of measuring. The rats received a dosage of fifty mg/kg of ginkgo mixed with three ml of water for four days preceding and during Stages 2 and 4 of this research. Water intake was recorded daily. Animals were fed Hartz Guinea Pig Pellets.

Procedure

Animals were initially weighed in order to determine approximate body weight. For 2 weeks food amount was measured in order to determine how much each rat actually consumed. Once intake baseline was obtained food deprivation was initiated. Daily food amounts were reduced until animals reached 90% of their initial body weight. During the experiment animals were maintained at this weight.

An A-B-A-B reversal design was utilized in this study. During Stage 1 the rats were tested on the first maze (Ml) design without any ginkgo. Upon completion of Stage 1 ginkgo was made available to each subject. The water was changed to include a dosage of fifty mg/kg of ginkgo mixed with three ml water. This ginkgo and water mixture was available during the four nonrunning days that separated Stages 1 and 2 and the four days of maze running in Stage 2. A different maze (M2) design, similar in length and turns to the first one, was used to measure maze performance.

At the conclusion of Stage 2 the ginkgo and water mixture was replaced by plain water. The water was available for four nonrunning days, which initiated Stage 3. At the conclusion of these four days another maze (M3) design, similar in length and turns to those used in Stages 1 and 2, was used to measure maze performance for four days.

Following maze testing the water was again replaced with the water and ginkgo mixture. This mixture was available during four non-running days of Stage 4. A fourth and final maze (M4) pattern, similar in length and turns to those used previously, was used to measure maze performance for four more days. After this final session animals were returned to food and water ad lib.

Results

The dependent variable in this study was number of wrong turns in a maze. The independent variables were experimental stages and whether or not ginkgo was available in the subject's water supply. Two performance measures were calculated. The total number of wrong turns for each rat for each of the 16 trials in all four stages appears in Figure 1. These data were simply determined by adding the number of wrong turns for each subject in each stage. For each subject there were four values. The investigator also calculated the increase and decrease in the number of errors between the stages. These data appear in Table 1. Difference scores were obtained by subtracting the total number of wrong turns for the given stage from the total number of wrong turns in the subsequent stage (B1-Al, A2-B1, and B2-A2). This score was then divided by the total number of wrong turns in the previous stage and rounded to two decimal places. Negative scores indicated an improvement from the previous stage.

Subject 1 performed a total of 260 wrong turns for all four stages with a mean of 65.00 and a standard deviation of 27.43. Subject 2 performed a total of 300 wrong turns for all four stages with a mean of 75.00 and a standard deviation of20.12. Both subjects performed a total of 165 wrong turns for Stage 1 with a mean of 82.50 and a standard deviation of 13.44. For Stage 2 both subjects performed a total of 1 78 wrong turns for with a mean of 89.00 and a standard deviation of 2 1 .2 1. Both subjects performed a total of 90 wrong turns for Stage 3 with a mean of 45 .00 and a standard deviation of 25 .46 and for Stage 4 both subjects performed a total of 127 wrong turns with a mean of 63.50 and a standard deviation of 4.95. For Subject 1 the mean difference score was .21 and the standard deviation of 1.12. For Subject 2 the mean different score was -.01 and the standard deviation of .41. A one-way repeated-measures analysis of variance (ANOVA) was calculated to examine the total number of wrong turns for all four stages. The results of the analysis did not reveal a significant effect for number of wrong turns in each stage of maze performance as a function of availability of ginkgo in water, $F(3, 4) = 2.42$, $p > .05$.

## DISCUSSION

Subjects performed maze running in four different mazes with or without the availability of ginkgo biloba. Several observations were made in reviewing the individual performances of the subjects (see Figure 1). The first subject showed an improvement in performance when ginkgo was first introduced into the water supply during Stage 2. The number of wrong turns between Stages Al and B1 decreased by 18. On the other hand, subject 2 showed a decline in maze performance between the same stages. The total number of wrong turns between Stages Al to B1 increased by 31. Secondly both subjects showed improved performance when ginkgo was removed from their drinking water (B 1 to A2). The number of wrong turns for subject 1 decreased by 47. Similarly, the number of wrong turns for subject 2 decreased by 41. Also both subjects showed a difference in maze performance when ginkgo was reintroduced a second time. Number of wrong turns for subject 1 increased by 40 and this value for subject 2 decreased by 3. It should be noted that subject 1 showed a 148% increase in errors between Stages A2 and B2. Gajewski and Hensch (1999) tested maze performance on rats using five subjects. The researchers observed that there was improved performance when ginkgo was introduced, which was predicted. They also noted that when ginkgo was not available in the water supply, all five subjects showed a decline in performance as predicted. A final finding was that four of the five subjects improved performance when ginkgo was reintroduced.

Although the results of this study were not statistically significant, the effects of ginkgo seemed to differentially effect the two subjects in this study. Maze performance for subject improved in each of the first three stages (Al to B 1, B 1 to A2) but then errors

increased during the final stage (see Table 1). Subject 1 made 27 errors in Stage 3 and 67 errors in Stage 4. The subject's percent increase in errors may have been caused by a ceiling effect, which perhaps limited the possibility of improvement. In contrast, initial maze performance for subject 2 was very poor with respect to number of errors but gradually improved in the final three stages (B 1 to A2, A2 to B2). According to Gajewski and Hensch (1 999) "If poor initial performance on the task were due to poor circulation in the brain, the largest improvements would be expected for mice whose initial performance was the worst" (p.483).

Due to time constraints many confounds in this study could not be avoided or resolved. This researcher intended to test five subjects. However prior to maze performance, three subjects died. The original food deprivation procedure used in this study involved reducing subjects body weight to eighty percent of their initial weight. Food reduction, plus stress and age may have contributed to the deaths of the three subjects. Perhaps all five subjects could have been used in data collection if food deprivation had initially involved reducing subjects body weight to ninety percent of their original weight.

The weight of the rats prior to maze testing may have been another confound. While weights ranged from 284.2 g to 223.4 g, rats were food deprived by the same amount of food in order to reach ninety percent of their body weight. This deprivation procedure may have been stressful for heavier rats who were accustomed to eating a certain amount of food. Stress may have contributed to greater anxiety during maze performance, thus causing an increased number of wrong turns during maze performance. The Yerkes-Dodson law states, ". . .that easy tasks are best accomplished with a relatively high level of [ but more difficult tasks are better accomplished with a low or moderate level.. ." (Ormrod, 1999, p. 424). In order to reduce the effect of this confound anxiety levels within subjects should be reduced. Animals of similar weight should have been used in the research.

Other methodological problems involved the apparatus and equipment. The researcher maintained all maze pieces in the condition in which they were received. As shown in Appendix A, only start and goal boxes had wire bottoms. The wiring may have affected the results of the research by serving as a marker for the start and end of the maze. This confound could have been corrected by removing the wire from the start and goal box. Installing wire on all maze parts would have been problematic since parts had to be moved to create different maze designs.

During Stages 1 through 4 the amount of water or ginkgo mixture consumed was recorded. However, during the initial placement of water bottles into rat cages the researcher observed that the contents of the water bottle would drip for several seconds. This water loss could not be recorded or controlled for due to the construction of the water bottles. No solution to this problem could be developed to avoid this confound.

Another problem in this study involved testing times. Data collection during days of maze performance occurred at different times of the day depending on the researcher's schedule. The majority of the data was collected during the late afternoon. The researcher observed that subjects seemed livelier during certain times of the day. When maze performance was measured during the early morning or late afternoon rats were constantly exploring the maze parts. When they reached the goal box and then were placed back in the start box, subjects appeared quick to search for the goal box again. On the other hand, maze performance during the early afternoon was different. Rats appeared unmotivated to explore the maze parts. When they finally reached the goal box and were placed back in the start box, the food reward did not seem to motivate them to search for food again. It seems reasonable to assume that activity levels vary according to some circadian cycle or other biological cycle. Thus maze performance could have been affected by time of day for testing since this variable was not controlled for.

Extraneous noises may have interfered with subjects' performance in this study. During maze running the researcher placed a notice on the door to prevent people from entering the testing area. In addition, all appliances capable of producing sounds were

unplugged to prevent unnecessary noises. However, not all sounds could be prevented. The testing area was located next to a main hall that led to a main entrance to the building. Also, the men's restroom was located next to the testing area. The researcher heard the restroom doors open and close during times of heavy traffic in the halls.

A final problem with this study was the fact that rats had been used in previous research. This may have confounded the results. It is unknown to the researcher as to what types of research the rats had previously participated in or the outcomes of that research.

Some data were collected in this study but not statistically analyzed. The amount of food distributed, leftover and consumed was recorded daily for each subject. In addition, the researcher recorded the amount of water or ginkgo mixture consumed. Future researchers should maintain careful records of this data and include it in statistical analyses.

It is important to continue research in this area because there is no available cure for dementia. Alzheimer's Disease is the most common form of primary degenerative dementia and the fourth leading cause of death in persons 65 years of age and older. Cognex, an FDA approved acetycholine enhancing drug is currently used to slow the process of Alzheimer's. However, it is not easily tolerable because of unpleasant side effects. While it is unclear whether or not ginkgo biloba has the ability to enhance memory or slow the dementia process, research should continue with emphasis "...to identify the neurochemical mechanisms responsible for this possible enhancement of memory.. ." (Gajewski and Hensch, 1999, p. 484).

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