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Abstract

The effects of chronic oral administration of a standardized Ginkgo biloba extract (GBE) on the performance of two classical memory tasks was investigated. Long-Evans rats, 3 and 16 months of age, received daily doses of diets with or without GBE (100 mg/kg) for a period of 6 weeks before testing and throughout the experiment. A 2 x 2 (Age x Treatment) ANOVA indicated that GBE treatment prevented learning on a radial maze task as measured by number of days to criterion, $F(1, 28) = 5.73$, $p < .05$. *A 2 x 2 x 19 (Age x Treatment x Day) ANOVA was used to analyze the number of correct responses from Days 1 to 19. The results showed an interaction between Age and Treatment* $F(1, 28) = 6.34$, $p < .05$; *namely young treated rats performed worse than other groups. Analysis of response frequency by a 2 x 2 (Age x Treatment) ANOVA revealed a significant interaction* $F(1, 28) = 4.41$, $p < .05$, *and a subsequent post hoc test indicated that the young treatment group responded notably more than the old groups. Measured performance on a one-way passive avoidance task indicated that neither treatment nor age had an effect. The results suggest that at the prescribed dose level and duration, GBE impairs performance on a radial maze task, particularly in young rats.*

The *Ginkgo biloba* tree is one of the oldest species of trees alive today. For over 5000 years, Chinese medicine has recognized the medicinal uses of *Ginkgo biloba* leaves to "benefit the brain" and allay a variety of pathological symptoms (Murray, 1990). Pharmacological and clinical studies published over the last few years have demonstrated the efficacy of a standardized *Ginkgo biloba* extract (GBE) for the treatment of a wide variety of age related conditions like edema, impotence, vertigo, tennitis, vigilance, and a number of pathologies associated with memory disorders. Currently, GBE is one of the most prescribed medicines in Europe for the treatment of pathological conditions.

GBE's are made from the green leaves of the *Ginkgo biloba* tree. The active constituents of the standardized extracts include 24% glucoflavonoids, 6% terpenes (bilobalides and ginkgolides), and proanthocyanidies. Organic acids are added primarily to increase the compounds solubility and facilitate circulatory transportation to vital organs (Drieu, 1988). In regards to behavioral dysfunctions associated with memory, studies (e.g., Taylor, 1988) have suggested that GBE causes numerous morphological and biochemical changes in the brain which improve the memory of individuals suffering from Alzheimer's Disease, cerebral vascular insufficiency, and those affected by normal senescent memory loss. It is well known that memory dysfunctions result from a decline of cholinergic muscarinic activity in the hippocampus (Deutch, 1971; Gold & McGraugh, 1975).

Numerous studies have cited similar declines in the brains of those suffering from forms of presenile and senile dementia (Bartus, Dean, Beer, & Lippa, 1982; Davis & Moloney, 1976). An inverse relationship between age and acetylcholine transmission has also been identified in animals and humans (Bartus et al., 1982; Taylor, 1988). Taylor has demonstrated that chronic oral administration of GBE increases the number of post-synaptic muscarinic acetylcholine receptors in the hippocampus regardless of age, but more so in aged rats. Cognitive deficits can also result from a reduction in cerebral blood flow, and GBE has been recognized for its ability to dilate arteries leading to the brain, thereby restoring cerebral circulation of glucose and oxygen which is essential for proper brain function (Karcher, Zageman, & Krieglstein, 1984; Rapin, Lamproglou, Dreiu, & Defeudis, in press).

Serotonin is another neurotransmitter that has been implicated as playing a critical role in memory; however, the correlation between serotonin levels and memory is less clear. In man and rodents, serotonin levels decrease in the cerebral cortex with age (Gottfries, 1979; Huguet, Drieu, & Piriou, 1994). Lower serotoninergic activity has been identified as a major contributor to mood disorders like depression, which are more prevalent with age. Coupling the facts that one of the primary symptoms of depression is memory impairment, and 5-HT-ergic agonists (e.g., tricyclics and MAO inhibitors) alleviate this symptom, the correlation between cerebral serotonin and memory appears to be positive. In this regard, recent studies by Huguet et al. (1994) demonstrated that treatment of GBE increases the number of 5-HT1A receptors of the cerebral cortex in senescent rats; nonsignificant increases in young rats were also found.

Elucidation of the mechanisms by which GBE restores cerebral neuronal systems is not complete. Flavonoid derivatives are known to exhibit free radical scavenger activity which prevents lipid peroxidation of cell membranes (Pincemail & Deby, 1988). Neuronal membranes are uniquely more susceptible to peroxidation due to their high density of polyunsaturated fatty lipids. This scavenger activity alone maintains the relationship between neuronal membranes and receptors and also maintains membrane fluidity. Furthermore, it is believed that GBE enhances receptor density by restoring essential protein synthesis (Huguet et al., 1994).

In addition to cerebral modulation, a few recent studies indicate that GBE affects the endocrine system by reducing plasma corticoids and catecholamines released by the adrenal cortex and medulla, respectively (Rapin et al., in press). Since elevated levels of these hormones are associated with anxiety-like activity and the disruption of learning performance (Hurwitz, Robinson, & Barofsky, 1971), GBE is said to exert "anti-stress" activity. By decreasing anxiety levels, GBE can prevent a number of maladaptive learning behaviors ranging from hyperactivity to an inability to make decisions (LePoncin, Aymonod, Brillon, Pesquies, & Rapin, 1980). In humans, the detrimental effects of anxiety are more pronounced in older subjects and can be particularly deleterious to learning.

Clinical studies have demonstrated that chronic GBE treatment alleviates behavioral deficits associated with memory in individuals suffering from senility and many types of dementia (GeBner, Voelp, & Klasser, 1985;; Taillandier et al., 1988; Warburton, 1988). Most positive effects of GBE result after subjects receive treatment for at least 3-4 weeks, and the duration of treatment is positively correlated with the beneficial effects (e.g., Taillandier et al., 1988). A smaller number of studies have shown that treatments can also improve the memory and performance on tasks in normal young volunteers (Subhan & Hindmach, 1984).

Although many studies have examined the biochemical and morphological changes in rodents, few have investigated how these effects determine behavior. As a whole, the cited studies indicate that GBE primarily causes restorative physical changes in older subjects in biological systems important to the memory process. The present study was aimed at investigating the efficacy of chronic oral treatment of GBE on performance of memory tasks in young and old rats. Given this information, one would also expect to witness improved performance on memory tasks in older subjects.

Experiment 1

Method

Subjects. The subjects were 32 experimentally naive female Long-Evans rats weighing between 181-359 g at the beginning of the experiment and were classified as either young or aged, approximately 3 and 16 months old, respectively. Rats were housed separately on a 12 hr light-dark cycle in a temperature controlled room (23 °C). One

week prior to shaping, subjects were put on a 23 hr food deprivation schedule. Weights were monitored, and animals were given additional rations if their body weight fell below 85% of free-feeding weight.

GBE Treatment. Six weeks prior to shaping, both young and aged rats were assigned to either an experimental or control group. The young experimental (YE) and old experimental (OE) groups were placed on a GBE-rich diet; each rat received 100 mg/kg of GBE daily. The supplement, marketed by Enzymatic Therapy®, is standardized to contain 24% Ginkgoheterosides. Weighed doses were masked in approximately 2 g of instant whipped potatoes and 0.2 g of sucrose. Young control (YC) and aged control (AC) groups received the potato-sucrose mixture without added GBE. Subjects were fed the supplement at 8:00 am daily for the duration of the experiment (approximately 11 weeks) and were weighed every third day to verify appropriate dose. Dose levels were based on previous studies (e.g., Taylor, 1988).

Materials. The rats were tested on an elevated radial maze similar to the one described by Olton and Samuelson (1976). This apparatus has long been recognized as a valid tool for investigating the influence of pharmacological compounds on memory (Olton, 1987). The maze was elevated 94 cm above the floor. Each arm was 7 cm wide and extended 85 cm from the center platform; a 2.5 cm wall surrounded each arm, and a small cup was placed in each arm. An enclosed box over the center platform could be raised or lowered to control entry into all arms. The experimenter regulated this box from a separate room via a system of fishlines; rats were visually monitored by camera.

Procedures. Each rat was randomly assigned four different arms which were baited at the beginning of each trial with two Bioserve® pellets. These assigned arms remained constant for each rat throughout shaping and test trails. Testing occurred 7 days a week between 2 p.m. - 6 p.m. The order of testing each group (i.e., YC, OC, YE, and OE) was rotated each day, and the maze was washed daily with dilute ammonia water at the end of testing.

Shaping trials were conducted on Days 1-10, during which all cups (baited and nonbaited), were daily placed successively farther away from the central platform. Five Bioserve® pellets were placed in the arms with the baited cups; leading from the center platform to each cup. At the beginning of a trial, each animal was placed on the central platform under the enclosed box. When the box was raised, the rat was allowed to explore the maze for 3 min, during which the number of arm entries was recorded. The criterion for arm entry was placement of all 4 feet into an arm. On Days 5-10, subjects explored the maze for 3 min or until all arms were entered. Those subjects with fewer than two entries per session, were physically placed at various locations in the maze and encouraged to explore the maze. By Day 10 all rats were responding with approximately the same frequency.

Testing was conducted from Day 11-41. All cups were positioned at the distal end of each arm, and no Bioserve® tablets led to baited arms. Testing procedures were similar to shaping trials, however each rat was allowed only four arm entries; upon return to the central platform after the forth entry, the enclosed box was lowered. All subjects were tested from Days 11-30, during which the number of correct responses was recorded. The number of days to criterion for each individual subject was also recorded. A subject reached criterion when she made three of four correct responses for three consecutive days. After Day 30, testing continued only for those subjects who did not reach the criterion. By Day 41, all subjects had reached the criterion.

Results

Results from a 2 x 2 (Age x Treatment) analysis of variance on the response frequency during Days 1 to 5, demonstrated significant age effects, $F(1, 28) = 16.13$, $p < .001$, and a significant interaction, $F(1, 28) = 4.41$, $p < .05$. Cell

means for the YC, YE, OC, and OE groups were 42.0, 59.6, 31.8, and 26.9,

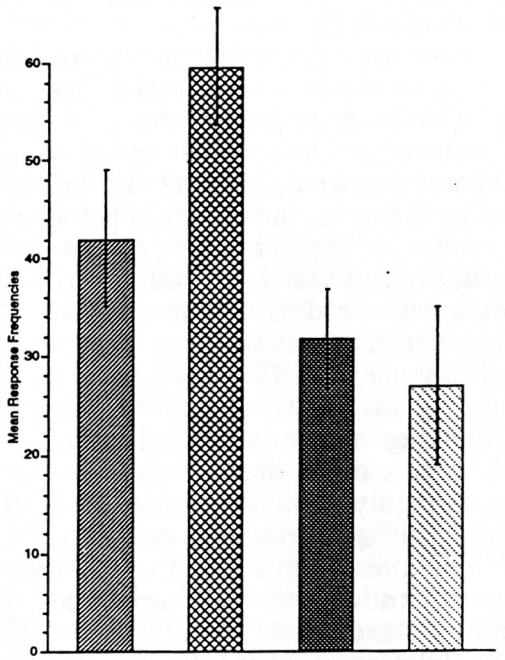

Figure 1. Mean response frequencies during shaping phase of radial maze task.

respectively (See Figure 1). A Scheffe post hoc test ($p < .05$) showed that the YE group response frequency was

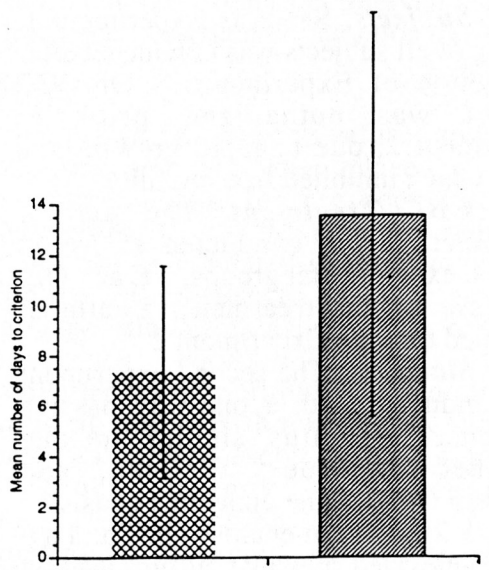

Figure 2. Mean number of days to criterion on radial maze task.

significantly higher than the OE and OC, $F(3, 28) = 7.26, p < .05$.

The mean number of days to criterion for each group is displayed in Figure 2. Means of 7.38 and 13.56 days, respectively, were needed for the control and experiment groups to reach criterion. A 2 x 2 (Age x Treatment) analysis of variance verified that the experimental group needed significantly more days than the control group to reach criterion, $F(1, 28) = 5.73, p < .05$. The mean days to criterion for individual groups were as follows: YC, 4.88; YE, 15.50; OC, 9.88; and OE, 11.63.

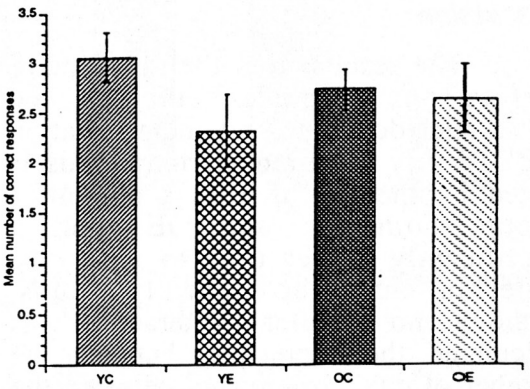

Figure 3. Overall mean number of correct responses per day during test phase of radial arm maze.

Analysis of the number of correct responses from Days 1 to 19 was conducted by a 2 x 2 x 19 (Age x Treatment x Day) split-plot analysis of variance. The results showed significant

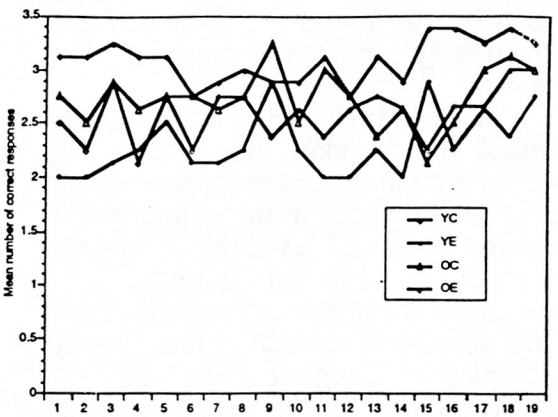

Figure 4. Mean number of correct responses during test phase of radial arm maze, Days 1 - 19.

treatment effects, $F(1, 28) = 10.43$, $p < .01$ and a significant two-way interaction between age and treatment $F(1, 28) = 6.34$, $p < .05$. Cell means for the YC, YE, OC, and OE groups were 3.06, 2.32, 2.73, and 2.64, respectively (See Figure 3). A Scheffe post hoc test ($p < .05$) showed that the YE group's average number of correct responses during the 19-day test period was significantly lower than the other three groups, $F(2, 54) = 14.18$, $p < .001$ (See Figure 4).

Discussion

The results from the radial maze testing phase indicate that at the prescribed dose and treatment duration GBE clearly impaired learning. This is noted by the fact that as a combined group, both YE and OE took a significantly longer time to reach the criterion than the control groups. Although no significant interaction was calculated, the discrepancy between the number of days to criterion between the YE and YC was much larger than between OE and OC, suggesting that GBE had a more deleterious effect on the young rats than the old. Evaluation of the group performance over 19 days of testing concludes that the YE performed significantly worse than the other groups. This selective effect is contrary to expectations based on past studies which indicate that at this dose, critical biochemical and morphological changes occur primarily in old rats, which should theoretically improve memory and learning.

A plausible explanation can be surmised if one takes into account the positive correlation between treatment and activity levels found in the young rats of this study. It is well established that the relationship between arousal and performance is curvilinear, and although a certain amount of arousal is required for the display of adaptive behaviors, too much arousal can lead to overactivity and poor performance. LePoncin et al. (1980) revealed that over arousal can also lead to hypoactivity and poor performance. This over arousal may explain why the OE group took longer to reach the criterion, and their response frequency tended to be lower than other groups.

Another possible explanation for the adverse effects of treatments may be related to dose levels. As with any drug used to treat behavioral or physical problems, proper dose levels are critical for desired results; in fact, improper levels can cause adverse conditions. In this regard, studies that have found positive effects on elderly humans (e.g., Taillandier et al., 1988) have used daily doses ranging from 120-600 mg/day or 2-12 mg/kg of body weight. In contrast, most studies on the biological effects of GBE in rats use a much higher dose of approximately 50-100 mg/kg of body weight. It is quite possible that although GBE had a restorative effect on neuronal systems known to be important in memory, excessive levels of GBE adversely affected other biological factors important for proper memory, performance functions, or both.

Experiment 2

Method

Subjects. Same as Experiment 1. Testing of all subjects was conducted after completion of Experiment 1. One OE subject was euthanized prior to Experiment 2, due to rapid growth of a tumor which inhibited free mobility.

GBE Treatment. The start of Experiment 2 was conducted 11 weeks after the experimental groups, YE and OE, were placed on treatment; treatment continued through Experiment 2.

Materials. The second experiment was conducted with a one-way passive avoidance apparatus similar to one described by Ader, Weijnen, and Moleman (1972). The apparatus consisted of a 24 x 28 x 38 cm enclosed box and a 6 x 24 cm elevated runway platform leading to a 6 x 6 cm opening into the box. Entrance into the box was controlled by a guillotine door. The runway was covered with wire mesh and the floor of the enclosed box was constructed with 4 mm

steel rods spaced 1.4 cm apart from each other. The box was elevated 94 cm above the floor and maintained in a dark room with the exception of a 40-W lamp fixed 35 cm above the runway.

Procedures. Testing was conducted over the course of two days. On the first day, each animal was separately placed in the box with the door closed for a period of 2 min. This was followed by a single trial in which the rat was placed on an elevated runway (facing opposite the door) and allowed to enter the box for 10 s. Upon entry (4 feet on the interior grid floor), a guillotine door was lowered, leaving enough height for its tail.

On Day 2, each rat was given three such trials, during which the latency to enter the box was recorded. Between trials, rats were placed in an adjacent cage for a 30 s intertrial interval. On the third of these trials, the rat received a single 1 s, 0.50 mA electric shock through the grid floor of the cage immediately after entering. Shocks were produced by 25-V AC source delivered through a variable series resistor. Retention was tested when the rats were again placed on the elevated runway 30 s, 1 hr, and 20 hr after the shock trial. Subjects were allowed a maximum 120 s retention interval, and no shock was presented on retention trials.

Results

Two OC subjects refused to enter the darkened box when placed on the outside platform for preshock latency measurement, so their measurements could not be tabulated in the results. To control for differences in the preshock latency times, measurements of the postshock retention intervals were converted to proportions of retention by dividing the postshock latency minus preshock latency factor by 120 s minus preshock latency. All negative proportions, which resulted when postshock latencies were less than preshock latencies, were assigned a value of 0.0, since they reflected no retention. A 2 x 2 x 3 (Age x Treatment x Time) between within split-plot analysis of variance was used to analyze the proportion of retained learning. Significance was reached for the main effect of Time, $F(2, 50) = 34.93$, $p < .001$. The proportion of retained learning for each interval was as follows: 30 s .80; 1 hr .49; and 20 hr .19. A Scheffe post hoc test ($p < .05$) showed a significant difference in the proportions between all time intervals, $F(2, 84) = 20.08$, $p < .001$ (See Figure 5). Other main effects and interactions showed no significance.

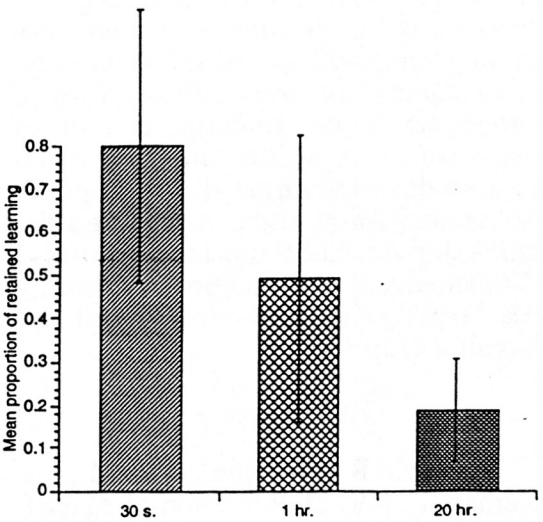

Figure 5. Mean proportion of retained learning over trial intervals.

Discussion

The significant differences in the proportions of retained learning suggest that over time, rats were less likely to remember the possible adverse effects of entering the dark box. Since no significant age differences were revealed between control groups in Experiment 1 or Experiment 2, one may conclude that either these tests are not sensitive to age differences in recall and memory or no memory differences exist between age groups. It is possible that at 16 months of age, the biological systems critical for proper memory function have not been significantly altered by the detrimental effects of aging. In fact, many of the studies investigating age differences in rats use subjects that are 18 months of age or older.

General Discussion

In conclusion, the present study suggests that repeated oral administration of a standardized GBE can have negative effects on the performance of a memory task in young and old rats. To date, no known studies have been published which demonstrate similar findings. In light of these results, it seems important that future studies determine the appropriate dose needed for positive results and the relationship between the morphological and biochemical modifications caused by GBE treatment and the resulting behavioral changes.

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