Electrophysiological Analysis of the Effects of Ginkgo Biloba on Visual Processing in Older Healthy Adults

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Several studies have tested the efficacy of ginkgo biloba using compromised visual systems and have found improvement in vision. We measured functional changes in the visual system of older, healthy adults to see if ginkgo extract EGb 761 would increase performance in the normal visual system. Two electrophysiological measures were taken during baseline, placebo, and treatment conditions: visual evoked potentials were used to assess changes in low-level functioning of the visual pathways, and P300 recognition responses were measured to assess higher order processing. No significant effect was found in the lower level visual pathways. However, when using regression analysis across age to assess higher order functioning, an improvement was found. The results suggest that the higher order processing stages, which may be influenced by cognition, decline more rapidly than do lower level processing stages in healthy adults as a function of age, and that the use of ginkgo biloba extract may improve the functioning of this system.

GINKGO biloba is a phytomedicine extract from the leaves of the ginkgo tree. It is believed to be beneficial as a nutritional supplement and for treating many age-related cognitive deficiencies (1,2). The ginkgo extract reportedly works for several reasons, most prominently by increasing the uptake of glucose and other select neurotransmitters by brain cells, which improves the transmission of nerve signals (3,4), and via its antioxidant abilities, which improves platelet and nerve cell functions and blood flow to the nervous system and brain (5–10). Clinical research trials have most commonly used a standardized form of ginkgo biloba known as EGb 761. Because EGb 761 has many different molecular mechanisms of action, there is currently no single component of the extract that can account for all of the observed clinical effects (11,12). It seems that several distinct effects, caused by various components of the extract, work together in a complementary manner to produce a range of health-related benefits.

Although some studies have found little or no effect (13–15), there are claims that ginkgo biloba can effectively treat such things as memory impairments and lack of concentration (16–18), cerebral vascular insufficiency (19–22), and many types of cognitive impairments including general aging effects (9,23–25) and cognitive and behavioral processes in demented patients (see 26 for a review). There is also experimental and clinical evidence to show that ginkgo biloba enhances neural functioning in the retina and optic nerve (20–22,27,28). These effects seem to be most pronounced when measuring compromised systems, possibly due to the fact that most studies test the effects of ginkgo biloba on populations with known deficiencies. Because of this, there is little normative data available. One of the goals of the present study was to see if EGb 761 could enhance vision-related neural functioning in a healthy population. To this goal, optic nerve functioning and visual attention mechanisms were measured in older, healthy adults using objective techniques that are considered to be sensitive measures of neural integrity (29). Both techniques are briefly described.

Visual Evoked Potential

The visual evoked potential (VEP) is often used to assess functional changes in lower level visual processing. In the VEP technique, electrodes are placed on the scalp to measure the electrical potentials of cortical activity using the electroencephalograph. Cortical signals are recorded in response to visual stimulation of the eye. The visual stimulation is time-locked to the VEP recording, producing consistent neural signals to repeated presentations of the stimuli. Therefore, the recorded response is the summed cortical activity generated by the pathway from the retina to the cortex (30, also see 31 for an in-depth description of this procedure).

Color-grating stimuli are used to elicit distinct VEP waveform responses. Color information is processed by at least two separate pathways in the optic nerve. The L-M pathway, named after the L and M cones, processes red/green opponent color information. It responds best to stimuli that selectively modulate the S cones (34). The S-(L+M) pathway processes blue/yellow opponent color information and responds best to chromaticities that selectively modulate the S cones (34). Cortical responses to the visual presentation of these two color gratings can be compared to quantify differences in the visual pathways. Additionally, responses from the same pathway can be analyzed for a treatment effect, such as differences attributable to ginkgo.

Two studies using the VEP have measured the effects of EGb 761 in alloxan diabetic and normal Swiss rats. In the first study (35), VEP recordings and optic nerve histopathology were used to assess changes in visual functioning. Both assessment techniques showed positive effects of EGb 761. The second study (36) recorded flash VEPs for visual assessment and again showed a significant effect of EGb 761.
on diabetic rats. Taken together, these studies demonstrate that the VEP is capable of detecting subtle changes in visual functioning due to the influence of EGb 761.

**P300**

The P300 recognition response is also measured using the electroencephalograph. The name is descriptive of the measurement—a positive (P) deflection in the response waveform at approximately 300 ms. Generally, the P300 response is elicited using an “oddball” task. In this paradigm, a participant is presented an auditory or visual train of standard stimuli with odd and random target stimuli infrequently imbedded in the train. If the target is detected, a P300 is elicited (37,38). However, if the participant is not attending to the task and the target is not detected, a P300 response will not be elicited (39). Because of this, the P300 is thought to reflect stimulus discrimination (and therefore, conscious detection) of the target (40).

At least two studies have used auditory stimuli to elicit P300 responses for measuring the effects of ginkgo biloba. However, neither study used the common extract EGb 761. The researchers in the first study (41) used the extract ginkobene to study patients diagnosed with age-associated memory impairment. They found no waveform amplitude differences between treatment and placebo, but they did find a significant decrease in latency in the treatment group compared to the placebo group. In the second study (42), researchers used the extract GK501 on healthy normal adults. The results showed a decrease in P300 latency during treatment, but this difference between treatment and placebo did not reach significance.

In the present study, VEPs and P300 recognition responses were recorded from an older, healthy population during baseline, while taking EGb 761, and while taking a matched placebo. If EGb 761 has an improving effect on the visual processing system, it was reasoned that this effect should be noticeable by decreases in response latency measurements and/or increases in response amplitudes. Failure to find these changes would suggest that EGb 761 does not increase neural efficiency in the visual system of normal, healthy adults.

**METHODS**

**Participants**

Thirty volunteers from the Reno, Nevada community gave written informed consent to participate in this study. They were recruited through newspaper advertisement and by word of mouth. Participants ranged from 41 to 83 years of age. Only participants with normal or corrected-to-normal visual acuity of 20/30 or better (determined using the Snellen eye chart), normal color vision (determined using the Ishihara 38-plate test), and self-report of good health (determined using a health questionnaire) were included in the study.

**VEP Stimuli**

Horizontal sinusoidal gratings (1.0 c/deg) were generated on a personal computer using a Cambridge Instruments graphics board (Cambridge Research Systems Ltd., Cambridge, U.K.) and displayed on a computer monitor. The monitor was calibrated using a Photo Research PR650 spectroradiometer (Photo Research, Inc., Chatsworth, CA). Stimuli were viewed at a distance of 57 cm subtending a visual angle of 21°. Chromatic gratings that selectively modulate the L-M channel (red/green color opponent pathway) and the S-(L+M) channel (blue/yellow color opponent pathway) were presented in an onset/offset timing sequence (100 ms on, 400 ms off). The color axes were in the isoluminant plane of the MBDKL color space (43,44), the cardinal axes of which selectively activate these opponent channels. Average chromaticity (International Commission on Illumination [CIE] 1931: x = 0.290, y = 0.304) and luminance (42.2 cd/m²) were held constant. Stimuli were modulated around the mean chromaticity and displayed at the monitor’s maximum contrast (cone contrast for S = 84.8%; for LM: L = 5.5%, M = 10.6%). The endpoints of the chromatic axes in 1931 CIE coordinates were: L-M (x = 0.358, y = 0.294; x = 0.254, y = 0.342) and S-(L+M) (x = 0.272, y = 0.227; x = 0.395, y = 0.515).

**Recordings**

Evoked signals were recorded using Grass gold cup surface electrodes and Grass amplifiers (Grass-Telefactor, West Warwick, RI) input into a National Instruments I/O board (National Instruments Corp., Austin, TX) in a personal computer. Electrodes were fixed along the midline in accordance with International 10–20 system standards. The active, reference, and ground electrodes were attached at Oz, Fz, and Cz, respectively. All electrode sites were scrubbed with scalp cleanser prior to attachment. The electrodes were attached using a conductive paste with impedances kept below 10.0 KΩ (measured at 30 MHz).

**Analysis**

Analyses were performed offline. Visual evoked potential amplitudes were determined by calculating the difference between the largest negative wave deflection between 100 and 300 ms and the largest positive wave deflection between 150 and 350 ms. Latencies were measured within these same time periods and determined at the first major negative trough. Each waveform was also visually inspected to make sure responses fell within the above time windows. Amplitudes and latencies associated with the P300 measurement were determined by calculating the difference between the largest negative wave deflection between 200 and 500 ms and the largest positive wave deflection between 200 and 600 ms. Latencies were determined at the largest positive peak within these same time windows.

**Procedures**

VEPs and P300 responses were measured for each participant. Measurements were taken prior to ginkgo or placebo (baseline), after taking 240 mg/day of EGb 761 for 4 weeks, and after taking a matched placebo for 4 weeks. The order of treatment and placebo conditions was balanced across participants. Placebo and EGb 761 tablets were matched in color, size, and weight and were purchased together from Vitamin Research Products, Inc. (Carson City, NV). They were separated into identical containers in the laboratory by experimenters. Containers were unlabeled except for either the letter “A” or “Z” written in marker on the cap to identify the experimental condition. Participants were instructed to take one 120-mg tablet in the morning and one in the evening every day for 28 days in each experimental condition. There were no washout periods between experimental conditions. This cross-over study was double-blind; neither the...
participant nor the experimenter knew which condition was being tested until after the 8-week testing period.

VEP.—Participants were instructed to visually fixate on the center of the monitor while grating stimuli were presented. The different stimulus conditions were presented 60 times each for averaging, and were randomly interleaved to reduce the potential of unequal fatigue effects. VEPs were recorded only while participants were attentive. Recording lasted approximately 2 minutes.

P300.—After the VEP recording session, participants were visually presented a series of “X” and “O” letters temporally separated by 1 second. Each letter was visible for 250 ms. Eighty-five percent of the time the letter “O” was presented; the letter “X” was presented 15% of the time. Presentation order was randomized. Participants were instructed to push corresponding keys on a computer keyboard depending on which letter was presented and were encouraged to respond as quickly and accurately as possible. Behavioral responses to this task were not recorded; the task objective was simply to ensure that participants were paying close attention to the presentation of the letters as the P300 measurement depends on the recognition of the oddball stimulus (37–40). Because a P300 response is most apparent in traces associated with the oddball stimulus, cortical potentials were recorded only when the letter “X” was presented. The task continued until the oddball stimulus had been presented 60 times. Each recording session lasted approximately 7 minutes.

RESULTS

VEP

One participant was excluded from the analysis because of abnormalities in the VEP waveform during placebo and treatment conditions in both visual pathways. A two-factor repeated measures analysis of variance (ANOVA) was conducted on the VEP response data from the remaining 29 participants for the main effects of visual pathways and experimental conditions. There were no significant results of main effects (visual pathway latency: $F = 3.77$; amplitude: $F = 3.40, F_a = 4.20$ at $\alpha = 0.05$; experimental condition latency: $F = 0.76$; amplitude: $F = 1.38, F_a = 3.34$ at $\alpha = 0.05$) or interaction effects (latency: $F = 1.59$; amplitude: $F = 1.06, F_a = 3.34$ at $\alpha = 0.05$) when using either latency or amplitude as the dependent variable (see Figure 1 for averaged response waveforms from each condition). Averaged latencies for the L-M pathway during baseline, placebo, and treatment were 120.2 ms ($\text{SD} = 20.0$), 124.4 ms ($\text{SD} = 21.6$), and 119.0 ms ($\text{SD} = 18.1$), respectively. Averaged amplitudes for baseline, placebo, and treatment were 40.0 $\mu$V ($\text{SD} = 38.2$), 33.8 $\mu$V ($\text{SD} = 33.0$), and 34.3 $\mu$V.
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Figure 2. Averaged P300 waveforms. P300 response waveforms are shown for each experimental condition. The vertical line is used to show a trend towards decreased latencies in the treatment condition.

\((SD \,33.6)\) for the L-M pathway and 35.7 \(\mu V \,(SD \,20.0), \,31.5 \,\mu V\) (SD 17.9), and 33.4 \(\mu V \,(SD \,18.8)\) for the S-(L+M) pathway.

Research has shown that pathway responses change with age (e.g., 45–47); therefore, the variance in experimental conditions across age for each of the visual pathways was tested using regression analysis. In all conditions it was statistically determined that the linear model was the best fit for the response data. For the L-M pathway, latency values showed no significant change across age in any experimental condition (baseline showed an increase of 23 ms from 111 to 134 ms; \(r=0.321, p=.090\); for placebo, there was an increase of 24 ms from 115 to 139 ms: \(r=0.315, p=.096\); and for treatment, there was an increase of 18 ms from 112 to 130 ms: \(r=0.276, p=.148\)). Latency values for the S-(L+M) pathway showed an increase in all experimental conditions across age (an increase of 27 ms from 119 to 146 ms at baseline: \(r=0.428, p=.021\); for placebo, an increase of 29 ms from 118 to 147 ms: \(r=0.399, p=.032\); and an increase of 42 ms from 114 to 156 ms for treatment: \(r=0.493, p=.004\)), but changes were not significantly different. Regression analysis of amplitude measures showed no significant change in any of the conditions in either visual pathway (L-M pathway baseline: a decrease of 8 \(\mu V\) from 43 to 35 \(\mu V\), \(r=0.179, p=.352\); placebo: a decrease of 5 \(\mu V\) from 36 to 31 \(\mu V\), \(r=0.071, p=.716\); treatment: a decrease of 10 \(\mu V\) from 38 to 28 \(\mu V\), \(r=0.276, p=.149\). S-(L+M) pathway baseline: a decrease of 11 \(\mu V\) from 40 to 29 \(\mu V\), \(r=0.253, p=.185\); placebo: a decrease of 9 \(\mu V\) from 35 to 26 \(\mu V\), \(r=0.428, p=.096\); treatment: a decrease of 11 \(\mu V\) from 38 to 27 \(\mu V\), \(r=0.308, p=.103\).

**P300**

In addition to the one participant removed from the analysis in the VEP experiment, two participants were excluded from the P300 analysis for indeterminable responses. A single factor repeated measures ANOVA was conducted on the data from the remaining 27 participants to test for treatment effects. There were no significant treatment effects found using either latency (\(F = 0.35, F_g = 3.14\) at \(\alpha = 0.05\)) or amplitude (\(F = 1.30\) as the dependent variable (although a trend was present; see Figure 2 for averaged response waveforms). Using response latency as the measure, there was a slight but insignificant difference between experimental conditions (baseline: 361.7 ms [SD 57.8]; placebo: 364.6 ms [SD 59.7]; and treatment: 353.9 ms [SD 55.6]). Response amplitudes also showed small differences (baseline: 34.4 \(\mu V\) [SD 9.8]; placebo: 40.0 \(\mu V\) [SD 16.8]; and treatment: 38.8 \(\mu V\) [SD 12.8]), but the differences did not reach significance.

A regression analysis using response latency to test for treatment effects across age was subsequently conducted. It was statistically determined that a linear model was the best fit of the data. There was a significant increase in latency across age during the baseline condition (a trend line increase of 90 ms from 328 to 418 ms; \(r=0.412, p=.033\)) and the placebo condition (a trend line increase of 86 ms from 332 to 418 ms; \(r=0.382, p=.049\)), but no significant change with age was found during the treatment condition (a trend line increase of 28 ms from 343 to 371 ms; \(r=0.140, p=.486\)). Results are plotted in Figure 3. As can be seen, trend lines for the baseline and placebo conditions, although different, are nearly identical (i.e., difference in average change across age is only 4 ms). For amplitude values, regression analysis showed that there were no significant changes with age in any of the experimental conditions (baseline: \(r=0.102, p=.613\); placebo: \(r=0.138, p=.491\); and treatment: \(r=0.316, p=.108\)).

**DISCUSSION**

Previous studies (35,36) have shown the VEP technique to be sensitive enough to detect subtle changes in nerve functioning using EGB 761. Therefore, any potential treatment effect of EGB 761 on VEP responses in the present study should also be detectable. Cortical responses to stimuli modulating the L-M and the S-(L+M) visual pathways were recorded for each participant. Response latency and amplitude values were found to be within the normal range for this age group (46). However, no differences between experimental conditions were found that could be attributed to EGB 761. This suggests that EGB 761 does not have an improving effect on neural efficiency in the optic nerve of normal, healthy adults. Other studies, for example testing the efficacy of EGB 761 in normal tension glaucoma (22), have found an improving effect. This discrepancy may be due to measurements in the current study being taken at or near ceiling in the functioning of the visual system. Because participants were all screened for healthiness, the low-level visual system may already have been operating at optimum levels.

Additional evidence for this reasoning comes from informal exit interviews of 18 of the 30 participants. Sixteen of the 18
correctly identified which condition was treatment and which was placebo. Although two participants refused to speculate as to which condition was which, no person made an incorrect guess. All 16 participants reported feeling more energy and described feeling “mentally sharper” while taking the ginkgo biloba tablets. (This is only reported as anecdotal evidence because it was not measured experimentally.) The fact that no differences were found in the low level visual system despite the self-report of mental improvement, again, suggests that this lower level system may be operating at or near ceiling.

The potential efficacy of EGb 761 was also tested using P300 responses to visual stimuli. An ANOVA test found no significant effects of treatment. Failure to see differences in the overall P300 ANOVA may again be due to the younger participants’ neural systems operating at or near ceiling. In other words, their systems may already be operating efficiently enough so that any potential improvements from EGb 761 are lost to the measurement (the system can only perform so well). The initial experimental design did not include an analysis across age, so age was not balanced in the present study. Because there were fewer older participants, changes with age may be hidden in the overall ANOVA. It is also possible that some participants had health issues that they failed to report because the health screening was a self-report questionnaire. But even if there were unreported health problems in this study, it remains unclear how this would affect the results because all participants were tested in all conditions (i.e., they were compared to themselves).

It is interesting that a treatment effect for P300 responses was found when considering age. When we used regression analysis to measure changes across age, a significant increase in latency was found during baseline and placebo conditions but not during the treatment condition. This suggests that EGb 761 may indeed improve higher order system functioning as assessed using the recognition response. However, because half of the participants received the extract first followed by the placebo, and the placebo latencies were not statistically different from baseline, the improving effects of EGb 761 are probably relatively short-lived. Future studies should assess how long-lasting this beneficial improvement in functioning is (which may also hint at the potential of ginkgo to improve cognitive functioning more generally).

We found interesting effects from two participants who were excluded from the present study for failing to meet screening requirements. According to health questionnaires and subsequent informal interviews, one participant had a long history of drug and alcohol abuse and the other had a history that included multiple strokes. Results from both participants showed large decreases in latency responses in the treatment condition when compared to baseline and placebo for both VEP and P300 responses. This finding is in line with other studies showing treatment effects of ginkgo in neurally compromised systems and may be further evidence to support the interpretation that the failure to find a prominent effect in the current study was due to the general good health and neural efficiency of the participants.

Conclusion

The present study shows that EGb 761 has no measurable improving effect on the physiological functioning of the early visual pathways in older healthy adults. However, when assessing higher order neural changes across age, a significant improvement for recognition responses was found using the P300 measure. The lack of such an effect in the early visual pathways may be due to the neural integrity of the early visual system being more resistant to aging than the higher order
visual system, as the higher order visual system relies on additional cognitive processing. The P300 responses are believed to include cognitive aspects as attention, recognition, and memory are necessary to perform the measurement task (37–40). Therefore, the difference in findings when comparing measurements using the VEP and the P300 implies that the higher order cognitive functions of participants in this study may be beginning to decline at a faster rate than the lower level physiological functions of the visual system. In addition, the results demonstrate that the P300 measure is sensitive to subtle changes in neural functioning.

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