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ORIGINAL INVESTIGATION

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Differential cognitive effects of *Ginkgo biloba* after acute and chronic treatment in healthy young volunteers

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Abstract *Rationale:* Acute doses of *Ginkgo biloba* have been shown to improve attention and memory in young, healthy participants, but there has been a lack of investigation into possible effects on executive function. In addition, only one study has investigated the effects of chronic treatment in young volunteers. *Objectives:* This study was conducted to compare the effects of ginkgo after acute and chronic treatment on tests of attention, memory and executive function in healthy university students. *Methods:* Using a placebo-controlled double-blind design, in experiment 1, 52 students were randomly allocated to receive a single dose of ginkgo (120 mg, n=26) or placebo (n=26), and were tested 4 h later. In experiment 2, 40 students were randomly allocated to receive ginkgo (120 mg/day; n=20) or placebo (n=20) for a 6-week period and were tested at baseline and after 6 weeks of treatment. In both experiments, participants underwent tests of sustained attention, episodic and working memory, mental flexibility and planning, and completed mood rating scales. *Results:* The acute dose of ginkgo significantly improved performance on the sustained-attention task and pattern-recognition memory task; however, there were no effects on working memory, planning, mental flexibility or mood. After 6 weeks of treatment, there were no significant effects of ginkgo on mood or any of the cognitive tests. *Conclusions:* In line with the literature, after acute administration ginkgo improved performance in tests of attention and memory. However, there were no effects after 6 weeks, suggesting that tolerance develops to the effects in young, healthy participants.

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Introduction

Ginkgo biloba is an ancient fossil tree which has been used as a food and for medicinal purposes in China and the Far East for millennia. It was traditionally used for cardiovascular disease and asthma, but more recently its cognitive enhancing properties have been recognised in the West. Extracts are obtained from the dried leaves, and the main active constituents are generally considered to belong to two distinct chemical groups: the biflavone glycosides such as ginkgetin, isoginkgetin, bilobetin and related compounds and the terpene lactones known as ginkgolides A, B, C, etc. and a sesquiterpene trilactone bilobalide (DeFeudis 1998; Joyeux et al. 1995). Most of the pharmacological and clinical work carried out on ginkgo has used an extract containing both these classes of compounds, and it has been shown that such extracts are antioxidants and vasodilators and can increase cerebral blood flow in animals (DeFeudis 1998; Bridi et al. 2001; Bastianetto et al. 2000). Extracts also possess neuroprotective potential, thought to be mediated via inhibition of nitric oxide synthesis (Calapai et al. 2000). It is not yet clear which individual classes of compounds are responsible for those effects relevant to memory enhancement; although each class has some differing effects, they also have others in common, in particular free radical scavenging and antioxidant effects (Joyeux et al. 1995). The ginkgolides are anti-inflammatory due to their specific platelet-activating factor antagonist properties (Braquet 1987) and also have complex effects on neurotransmitter uptake and certain neurotransmitter receptors in cerebral ischaemia and neuronal injury (DeFeudis 1998). Bilobalide has a protective effect against reactive oxygen species-induced apoptosis in PC12 cells (Zhou and Zhu 2000) and, more importantly, is now known to act as an antagonist at recombinant $GABA_A$ receptors (Huang et al. 2003). There is now a body of literature pertaining to the

cognitive enhancing properties of ginkgo in clinical populations suffering from vascular dementia (Kanowski et al. 1996; Le Bars et al. 1997) or Alzheimer's disease (Oken et al. 1998; Le Bars et al. 1997; Kanowski et al. 1996). The most recent meta-analysis of studies conducted in patients with dementia and cognitive impairment concluded that although there were promising effects of ginkgo for both cognitive and noncognitive symptoms, there is still the need for a modern, large-scale trial to resolve inconsistencies across more recent studies (Birks et al. 2002).

There is also some evidence of improved cognition in healthy ageing populations, although this is somewhat inconsistent. Rigney et al. (1999) investigated the effects of four doses of ginkgo (120–300 mg) in healthy volunteers (30–59 years) over a 1- to 2-day treatment period. They reported 120 mg had the most potent effects of all doses, with improvement on the Sternberg memory scanning test after both 1 and 2 days of treatment, which was more pronounced for the 50- to 59-years age group. Hartley et al. (2003) found improvements on tests of short-term memory, mental flexibility and sustained attention after 1 week of ginkgo (120 mg/day) treatment in postmenopausal women (aged 53–65 years). Since the participants in this study were not tested after an acute dose, it is not possible to determine whether the effects after 1 week differ from those after an acute dose. However, in a small cross-over study, Nathan et al. (2002) found no effects of a single dose of ginkgo (120 mg) in older participants (aged 50–70 years) on tests of working memory, attention, visual or auditory episodic memory. The lack of effects in this study may be due to the small sample size (n=11 per cell) or the time of testing after the dose (90 min).

Furthermore, the effects of ginkgo after chronic treatment periods in healthy older populations are equally equivocal. Mix and Crews (2002) reported that ginkgo (180 mg/day) administered for 6 weeks improved performance on the selective reminding test and the Weschler Memory Scale in participants aged 60 years and older. In a similar study, ginkgo (180 mg/day) administered for 6 weeks improved performance on the colour-naming component of the Stroop task and showed a trend towards an improvement on the visual reproduction component of the Weschler Memory Scale in participants aged 55–86 (Mix and Crews 2000). These effects were not replicated by Cieza et al. (2003) and Solomon et al. (2002), who found no beneficial effects of ginkgo on tests of attention or memory in elderly populations after treatment periods of 4–6 weeks. The only study to investigate a longer treatment interval reported no effects of ginkgo on tests of memory in elderly participants who had taken ginkgo supplements for periods of 5 months or 2 years or more (Persson et al. 2004). However, there were limitations to this study due to a lack of double-blinding, standardised dosing or a standardised treatment period.

As the effects of ginkgo may differ depending on the age range of participants, it seems paramount to use well-defined cohorts. An important question is whether ginkgo, in addition to its beneficial effects in clinical, and to a lesser extent, in healthy ageing populations, can improve cognitive performance in younger populations who are at or near to their peak of cognitive ability. Four studies have investigated the effects of acute/subchronic doses of ginkgo in university students. Kennedy et al. (2000) reported improvement in a factor of 'quality of memory' with an acute dose of ginkgo (120 mg) and improvement in a factor of 'speed of attention' with 240- and 360-mg doses. Furthermore, all three doses of ginkgo were found to improve performance on serial subtraction tasks (Scholey and Kennedy 2002), and a 360-mg dose improved performance on a factor of 'secondary memory' and, to a lesser extent, on a factor of attention (Kennedy et al. 2002). Moulton et al. (2001) investigated the effects of short-term treatment with ginkgo (120 mg for 5 days) in male college students (mean age, 20.5 years). The only effect was in the Sternberg memory test in which the ginkgo group performed better than the placebo group at the easiest stage of the task, whereas the ginkgo group performed worse than placebo at the hardest stage. There were no other effects on tests of prose memory, digit span or reading span. Stough et al. (2001) is the only study to investigate chronic effects in a younger population. They reported improvement in working memory and memory consolidation in participants (aged 18–40 years) after 30 days of treatment with ginkgo (120 mg).

Since Stough et al. (2001) was the only study to investigate the chronic effects of ginkgo in a younger population and the age range of participants used was fairly wide, the aim of the present study was to compare the effects of ginkgo after acute and chronic treatment in a well-defined population of healthy university students, using two parallel studies. In addition to testing attention and memory, tests of executive function were included since this has received limited investigation.

Materials and methods

Participants

Ninety-two university students aged between 18 and 26 years were recruited by word of mouth and poster advertisement from King's College and The School of Pharmacy, University of London. Fifty-two participants took part in experiment 1 (26 men and 26 women) and 40 participants took part in experiment 2 (21 men and 19 women). The study was approved by King's College Ethics Committee and all participants gave written informed consent. All participants were required to fill out a screening questionnaire prior to the study and reported that they were currently healthy. Exclusion criteria were use of psychoactive or anticoagulant medication, alcohol or drug dependence, pregnancy or lactation or use of ginkgo, ginseng or soya isoflavone supplements within the month preceding participation in the study.

Supplement

The *G. biloba* supplement used was Ginkyo one-a-day tablets (Lichtwer Pharma UK, Mere Park, Marlow, Bucks, UK), containing 120 mg of the standardised extract LI 1370 from the leaves of the *G. biloba* tree. This extract contains 25% total ginkgo flavonoids and 6% total terpene lactones. Placebo tablets (also supplied by Lichtwer) were identical in appearance. The drug allocation was performed by a third party not involved in the testing. Peak plasma levels of the terpene lactones are reached at 1–4 h and the flavonoid glycosides at 2–3 h after administration (Upton 2003). Four hours is the time point at which cognitive improvement and EEG effects have been reported following acute ginkgo administration (Kennedy et al. 2000, 2003).

Procedure

Experiment 1

Participants were initially required to attend a screening session on the morning of participation, when they completed a screening questionnaire, the Hospital Anxiety and Depression Scale (HAD; Zigmond and Snaith 1983) in order to determine levels of trait anxiety and depression, and the National Adult Reading Test-Revised (NART-R; Nelson and Willison 1991) in order to provide an estimate of verbal IQ. In addition, participants were asked to complete alcohol and caffeine diaries in order to determine habitual levels of consumption. Participants were randomly allocated to the ginkgo (120 mg, n=26) or placebo (n=26) groups, and received one tablet on the morning of participation, 4 h before the test session. Participants were instructed to abstain from caffeine-containing drinks during the 4-h period before the test session and had been instructed to abstain from alcohol the day before the test session. Smokers were not asked to abstain from smoking since nicotine withdrawal has been shown to impair cognitive performance in smokers (Snyder and Henningfield 1989). For the 4-h period before the test session, participants were instructed to remain on the university campus and attend to their usual academic activities. The test session took place in the afternoon and lasted approximately 1 1/4 h. Before the test session participants were administered a practice session in order to familiarise themselves with the Cambridge Neuropsychological Test Automated Battery (CANTAB, CeNeS, Cambridge, UK).

Experiment 2

Participants were required to fill out a screening questionnaire prior to participation and were randomly allocated to the ginkgo (n=20) or placebo group (n=20). Participants were required to attend two 1 1/4-h testing sessions: the first one before starting treatment (baseline) and the second after 6 weeks of treatment. Participants took one tablet containing ginkgo (120 mg) or placebo each morning for the 6-week period. On the second test occasion participants were tested 3–5 h after their last dose. On each test day, participants were instructed to abstain from caffeine-containing drinks for 4 h before the test session and to abstain from alcohol the day before the test session. Immediately prior to the baseline test session participants were given a practice session to familiarise themselves with the computerised test battery. An estimate of verbal IQ was obtained using the NART-R. Trait anxiety and depression were measured with the HAD scales. Participants completed alcohol and caffeine diaries so levels of habitual consumption could be determined.

Cognitive test battery

The test order is presented in Table 1.

Sustained attention

The Paced Auditory Serial Addition Task (PASAT) was used as a measure of sustained attention (Spreen and Strauss 1991). Participants were required to add together successive pairs of single digits that were read from a list. There were four digit-presentation speeds, with digits presented every 2.4, 2.0, 1.6 and 1.2 s. The first two trials consisted of 31 digits and were used as practice, and the two fastest trials comprised 61 digits and were used to assess performance. The number of correct responses were recorded from a score out of 60 for the two fastest presentation speeds.

Table 1 Order of test battery

	Test order
Pretest session	National Adult Reading Test-Revised (NART-R)
	Hospital Anxiety and Depression Scale (HAD)
Practice session	Intra Dimensional/Extra Dimensional set shifting task (IDED)
	Stockings of Cambridge (SoC)
	Spatial working memory (SWM)
Test session	Word presentation
	Picture presentation
	Mood rating scale
	Alcohol and caffeine diaries
	IDED
	SoC
	SWM
	Pattern recognition memory (PRM)
	Spatial recognition memory (SRM)
	Word recall
	Picture recall
	Paced Auditory Serial Addition Task (PASAT)

Episodic memory

Pattern recognition memory

The pattern recognition memory (PRM) task is taken from CANTAB (Owen et al. 1995). In this task, participants were presented with a series of patterns which they were instructed to memorise. In the recognition phase they had to choose between the pattern they recognised and a distracter pattern. There were two test blocks, each with 12 patterns. The percentage of correct responses and the latency to correctly respond were recorded.

Spatial recognition memory

The spatial recognition memory (SRM) test was taken from the CANTAB (Owen et al. 1995). The test involved the presentation of five white boxes at different positions on the screen, one after the other. In the recognition phase the subject had to choose between the box which they recognised to be in the same position as earlier and a distracter box. There were four test blocks, each with five trials. The percentage correct responses and latency to respond were recorded.

Delayed recall of words and pictures

Participants were visually presented with a series of 36 words belonging to two semantic categories (Weingartner et al. 1993) on a computer monitor with a stimulus duration of 3 s and interstimulus interval of 200 ms. Participants were instructed to try and remember as many words as they could, as they would be asked for their recall later on in the session. Immediately afterwards participants were presented with a series of 20 pictures of common objects (Snodgrass and Vanderwart 1980) on a computer screen with a stimulus duration of 5 s and interstimulus interval of 200 ms. Participants were instructed to verbalise each picture and to remember as many pictures as possible, as they would be asked for their recall later on in the session. After a filled delay of 25 min (during which the CANTAB tests were completed), participants were required to write down as many words and as many pictures as they could remember, and the number of correctly recalled items was scored.

Executive function

Spatial working memory

The spatial working memory (SWM) test was taken from the CANTAB. In this test, participants are required to search through an array of boxes for 'blue tokens' to 'fill up' the column at the side of the screen, the key instruction being not to return to a box where a blue token has already been hidden. This test has strategic and mnemonic elements and is sensitive to frontal lobe damage (Owen et al. 1990). There were three stages of the task, involving four, six or eight boxes. Two types of errors are possible on this task: returning to a box where a token has already been found ('between errors') or returning to a box that was previously visited and shown to be empty ('within errors'). The summation of between errors and within errors across all stages of the task was recorded. The use of strategy was also recorded (a lower strategy score indicates a better strategy).

Mental flexibility

The Intra Dimensional/Extra Dimensional set shifting task (IDED) was taken from the CANTAB and assesses mental flexibility, which is particularly impaired by frontal lobe lesions (Owen et al. 1991) and has been shown to activate the prefrontal cortex (Rogers et al. 2000). In this task there are nine stages in which participants have to learn rules, reverse rules and shift rules. The number of stages completed were recorded for the whole group, and the number of errors before the extradimensional shift (Pre-EDS errors) and errors at the extradimensional shift (EDS errors) were recorded for those subjects who were able to complete the whole task.

Planning

The Stockings of Cambridge (SoC) task was taken from the CANTAB and is a measure of planning ability. This test is sensitive to frontal lobe damage and was shown to activate the dorsolateral prefrontal cortex (Owen et al. 1990, 1996). In this task, participants were presented with an arrangement of three coloured balls housed in 'stockings' that had to be rearranged in the fewest moves possible to match a goal arrangement at the top of the screen. The participants were instructed to plan their moves before starting, and task difficulty varied from two to five moves. The first two stages were used for practice. The initial and subsequent thinking times and the number of moves to complete the task were recorded for the two most difficult stages.

Visual analogue mood scales

Self-ratings of mood were taken at the beginning of the test session. The scale consisted of 16 opposed adjectives separated by a 100-mm line, and participants were instructed to place a perpendicular mark across each line to indicate how they were feeling at the time between the two extremes. Three individual factors of 'anxiety', 'alertness' and 'contentedness' have been derived from the mood rating scale (Bond and Lader 1974).

Statistical analysis

All the data for experiment 1, except for PASAT, were analysed by two-tailed independent samples *t* tests. The data for PASAT were analysed by a repeated measures analysis of variance (ANOVA), with 'speed' as the within-group factor and 'treatment' as the between-group factor. The data for experiment 2 were analysed by two-way repeated measures ANOVAs, with 'treatment' as the between-group factor and 'week' as the repeated measure factor (baseline and after 6 weeks of treatment). For PASAT, the data were analysed by a three-way repeated measures ANOVA, with 'week' and 'speed' as the within-group factors and 'treatment' as the between-group factor. Where effects reached, or were close to, significance the *t* value and probability levels are given in the text; otherwise, values are given in the tables. All data were analysed using Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) version 10 for Windows.

Results

Experiment 1: effects of acute treatment

Group characteristics

The two treatment groups were well matched and did not differ significantly in age, body mass index, estimated verbal IQ score, daily caffeine intake, weekly alcohol consumption or trait anxiety and depression ratings on the HAD scale (Table 2). Eight of the participants were smokers: five in the placebo group smoking a mean of 6.2 cigarettes per day and three in the ginkgo group smoking a mean of 10.0 cigarettes per day.

Sustained attention

There was a significant Treatment×Speed interaction on PASAT ($F_{1,50}$=7.1, P<0.02), which was because the ginkgo group were better than placebo as the task became more difficult. Furthermore, there was a significant effect on the most difficult stage of the task (t_{50}=2.1, P<0.04) because the ginkgo group scored significantly more correct responses than the placebo group (Fig. 1).

Episodic memory

There was a significant effect of ginkgo on the percentage of correct responses on the PRM task (t_{50}=2.0, P=0.05), because the ginkgo group made more correct responses than the placebo group (Fig. 2a). There were no effects on the latency to correctly respond on this task or on the SRM task (Table 3).

The ginkgo group recalled more words than the placebo group, although this just missed significance (t_{50}=1.8, P=0.07; see Fig. 2b). There was no effect of treatment on the picture recall task (Table 3).

Executive function

There were no effects of ginkgo on the SWM, IDED or SoC tasks (Table 3).

Visual analogue mood ratings

There were no significant effects of ginkgo on the factors of anxiety or contentedness (Table 3). However, the ginkgo group rated themselves as more alert than the placebo group, but this was not significantly different (Table 3).

Experiment 2: effects of chronic treatment

Group characteristics

It can be seen from Table 2 that the ginkgo and placebo groups did not differ in age, body mass index, estimated verbal IQ scores, caffeine or alcohol consumption, smoking habits, or trait anxiety and depression scores on the HAD scale. There were eight smokers: four in the placebo

Table 2 Mean (±SEM) age, body mass index (BMI), estimated verbal IQ (based on the National Adult Reading Test-Revised), daily consumption of caffeinated drinks, weekly alcohol consumption, and scores on the hospital anxiety and depression scales (HAD_A and HAD_D), number of smokers and mean cigarettes smoked per day of the subjects allocated to placebo and ginkgo groups

	Experiment 1				Experiment 2			
	Placebo	Ginkgo	*t*	*P*	Placebo	Ginkgo	*F*	*P*
Age	21.7±0.4	21.3±0.3	−0.9	0.38	21.5±0.3	21.2±0.3	0.27	0.61
BMI	22.3±0.4	22.3±0.7			22.7±0.5	22.4±0.6	0.13	0.72
Verbal IQ	106.4±1.3	108.0±1.2	0.9	0.38	104.3±1.2	105.5±1.6	0.38	0.54
Daily caffeinated drinks (cups)	2.7±0.4	2.2±0.3	−0.9	0.38	3.7±0.5	3.1±0.6	0.61	0.44
Weekly alcohol consumption (units)	11.0±2.2	10.8±1.8	−0.1	0.93	7.6±2.5	12.0±2.6	1.47	0.23
HAD_A	6.0±0.6	4.8±0.4	−1.5	0.13	7.2±0.8	5.8±0.7	1.87	0.18
HAD_D	2.7±0.4	2.2±0.4	−0.9	0.39	3.4±0.6	3.3±0.5	0.04	0.85
No of smokers/mean cigarettes per day	5/6.2	3/10.0			4/5.1	4/8.5		

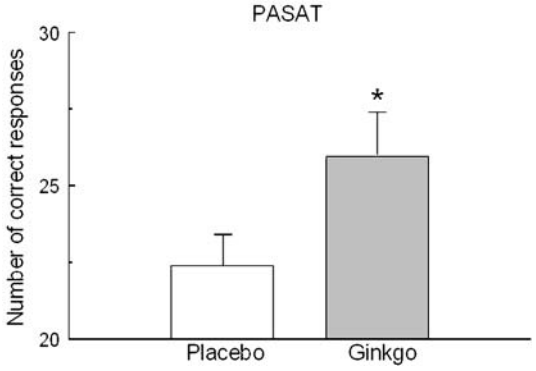

Fig. 1 Mean (±SEM) number of correct responses on PASAT (1.2-s presentation speed) after a single dose of placebo or ginkgo. *P<0.04

group smoking a mean of 5.1 cigarettes per day and four in the ginkgo group smoking a mean of 8.5 cigarettes per day.

Sustained attention

There was no effect of ginkgo on PASAT (Week×Speed×Treatment: $F_{1,38}$=0.1, not significant), but there was a significant effect of week ($F_{1,38}$=30.1, P<0.0001 for both stages), because all participants made more correct responses on the second test occasion (Table 4).

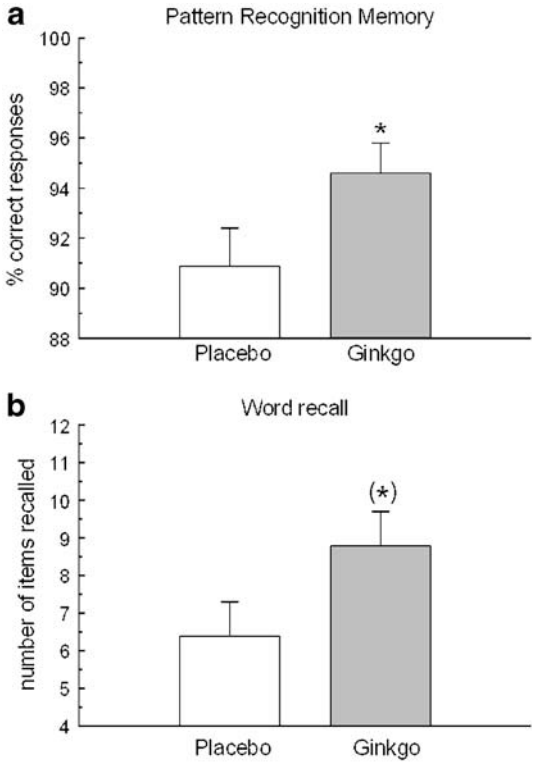

Fig. 2 **a** Mean (±SEM) percentage of correct responses on the PRM task after a single dose of placebo or ginkgo (*P=0.05). **b** Mean (±SEM) number of delayed recall of words after a single dose of placebo or ginkgo (*P=0.07)

Table 3 Mean (±SEM) scores on cognitive tests and mood rating scales of subjects in experiment 1, allocated to receive a single dose of placebo or ginkgo

	Placebo	Ginkgo	t	P
PASAT (1.6 s)				
Number correct	29.3±1.4	29.1±1.2	−0.12	0.90
Picture recall				
Number correct	6.7±0.7	6.6±0.4	−0.92	0.32
PRM				
Mean correct latency (ms)	1,904±67	1,855±69	−0.51	0.62
SRM				
Mean correct latency (ms)	2,119±101	2,067±105	−0.36	0.72
Correct responses (%)	81.0±2.0	83.3±1.9	0.84	0.41
SWM				
Between errors	16.3±3.1	19.2±3.1	0.67	0.51
Within errors	0.5±0.2	0.8±0.3	1.28	0.20
Strategy	30.7±1.0	30.8±1.2	0.02	0.98
IDED				
Stages completed	8.9±0.4	8.7±0.1	−1.0	0.31
Pre-EDS errors	6.2±0.7	5.9±0.3	−0.4	0.69
EDS errors	5.1±0.9	4.8±1.2	−0.2	0.82
SoC				
Mean initial thinking time (ms)				
4 Moves	8,839±1,256	7,456±784	−0.93	0.36
5 Moves	9,772±1,223	8,955±1,256	−0.47	0.64
Mean subsequent thinking time (ms)				
4 Moves	875±154	803±133	−0.36	0.72
5 Moves	551±132	800±170	1.16	0.25
Number of moves				
4 Moves	5.2±0.2	5.4±0.2	0.74	0.46
5 Moves	6.2±0.3	6.4±0.3	0.57	0.57
Mood				
Alertness	54.7±2.2	58.5±1.8	−1.31	0.19
Anxiety	50.4±2.4	51.5±2.2	0.35	0.73
Contentedness	45.3±2.9	49.2±2.5	−1.00	0.32

Episodic memory

There were no significant Week×Treatment interactions on any of the tests of episodic memory (Table 4). There was, however, a significant effect of week for the latency to respond on the PRM task ($F_{1,38}$=11.8, P<0.002), the SRM task ($F_{1,38}$=5.6, P<0.03) and on the number of recalled words ($F_{1,38}$=31.2, P<0.0001) and pictures ($F_{1,38}$=4.9, P<0.04) because all participants became better at the tests on the second test occasion, regardless of treatment.

Executive function

There were no effects of ginkgo on the SWM, IDED or SoC tasks (Table 4). There was, however, a significant effect of week for the number of moves to complete the four-move problem ($F_{1,38}$=10.1, P<0.004) and the subsequent thinking time for both the four-move ($F_{1,38}$=14.8, P<0.0001) and five-move ($F_{1,38}$=5.7, P<0.03) problems

Table 4 Experiment 2: mean (±SEM) scores on cognitive tests and mood rating scales of subjects at baseline and after 6 weeks of treatment with placebo or ginkgo

	Placebo		Ginkgo		*F*	*P*
	Baseline	Week 6	Baseline	Week 6		
PASAT (1.2 s)						
Number correct	25.5±1.5	31.3±1.8	20.8±1.6	25.2±1.8	0.49	0.49
PASAT (1.6 s)						
Number correct	32.2±1.8	37.4±2.1	27.2±2.1	31.7±1.9	0.12	0.74
Word recall						
Items recalled	7.0±1.0	11.3±0.9	6.8±1.0	10.1±0.7	0.48	0.50
Picture recall						
Items recalled	6.4±0.6	7.3±0.4	6.6±0.6	7.5±0.5	0.004	0.95
PRM						
Mean correct latency (ms)	1,924±78	1,754±80	1,933±70	1,714±72	0.19	0.67
Correct response (%)	92.3±1.9	96.0±1.0	94.0±1.9	93.5±2.0	3.13	0.09
SRM						
Mean correct latency (ms)	2,143±123	1,904±78	2,072±157	1,966±148	0.84	0.37
Correct responses (%)	79.1±4.2	82.0±3.1	80.8±2.9	86.0±2.2	0.22	0.64
SWM						
Within errors	2.4±1.7	0.7±0.4	1.3±0.6	1.3±0.6	0.64	0.43
Between errors	14.7±2.3	12.4±2.5	17.0±3.6	12.9±3.4	0.27	0.61
Strategy	30.8±1.2	29.6±1.3	29.9±1.3	29.0±1.4	0.06	0.81
IDED						
Stages completed	8.4±0.4	8.8±0.6	8.7±0.7	8.8±0.1	1.21	0.28
Pre-EDS errors	7.9±1.3	6.6±0.8	6.6±0.9	5.2±0.4	0.02	0.88
EDS errors	3.2±0.5	2.9±0.6	3.2±0.7	4.4±1.3	0.73	0.40
SoC						
Mean initial thinking time (ms)						
4 Moves	6,263±633	6,428±1,204	6,506±871	6,817±1,181	0.01	0.92
5 Moves	7,492±1,088	7,678±1,321	8,437±1,526	7,633±1,131	0.47	0.50
Mean subsequent thinking time (ms)						
4 Moves	722±136	440±94	1,056±211	370±129	2.58	0.12
5 Moves	519±115	365±101	1,055±296	519±108	1.75	0.19
Number of moves						
4 Moves	5.3±0.2	4.8±0.2	5.6±0.2	4.8±0.2	0.62	0.44
5 Moves	6.3±0.3	6.3±0.3	6.8±0.3	6.3±0.3	1.50	0.23
Mood						
Alertness	59.0±2.3	58.4±2.4	59.6±2.0	60.9±2.2	0.20	0.66
Anxiety	52.4±2.2	50.8±2.2	51.1±2.3	49.4±1.8	0.01	0.97
Contentedness	49.4±2.9	47.6±3.0	45.2±2.3	48.3±2.9	1.07	0.31

on the SoC task, as all participants performed better on the second test occasion.

Visual analogue mood ratings

There was no significant effect of ginkgo on the three mood factors of alertness, anxiety or contentedness (Table 4).

Discussion

This study has shown that a single dose of ginkgo improved performance on tests of sustained attention and PRM in healthy young volunteers. However, the positive results on the PASAT should be viewed with some caution, since the two groups in experiment 2 differed at baseline to a similar extent to the effect of ginkgo in experiment 1. Of the studies that investigated the effects of acute doses of ginkgo, improvements were found in tests of attention and/or memory, and thus the results of this study are generally consistent with the literature. Sustained attention (as measured by the PASAT) was also improved after 1 week's treatment with ginkgo (120 mg/day) in postmenopausal women (Hartley et al. 2003). In young volunteers, Scholey and Kennedy (2002) found acute doses of ginkgo (120, 240 and 360 mg) to improve performance on serial subtraction tasks (which have components of attention and working memory). PASAT is similar to the serial subtraction tasks in that it is based on an arithmetic paradigm involving elements of sustained attention and working memory. However, the effects on more simple attentional tasks are not as convincing. Although Kennedy et al. (2000) found acute administration of ginkgo (240 and 360 mg) to

improve a speed of attention factor (derived from measures of digit vigilance, simple reaction time and choice reaction time) in young volunteers, this improvement was not replicated in a similar study in the same population (Kennedy et al. 2002). Other studies that have used simple reaction time or choice reaction time have found no effects of acute doses of ginkgo in these tasks (Rigney et al. 1999; Subhan and Hindmarch 1984; Nathan et al. 2002). Thus, this suggests that ginkgo is more effective in improving performance in complex attentional tasks that involve working memory, rather than simpler tasks such as simple and choice reaction time which only measure attention.

With regard to memory, the present study showed an improvement in short-term visual recognition memory. Performance on a similar test of short-term visual-recognition memory, delayed matching to sample, was found to be improved after 1 week's treatment with ginkgo in postmenopausal women (Hartley et al. 2003). Kennedy et al. (2002) found ginkgo (360 mg) to improve performance on specific episodic memory tests of delayed word recall and delayed picture recognition. Mix and Crews (2002) found 6 weeks of ginkgo (180 mg) to improve performance on both delayed recall of verbal material and recognition of visual material, and Stough et al. (2001) found improvement on a delayed verbal recall task. Although we found improved delayed verbal recall in the present study, this just failed to reach significance. It therefore seems that there is a pattern of ginkgo's effects on memory tasks, with improvement in visual recognition and delayed verbal recall tasks. Like Stough et al. (2001), we used a dosage of 120 mg in the present study, and although there is the possibility that a higher dose would have been more effective, there is no consistent evidence for this in the literature.

Ginkgo has been found to improve performance on numerical working memory tasks such as the Sternberg task (Subhan and Hindmarch 1984; Rigney et al. 1999) and digit span backwards (Stough et al. 2001). We did not use a numerical or verbal working memory task that can be directly compared with these tests; however, we found no effect of ginkgo on the SWM task. In support of our finding, Kennedy et al. (2002) found no effect of ginkgo (360 mg) in a similar test of SWM to that used in the present study. It is therefore possible that ginkgo may only improve performance on tests of numeric memory, rather than SWM. Furthermore, there was no effect of ginkgo on the SRM or SoC tasks, which supports the contention that ginkgo is without effect on tasks with a spatial component.

Despite the aim of the study to investigate the possible effects of ginkgo on executive function, we found no evidence for this in the student population after acute or chronic treatment. These results contrast with our previous findings of improved mental flexibility in postmenopausal women after 1 week (Hartley et al. 2003) and 6 weeks (Elsabagh et al. 2005) of treatment. A possible explanation for this discrepancy is that there was a ceiling effect on the IDED task within the present study, preventing the possibility of improvement with ginkgo. IDED is particularly sensitive to the effects of ageing, and deterioration in performance on this task has been detected within age groups as young as 55–59 years (Robbins et al. 1998; Elsabagh et al. 2005). Thus, the effects we found in postmenopausal women may demonstrate the requirement for a certain degree of impairment in the task in order for ginkgo to have an effect. This contention is supported by comparison of scores across studies: in the present study only one subject out of 92 was unable to reach the critical extradimensional shift stage, compared with around 15% of the participants in the postmenopausal studies (Hartley et al. 2003; Elsabagh et al. 2005). Furthermore, there was no practice effect on IDED in experiment 2 of the present study, indicating that participants were at their peak of performance at baseline.

Although there was a trend towards increased ratings of subjective alertness after acute ginkgo treatment in the present study, this effect was not significant. Kennedy et al. (2002) found an improvement in the factors of alertness and contentedness after an acute dose of ginkgo (360 mg) in healthy young volunteers. However, this was not replicated in a similar study that investigated three doses of ginkgo (120, 240 and 360 mg) in the same population (Kennedy et al. 2000). Furthermore, a recent study reported the failure of ginkgo to ameliorate the decrease in subjective ratings of alertness associated with the post-lunch dip in young volunteers (Mattes and Pawlik 2004). Overall, this suggests that the effects of ginkgo on mood are not as robust as the effects on cognition.

Despite the improvements in attention and memory in experiment 1, these effects could not be detected after 6 weeks of ginkgo treatment. This failure to detect any effects after chronic treatment may have been due to the marked practice effects on the tests. Indeed, the practice effect on word recall and PASAT were particularly pronounced ($P<0.0001$) despite the gap of 6 weeks between testing sessions. Stough et al. (2001) demonstrated improvements with ginkgo in a slightly wider age group after a shorter period of 4 weeks, but did not report the practice effects in the study. As the groups used in both experiments were from the same population of university students, it is unlikely that differences in subject characteristics between the two experiments could have accounted for the lack of effects after 6 weeks of treatment. Another explanation that has been suggested by our own findings in postmenopausal women and results from EEG studies is that tolerance may develop to the effects of ginkgo after chronic periods of treatment. Although improvements were found in attention, short-term memory and mental flexibility after 1 week's ginkgo treatment in postmenopausal women (Hartley et al. 2003), after 6 weeks the only remaining improvement was in mental flexibility, and this was limited to an older age group with more impaired performance (Elsabagh et al. 2005). Similarly, EEG recordings after single doses in healthy volunteers have indicated that ginkgo induces a decrease in theta and beta power (Kennedy et al. 2003) or an increase in alpha power (Itil et al. 1996; Luthringer et al. 1995), which indicates a more alert or activated state. However, in older volunteers after chronic treatment of 12 weeks there was no effect on the EEG profile, except for a subgroup of participants who had more impaired vigilance

at baseline (Gessner et al. 1985). However, the beneficial effects of ginkgo are sustained after long-term treatment periods in patients with dementia (Kanowski et al. 1996; Oken et al. 1998; Birks et al. 2002), which suggests that there may be different mechanisms of action in healthy and clinical populations.

Many mechanisms have been attributed to the cognitive enhancing effects of ginkgo such as improving cerebral blood flow (Krieglestein et al. 1986), antioxidant effects (Oyama et al. 1996; Sastre et al. 1998) and direct effects upon neurotransmitter systems (Brunello et al. 1985; Rapin et al. 1991). In the literature, tolerance to psychotropic drugs is commonly associated with adaptation of neurotransmitter systems. For example, chronic administration of caffeine is associated with changes in adenosine receptors (Svenningsson et al. 1999). Furthermore, there is some evidence from an early study on the effects of ginkgo in the rat that may indicate an adaptation to increased stimulation of the cholinergic system. It was demonstrated in young (4 months old) rats that a single dose of ginkgo increased the synthesis rate of acetylcholine (ACh) in the hippocampus, suggesting increased ACh release. Although this effect was maintained after 21 days treatment, there was a concurrent decrease in muscarinic cholinergic receptor binding in brain areas including the hippocampus (Rapin et al. 1991). Although the detailed mechanisms were not described in this report, it is probable that whilst the stimulatory effect on ACh release was maintained, the overall effect would have been less pronounced due to reduced postsynaptic receptor binding. We cannot say whether this is the mechanism that underlies tolerance to the cognitive effects of ginkgo, although it does suggest that adaptive mechanisms to the stimulatory effects of ginkgo do occur in the brain. Interestingly, a similar study in aged rats (24 months) demonstrated that chronic ginkgo treatment (28 days) reversed the age-related decrease in muscarinic receptor binding in the brain and restored levels to that of young rats (Taylor 1991). This may demonstrate different effects of ginkgo in young and ageing/clinical populations.

Recently, bilobalide was shown to be an antagonist at human recombinant $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ receptors (Huang et al. 2003), which could provide a basis for the direct effect of the leaf extract on memory enhancement and increased alertness, and would be more relevant to the short-term effects of ginkgo. However, it is most likely that the overall effect of ginkgo on cognition is the result of several mechanisms as well as a combination of phytochemicals. In support of this premise, synergism has been described for some of the components of ginkgo (Wagner 1999; Williamson 2000), although these interactions were more pertinent to anti-inflammatory activity than enhancement of cognition.

In summary, this study has shown that after an acute dose, ginkgo improved sustained attention and episodic memory in healthy young volunteers, but that these effects did not persist after a chronic period of 6 weeks of treatment. This indicates that ginkgo may have some application to real-life situations pertinent to this age group, such as sitting of examinations, but not after any sustained period of treatment.

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