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The Influence of Soy-derived Phosphatidylserine on Cognition in Age-Associated Memory Impairment

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Phosphatidylserine (PS) is a phospholipid widely sold as a nutritional supplement. PS has been claimed to enhance neuronal membrane function and hence cognitive function, especially in the elderly. We report the results of a clinical trial of soybean-derived PS (S-PS) in aging subjects with memory complaints.

Subjects were 120 elderly (>57 years) of both sexes who fulfilled the more stringent criteria for age-associated memory impairment (AAMI); some also fulfilled the criteria for age-associated cognitive decline. Subjects were allocated at random to one of the three treatment groups: placebo, 300 mg S-PS daily, or 600 mg S-PS daily. Assessments were carried out at baseline, after 6 and 12 weeks of treatment, and after a wash-out period of 3 weeks.

Tests of learning and memory, choice reaction time, planning and attentional functions were administered at each assessment. Delayed recall and recognition of a previously learned word list comprised the primary outcome measures.

No significant differences were found in any of the outcome variables between the treatment groups. There were also no significant interactions between treatment and 'severity of memory complaints'.

In conclusion, a daily supplement of S-PS does not affect memory or other cognitive functions in older individuals with memory complaints.

Keywords: Age-associated memory impairment, Cognitive functioning, Neuronal membrane function, Nutritional supplement, Phosphatidylserine, Phospholipid

INTRODUCTION

Cognitive aging, age-associated cognitive decline, and mild cognitive impairment are all defined as the decline of cognitive functioning with age. The aging of the population results in an increasing prevalence of diminished cognitive functioning, and widespread requests for therapeutic agents to attenuate cognitive decline. Despite worldwide concern about the increasing problem of cognitive decline in the aging population,

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not much research has been devoted to empirically studying the influence of nutritional supplements on human cognitive functioning, particularly in the elderly. There are few placebo-controlled studies of nutritional supplements in the elderly in the recent literature (Riedel and Jorissen, 1998). Nonetheless, many nutritional supplements are advocated on the grounds that they might enhance cognitive function, especially in the elderly. One such example, phosphatidylserine (PS), is a naturally occurring phospholipid, the daily intake of which is about 0.1 mmol (approximately 75 mg) in human diet (Bruni *et al.*, 1989). PS can be derived from several sources. Bovine cortex PS (BC-PS) has until recently been used in all PS efficacy studies. However, because of the obvious safety considerations for products of animal origin, alternative sources of PS have been developed, for example, soybean (S-PS) or eggs (E-PS).

PS influences the functioning of neuronal membranes, for example, the release of vesicles containing neurotransmitters from the presynaptic terminal (Nishizuka, 1984), signal transduction (Nishizuka, 1984), cell to cell communication, preservation of the cellular sodium–potassium and calcium–magnesium balance (Toffano, 1987) and regulation of cell growth (Nunzi *et al.*, 1990). In addition PS can restore the diminished acetylcholine release seen in cortical slices obtained from aging rats (Vannucchi and Pepeu, 1987). PS is also capable of counteracting scopolamine-induced amnesic effects in rats (Furushiro *et al.*, 1997; Zanotti *et al.*, 1986). This implies that PS may affect the cholinergic system. Finally, it has been shown that BC-PS elevates the reduced NMDA-receptor density in aged mice by approximately 25% (Cohen and Muller, 1992), and generates an increase in the efficacy of hippocampal synaptic transmission (Borghese *et al.*, 1993). Since the content of PS, expressed as percentage of total phospholipids, is higher in human brain than in other human tissues, and this percentage decreases with age (White, 1973), the administration of PS may be beneficial in preventing and treating cognitive impairment in the elderly. This is consistent with 'the membrane hypothesis of aging' (Sun and Sun, 1979), which states that age-related changes in the molecular structure and lipid–protein interactions of biological membranes in the central nervous system may impair their function. These changes may thus contribute to acceleration of the cognitive aging process.

Double-blind, placebo-controlled studies showed that BC-PS improved cognitive function in Alzheimer patients (Amaducci, 1988; Delwaide *et al.*, 1986; 1989), in senile mentally deteriorated patients (Palmieri *et al.*, 1987), and in subjects with mild to severe cognitive deterioration (Cenacchi *et al.*, 1993; Villardita *et al.*, 1987) such as in Age-Associated Memory Impairment (AAMI) (Crook, 1998; Crook *et al.*, 1991; Gindin *et al.*, 1993). The diagnostic term AAMI refers to complaints of memory impairment in tasks of daily life. People with AAMI also have an impaired performance on psychological tests (Crook *et al.*, 1986). On the basis of these double-blind studies and several open trial studies (Allegro *et al.*, 1987; Caffarra and Santamaria, 1987; Granata, 1987; Sinforiani *et al.*, 1987), it has been suggested that BC-PS is effective in the treatment of cognitive impairment in the elderly. Crook and colleagues (1991) found improvement of memory and learning after 12 weeks of treatment with 300 mg BC-PS in a double-blind placebo-controlled study with 149 AAMI subjects. Additional analyses showed that this effect was only present in a subgroup of patients with lower memory performance at baseline.

Since its introduction, only one placebo-controlled study into the cognition-enhancing effects of S-PS has been reported as an abstract (Gindin *et al.*, 1993). In this study with initially 72 AAMI-subjects memory and mood improved significantly in the 300 mg S-PS treatment group compared to placebo. However, in contrast to the findings of Crook *et al.* (1991), this effect was stronger in a subgroup of subjects with a better memory performance at baseline. These

TABLE I Exclusion criteria

- evidence of delirium, confusion, or other disturbances of consciousness;
- any neurological disorder that could produce cognitive deterioration as determined by history, clinical neurological examination, or neuroradiological examination;
- history of any infective or inflammatory brain disease;
- evidence of significant cerebral vascular pathology as determined by a Hachinski Ischemic Score (HIS) over 4 (Small, 1965);
- history of head injury;
- current psychiatric diagnosis according to DSM-IV criteria of a major psychiatric disorder;
- current diagnosis or history of alcoholism or drug dependence;
- any medical disorder that could produce cognitive deterioration;
- use of any psychotropic drug or any other drug that may significantly affect cognitive function during the month prior to psychometric testing;
- known hypersensitivity of PS.

findings at least cast some doubt on the efficacy of PS in AAMI.

The present study evaluated the efficacy of two doses of S-PS (300 mg or 600 mg daily), in a double-blind, placebo-controlled study of subjects with AAMI. The primary objective of this study was to ascertain the possible dose-related enhancing effects of 300 mg S-PS, 600 mg S-PS, compared to placebo on memory and other cognitive functions after 12 weeks of treatment. The secondary objective was to investigate a possible differential effect of S-PS between subgroups of subjects with AAMI because previous clinical studies with BC-PS (Crook *et al.*, 1986) and also of other treatments of AAMI (Israel *et al.*, 1994) have shown that the treatment response is correlated positively to the severity of the cognitive impairment at baseline.

METHODS

Subjects

Subjects were recruited through advertisements in the local newspaper and local television, and through posters in general practitioners' waiting rooms and at places where the elderly meet, such as sport or recreation centers for the elderly. Subjects were older than 57. All subjects fulfilled the criteria for AAMI (Crook *et al.*, 1986). They had complaints of memory impairment in every day life (a score of over 24 on the memory complaints questionnaire (MAC-Q) (Crook *et al.*, 1992)) and their memory test performance was at least one standard deviation below the mean established for young adults on at least one of the following standardized tests: Benton Visual Retention Test (Benton, 1975), Logical Memory Subtest of the Revised Wechsler Memory Scale (Wechsler, 1974) or the Paired Associates Learning Subtest of the Revised Wechsler Memory Scale (Wechsler, 1974). Their intellectual function was adequate, as determined by an IQ score equivalent to 90 or more (raw score of at least 32), assessed with the Vocabulary Subtest of the Wechsler Adult Intelligence Scale (WAIS) (Wechsler, 1955). They did not suffer from dementia, as determined with the Mini-Mental State Examination (Folstein *et al.*, 1975) (MMSE score > 24); or depression, as assessed with Geriatric Depression Scale (score < 15) (Yesavage and Brink, 1983). The exclusion criteria are listed in Table I. A shortened version of the Groningen Intelligence Test (Luteijn and van der Ploeg, 1983) (GIT), with three subtasks, was used to estimate the IQ score. A subgroup of AAMI subjects also fulfilled the criteria for Age-Associated Cognitive Decline (AACD) (Rediess and Caine, 1996). This AACD subgroup had memory scores (delayed recall at intake) of at least one standard deviation below the norm for their own age group (Houx, 1991). The AACD subgroup is in this study referred to as the 'moderate' group, and the Non-AACD subgroup is referred to as the

TABLE II Subject characteristics at baseline

	Placebo	300 mg PS	600 mg PS
Number of subjects	39	40	41
Number of females	19	21	21
Number of males	20	19	20
Number of AACD*	9	18	14
Mean Age (S.E.)	64.6 (0.9)	65.3 (0.9)	65.8 (1.1)
Mean IQ (S.E.)	120.3 (1.9)	115.1 (1.7)	117.7 (1.9)

*AACD stands for Age-Associated Cognitive Decline.

'mild' group. The Medical Ethics Committee of the University Hospital of Maastricht approved the study and all subjects gave written informed consent.

In all, 546 subjects were recruited, of whom 132 entered the study. Of these, 120 subjects completed the treatment according to protocol (placebo: $n = 39$, 300 mg S-PS: $n = 40$ and 600 mg S-PS: $n = 41$). The treatment groups were not significantly different in IQ, age or sex. The subjects' characteristics at baseline are summarized in Table II. Drop-outs were distributed equally over the three groups: four in the placebo group, five in the 300 mg S-PS group and three in the 600 mg S-PS group. The reasons for drop-out were not related to the treatment according to an independent physician.

Study Design

The study was conducted according to a randomized, double-blind, placebo-controlled, parallel group design. Subjects were consecutively assigned to the placebo, the 300 mg S-PS, or the 600 mg S-PS group following a predetermined order based on a randomization schedule using balanced blocks of six subjects. After psychological and medical screening, subjects underwent a training session followed by a 1-week placebo lead-in, 12 weeks of treatment (placebo, 300 mg or 600 mg S-PS), and a 3-week placebo washout (see Figure 1). The neuropsychological test assessments took place at baseline, after 6 weeks and 12 weeks of treatment, and after washout.

Treatment

Phosphatidylserine Leci-PS-40P, a substance of food-grade quality (supplied by Novartis Consumer Health SA, Nyon, Switzerland), is produced from soya lecithin by enzymatic transesterification. The product Leci-PS-40P is a powder and contains 40% PS, 13% phosphatidylcholine, 9% phosphatidylethanolamine, 5% phosphatidylinositol, 5% phosphatidic acid, and 28% polyunsaturated fatty acids.

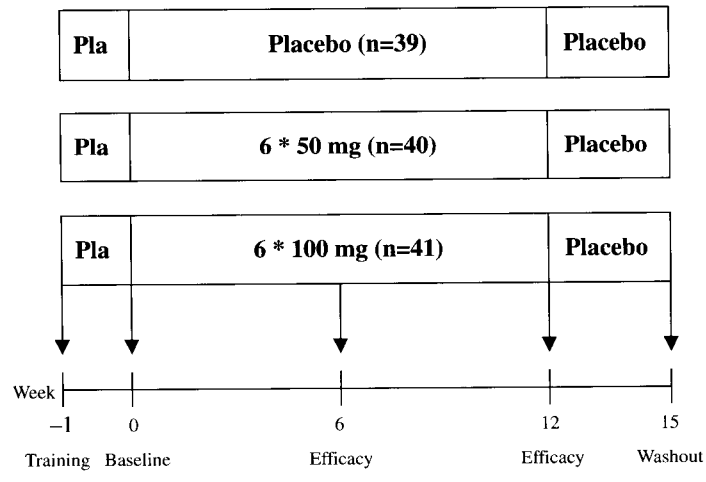

FIGURE 1 Treatment Schedule of the study to the influence of a 12-weeks treatment with two dosages phosphatidylserine (PS) or placebo on cognition in subjects with Age-Associated Memory Impairment.

The study substance was packaged in 7-day packs containing three blister trays, each containing 14 soft gelatin capsules. Each active capsule contained a phospholipid mixture of the composition described above in an amount equivalent to 0 mg (placebo), 50 mg or 100 mg pure S-PS. The mixture was diluted with medium chain triglycerides (MCT) oil to fill the remainder of the soft gelatin capsule. The MCT oil contained 95% polar lipids (coconut and palm oil) and 5% carbohydrates. Two capsules were taken three times daily: two at breakfast, two at lunch, and two at dinner. S-PS 50 mg, S-PS 100 mg, and placebo capsules looked and tasted the same.

Outcome Measures

Visual Verbal Learning Test (VVLT)

This test is an improved version of one originally devised by Rey (Brand and Jolles, 1985; Rey, 1964). During this test 15 monosyllabic words are presented for 1 second on a computer monitor. There is a 2-second inter-stimulus interval between words. After word presentation the subjects are requested to report in any order as many words they can remember (immediate recall). This procedure is repeated five times. The total immediate recall is the sum of the immediate recall measured in the five different trials. After 20 minutes, delayed recall and delayed recognition are tested. During the delayed recognition test a list of 30 words is presented with 15 words from the learned list and 15 new (but comparable) words. Each word is presented for 1 second, and the maximum time interval between two words is 3 seconds. The subjects are requested to push one of two hand-held buttons: green for yes with the preferred hand if they recognize the word, and red for no with the other hand. The number of correctly recognized words, corrected for the subjects' response tendency (sensitivity measure A'), is determined by means of the following formula: $A'=1-1/4(FR/CR+(1-CR)/(1-FR))$. CR is the number of correctly recognized words (hits) and FR is the number of falsely recognized words (misses) (Pollack and Norman, 1964). A' was arcsin transformed before it was used in statistical analyses because of its skewed distribution. Delayed recognition reaction time was also measured.

Memory Scanning Test

Memory scanning measures the speed of the memory search process. The underlying principle is that the extra time needed to complete a test in which there is a stepwise increase in the amount of information to be kept in memory, is a measure of the ease with which information is processed in working memory (Sternberg, 1975). Briefly, 20 items are to be crossed out on four test sheets containing matrices of letters in 10 lines by 12 columns. The items to be crossed out have to be memorized before the subtask is started. In the first subtask, the memory set contains one item, in the second subtask two items, and so on. With increasing memory load, each task invariably takes more time to complete. Individual slopes, intercepts, and linearities of the completion time are calculated as a function of memory load as measures of the speed of processing information in working memory. Memory scanning slopes and intercepts were used in this study.

Verbal Fluency test

The fluency test can be regarded as a measure of strategy-driven retrieval of information from semantic memory (Luteijn and van der Ploeg, 1983). The subjects are asked to produce, within 1 minute, as many four-letter words as possible starting with a given letter. The number of correct responses and errors are recorded. Nonsense words are not accepted, but names, conjugations and plurals are allowed. Starting letters were H, L, R and M. These letters yielded a similarly high number of correct responses (average: 9.5 to 10.7 words) in a population aged between 58 and 83 years with an average age of 70 (Houx, unpublished data).

Stroop Color Word Test (SCWT)

The SCWT has often been used to test selective attention (Houx *et al.*, 1993). The test involves three cards each displaying a hundred stimuli: color names, colored patches, and color names printed in incongruously colored ink. The amount of extra time needed to discard irrelevant but very salient information (verbal) in favor of a less obvious aspect (color naming) is recorded. The outcome parameters of this test are the time needed to complete each subtest. The interference denotes the percentage of extra time needed to complete card III, relative to the average of cards I and II: (time card III/((time card I + time card II)/2)) * 100%.

Signal Detection Test (SDT)

The SDT measures the ability to continuously scan the entire computer screen, searching for rarely occurring 'signals' (Schuhfried, 1991). During the task, 20 dots are presented on the screen in a random fashion. Every second three dots change position. When four dots form a square, the subject has to push a button as quickly as possible (within 2 seconds). In this study the perceptual sensitivity measure A′ and the reaction time were taken as dependent variables.

Motor Choice Reaction Time (MCRT)

Speed of information processing was assessed by measuring reaction times (RTs) as a function of task complexity (Houx and Jolles, 1993). The test consists of a simple, choice and incompatible reaction time test. This yields RTs consisting of an initiation phase (time from stimulus onset until release of a hold button) and a movement phase (time from release of the hold button until the response button is pushed). The measures used for analysis are the median initiation RTs of the simple, the choice, and the incompatible condition.

Concept Shifting Test (CST)

The CST consists of three parts (Jolles *et al.*, 1995). On each test sheet, 16 small circles (diameter = 15 mm) are grouped in a larger circle, with a radius of 8 cm. In the smaller circles, the test items (numbers (A), letters (B), or both (C)) appear in a fixed random order. Subjects are requested to cross out the items in the right order. In parts A and B, the subjects have to connect the numbers (1–2–3-etc.) and the letters (A–B–C-etc.) respectively. In part C, the subject is requested to alternate between these sequences (1–A–2–B-etc.). An exact estimate of the slowing due to shifting between concepts can be obtained by calculating the concept shifting interference, by comparing part C (digits and letters) with part A and part B.

Tower of London (TOL)

The TOL is a test of planning (Shallice, 1982). The test consists of three colored balls, which must be arranged on three sticks to match a picture with the goal positions. Varying the minimum number of moves to reach the goal positions alters the complexity of the problem. Each trial consists of a 2-, 3-, 4-, 5-, 6- and 7-move problem. Prior to each problem the subjects are told the minimum number of moves in which the problem can be solved. The number of moves, time to solve the problem, and time between presentation of the goal positions and the first move (decision time) are recorded. In this study the TOL was only assessed twice: at baseline and after 12 weeks of treatment. The maximum number of steps were used in this study.

Primary and Secondary Outcome Measures

The Visual Verbal Learning Test provided the primary outcome measures, namely, delayed recall, delayed recognition reaction time, and delayed recognition sensitivity. These three

primary variables give an indication of long-term memory performance. Total immediate recall of the verbal learning test, slope and intercept of the memory scanning, fluency, stroop interference, sensitivity and reaction time of the signal detection test, reaction times of the MCRT (simple, choice and incompatible condition), the concept shifting interference, and the maximum number of steps of the TOL were the secondary outcome measures.

STATISTICAL ANALYSIS

Data collected at the end of the placebo lead-in were taken as baseline scores, and data collected after 6 and 12 weeks of treatment were taken as the outcome measures. The MANOVA procedure was used to analyze the data in SPSS 8.0 for Windows. For the outcome measures collected after 6 weeks of treatment and at the end of the active treatment period (week 12), its corresponding baseline scores were entered as covariates in a two-way 3×2 analysis of variance, using treatment (0, 300, 600 mg) and the 'severity of memory decline' (mild versus moderate) as between-subjects factor and time as within-subjects factor in a repeated measures design. All outcome measures were analyzed separately and possible significant differences were corrected with Bonferroni–Holme correction for multiple hypothesis testing (Holm, 1979). Similar analyses were carried out to evaluate changes during the wash-out period. The outcome scores assessed at week 15 were analyzed in a MANOVA with the baseline scores as covariate.

RESULTS

Primary Outcome Variables

The means and standard errors of the primary outcome variables and the secondary outcome variables are listed in Table III. There were no effects of treatment on long-term memory performance for delayed recall ($F(2,113) = 1.25$, ns), delayed recognition sensitivity ($F(2,113) = 0.15$, ns) and delayed recognition reaction time ($F(2,113) = 1.33$, ns) after 6 and 12 weeks of S-PS treatment (see Figure 2 for delayed recall). There was a significant effect of 'severity of memory decline' for delayed recall ($F(1,113) = 23.29$, $p < .001$) and delayed recognition sensitivity ($F(1,113) = 17.64$, $p < .001$). Subjects with moderate memory decline performed lower on delayed recall and delayed recognition sensitivity compared with that of subjects with mild memory decline. There was no 'severity of memory decline' effect for delayed recognition reaction time ($F(1,113) = 0.15$, ns).

After the wash-out period there were no treatment effects for delayed recall ($F(2,113) = 0.71$, ns), delayed recognition sensitivity ($F(2,113) = 0.95$, ns) and delayed recognition reaction time ($F(2,113) = 0.80$, ns). There were no 'severity of memory decline' effects for delayed recall ($F(1,113) = 0.61$, ns), delayed recognition sensitivity ($F(1,113) = 8.52$, ns after Bonferroni-Holme correction with $p = .004$) and delayed recognition reaction time ($F(1,113) = 0.51$, ns) after the washout-periods.

None of the MANOVA results for the primary outcome variables showed interaction effects between treatment and 'severity of memory decline', or between treatment and time.

Secondary Outcome Variables

The MANOVA analysis of the secondary outcome measures revealed the same pattern of results as the primary outcome measures did. There were no effects of treatment at week 6 and week 12 for total immediate recall ($F(2, 113) = 0.54$, ns), memory scanning intercept ($F(2,113) = 1.28$, ns), memory scanning slope ($F(2,113) = 2.82$, ns), fluency ($F(2,113) = 0.48$, ns), stroop interference ($F(2,113) = 1.40$, ns), signal detection sensitivity ($F(2,113) = 1.77$, ns), signal detection reaction time ($F(2,113) = 1.82$, ns),

TABLE III Means (± SE) of the primary and secondary variables for the treatment groups at baseline, at 6 and 12 weeks after treatment (wk 6 and wk 12), and after a wash-out period of 3 weeks (wk 15). The number of subjects (*n*) is 39 in the placebo group, 40 in the 300 mg PS group and 41 in the 600 mg PS group

	Treatment group	Mn (se) Wk 0 baseline	Mn (se) Wk 6 efficacy	Mn (se) Wk 12 efficacy	Mn (se) Wk 15 washout
Primary outcome variables					
Delayed recall (# words)	Placebo	8.4 (0.4)	9.5 (0.4)*	10.3 (0.5)*	9.3 (0.5)
	300 mg PS	7.7 (0.6)	8.8 (0.5)*	8.6 (0.6)*	8.3 (0.50)
	600 mg PS	8.2 (0.5)	8.8 (0.5)*	9.4 (0.5)*	9.3 (0.5)
Recognition sensitivity (%)	Placebo	95.4 (0.7)	95.8 (0.6)*	96.7 (0.4)*	97 (0.4)
	300 mg PS	93.7 (0.8)	95.1 (0.7)*	95 (0.8)*	94.2 (1.1)
	600 mg PS	94.6 (0.7)	95.6 (0.5)*	95.7 (0.6)*	95.4 (0.8)
Recognition RT (msec)	Placebo	770.2 (18.5)	729.7 (15.5)	714.8 (15.4)	713.6 (13.6)
	300 mg PS	764.8 (16.9)	732.1 (13)	740.5 (13.1)	722 (13.5)
	600 mg PS	788.7 (20)	758.6 (16.7)	745.9 (14.7)	739.8 (16.5)
Secondary outcome variables					
Immediate total recall (# words)	Placebo	44.7 (1.5)	47.5 (1.3)*	49.9 (1.4)*	49.2 (1.4)
	300 mg PS	41.2 (1.5)	45 (1.3)*	44.4 (1.7)*	45.4 (1.5)
	600 mg PS	44.2 (1.6)	46.3 (1.6)*	47.3 (1.2)*	48.2 (1.3)
Memscan slope	Placebo	13.3 (0.8)	12.9 (0.8)	13.9 (0. 8)	14.1 (1)
	300 mg PS	13.8 (1.1)	14.2 (0.9)	15.7 (1.2)	14.7 (1)
	600 mg PS	14.4 (1)	14 (0.8)	13.8 (0.8)	14.4 (0.8)
Memscan intercept (sec)	Placebo	27.3 (0.9)	27.6 (0.9)	27.7 (0.7)	27.7 (1)
	300 mg PS	30.7 (1)	32 (1.3)	33.6 (1.5)	32.6 (1.3)
	600 mg PS	30.5 (1.1)	31 (1.1)	32 (1.2)	32.4 (1.3)
Fluency (# words)	Placebo	11.5 (0.8)	12.3 (0.7)	12.1 (0.7)	13 (0.7)
	300 mg PS	9.8 (0.5)	10.6 (0.7)	11.2 (0.6)	10.8 (0.8)
	600 mg PS	10.7 (0.6)	11.1 (0.6)	10.9 (0.7)	12.2 (0.6)
Stroop interference (sec)	Placebo	83.8 (4.4)	77.8 (3.7)	78.4 (3.8)	73.1 (3.3)
	300 mg PS	87.5 (4.9)	83.2 (4.4)	76.4 (4.5)	74.2 (4.1)
	600 mg PS	92.3 (4.8)	91.3 (5.1)	86.1 (3.6)	82.3 (3.6)
SDT sensitivity (%)	Placebo	0.7 (0.02)	0.7 (0.02)	0.7 (0.02)	0.7 (0.02)
	300 mg PS	0.7 (0.01)	0.7 (0.02)	0.7 (0.01)	0.7 (0.02)
	600 mg PS	0.6 (0.02)	0.7 (0.02)	0.7 (0.02)	0.7 (0.02)
SDT RT (msec)	Placebo	843.3 (14.7)	855.4 (12.8)	830.5 (14.2)	842.7 (16.4)
	300 mg PS	877.5 (15.8)	892.2 (14.7)	880.8 (15.1)	873.4 (22.4)
	600 mg PS	946.0 (17.4)	945.2 (22.2)	889.9 (14.7)	913.8 (20.5)
MCRT SimpleRT (msec)	Placebo	307.5 (5.5)	310.1 (5.9)	312.6 (6.3)	308.4 (4.9)
	300 mg PS	325.5 (6.3)	323.1 (7.7)	320.4 (7.9)	318.3 (7.1)
	600 mg PS	333.7 (7.5)	329.3 (7.9)	328.2 (7.6)	328.2 (7.5)
MCRT ChoiceRT (msec)	Placebo	54.7 (4.4)	51.4 (4.5)	51.7 (5.7)	56.8 (4.3)
	300 mg PS	38.6 (3.9)	48.7 (3.7)	43.7 (5.3)	53.6 (3.8)
	600 mg PS	54.7 (4.8)	53.7 (5.4)	62.1 (4. 6)	55.7 (4.3)
MCRT IncompRT (msec)	Placebo	128.8 (7)	126 (7.2)	114.4 (6.6)	107 (5.4)
	300 mg PS	129.4 (7.7)	117.7 (7.4)	113.8 (7.7)	96.9 (7.8)
	600 mg PS	144.5 (8.8)	132.3 (7.1)	127.2 (6.9)	127 (6.3)
CST Interference (sec)	Placebo	52.44 (6.1)	47.9 (5)	35.9 (3.9)	44.6 (4.3)
	300 mg PS	51.70 (7.2)	55.3 (6)	48.1 (4.9)	46.9 (5.4)
	600 mg PS	57.34 (6.4)	61.2 (6.1)	55.8 (5)	55.8 (6.2)
Tower of London	Placebo	6.82 (0.1)		6.7 (0.1)	
	300 mg PS	6.53 (0.2)	Not assessed	6.6 (0.2)	Not assessed
	600 mg PS	6.80 (0.1)		6.9 (0.1)	

* denotes a significant 'severity of memory' effect, which indicates a difference in performance between subjects with moderate memory decline and subjects with mild memory decline.

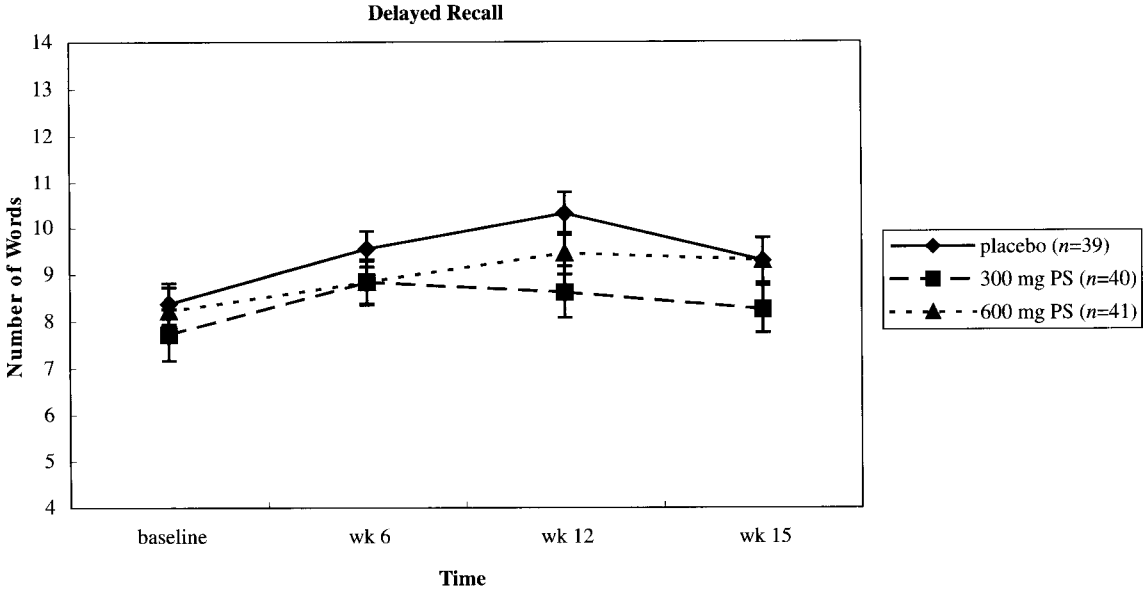

FIGURE 2 Mean (±SE) delayed recall scores of the VVLT at baseline, at 6 and 12 weeks after treatment (wk 6 and wk 12), and after a wash-out period of 3 weeks (wk 15). The number of subjects (n) is 39 in the placebo group, 40 in the 300 mg PS group and 41 in the 600 mg PS group.

MCRT simple reaction time ($F(2, 113) = 0.40$, ns), MCRT choice reaction time ($F(2, 113) = 0.87$, ns), MCRT incompatible reaction time ($F(2, 113) = 0.85$, ns) and for concept shifting interference ($F(2, 113) = 3.32$, ns after Bonferroni–Holme correction with $p = .040$). There was also no treatment effect for the TOL maximum number of steps ($F(2, 113) = 1.38$, ns) after week 12. 'Severity of memory decline' effects on total immediate recall were present. Immediate recall performance was worse in the moderate subgroup than in the mild subgroup ($F(1, 113) = 19.84$, $p < .001$). The other secondary variables did not show 'severity of memory decline' effects after 6 and 12 weeks of treatment.

No significant treatment differences were measured after the washout period for total immediate recall ($F(2, 113) = 0.29$, ns), memory scanning intercept ($F(2, 113) = 1.15$, ns), memory scanning slope ($F(2, 113) = 1.03$, ns), fluency ($F(2, 113) = 2.77$, ns), stroop interference ($F(2, 113) = 0.20$, ns), signal detection sensitivity ($F(2, 113) = 0.06$, ns), signal detection reaction time ($F(2, 113) = 1.08$, ns), MCRT simple reaction time ($F(2, 113) = 1.53$, ns), MCRT choice reaction time ($F(2, 113) = 0.95$, ns), MCRT incompatible reaction time ($F(2, 113) = 3.74$, ns after Bonferroni–Holme correction with $p = .027$) and for concept shifting interference ($F(2, 113) = 0.14$, ns). There were no 'severity of memory decline' effects after the washout period.

Furthermore there were no significant interactions between treatment and 'severity of memory decline' or between treatment and time at week 6, week 12 or after the wash-out period.

DISCUSSION

This double-blind, placebo-controlled study showed that S-PS treatment did not have any effect on cognitive performance in subjects older than 57 years of age with age-associated memory impairment.

We will first discuss some methodological issues that might have influenced the results of this study (for example due to sampling error).

There might have been a confounding factor present in the distribution of the subjects between treatment groups. Although there were no significant differences in IQ, age, or sex, mean IQ appeared higher in the placebo group (mean IQ = 120) than in the treatment groups (mean IQ = 115 for 300 mg S-PS and 118 for 600 mg PS). However, none of the outcome measures at baseline, with the exception of the Concept Shifting Test and the Fluency Test scores, were significantly associated with IQ scores. Even if there was a significant group difference, the baseline correction which we applied in our analyses should have dealt with this problem.

Another factor that could have influenced the results concerns the sensitivity of the cognitive tests to detect treatment effects, defined as the test–retest reliability. The latter has been identified as the most appropriate single summary measure of reliability (Parrott, 1991). Average intraclass correlation coefficients, which are overall correlations of multiple assessments, were analyzed over five assessments (including training). The analyses revealed an adequate test–retest reliability higher than 0.8 for the primary variables to detect treatment effects. The test–retest reliabilities was higher than 0.8 for the secondary variables, except for choice reaction time (0.7), concept shifting interference (0.6), and the number of steps of the tower of London (0.3) which was only assessed at baseline and at week 12. This indicates that only the concept shifting test and the tower of London test may not have been sensitive enough to detect a treatment effect.

Concerning the sample size, a priori power analysis showed that 117 subjects were the minimum total sample size in a three group design, in order to detect a treatment difference on delayed recall of 1.25 (with standard deviation of 2) with a power of 80%. In case of a smaller effect size, indeed, power would have been too low. However, a smaller effect then should have emerged as a trend in the data.

A potentially important treatment-related issue is the presence of small amounts of phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylinositol (PI) in the phosphatidylserine substance used in our study. It would have been better had S-PS been the only phospholipid in the capsules because the other phospholipids may have influenced the treatment effect of S-PS. In rat studies it has been shown that the administration of PS liposomes increases calcium-dependent acetylcholine release from the cerebral cortex in anaesthetized rats, with PE being about half as active as PS whereas PC was inactive in this respect (Casamenti *et al.*, 1979). Despite these less pronounced effects of PC and PE on acetylcholine release, the mechanisms of action of the phospholipids may have interacted with those of the S-PS treatment in our study. Eagger *et al.* (1991) showed that 150 mg tacrine (a cholinesterase inhibitor) in combination with 10.8 g lecithin (containing 15% PC) also improved cognitive function in Alzheimer patients, although the authors mentioned that lecithin was unlikely to have an influence because the treatment had no effect on plasma choline concentrations. The lecithin dose was about 30 times higher than the effective dose of BC-PS. Therefore, although in principle we cannot exclude effects of PC and PE, we doubt whether they would have had an effect because they were present in very small amounts.

We assumed that the AAMI criteria are suitable to demonstrate a treatment effect in normally aging individuals. Crook *et al.* (1986) published the diagnostic criteria for AAMI to stimulate research into the epidemiological, clinical characterization, and treatment aspects of 'normal' later-life memory loss. Several drugs have been tested for their cognition-enhancing effects on AAMI or age-associated cognitive decline (AACD), although no cognition enhancer has reliably and repeatedly been demonstrated to be effective (Riedel and Jolles, 1996). Subjects with AAMI may not be suitable as a clinical population in studies of drugs or nutrients.

However, as most nutritional supplements are aimed at normally aging subjects, this argument may not hold for nutrient studies. AAMI-subjects hardly differ from 'normal-aging' subjects and for this purpose, application of the AAMI criteria may be a well-tuned pragmatic approach in efficacy studies of nutritional supplements for the elderly. As such, the AAMI criteria fulfill the function of excluding successfully aging subjects from study participation. Smith *et al.* (1991) discussed some problems of reliability and expressed concerns regarding the AAMI criteria. One of their suggestions was the use of age-appropriate norms, since the AAMI criteria do not consider the discontinuity of normal test performance between the younger old and the older old. We used the AAMI criteria so that we could compare our results with those of previous PS studies (Crook, 1998; Crook *et al.*, 1991; Gindin *et al.*, 1993). We also used age-appropriate norms to define the group with more severe memory decline.

Finally, we assumed that ingested S-PS would be available in the brain. Whereas nothing is known about the amount of BC-PS or S-PS that passes through the gastrointestinal tract and the blood–brain barrier after oral administration in humans. In rats, orally administered radioactive-labeled BC-PS (^{14}C-PS) passes the gastrointestinal tract very slowly, and an intravenously injected dose was halved very rapidly and only 0.25% of an injected dose reached the brain tissue after 20 minutes (Orlando *et al.*, 1987). However in humans, Rosadini *et al.* (1990) using indirect quantitative EEG methods, showed an increase of the power on the 'alpha' frequency at the anterior electrode after a 50-mg dose of intravenously administered BC-PS, and over the whole scalp after a 75-mg dose of BC-PS. This implies that BC-PS induced cerebral activity after intravenous administration. Plasma concentrations of PS after oral administration in humans have not been reported in the literature.

In future studies, attention should be paid to the fatty-acid content of PS. Essential fatty acids may control the modulation of neuronal membrane fluidity and thus might influence cognitive functions (Yehuda *et al.*, 1999). Treatment with a 1:4 ratio of n-3 and n-6 fatty acids improved mood, cooperation, appetite, sleep, ability to navigate in the home, and short-term memory in a placebo-controlled trial with 100 Alzheimer patients (Yehuda *et al.*, 1996). In our study, linoleic acid (n-6) accounted for approximately 58% of the polyunsaturated fatty acids in and linolenic acid (n-3) for 7%. No information is available of the fatty acid content of PS used in other human studies. In animal studies (Blokland *et al.*, 1999; Sakai *et al.*, 1996), there was a large difference in fatty acid content of the S-PS and BC-PS formulas used, and even between the different S-PS formulas. This might be important for the efficacy of specific PS formulas.

Another aspect that needs to be addressed is the way of production of S-PS by enzymatic conversion. Some enzyme may be included in the final product, which may lead to a partial degradation of S-PS with time. In our study, the capsules contained 50% of the initial value of S-PS after 15 months. This final level was still higher than the level of supplementation used in studies with BC-PS, and the last treatment was finished 11 months after the capsules were prepared.

Despite these reservations, PS would appear to have only doubtful cognitive enhancing effects in subjects with AAMI. PS did not influence cognitive functions in our AAMI population, whereas Crook and colleagues showed that S-PS and BC-PS enhanced both name recall immediately and an hour after introduction, and learning and recall of written information in AAMI subjects (Crook, 1998). However, this effect was only present in the subgroup of patients with the most severe cognitive impairment. Crook compared the placebo and BC-PS group from the 1991 study (Crook *et al.*, 1991) with a new S-PS group, without introducing a new placebo group. Thus the data were not analyzed according to a double-blind procedure, which is very important

in clinical trials. Gindin and colleagues showed, in a double-blind, placebo-controlled study, a significant improvement of memory and mood in 72 AAMI-subjects (Gindin *et al.*, 1993). In contrast to the study by Crook *et al.*, subjects with higher baseline scores had an improved memory function after treatment.

The behavioral effects of S-PS have also been studied in animals. Blokland *et al.* (1999) compared the behavioral effects of an intraperitoneal injection of BC-PS with S-PS and E-PS in a placebo controlled rat study. BC-PS and S-PS had similar effects on tests of avoidance learning, but not on tests of spatial discrimination learning. Sakai *et al.* (1996) also found that orally administered S-PS and BC-PS improved scopolamine-induced deterioration of passive avoidance. Compared to the cognition-enhancing effects in rats of other cholinesterase inhibitors, such as metrifonate (Blokland *et al.*, 1995; van der Staay *et al.*, 1996), the magnitude of the effects obtained with BC-PS and S-PS were only marginal.

Thus while BC-PS and S-PS may have cognition-enhancing effects in animals, their effects in humans are not yet clear. This difference in effect might be caused by the difference between animal and human research. In animal research the methodological conditions can be controlled more accurately. For instance, the diet of the animals is the same, whereas in human PS studies, including our study, the diet of the free-living volunteers was not controlled. The diet composition, for example, a high fat versus a low fat diet, might influence the uptake of S-PS in the gastrointestinal tract.

In summary, this study showed that oral administration of S-PS did not improve memory or other cognitive functions in people suffering from AAMI. Since others (Crook *et al.*, 1991; Gindin *et al.*, 1993) have shown cognition-enhancing effects of BC-PS in different subgroups of AAMI subjects, and the plasma concentration of PS after oral administration has not been measured in humans, the efficacy of PS is still questionable. Future research could benefit from the development of methods to assess changes in the relatively low plasma concentrations of PS after its oral administration in humans.

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References

Allegro, L., Favaretto, V. and Ziliotto, G. (1987) Oral phosphatidylserine in elderly patients with cognitive deterioration: An open trial. *Clin. Trials J.* **24**, 104–108.

Amaducci, L. (1988) Phosphatidylserine in the treatment of Alzheimer's disease: results of a multicenter study. *Psychopharmacol. Bull.* **24**, 130–134.

Benton, A. (1975) The Revised Visual Retention Test: Clinical and Experimental Applications (New York: Psychological Corporation).

Blokland, A., Hinz, V. and Schmidt, B.H. (1995) Effects of Metrifonate and Tacrine in the Spatial Morris Task and Modified Irwin Test: Evaluation of the Efficacy/Safety Profile in Rats. *Drug Development Research* **36**, 166–179.

Blokland, A., Honig, W., Brouns, F. and Jolles, J. (1999) Cognition-Enhancing Properties of Subchronic Phosphatidylserine (PS) Treatment in Middle-Aged Rats: Comparison of Bovine Cortex PS with Egg PS and Soybean PS. *Nutrition* **15**, 778–783.

Borghese, C.M., Gomez, R.A. and Ramirez, O.A. (1993) Phosphatidylserine increases hippocampal synaptic efficacy. *Brain Res. Bull.* **31**, 697–700.

Brand, N. and Jolles, J. (1985) Learning and retrieval rate of words presented auditorily and visually. *J. of Gen. Psychol.* **112**, 201–210.

Bruni, A., Mietto, L., Bellini, F., Boarato, E. and Toffano, G. (1989) Pharmacological and autopharmacological action of phosphatidylserine. In: Bazan, N.G., Horrocks, L.A. and Toffano, G. (Eds), *Phospholipids in the Nervous System: Biochemical and Molecular Pathology* (Padova: Liviana Press), Vol. 17, pp. 217–224.

Caffarra, P. and Santamaria, V. (1987) The effects of phosphatidylserine in patients with mild cognitive decline: An open trial. *Clin. Trials J.* **24**, 109–114.

Casamenti, F., Mantovani, P., Amaducci, L. and Pepeu, G. (1979) Effect of phosphatidylserine on acetylcholine output from the cerebral cortex of the rat. *J. Neurochem.* **32**, 529–533.

Cenacchi, T., Bertoldin, T., Farina, C., Fiori, M.G. and Crepaldi, G. (1993) Cognitive decline in the elderly: a double-blind, placebo-controlled multicenter study on efficacy of phosphatidylserine administration. *Aging Milano* **5**, 123–133.

Cohen, S.A. and Muller, W.E. (1992) Age-related alterations of NMDA-receptor properties in the mouse forebrain: partial restoration by chronic phosphatidylserine treatment. *Brain Res.* **584**, 174–180.

Crook, T., Bartus, R.T., Ferris, S.H., Whitehouse, P., Cohen, G.D. and Gershon, S. (1986) Age-associated memory impairment: propose diagnostic criteria and measures of clinical change – report of a National Institute of Mental Health Work Group. *Dev. Neuropsychol.* **2**, 262–276.

Crook, T.H. (1998) Treatment of age-related cognitive decline: effects of phosphatidylserine. In: Klatz, R.M. and Goldman, R. (Eds), *Anti-aging Medical Therapeutics* (California: Harina del Rey), Vol. II, pp. 20–28.

Crook, T.H., Feher, E.P. and Larrabee, G.J. (1992) Assessment of memory complaint in age-associated memory impairment: the MAC-Q. *Int. Psychogeriatr.* **4**, 165–176.

Crook, T.H., Tinklenberg, J., Yesavage, J., Petrie, W., Nunzi, M.G. and Massari, D.C., (1991) Effects of phosphatidylserine in age-associated memory impairment. *Neurology* **41**, 644–649.

Delwaide, P.J., Gyselynck Mambourg, A.M., Hurlet, A. and Ylieff, M. (1986) Double-blind randomized controlled study of phosphatidylserine in senile demented patients. *Acta. Neurol. Scand.* **73**, 136–140.

Delwaide, P.J., Maertens de Noordhout, A., De Paqua, V., Ylieff, M., Gyselinkc-Mambourg, A.M. and Hurlet, A. (1989) Effects of phosphatidylserine (BC-PS) on aged brain in normal subjects and senile demented patients. In: Bazan, N.G., Horrocks, L.A. and Toffano, G. (Eds), *Phospholipids in the Nervous System: Biochemical and Molecular Pathology* (Padova: Liviana Press), Vol. 17, pp. 261–268.

Eagger, S.A., Levy, R. and Sahakian, B.J. (1991) Tacrine in Alzheimer's disease [see comments]. *Lancet* **337**, 989–992.

Folstein, M.F., Folstein, S. and McHugh, P.R. (1975) Mini-Mental State: A practical method for grading the cognitive state of patients for the clinician. *J. Psychiat. Res.* **12**, 189–198.

Furushiro, M., Suzuki, S., Shishido, Y., Sakai, M., Yamatoya, H., Kudo, S., Hashimoto, S. and Yokokura, T. (1997) Effects of oral administration of soybean lecithin transphosphatidylated phosphatidylserine on impaired learning of passive avoidance in mice. *Jpn. J. Pharmacol.* **75**, 447–450.

Gindin, J., Kedar, D., Naor, S., Novikov, M., Walter-Ginzburg, A. and Levi, S. (1993) The effect of herbal phosphatidylserine on memory and mood in community elderly. *Gerontologist* **33**, 230 (Abstract).

Granata, Q.a.D.M., J. (1987) Phosphatidylserine in elderly patients, an open trial. *Clin. Trial J.* **24**, 99–103.

Holm, S. (1979) A simple sequentially rejective multiple test procedure. *Scand. J. Statist.* **6**, 65–70.

Houx, P. (1991) Cognitive Aging and Health-Related Factors (Rijksuniversiteit Limburg, Maastricht).

Houx, P.J. and Jolles, J. (1993) Age-related decline of psychomotor speed: effects of age, brain health, sex and education. *Perceptual Motor Skills* **76**, 195–211.

Houx, P.J., Vreeling, F.W. and Jolles, J. (1993) Stroop interference: aging effects assessed with the Stroop Color-Word Test. *Aging Res.* **19**, 209–224.

Israel, L., Melac, M., Milinkevitch, D. and Dubois, G. (1994) Drug therapy and memory training programs: A double-blind randomized trial of general practice patients with age-associated memory impairment. *Int. Psychogeriatr.* **6**, 155–170.

Jolles, J., Houx, P.J., van Boxtel, M.P.J. and Ponds, R.W.H.M. (1995) The Maastricht Aging Study, Determinants of cognitive aging. (Maastricht: Maastricht University).

Luteijn, F. and van der Ploeg, F.A.E. (1983) Manual Groningen Intelligence Test (GIT) (Lisse, The Netherlands: Swets and Zeitlinger).

Nishizuka, Y. (1984) Turnover of inositol phospholipids and signal transduction. *Science* **225**, 1365–1370.

Nunzi, M.G., Milan, F., Guidolin, D., Zanotti, A. and Toffano, G. (1990) Therapeutic properties of phosphatidylserine in the aging brain. In: Hanin I. and Pepeu, G. (Eds), *Phospholipids: Biochemical, Pharmaceutical, and Analytical Considerations* (New York: Plenum Press), pp. 213–218.

Orlando, P., Battistella, A. and Toffano, G. (1987) Pharmacokinetics of radiolabelled brain phosphatidylserine. *Clinical Trials Journal* **24**, 18–24.

Palmieri, G., Palmieri, R., Inzoli, M.R., Lombardi, G., Sottini, C., Tavolato, B. and Giometto, B. (1987) Double-blind controlled trial of phosphatidylserine in patients with senile mental deterioration. *Clin. Trials J.* **24**, 73–83.

Parrott, A.C. (1991) Performance tests in human psychopharmacology: I. Test reliability and standardization. *Hum. Psychopharmacol.* **6**, 1–9.

Pollack, I. and Norman, D.A. (1964) A non-parametric analysis of recognition experiments. *Pscychonomic. Sci.* **1**, 125–126.

Rediess, S. and Caine, E.D. (1996) Aging, cognition and DSM-IV. *Aging, Neuropsychol., and Cognit.* **3**, 105–117.

Rey, A., (1964) L'examen psychologique dans les cas d'encéphalopathie traumatique (Paris: Presses Universitaires de France).

Riedel, W.J. and Jolles, J. (1996) Cognition enhancers in age-related cognitive decline. *Drugs Aging* **8**, 245–274.

Riedel, W.J. and Jorissen, B.L. (1998) Nutrients, age and cognitive function. *Curr. Opin. Clin. Nutr. Metab. Care* **1**, 579–585.

Rosadini, G., Sannita, W.G., Nobili, F. and Cenacchi, T. (1990) Phosphatidylserine: quantitative EEG effects in healthy volunteers. *Neuropsychobiology* **24**, 42–48.

Sakai, M., Yamatoya, H. and Kudo, S. (1996) Pharmacological effects of phosphatidylserine enzymatically synthesized from soybean lecithin on brain functions in rodents. *J. Nutr. Sci. Vitaminol. Tokyo* **42**, 47–54.

Schuhfried, G. (1991) Wiener Testsystem: Signal Detektion [Vienna Test System: Signal Detection Test] (Mödling, Austria: Schuhfried GmbH).

Shallice, T. (1982) Specific impairments of planning. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **298**, 199–209.

Sinforiani, E., Agostinis, C., Merlo, P., Gualtieri, S., Mauri, M. and Mancuso, A. (1987) Cognitive decline in ageing brain: Therapeutic approach with phosphatidylserine. *Clin. Trials J.* **24**, 115–124.

Small, G. (1965) Revised ischemic score for diagnosing multi-infart dementia. *J. of Clin. Psychiatr.* **46**, 514–517.

Smith, G., Ivnik, R.J., Petersen, R.C., Malec, J.F., Kokmen, E. and Tangalos, E. (1991) Age-associated memory impairment diagnoses: problems of reliability and concerns for terminology. *Psychol. Aging* **6**, 551–558.

Sternberg, S. (1975) Memory scanning: New findings and current controversies. *Q. J. Exp. Psychol.* **27**, 1–32.

Sun, A.Y. and Sun, G.Y. (1979) Neurochemical aspects of the membrane hypothesis of aging. In: Meier-Ruge, W. (Ed), *CNS Aging and its Neuropharmacology, Experimental and Clinical Aspects* (Basel: S. Karger), Vol. 15, pp. 34–53.

Toffano, G. (1987) The therapeutic value of phosphatidylserine effect in the aging brain. In: Hanin, I. and Ansell, G.B. (Eds), *Lecithin: Technological, Biological, and Therapeutic Aspects* (New York: Plenum Press), pp. 137–146.

van der Staay, F.J., Hinz, V.C. and Schmidt, B.H. (1996) Effects of metrifonate, its transformation product dichlorvos, and other organophosphorus and reference cholinesterase inhibitors on Morris water escape behavior in young-adult rats. *J. Pharmacol. Exp. Ther.* **278**, 697–708.

Vannucchi, M.G. and Pepeu, G. (1987) Effect of phosphatidylserine on acetylcholine release and content in cortical slices from aging rats. *Neurobiol. Aging* **8**, 403–407.

Villardita, C., Grioli, S., Salmeri, G., Nicoletti, F. and Pennisi, G. (1987) Multicentre clinical trial of brain phosphatidylserine in elderly patients with intellectual deterioration. *Clin. Trials J.* **24**, 84–93.

Wechsler, D. (1955) Manual for the Wechsler Adult Intelligence Scale (New York: The Psychological Corporation).

Wechsler, D. (1974) Wechsler Memory Scale Manual (New York: The Psychological Corporation).

White, D.A. (1973) The phospholipid composition of mammalian tissues. In: G.B. Ansell, J.N. Hawthorne and R.M.C. Dawson, (Eds), *Form and Function of Phospholipids* (Amsterdam: Elsevier Scientific Publishing Company), Vol. 3, pp. 441–482.

Yehuda, S., Rabinovitz, S. and Mostofsky, D.I. (1999) Essential fatty acids are mediators of brain biochemistry and cognitive functions. *J. Neurosci. Res.* **56**, 565–570.

Yehuda, S., Rabinovtz, S., Carasso, R.L. and Mostofsky, D.I. (1996) Essential fatty acids preparation (SR-3) improves Alzheimer's patients quality of life. *Int. J. Neurosci.* **87**, 141–149.

Yesavage, J. and Brink, T. (1983) Development and validation of a geriatric depression scale: A preliminary report. *J. of Psychiatr. Res.* **17**, 37–49.

Zanotti, A., Valzelli, L. and Toffano, G. (1986) Reversal of scopolamine-induced amnesia by phosphatidylserine in rats. *Psychopharmacology Berl.* **90**, 274–275.