International Psychogeriatrics (2012), Vol. 24, Supplement 1, S25–S34 © International Psychogeriatric Association 2012
doi:10.1017/S1041610212000567

Ginkgo biloba leaf extract (EGb 761®) and its specific acylated flavonol constituents increase dopamine and acetylcholine levels in the rat medial prefrontal cortex: possible implications for the cognitive enhancing properties of EGb 761®

J. Kehr,[1,2] S. Yoshitake,[2] S. Ijiri,[3] E. Koch,[4] M. Nöldner[4] and T. Yoshitake[1]

[1] *Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden*
[2] *Pronexus Analytical AB, Stockholm, Sweden*
[3] *School of Pharmacy, International University of Health and Welfare, Tochigi, Japan*
[4] *Preclinical Research, Dr. Willmar Schwabe GmbH & Co. KG, Karlsruhe, Germany*

ABSTRACT

Experimental and clinical data suggest that the *Ginkgo biloba* standardized extract EGb 761® exerts beneficial effects in conditions which are associated with impaired cognitive function. However, the neurochemical correlates of these memory enhancing effects are not yet fully clarified. The aim of this study was to examine the effect of repeated oral administration of EGb 761® and some of its characteristic constituents on extracellular levels of dopamine (DA), noradrenaline (NA), serotonin (5-HT), acetylcholine (ACh) and the metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) in the medial prefrontal cortex (mPFC) of awake rats by use of *in vivo* microdialysis technique.

Subacute (14 days, once daily), but not acute, oral treatment with EGb 761® (100 and 300 mg/kg) or the flavonoid fraction, which represents about 24% of the whole extract caused a significant and dose-dependent increase in extracellular DA levels in the mPFC. Repeated administration of EGb 761® also caused a modest but significant increase in the NA levels, whereas the concentrations of 5-HT and those of the metabolites DOPAC, HVA and 5-HIAA were not affected. The same treatment regimen was used in a subsequent study with the aim of investigating the effects of two Ginkgo-specific acylated flavonols, 3-O-(2''-O-(6'''-O-(p-hydroxy-trans-cinnamoyl)-β-D-glucosyl)-α-L-rhamnosyl)quercetin (Q-ag) and 3-O-(2''-O-(6'''-O-(p-hydroxy-trans-cinnamoyl)-β-D-glucosyl)-α-L-rhamnosyl)kaempferol (K-ag). Both compounds together represent about 4.5% of the whole extract. Repeated oral treatment with Q-ag (10 mg/kg) for 14 days caused a significant increase in extracellular DA levels of 159% and extracellular acetylcholine (ACh) levels of 151% compared to controls. Similarly, administration of K-ag (10 mg/kg) induced a significant rise of DA levels to 142% and ACh levels to 165% of controls, whereas treatment with isorhamnetin, an O-methylated aglycon component of EGb 761® flavonol glycosides had no effect. None of the tested flavonoids had a significant effect on extracellular DOPAC and HVA levels.

The present findings provide evidence that the subacute treatment with EGb 761® and its flavonol constituents increases DA and ACh release in the rat mPFC, and suggest that the two Ginkgo-specific acylated flavonol glycosides Q-ag and K-ag are active constituents contributing to these effects. As seen for isorhamnetin, the effect on neurotransmitter levels seems not to be a general effect of flavonols but rather to be a specific action of acylated flavonol glycosides which are present in EGb 761®. The direct involvement of these two flavonol derivatives in the increase of dopaminergic and cholinergic neurotransmission in the prefrontal cortex may be one of the underlying mechanisms behind the reported effects of EGb 761® on the improvement of cognitive function.

Key words: Ginkgo biloba, neurotransmitter release, microdialysis, rat

Introduction

The standardized extract EGb 761® from the leaves of *Ginkgo biloba* contains 24% flavonol glycosides, 6% terpene trilactones and other substances including proanthocyanidins and organic acids (DeFeudis and Drieu, 2000; DeFeudis, 2003; Chan *et al.*, 2007).

The major flavonol constituents of *Ginkgo biloba* are essentially flavonol-O-glycosides of quercetin, kaempferol and isorhamnetin forming

Correspondence should be addressed to: Dr. Jan Kehr, Pronexus Analytical AB, Karolinska Institutet Science Park, Fogdevreten 2a, 171 77 Stockholm, Sweden. Phone: +46 8 403 90125; Fax: +46 8 5248 7234. Email: jk@pronexus.se

mono-, di- and triglycosides with D-glucose and L-rhamnose (Hasler, 2000; Bedir *et al.*, 2002). Besides differences in their basic structure and the glycosylation pattern, flavonoids exhibit an enormous diversity due to glycosylation, methylation and acylation. In particular, acylation has been shown to significantly modify physico-chemical and biological properties of these substances. Acylation of flavonoids with malonic, coumaric, caffeic or ferulic acid is common, especially among anthocyanins, but is less frequent for flavonols (Viskupicova *et al.*, 2009). The acylated flavonol glycosides in *Ginkgo biloba* consist of di- and triglycosides which are esterified with coumaric acid. They are substitued with a diglycoside unit (biloside) at C3. Acylated quercetin- and kaempferol-3-O-bilosides are specific constituents of Ginkgo leaves (Hasler, 1993). The two acylated flavonol glycosides, 3-O-(2''-O-(6'''-O-(p-hydroxy-trans-cinnamoyl)-β-D-glucosyl)-α-L-rhamnosyl)quercetin (Q-ag) and 3-O-(2''-O-(6'''-O-(p-hydroxy-trans-cinnamoyl)-β-D-glucosyl)-α-L-rhamnosyl)kaempferol (K-ag) (Figure 3), represent about 4.5% of EGb 761® content (Dr. F. Lang, Dr. Willmar Schwabe GmbH & Co. KG, personal communication).

The terpene trilactone constituents include ginkgolides A, B, C and J, which together account for 3.1%, and bilobalide, which comprises 2.9% of the total EGb 761® extract. The *Ginkgo biloba* extract and its numerous individual constituents have, among other activities, shown to protect and improve mitochondrial energy production, to ameliorate microcirculation, and to possess antioxidant, radical scavenging and neuroprotective properties (Wu and Zhu, 1999; DeFeudis and Drieu, 2000; Ahlemeyer and Krieglstein, 2003; Ahmad *et al.*, 2005; Abdel-Kader *et al.*, 2007; Rojas *et al.*, 2008).

Experimental and clinical data suggest that EGb 761® exerts beneficial effects as a cognitive enhancer and in the therapy of age-related neurological disorders including Alzheimer´s disease (AD) (for review, see DeFeudis and Drieu, 2000; DeFeudis, 2003; Weinmann *et al.*, 2010). In animal experiments, *Ginkgo biloba* extract was shown to improve spatial memory deficits in a transgenic mouse model of AD (Stackman *et al.*, 2003), as well as to improve acquisition of working memory in young (Satvat and Mallet, 2008) and aged rats (Wang *et al.*, 2006; Blecharz-Klin *et al.*, 2009). However, the precise neurochemical correlates of these behavioral effects of *Ginkgo biloba* extract are not completely understood. A number of clinical studies have demonstrated that *Ginkgo biloba*, and particularly EGb 761®, ameliorate cognitive deficits associated with mild to moderate AD and vascular dementia (Wang *et al.*, 2010; Weinmann *et al.*, 2010) and improve memory function in patients with very mild cognitive impairment (Grass-Kapanke *et al.*, 2011) as well as in middle-aged healthy volunteers (Kaschel, 2011). Based on the evidence from randomized and controlled clinical trials, the German Institute for Quality and Efficacy in Health Care (IQWiG) concluded that EGb 761® is beneficial in AD (IQWiG, 2008). Two large-scale prospective cohort studies have suggested that long-term intake of EGb 761® might protect against development of dementia (Andrieu *et al.*, 2003; Dartigues *et al.*, 2007). A randomized placebo controlled pilot study highlighted that delay of conversion to dementia can only be expected with good compliance in long-term treatment (Dodge *et al.*, 2008).The Ginkgo Evaluation of Memory (GEM) study was carried out to test whether *Ginkgo biloba* is effective in prevention of dementia (and especially AD) in elderly individuals with normal cognition or those with mild cognitive impairment (DeKosky *et al.*, 2008). The interpretation of this randomized, double-blind, placebo controlled clinical trial of 3069 participants aged 72–96 years is limited by the very poor treatment compliance of this tertiary care center trial (Jerant *et al.*, 2011). A primary care based long-lasting clinical study on the use of EGb 761® for secondary prevention of AD involving participants without dementia aged 70 years or older who had spontaneously complained of memory problems (GuidAge) has been performed in Europe (Andrieu *et al.*, 2008). During the study 4.3% of the patients in the EGb 761® group developed AD compared to 5.2% in the placebo group. Because the dementia rate in this trial (4.8%) was much lower than expected for sample size planning (13.8%), this trend did not reach statistical significance. However, a clear difference was observed in a preplanned analysis of subjects treated for at least four years as only 1.6% of the EGb 761® treated participants converted to AD in comparison to 3.0% in the placebo group (Vellas *et al.*, 2010).

Besides the proposed anti-dementia and pro-cognitive effects of *Ginkgo biloba*, a growing body of clinical evidence exists for the efficacy of EGb 761® in the treatment of neuropsychiatric symptoms of dementia (Bachinskaya *et al.*, 2011; Ihl *et al.*, 2011). Thus, EGb 761® at a daily dose of 240 mg effectively alleviated behavioral and neuropsychiatric symptoms including apathy/indifference, sleep/night-time behavior, irritability/lability, depression/dysphoria, and aberrant motor behavior in patients with mild to moderate dementia (Bachinskaya *et al.*, 2011). A review and meta-analysis

https://doi.org/10.1017/S1041610212000567

of the usage of Ginkgo extract as an adjunct therapy in chronic schizophrenia showed that its use as an add-on therapy to antipsychotic medication produced a significant improvement in total and negative symptoms of chronic schizophrenia (Singh *et al.*, 2010). In addition, EGb 761® was effective in reducing the symptoms of tardive dyskinesia in schizophrenia patients (Zhang *et al.*, 2011).

The clinical studies demonstrating the efficacy of EGb 761® in alleviating neuropsychiatric symptoms in dementia, as well as the negative and cognitive symptoms in schizophrenia, suggest that the extract contains one or more active constituents, which may exert direct modulatory actions on central neurotransmitter systems. Of those, the most interesting candidates are the cholinergic and monoaminergic projections to the prefrontal cortex and hippocampus, the neuroanatomical structures strongly implicated in cognitive function and affective behavior. Indeed, cholinergic replacement therapy using the cholinesterase inhibitors (donepezil, rivastigmine, or galantamine) is currently the prevailing strategy for treatment of mild to moderate AD (O'Brien and Burns, 2011). The new (second and third) generations of antipsychotic drugs aim to reduce the negative symptoms and ameliorate cognitive deficits in schizophrenia preferentially by increasing the cholinergic (Terry, 2008) and dopaminergic transmission (Meltzer and Huang, 2008; Kim *et al.*, 2009) in the frontal cortical structures. In view of these facts, it is hypothesized that the systemic administration of EGb 761® may modulate the levels of dopamine (DA) and acetylcholine (ACh) neurotransmitters in the prefrontal cortex. In order to investigate this hypothesis, the microdialysis technique, which allows monitoring of extracellular levels of neurotransmitters including DA, ACh, as well as noradrenaline (NA), serotonin (5-HT) and the metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) was applied in awake rats.

Effects of acute and chronic treatment with EGb 761® on monoamines and their metabolites in the rat brain

In an initial study, the effects of acute and subacute (14 days, once daily) treatment with EGb 761® on extracellular levels of the monoamines DA, NA and 5–HT and their acidic metabolites DOPAC, HVA and 5-HIAA in the medial prefrontal cortex of awake rats were examined. A single oral administration of EGb 761® (100 mg/kg) had no effect on basal extracellular levels of monoamines and their metabolites (Yoshitake *et al.*, 2010).

This finding is in disagreement with some earlier *in vitro* and *ex vivo* whole tissue studies, which have suggested that *Gingko biloba* extract reduces the activity of both MAO A and MAO B in the rat (White *et al.*, 1996) and mouse brain (Wu and Zhu, 1999; Pardon *et al.*, 2000). On the other hand, a number of microdialysis studies, including our earlier data (Yoshitake *et al.*, 2004a), have demonstrated that the MAO inhibitors increase extracellular levels of all three monoamines and decrease the levels of their respective metabolites even after a single acute administration. These findings are also in agreement with the report by Shah and co-workers who found that an acute dose of *Gingko biloba* extract had no effect on brain tissue levels of monoamines and reduced the stress-induced increases in whole brain levels of catecholamines and 5-HT in the rat (Shah *et al.*, 2003).

However, strikingly different effects were observed following subacute daily treatment with EGb 761® for 14 days. Here, the EGb 761® challenge at the doses of 100 and 300 mg/kg p.o. caused a marked elevation in extracellular DA levels within 40–180 minutes. The lower dose of EGb 761® caused a maximal increase of the DA levels to $185 \pm 10\%$ (mean ± SEM, $p < 0.001$) of the corresponding value of the control group at 160 minutes, whereas the dose of 300 mg/kg EGb 761® increased the DA levels to $234 \pm 6\%$ ($p < 0.001$) at 100 minutes after drug administration (Figure 1). The low dose (30 mg/kg) of EGb 761® had no effect on extracellular DA levels. In an earlier study (Yoshitake *et al.*, 2010), it was shown that subacute treatment with EGb 761® had only a minor effect on the NA levels, while the levels of 5-HT were not affected. Interestingly, in spite of increased DA levels following the chronic treatment with EGb 761®, the concentrations of metabolites DOPAC and HVA were unchanged at any dose of EGb 761® (Yoshitake *et al.*, 2010). In the rat striatum, the subacute treatment with EGb 761® at the highest dose caused only a minor increase in the DA levels to $125 \pm 4\%$ of the vehicle-treated group at 120 minutes after the EGb 761® administration. This finding is in agreement with behavioral recordings where no significant increase in locomotor activity was recorded. The EGb 761® doses of 100 and 300 mg/kg used in the study are considered to be relevant to the currently recommended clinical doses of 240 mg/day (Schwabe, Tebonin® konzent, Summary of Product Characteristics, European Medicines Agency, 2011).

https://doi.org/10.1017/S1041610212000567

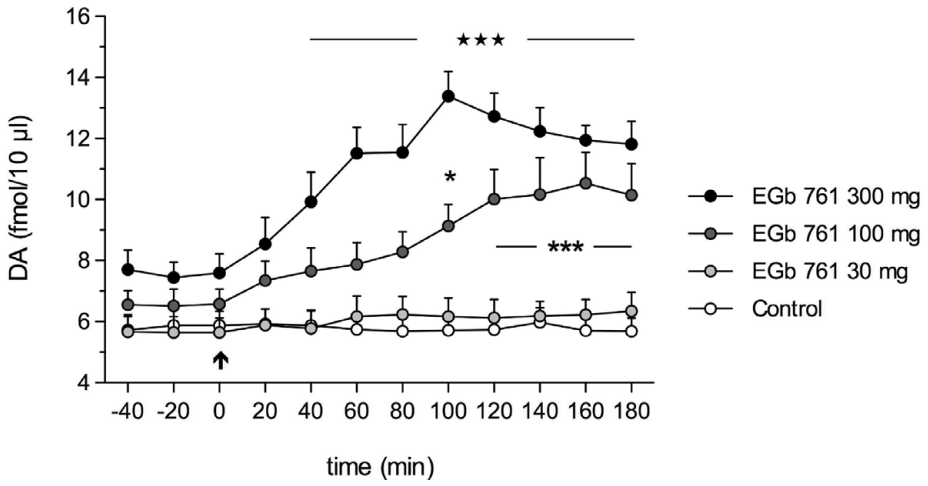

Figure 1. Effects of subacute (14 days, once daily) treatment with EGb 761® (30, 100 and 300 mg/kg p.o.) on extracellular concentrations of dopamine in the mPFC of awake rat. The arrow indicates the time of EGb 761® administration on day 14, the levels are expressed as mean ± SEM, n = 5; (***) $p < 0.001$; (*) $p < 0.05$; two-way repeated measures ANOVA followed by Bonferroni multiple comparison test.

Effects of EGb 761® main constituents on extracellular levels of DA: a major role of the flavonol glycosides

The following studies aimed to evaluate a potential role of the major constituents of EGb 761® on the observed effects of increased DA transmission in the rat mPFC. Three main constituent groups of the extract were tested: flavonol glycosides, ginkgolides, and bilobalide using the same dosing regimen as used for the whole extract. In separate groups of rats, each constituent was administered orally, once daily for 14 days at a dose corresponding to its respective content in 300 mg/kg of EGb 761®: flavonol glycosides 72 mg/kg, ginkgolides 8.7 mg/kg and bilobalide 9.3 mg/kg. Subacute treatment with flavonol glycosides caused a moderate increase in extracellular DA levels to a maximal value of 134 ± 14% at 120 min, as compared to the vehicle-treated group (Figure 2). Correspondingly, repeated administration of ginkgolides or bilobalide had only a minor or no effect on extracellular levels of DA (Yoshitake *et al.*, 2010). The relative $AUC_{(0–180\ min)}$ value calculated as the sum of the individual $AUC_{(0–180\ min)}$ increments of all three constituents was 140% of the vehicle-treated group, which was similar to the value of 153% observed for the treatment with 300 mg/kg of EGb 761® (Yoshitake *et al.*, 2010). Previously, it was proposed that EGb 761® may exert synergistic effects or a "polyvalent" action (DeFeudis and Drieu, 2000), i.e. a potentiation of the effects caused by the multiple active constituents of the EGb 761®, a mechanism of action often proposed for herbal remedies. However, the present findings indicate that the effects of EGb 761® on the DA levels in the mPFC are more likely only additive and not synergistic, reflecting the sum of the individual contributions of the main groups of constituents.

Effects of EGb 761®-specific acylated flavonols on extracellular levels of DA and ACh in the rat mPFC

Ginkgo biloba leaves contain at least two acylated glycosides of the aglycones quercetin and kaempferol: 3-O-(2''-O-(6'''-O-(p-hydroxy-trans-cinnamoyl)-β-D-glucosyl)-α-L-rhamnosyl)quercetin (Q-ag) and 3-O-(2''-O-(6'''-O-(p-hydroxy-trans-cinnamoyl)-β-D-glucosyl)-α-L-rhamnosyl) kaempferol (K-ag). The chemical structures of Q-ag and K-ag are shown in Figure 3. Both Q-ag and K-ag are specific flavonols of *Ginkgo biloba*, as already described in the introduction section. The standardized extract EGb 761® contain typically 2.4–2.6% Q-ag and 1.8–2.2% K-ag (Dr. F. Lang, Dr. Willmar Schwabe GmbH & Co. KG, personal communication).

In the final experiments, the effects of chronic treatment with Q-ag, K-ag, and isorhamnetin (Irh) on extracellular levels of DA and ACh were evaluated in the mPFC of freely moving rats. Isorhamnetin, the O-methylated quercetin (Figure 3), was used as a reference substance. Q-ag, K-ag, and Irh were each administered at a dose of 10 mg/kg/day for 14 days and the microdialysis experiments were performed following Q-ag, K-ag or Irh application on the last day of treatment. At this point, the levels of DA increased within the first 30–60 minutes following treatment with Q-ag and K-ag, but not with Irh, as shown in Figure 4. The maximal increases to 181 ± 16% and 160 ± 14% (mean ± SEM, $p < 0.001$) for Q-ag-treated and K-ag-treated rats, respectively, were observed at 90 minutes after the drug administration

https://doi.org/10.1017/S1041610212000567

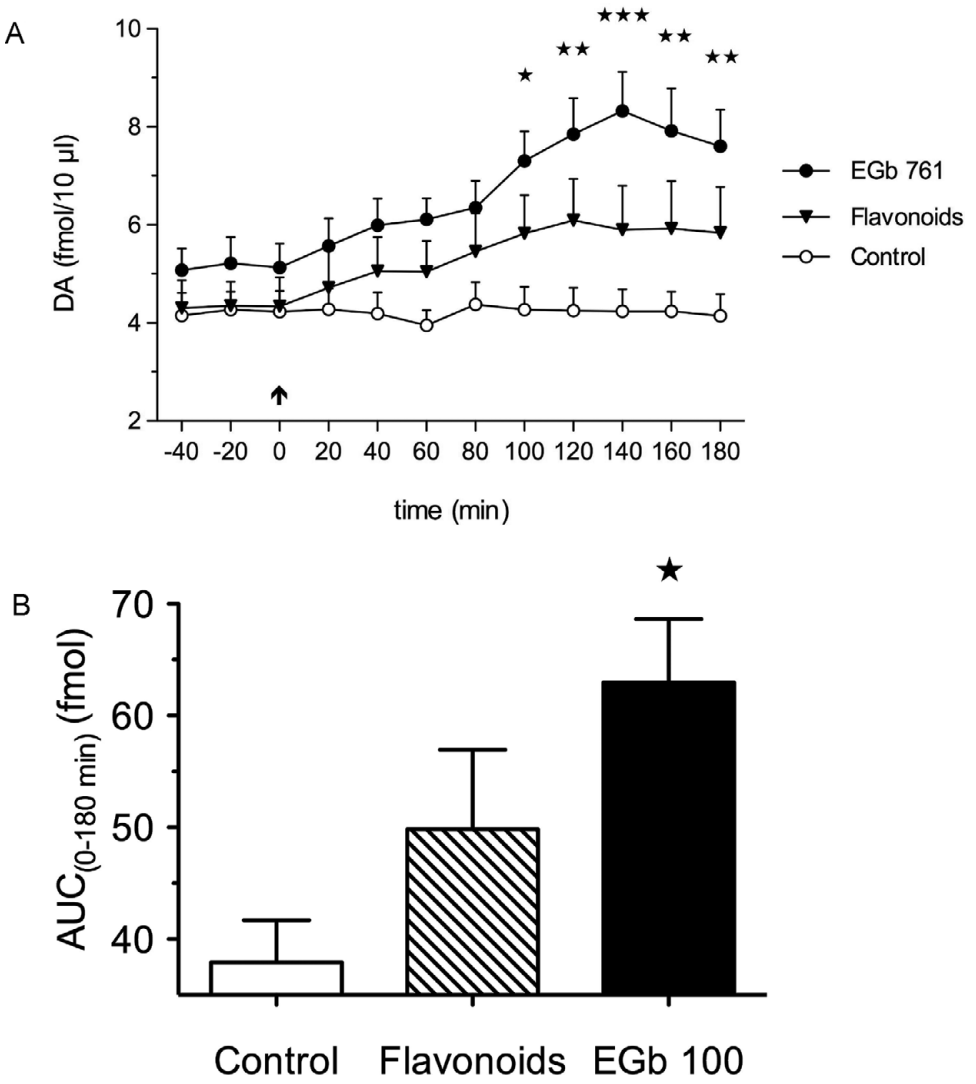

Figure 2. (A) Effects of subacute (14 days, once daily) treatment with EGb 761® (300 mg/kg, p.o.) extract and its flavonoid fraction (72 mg/kg, p.o.) on the extracellular concentrations of dopamine in the medial prefrontal cortex of awake rats. The arrow indicates the time of drug administration on day 14, the levels are expressed as mean ± SEM, n = 5; (***) $p < 0.001$; (**) $P < 0.01$; (*) $p < 0.05$; two-way repeated measures ANOVA followed by Bonferroni multiple comparison test. (B) The inset shows the overall effect of EGb 761® and flavonoids expressed as area-under-the-curve ($AUC_{(0–180\ min)}$) values for DA concentrations measured during the 180-min period after the treatment.

(Figure 4). The levels remained markedly increased even at the end (180 min) of the sampling period. Similar effects of Q-ag and K-ag treatment were observed for the extracellular ACh levels (Figure 5). Thus, Q-ag increased the ACh levels to a maximal value of 121 ± 25% of controls at 90 minutes and K-ag caused a rapid increase in ACh values to 158 ± 5% at 30 minutes after its administration. The overall effects of Q-ag, K-ag, and Irh expressed as relative $AUC_{(0–180\ min)}$ values calculated from the respective time courses (Figures 4 and 5) are depicted in Figure 6. As can be seen, Q-ag and K-ag increased the DA and ACh levels to about the same levels, whereas Irh had no effect on any of the neurotransmitters. Similarly, as in the case of the whole extract, the chronic treatment with the flavonols Q-ag, K-ag, and Irh did not affect the extracellular levels of the DA metabolites DOPAC and HVA.

Summary

A major finding of the study is that the subacute treatment with EGb 761® or its main constituent group the flavonols, as well as the individual acylated flavonol glycosides Q-ag and K-ag, increase the DA and ACh transmission in the mPFC of awake rats. Ginkgolides and bilobalide had only a minor or no effect on the extracellular levels

https://doi.org/10.1017/S1041610212000567

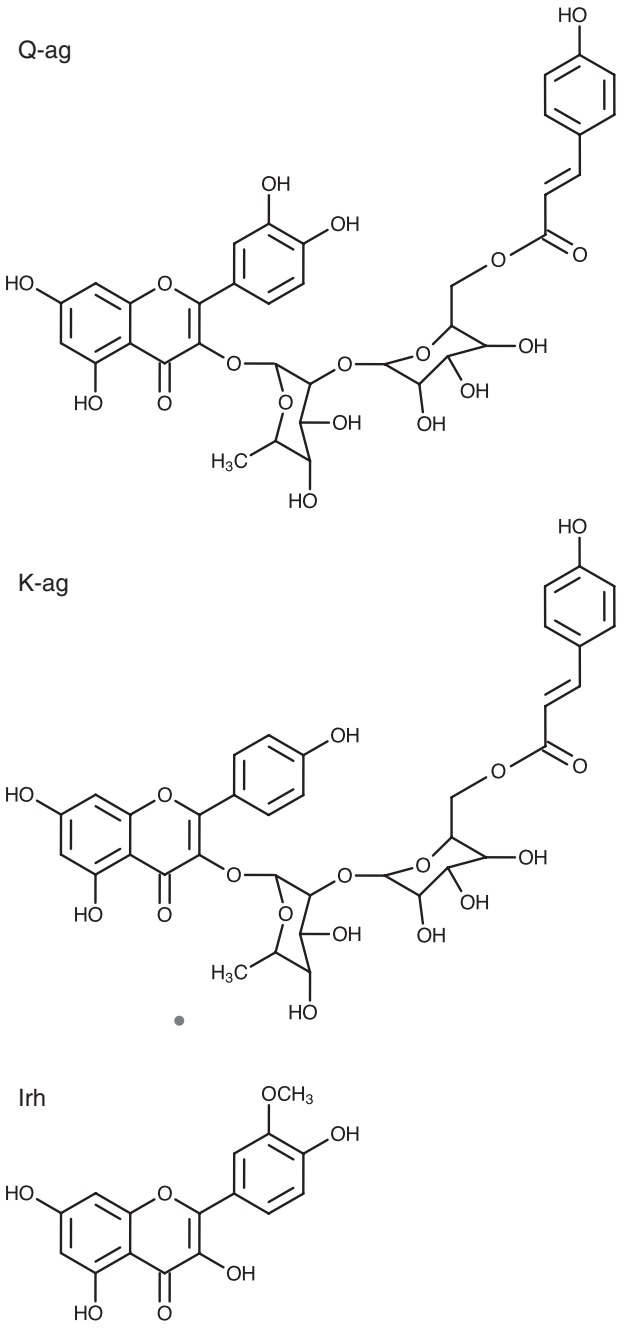

Figure 3. Chemical structures of acylated glycosides of quercetin, 3-O-(2″-O-(6‴-O-(p-hydroxy-trans-cinnamoyl)-β-D-glucosyl)-α-L-rhamnosyl)quercetin (Q-ag) and kaempferol, 3-O-(2″-O-(6‴-O-(p-hydroxy-trans-cinnamoyl)-β-D-glucosyl)-α-L-rhamnosyl)kaempferol (K-ag), as well as isorhamnetin (Irh).

of the monoaminergic neurotransmitters. These results suggest that the flavonol constituents and, in particular, the Ginkgo-specific acylated quercetin and kaempferol glycosides account for the direct modulatory effects of EGb 761® on the central neurotransmitters DA and possibly ACh in brain areas, which are neuroanatomically relevant to learning and memory processing.

Chronic treatment with EGb 761® had no effect on the activities of MAO-A or MAO-B in homogenates of mouse brains (Fehske *et al.*, 2009), which is also supported by the findings of the present microdialysis study showing unchanged extracellular levels of DOPAC and HVA in rat brains. One possible explanation of the effects exerted by the subacute treatment with EGb 761® and flavonol glycosides could involve desensitization or downregulation of receptors modulating the DA, NA, and ACh release in prefrontal cortical structures. Only limited data are available on the binding affinity of EGb 761® and its main constituents to central neurotransmitter receptors. Thus, the *Ginkgo biloba* extract reduced [^{3}H]ketanserin binding to 5-HT$_{2A}$ receptors in the frontal cortex of MAO-A knock-out mice (Shin *et al.*, 2000). Chronic treatment with EGb 761® increased binding (B_{max} values) to muscarinic acetylcholine receptors (Taylor, 1986) and α_2–adrenoceptors (Huguet and Tarade, 1992) in hippocampal membranes and 5-HT$_{1A}$ receptors (Huguet *et al.*, 1994) in cortical membranes of aged but not of young rats. Administration of EGb 761® to adult rats increased NA turnover in the cerebral cortex only after subacute treatment (Brunello *et al.*, 1985). In addition, it was shown that chronic but not acute treatment with sertraline caused an increase in the NA levels in the frontal cortex but not in the hippocampus of rats, most likely as a consequence of desensitization of cortical α_2–adrenoceptors (Thomas *et al.*, 1998). Blockade of α_2-adrenoceptors with the α_2–antagonist idazoxan was shown preferentially to increase extracellular DA levels in the PFC (Hertel *et al.*, 1999), to potentiate the effects of venlafaxine on DA and NA levels (Weikop *et al.*, 2004), and to raise cortical ACh levels (Tellez *et al.*, 1999). Modulation of other receptors such as 5-HT or muscarinic acetylcholine receptors by chronic treatment with EGb 761® or its flavonol constituents may also account for the observed increases in extracellular DA and ACh levels in the rat mPFC.

Cognitive decline is associated with natural processes of brain aging, but is pathologically accelerated in neurodegenerative diseases including AD or vascular type dementia and psychiatric disorders, such as schizophrenia or depression. Progressive impairment of learning and memory functions in AD is primarily attributed to the atrophy and loss of the cholinergic neurons in the basal forebrain (Bartus *et al.*, 1982; Coyle *et al.*, 1983; Terry and Buccafusco, 2003) and cholinergic replacement therapy is currently the prevailing strategy for treatment of mild to moderate AD (O'Brien and Burns, 2011). In addition, a body of evidence exists for the role of prefrontal cortical

https://doi.org/10.1017/S1041610212000567

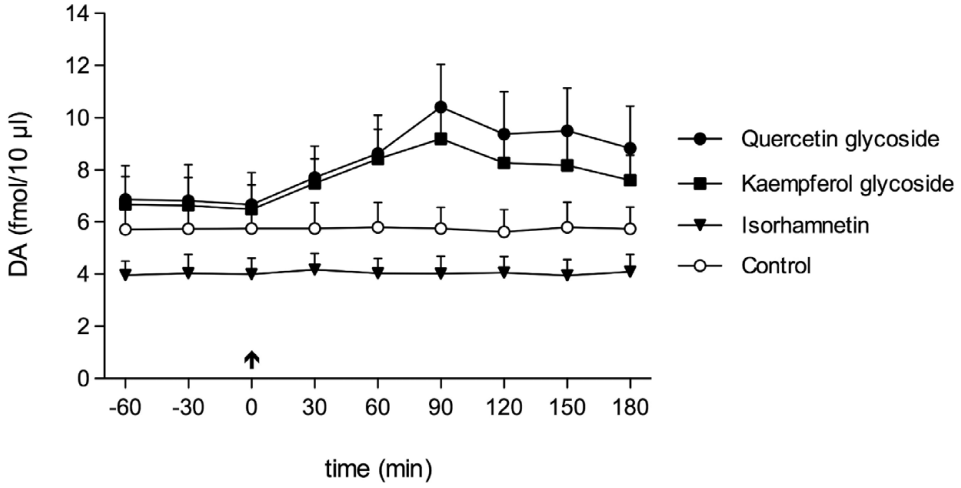

Figure 4. Effects of treatment with 10 mg/kg/day of quercetin glycoside (Q-ag), kaempferol glycoside (K-ag) and isorhamnetin (Irh) for 14 days on the extracellular concentrations of DA in the mPFC of awake rats. The arrow indicates the time of drug administration on day 14, the levels are expressed as mean ± SEM, n = 5.

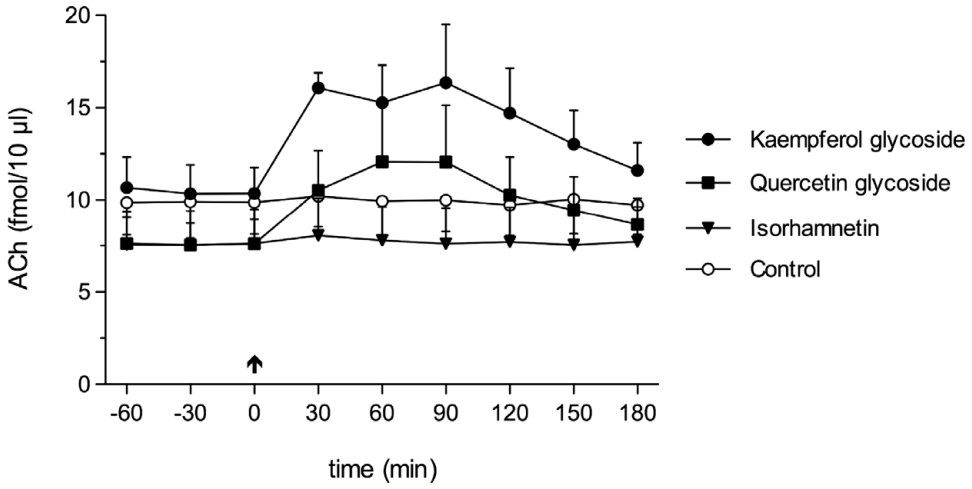

Figure 5. Effects of treatment with 10 mg/kg/day of quercetin glycoside (Q-ag), kaempferol glycoside (K-ag) and isorhamnetin (Irh) for 14 days on the extracellular concentrations of ACh in the mPFC of awake rats. The arrow indicates the time of drug administration on day 14, the levels are expressed as mean ± SEM, n = 5.

DA and DA receptors in modulating neuronal networks, which are essential for working memory (Castner and Goldman-Rakic, 2004; Goldman-Rakic, 1995; Goldman-Rakic *et al.*, 2000; Paspalas and Goldman-Rakic, 2005).

Taken together, these reports support the findings of the current study on a putative mechanism of action of EGb 761®. The present data demonstrate for the first time that repeated administration of the *Ginkgo biloba* extract EGb 761®, and in particular, its two specific acylated flavonol glycosides, results in increased release of DA and ACh in the rat prefrontal cortex. Increased dopaminergic and cholinergic transmission in the frontocortical brain areas may be one of the underlying mechanisms behind the clinically observed effects of EGb 761® on improved cognitive function in aged and healthy people, as well as in the treatment of neuropsychiatric symptoms of dementia.

Conflict of interest

S. Yoshitake and J. Kehr are employed by Pronexus Analytical AB, and E. Koch and M. Nöldner are employees of the company Dr. Willmar Schwabe GmbH & Co. KG. The study was supported by Dr. Willmar-Schwabe GmbH & Co. KG, Karlsruhe, Germany.

https://doi.org/10.1017/S1041610212000567

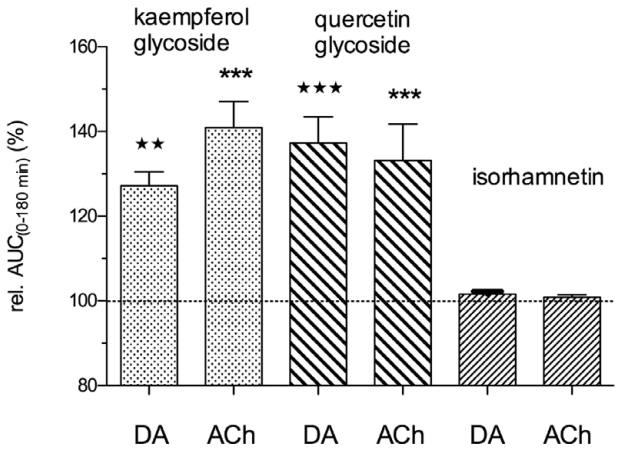

Figure 6. The overall effects of each 10 mg/kg/day of quercetin glycoside (Q-ag), kaempferol glycoside (K-ag) and isorhamnetin (Irh) for 14 days on the extracellular levels of DA and ACh in the mPFC of awake rats. The relative $AUC_{(0-180\ min)}$ values are expressed as percentage of the $AUC_{(0-180\ min)}$ value of the control group; mean ± SEM, n = 5; (***) $p < 0.001$; (**) $p < 0.01$; one-way ANOVA followed by Dunnett´s multiple comparison test.

Description of authors' roles

E. Koch, M. Nöldner, and J. Kehr designed the study and participated in the discussion of the results; S. Ijiri and S. Yoshitake performed the microdialysis experiments and, together with T. Yoshitake, the analysis of microdialysis samples; T. Yoshitake and J. Kehr carried out the data processing and statistical analysis; J. Kehr wrote the paper.

References

Abdel-Kader, R. *et al.* (2007). Stabilization of mitochondrial function by Ginkgo biloba extract (EGb 761). *Pharmacological Research*, 56, 493–502.

Ahlemeyer, B. and Krieglstein, J. (2003). Neuroprotective effects of Ginkgo biloba extract. *Cellular and Molecular Life Sciences*, 60, 1779–1792.

Ahmad, M. *et al.* (2005). Ginkgo biloba affords dose-dependent protection against 6-hydroxydopamine-induced parkinsonism in rats: neurobehavioural, neurochemical and immunohistochemical evidences. *Journal of Neurochemistry*, 93, 94–104.

Andrieu, S. *et al.* (2003). EPIDOS study. Association of Alzheimer's disease onset with ginkgo biloba and other symptomatic cognitive treatments in a population of women aged 75 years and older from the EPIDOS study. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, 58, 372–377.

Andrieu, S. and GuidAge study GROUP. (2008). GuidAge study: a 5-year double blind, randomised trial of EGb 761® for the prevention of Alzheimer's disease in elderly subjects with memory complaints. i. rationale, design and baseline data. *Current Alzheimer Research*, 5, 406–415.

Bachinskaya, N., Hoerr, R. and Ihl, R. (2011). Alleviating neuropsychiatric symptoms in dementia: the effects of Ginkgo biloba extract EGb 761®: findings from a randomized controlled trial. *Neuropsychiatric Disease and Treatment*, 7, 209–215.

Bartus, R. T., Dean, R. L. III, Beer, B. and Lippa, A. S. (1982). The cholinergic hypothesis of geriatric memory dysfunction. *Science*, 217, 408–414.

Bedir, E. *et al.* (2002). Biologically active secondary metabolites from Ginkgo biloba. *Journa of Agricultural and Food Chemistry*, 50, 3150–3155.

Blecharz-Klin, K., Piechal, A., Joniec, I., Pyrzanowska, J. and Widy-Tyszkiewicz, E. (2009). Pharmacological and biochemical effects of Ginkgo biloba extract on learning, memory consolidation and motor activity in old rats. *Acta Neurobiologiae Experimentalis (Warsaw)*, 69, 217–231.

Brunello, N., Racagni, G., Clostre, F., Drieu, K. and Braquet, P. (1985). Effects of an extract of Ginkgo biloba on noradrenergic systems of rat cerebral cortex. *Pharmacological Research Communications*, 17, 1063–1072.

Castner, S. A. and Goldman-Rakic, P. S. (2004). Enhancement of working memory in aged monkeys by a sensitizing regimen of dopamine D1 receptor stimulation. *Journal of Neuroscience*, 24, 1446–1450.

Chan, P. C., Xia, Q. and Fu, P. P. (2007). Ginkgo biloba leave extract: biological, medicinal, and toxicological effects. *Journal of Environmental Science and Health, Part C: Environmental Carcinogensis and Ecotoxicology Reviews*, 25, 211–244.

Coyle, J. T., Price, D. L. and DeLong, M. R. (1983). Alzheimer's disease: a disorder of cortical cholinergic innervation. *Science*, 219, 1184–1190.

Dartigues, JF, Carcaillon, L, Helmer, C, Lechevallier, N, Lafuma, A, Khoshnood, B (2007). Vasodilators and Nootropics as Predictors of Dementia and Mortality in the PAQUID Cohort. *J Am Geriatr Soc* 55: 395–399.

DeFeudis, F. V. (2003). A brief history of EGb 761® and its therapeutic uses. *Pharmacopsychiatry*, 36 (Suppl 1), S2–7.

DeFeudis, F. V. and Drieu, K. (2000). Ginkgo biloba extract (EGb 761®) and CNS functions: basic studies and clinical applications. *Current Drug Targets*, 1, 25–58.

DeKosky, S. T. *et al.* (2008). Ginkgo biloba for prevention of dementia: a randomized controlled trial. *JAMA*, 300, 2253–2262.

Dodge, H. H., Zitzelberger, T., Oken, B. S., Howieson, D. and Kaye, J. (2008). A randomized placebo-controlled trial of Ginkgo biloba for the prevention of cognitive decline. *Neurology*, 70, 1809–1817.

Fehske, C. J., Leuner, K. and Müller, W. E. (2009). Ginkgo biloba extract (EGb761®) influences monoaminergic neurotransmission via inhibition of NE uptake, but not MAO activity after chronic treatment. *Pharmacological Research*, 60, 68–73.

Grass-Kapanke, B., Busmane, A., Lasmanis, A., Hoerr, A. and Kaschel, R. (2011). Effects of Ginkgo biloba special extract EGb 761® in very mild cognitive impairment (vMCI). *Neuroscience & Medicine*, 2, 48–56.

Goldman-Rakic, P. S. (1995). Cellular basis of working memory. *Neuron*, 14, 477–485.

Goldman-Rakic, P. S., Muly, E. C. III and Williams, G. V. (2000). D(1) receptors in prefrontal cells and

circuits. *Brain Research. Brain Research Reviews*, 31, 295–301.

Hasler, A. (1993). Ginkgo, ein modernes Phytopharmakon? *Schweizerische Apotheker Zeitung*, 131, 315–316.

Hasler, A. (2000). Chemical constituents of Ginkgo biloba. In T. A. van Beek (ed.), *Ginkgo biloba* (pp. 109–142). The Netherlands: Harwood Academic.

Hertel, P., Nomikos, G. G. and Svensson, T. H. (1999). Idazoxan preferentially increases dopamine output in the rat medial prefrontal cortex at the nerve terminal level. *European Journal of Pharmacology*, 371, 153–158.

Huguet, F. and Tarrade, T. (1992). Alpha 2-adrenoceptor changes during cerebral ageing. The effect of Ginkgo biloba extract. *Journal of Pharmacy and Pharmacology*, 44, 24–27.

Huguet, F., Drieu, K. and Piriou, A. (1994). Decreased cerebral 5-HT1A receptors during ageing: reversal by Ginkgo biloba extract (EGb 761®). *Journal of Pharmacy and Pharmacology*, 46, 316–318.

Ihl, R. *et al.* (2011). Efficacy and safety of a once-daily formulation of Ginkgo biloba extract EGb 761® in dementia with neuropsychiatric features: a randomized controlled trial. *International Journal of Geriatric Psychiatry*, 26, 1186–1194.

IQWiG Institute for Quality and Efficacy in Health Care (2008). *Ginkgo in Alzheimer's disease. Executive Summary*. Commission No. A05-19B. Available at: https://www.iqwig.de/download/A05-19B_Abschlussbericht_Ginkgohaltige_Praeparate_bei_Alzheimer_Demenz.pdf (last accessed 27 Sept. 2011)

Jerant, A., Chapman, B., Duberstein, P., Robbins, J. and Franks, P. (2011). Personality and medication non-adherence among older adults enrolled in a six-year trial. *British Journal of Health Psychology*, 16, 151–169.

Kaschel, R. (2011). Specific memory effects of Ginkgo biloba extract EGb 761® in middle-aged healthy volunteers. *Phytomedicine*, 18, 1202–1207.

Kim, D. H., Maneen, M. J. and Stahl, S. M. (2009). Building a better antipsychotic: receptor targets for the treatment of multiple symptom dimensions of schizophrenia. *Neurotherapeutics*, 6, 78–85.

Meltzer, H. Y. and Huang, M. (2008). In vivo actions of atypical antipsychotic drug on serotonergic and dopaminergic systems. *Progress in Brain Research*, 172, 177–197.

Müller, W. E. and Chatterjee, S. S. (2003). Cognitive and other behavioral effects of EGb 761® in animal models. *Pharmacopsychiatry*, 36 (Suppl. 1), S24–S31.

O'Brien, J. T. and Burns, A. on behalf of the BAP Dementia Consensus Group (2011). Clinical practice with anti-dementia drugs: a revised (second) consensus statement from the British Association for Psychopharmacology. *Journal of Psychopharmacology*, 25, 997–1019.

Pardon, M. C., Joubert, C., Perez-Diaz, F., Christen, Y., Launay, J. M. and Cohen-Salmon, C. (2000). In vivo regulation of cerebral monoamine oxidase activity in senescent controls and chronically stressed mice by long-term treatment with *Ginkgo biloba* extract (EGb 761®). *Mechanisms of Ageing and Development*, 113, 157–168.

Paspalas, C. D. and Goldman-Rakic, P. S. (2005). Presynaptic D1 dopamine receptors in primate prefrontal cortex: target-specific expression in the glutamatergic synapse. *Journal of Neuroscience*, 25, 1260–1267.

Rojas, P., Serrano-García, N., Mares-Sámano, J. J., Medina-Campos, O. N., Pedraza-Chaverri, J. and Ögren, S. O. (2008). EGb 761® protects against nigrostriatal dopaminergic neurotoxicity in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced Parkinsonism in mice: role of oxidative stress. *European Journal of Neuroscience*, 28, 41–50.

Satvat, E. and Mallet, P. E. (2009). Chronic administration of a Ginkgo biloba leaf extract facilitates acquisition but not performance of a working memory task. *Psychopharmacology (Berlin)*, 202, 173–185.

Shah, Z. A., Sharma, P. and Vohora, S. B. (2003). Ginkgo biloba normalises stress-elevated alterations in brain catecholamines, serotonin and plasma corticosterone levels. *European Neuropsychopharmacology*, 13, 321–325.

Shin, J. C., Chen, K., Ridd, M. J. and Seif, I. (2000). Ginkgo biloba abolishes aggression in mice lacking MAO A. *Antioxidants & Redox Signaling*, 2, 467–471.

Singh, V., Singh, S. P. and Chan, K. (2010). Review and meta-analysis of usage of ginkgo as an adjunct therapy in chronic schizophrenia. *International Journal of Neuropsychopharmacology*, 13, 257–271.

Stackman, R. W., Eckenstein, F., Frei, B., Kulhanek, D., Nowlin, J, and Quinn, J. F. (2003). Prevention of age-related spatial memory deficits in a transgenic mouse model of Alzheimer's disease by chronic Ginkgo biloba treatment. *Experimental Neurology*, 184, 510–520.

Taylor, J. E. (1986). Neuromediator binding to receptors in the rat brain. The effect of chronic administration of Ginkgo biloba extract. (French). *La Presse Médicale*, 15, 1491–1493.

Tellez, S., Colpaert, F. and Marien, M. (1999). Alpha2-adrenoceptor modulation of cortical acetylcholine release in vivo. *Neuroscience*, 89, 1041–1050.

Terry, A. V. Jr. (2008). Role of the central cholinergic system in the therapeutics of schizophrenia. *Current Neuropharmacology*, 6, 286–292.

Terry, A. V. Jr. and Buccafusco, J. J. (2003). The cholinergic hypothesis of age and Alzheimer's disease-related cognitive deficits: recent challenges and their implications for novel drug development. *Journal of Pharmacology and Experimental Therapeutics*, 306, 821–827.

Thomas, D. N., Nutt, D. J. and Holman, R. B. (1998). Sertraline, a selective serotonin reuptake inhibitor modulates extracellular noradrenaline in the rat frontal cortex. *Journal of Psychopharmacology*, 12, 366–370.

Vellas, B. and Guidage Study Group. (2010). Results of GuidAge: a 5-year placebo-controlled study of the efficacy of EGb 761(R) 120mg to prevent or delay Alzheimer's dementia onset in elderly subjects with memory complaint. *Journal of Nutrition Health and Aging*, 14 (Suppl. 2), S23–S23.

Viskupicova, J., Ondrejovic, M. and Sturdik, E. (2009). The potential and practical applications of acylated flavonoids. *Pharmazie*, 64, 355–360.

Wang, Y., Wang, L., Wu, J. and Cai, J. (2006). The in vivo synaptic plasticity mechanism of EGb 761®-induced enhancement of spatial learning and memory in aged rats. *British Journal of Pharmacology*, 148, 147–153.

Wang, B. S. *et al.* (2010). Effectiveness of sstandardized *Ginkgo biloba* extract on cognitive symptoms of dementia with a six-month treatment: a bivariate random effect meta-analysis. *Pharmacopsychiatry*, 43, 86–91.

Weikop, P., Kehr, J. and Scheel-Krüger, J. (2004). The role of alpha1- and alpha2-adrenoreceptors on venlafaxine-induced elevation of extracellular serotonin, noradrenaline and dopamine levels in the rat prefrontal cortex and hippocampus. *Journal of Psychopharmacology*, 18, 395–403.

Weinmann, S., Roll, S., Schwarzbach, C., Vauth, C. and Willich, S. N. (2010). Effects of Ginkgo biloba in dementia: systematic review and meta-analysis. *BMC Geriatrics*, 10, 14. http://www.biomedcentral.com/1471-2318/10/14.

White, H. L., Scates, P. W. and Cooper, B. R. (1996). Extracts of Ginkgo biloba leaves inhibit monoamine oxidase. *Life Sciences*, 58, 1315–1321.

Yoshitake, T., Kehr, J., Yoshitake, S., Fujino, K., Nohta, H. and Yamaguchi, M. (2004). Determination of serotonin, noradrenaline, dopamine and their metabolites in rat brain extracts and microdialysis samples by column liquid chromatography with fluorescence detection following derivatization with benzylamine and 1,2-diphenylethylenediamine.*Journal of Chromatography: B, Analytical Technologies in the Biomedical and Life Sciences*, 807, 177–183.

Yoshitake, T., Yoshitake, S. and Kehr, J. (2010). The Ginkgo biloba extract EGb 761(R) and its main constituent flavonoids and ginkgolides increase extracellular dopamine levels in the rat prefrontal cortex. *British Journal of Pharmacology*, 159, 659–668.

Zhang, W. F., Tan, Y. L., Zhang, X. Y., Chan, R. C., Wu, H. R. and Zhou, D. F. (2011). Extract of Ginkgo biloba treatment for tardive dyskinesia in schizophrenia: a randomized, double-blind, placebo-controlled trial. *Journal of Clinical Psychiatry*, 72, 615–621.