

The Summer Meeting of the Nutrition Society, hosted by the Irish Section, was held at the University of Ulster, Coleraine on 16–19 July 2007

Symposium on ‘Diet and mental health’

Food for thought: the role of dietary flavonoids in enhancing human memory, learning and neuro-cognitive performance

Jeremy P. E. Spencer*

Molecular Nutrition Group, School of Chemistry, Food Biosciences and Pharmacy, University of Reading, Reading RG2 6AP, UK

Emerging evidence suggests that dietary-derived flavonoids have the potential to improve human memory and neuro-cognitive performance via their ability to protect vulnerable neurons, enhance existing neuronal function and stimulate neuronal regeneration. Long-term potentiation (LTP) is widely considered to be one of the major mechanisms underlying memory acquisition, consolidation and storage in the brain and is known to be controlled at the molecular level by the activation of a number of neuronal signalling pathways. These pathways include the phosphatidylinositol-3 kinase/protein kinase B/Akt (Akt), protein kinase C, protein kinase A, Ca–calmodulin kinase and mitogen-activated protein kinase pathways. Growing evidence suggests that flavonoids exert effects on LTP, and consequently memory and cognitive performance, through their interactions with these signalling pathways. Of particular interest is the ability of flavonoids to activate the extracellular signal-regulated kinase and the Akt signalling pathways leading to the activation of the cAMP-response element-binding protein, a transcription factor responsible for increasing the expression of a number of neurotrophins important in LTP and long-term memory. One such neurotrophin is brain-derived neurotrophic factor, which is known to be crucial in controlling synapse growth, in promoting an increase in dendritic spine density and in enhancing synaptic receptor density. The present review explores the potential of flavonoids and their metabolite forms to promote memory and learning through their interactions with neuronal signalling pathways pivotal in controlling LTP and memory in human subjects.

Flavonoids: Cognitive performance: Memory

Representing one of the most important lifestyle factors, diet can strongly influence the incidence and onset of CVD and neurodegenerative disorders, and thus a healthy diet is an essential factor for healthy ageing. Various phytochemical constituents of foods and beverages, in particular a class of photochemicals termed flavonoids, have been avidly investigated in recent years. A number of dietary intervention studies in human subjects and animals, in particular those using foods and beverages derived from *Vitis vinifera* (grape), *Camellia sinensis* (tea), *Theobroma cacao* (cocoa) and *Vaccinium* spp. (blueberry), have

demonstrated beneficial effects on vascular function and mental performance. While such foods and beverages differ greatly in chemical composition, macro- and micro-nutrient content and energy load per serving, they have in common that they are amongst the major dietary sources of flavonoids. Dietary intervention studies in several mammalian species, including man, using flavonoid-rich plant or food extracts have indicated that flavonoids are capable of improving both memory and learning^(1–7), via their ability to protect vulnerable neurons, enhance existing neuronal function and stimulate neuronal regeneration. In

Abbreviations: Akt, protein kinase B/Akt; BBB, blood–brain barrier; BDNF, brain-derived neurotrophic factor; CREB, cAMP-response element-binding protein; ERK, extracellular signal-regulated protein kinase; LTP, long-term potentiation; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; PI3K, phosphoinositide 3-kinase.

*Corresponding author: Dr Jeremy P. E. Spencer, fax +44 118 931 0080, email j.p.e.spencer@reading.ac.uk

addition, their neuroprotective potential is well reported and they have been shown to protect against neuronal death in both oxidative stress-induced⁽⁸⁾ and A β -induced neuronal-death models⁽⁹⁾. Furthermore, evidence supports the beneficial and neuromodulatory effects of flavonoid-rich ginkgo biloba (*Ginkgo biloba* L.) extracts, particularly in connection with age-related dementias and Alzheimer's disease^(9–11) and the citrus flavanone tangeretin has been observed to help maintain nigrostriatal integrity and functionality following lesioning with 6-hydroxydopamine⁽¹²⁾.

Historically, the biological actions of flavonoids have been attributed to their antioxidant properties⁽¹³⁾, through their ability to scavenge reactive species⁽¹⁴⁾ or through their influences on the intracellular redox status. However, it has been speculated that their classical H-donating antioxidant activity is not the explanation for the bioactivity of flavonoids *in vivo*, particularly in the brain where their levels are very low. Indeed, it has become evident that flavonoids are more likely to exert their neuroprotective actions by: the modulation of intracellular signalling cascades that control neuronal survival, death and differentiation; affecting gene expression; interactions with mitochondria^(15–17). The present review will highlight the impact of flavonoids on learning, memory and neuro-cognitive performance. In particular, it will highlight probable mechanisms that underpin such actions in the brain, including their interactions with neuronal intracellular signalling pathways pivotal in controlling long-term potentiation (LTP) and memory in human subjects.

Flavonoid structure, source and metabolism

Flavonoids comprise the most common group of polyphenolic compounds in the human diet and are found ubiquitously in plants. Major dietary sources of flavonoids include fruits, vegetables, cereals, tea, wine and fruit juices (for review, see Manach *et al.*⁽¹⁸⁾). Flavonoids consist of two aromatic C rings, benzopyran (rings A and C) and benzene (ring B), and may be divided in six subgroups based on the extent of the oxidation of ring C, the hydroxylation pattern of the ring structure and the substitution of the C-3-position (Fig. 1). The main dietary groups of flavonoids are: flavonols, e.g. kaempferol and quercetin, which are found in onions (*Allium cepa* L.), leeks (*Allium ampeloprasum* var. *porrum* (L.)) and broccoli; flavones, e.g. apigenin and luteolin, which are found in parsley (*Petroselinum crispum*) and celery (*Apium graveolens* L.); isoflavones, e.g. daidzein and genistein, which are mainly found in soyabean and soya products; flavanones, e.g. hesperetin and naringenin, which are mainly found in citrus fruit and tomatoes; flavanols, e.g. catechin, epicatechin, epigallocatechin and epigallocatechin gallate, which are abundant in green tea, red wine and cocoa; anthocyanidins, e.g. pelargonidin, cyanidin and malvidin, whose sources include red wine and berry fruits. Further information relating to the structure and classes of flavonoids may be found in the thorough review by Manach *et al.*⁽¹⁸⁾.

Although flavonoids display potent antioxidant capacity *in vitro*^(13,19,20), during absorption they are extensively

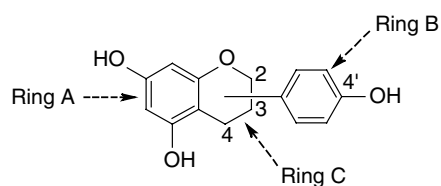
metabolised, resulting in substantial alteration of their redox potentials. For example, the majority of flavonoid glycosides and aglycones present in plant-derived foods are extensively conjugated and metabolised during absorption (for reviews, see Spencer *et al.*^(21,22)). In particular, they are subject to extensive phase I de-glycosylation and phase II metabolism of the resulting aglycones to glucuronides, sulfates and *O*-methylated forms during transfer across the small intestine^(21,23,24) and then again in the liver. Further transformation has been reported in the colon where the enzymes of the gut microflora degrade flavonoids to simple phenolic acids⁽²⁵⁾. In addition, flavonoids may undergo at least three types of intracellular metabolism: oxidative metabolism; P450-related metabolism; conjugation with thiols, particularly glutathione^(26,27). Circulating metabolites of flavonoids such as glucuronides, sulfates and conjugated *O*-methylated forms or intracellular metabolites such as flavonoid–glutathione adducts have greatly reduced antioxidant potential⁽²⁷⁾. Indeed, studies have indicated that although such conjugates and metabolites may participate directly in plasma antioxidant reactions and in scavenging reactive oxygen and nitrogen species in the circulation, their effectiveness is reduced relative to their parent aglycones^(28–32).

Flavonoid-induced improvements in memory, learning and cognitive performance

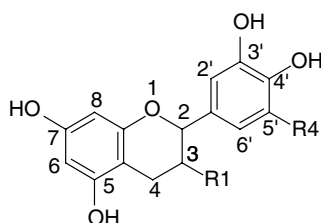
There is a growing interest in the potential of phytochemicals to improve memory, learning and general cognitive ability. A recent prospective study aimed at examining flavonoid intake in relation to cognitive function and decline, has provided strong evidence that dietary flavonoid intake is associated with better cognitive evolution, i.e. the preservation of cognitive performance with ageing⁽³³⁾. A total of 1640 subjects (aged ≥ 65 years) free from dementia at baseline and with reliable dietary assessment data were examined for their cognitive performance (mini-mental state examination, Benton's visual retention test, 'Isaacs' set test) four times over a 10-year period. After adjustment for age, gender and educational level flavonoid intake was found to be associated with significantly better cognitive performance at baseline and with a better evolution of the performance over time. In particular, subjects in the two highest quartiles of flavonoid intake (mg/d; 13·60–17·69 and 17·70–36·94) were found to have better cognitive evolution than subjects in the lowest quartile (0–10·39 mg/d), and after 10 years of follow-up subjects with the lowest flavonoid intake were found to have lost on average 2·1 points on the mini-mental state examination, whereas subjects with the highest quartile had lost only 1·2 points. Such data provides a strong indication that regular flavonoid consumption may have a positive effect on neuro-cognitive performance with ageing, although it does not provide information relating to the activity of specific flavonoid groups.

There has been much interest in the neuro-cognitive effects of soyabean isoflavones, primarily in post-menopausal women^(34–37). The rationale behind the potential of isoflavones to exert positive effects on cognitive

(A)

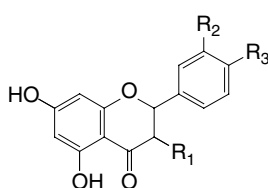


(C)



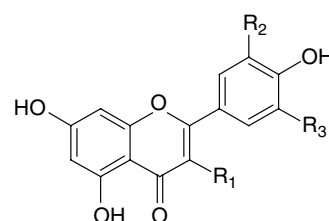
	R ₁	R ₄
Flavanols		
Catechin	OH	H
Epicatechin	OH	H
EGC	OH	OH
ECG	Gallate	H
EGCG	Gallate	OH

(E)



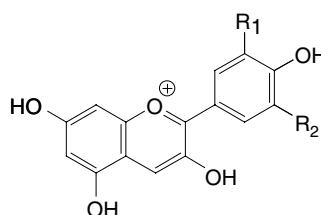
	R ₁	R ₂	R ₃
Flavanones			
Naringenin	H	H	OH
Hesperetin	H	OCH ₃	H
Flavanonols			
Taxifolin	OH	OH	OH
Astilbin	O-Rhamnosyl	OH	OH
Engeletin	O-Rhamnosyl	H	OH

(B)



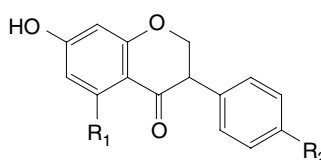
	R ₁	R ₂	R ₃
Flavonols			
Kaempferol	OH	H	H
Quercetin	OH	OH	H
Myricetin	OH	OH	OH
Isorhamnetin	OH	OCH ₃	H
Flavones			
Luteolin	H	OH	H
Apigenin	H	H	H

(D)



	R ₁	R ₂
Anthocyanidins		
Pelargonidin	H	H
Cyanidin	OH	H
Delphinidin	OH	OH
Paeonidin	OCH ₃	H
Petunidin	OCH ₃	OH
Malvidin	OCH ₃	OCH ₃

(F)



	R ₁	R ₂
Isoflavones		
Genistein	HO	OH
Daidzein	OH	H

Fig. 1. The structures of the main classes of flavonoids. The major differences between the individual groups reside in the hydroxylation pattern of the ring-structure, the extent of saturation of the C-ring and the substitution of in the 3-position: (A) general structure of flavonoids, (B) structure of flavonols and flavones, (C) structure of flavanols, also referred as flavan-3-ols, (D) structure of anthocyanidins, (E) structure of flavanones and flavanonols and (F) structure of isoflavones. EGC, epigallocatechin; ECG, epicatechin gallate; EGCG, EGC gallate.

function is believed to lie primarily in their potential to mimic the actions and functions of oestrogens in the brain⁽³⁸⁾. For example, epidemiological investigations have provided evidence that post-menopausal women who undertake oestrogen-replacement therapy have a significantly lower risk for the onset of Alzheimer's disease than women who do not⁽³⁹⁾. Furthermore, animal behavioural studies have shown that ovariectomy results in the development of cognitive dysfunction, which may be prevented by oestrogen replacement, suggesting that normal mammalian cognitive function is impaired by oestrogen reduction⁽³⁸⁾. Isoflavone supplementation (60 mg/d) has been observed to have a favourable effect on cognitive function⁽⁴⁰⁾, particularly verbal memory, in post-menopausal women⁽⁴¹⁾ and a 6-week and 12-week supplementation has been observed to have a positive effect of frontal lobe function^(42,43). However, other large intervention trials have reported that dietary isoflavone supplementation (50–100 mg/d) does not improve cognitive function^(34,44–46). If isoflavones do possess the potential to influence human memory and cognitive performance it is likely that their mechanism of action would include their role as weak oestrogens, their ability to inhibit tyrosine kinase-dependent signal transduction and their ability to act as weak antioxidants^(47,48).

Other flavonoid-rich foods, in particular those containing flavanols, have been observed to improve peripheral blood flow and surrogate markers of cardiovascular function in human subjects⁽⁴⁹⁾. In the context of the central nervous system brain-imaging studies in human subjects have demonstrated that the consumption of flavanol-rich cocoa may enhance cortical blood flow^(50–52). This finding is important as increased cerebrovascular function, especially in the hippocampus (a brain region important for memory), may facilitate adult neurogenesis⁽⁵³⁾. Indeed, new hippocampal cells are clustered near blood vessels, proliferate in response to vascular growth factors and may influence memory⁽⁵⁴⁾. As well as new neuronal growth, increases in neuronal spine density and morphology are considered vital for learning and memory⁽⁵⁵⁾. Changes in spine density, morphology and motility have been shown to occur with paradigms that induce synaptic as well as altered sensory experience and lead to alterations in synaptic connectivity and strength between neuronal partners, affecting the efficacy of synaptic communication. These events are controlled at the cellular and molecular level and are strongly correlated with memory and learning. The flavanol (–)-epicatechin, especially in combination with exercise, has been observed to enhance the retention of rat spatial memory in a water maze test⁽⁵⁶⁾. This improvement in spatial memory was shown to be associated with increased angiogenesis and neuronal spine density in the dentate gyrus of the hippocampus and with the up-regulation of genes associated with learning in the hippocampus.

There is also extensive evidence that berries, in particular blueberries, are effective at reversing age-related deficits in motor function and spatial working memory^(3,57–63). For example, the latency period to find a platform and the distance swum to a platform in a Morris water maze task are significantly reduced following blueberry

supplementation^(57,58). Such results may suggest favourable effects of the blueberry diet on locomotor activity in old animals^(64,65). However, reductions in the time taken to make a choice may also reflect an improved memory component, where rats 'remember' more rapidly and thus respond quicker. Animal studies with tea^(4,66), grape juice⁽⁶⁷⁾ or flavonols such as quercetin^(68,69) have provided further evidence that dietary flavonoids are beneficial in reversing the course of neuronal and behavioural ageing. Although such effects have been linked with antioxidant actions, it is more likely that these effects are mediated by a modulation of neurotransmitter release^(57,58), a stimulation of hippocampal neurogenesis⁽⁵⁹⁾ and changes in neuronal signalling^(61,62).

Cellular and molecular control of memory and learning

The laying down of long-term memory is usually divided into four distinct stages: learning (or acquisition of new information); consolidation; storage; retrieval (Fig. 2)^(70,71). Studies in patients with amnesia and experimental animals have demonstrated an important role for the hippocampus in consolidating labile short-term memory into a more stable long-term memory^(72–74). For example, studies have indicated that the disruption of the hippocampal structure affects recent memories preferentially^(75,76), whereas damage in neocortex affects more remote (long-term) memories⁽⁷⁷⁾. Thus, the general consensus is that the hippocampus plays a time-limited role in consolidating labile new memory into more stable long-term memory, and on the completion of hippocampal-dependent consolidation, these memories are eventually stored in the cortex without major hippocampal contribution^(72,76). Within brain regions LTP is widely considered to be one of the major mechanisms by which the brain learns and maintains memories. LTP refers to a persistent increase in the chemical strength of a synapse that can last from minutes to several days, and this process is thought to contribute to synaptic plasticity and increases in synaptic strength that are thought to underlie memory formation.

Studies into human mental retardation syndromes have led to new insights into the molecular underpinnings of human cognitive processing, in particular into mechanisms likely to contribute to learning and memory (for review, see Weeber & Sweatt⁽⁷⁸⁾, Dash *et al.*⁽⁷⁹⁾ and Tully⁽⁸⁰⁾). Such studies have highlighted the essential role of a number of neuronal signalling pathways in bringing about changes in LTP and therefore human memory and learning. It is known that the enhancement of both short-term and long-term memory is controlled at the molecular level in neurons⁽⁸¹⁾. Whereas short-term memory involves covalent modifications of pre-existing proteins, long-term memory requires the synthesis of new mRNA and proteins^(82–84) (Fig. 2). The rapid enhancement of the synthesis of a diverse array of neuronal proteins through such mechanisms provides the components necessary for persistent forms of LTP. Various signalling pathways have been linked with the control of *de novo* protein synthesis in the context of LTP and memory (Fig. 3): cAMP-dependent

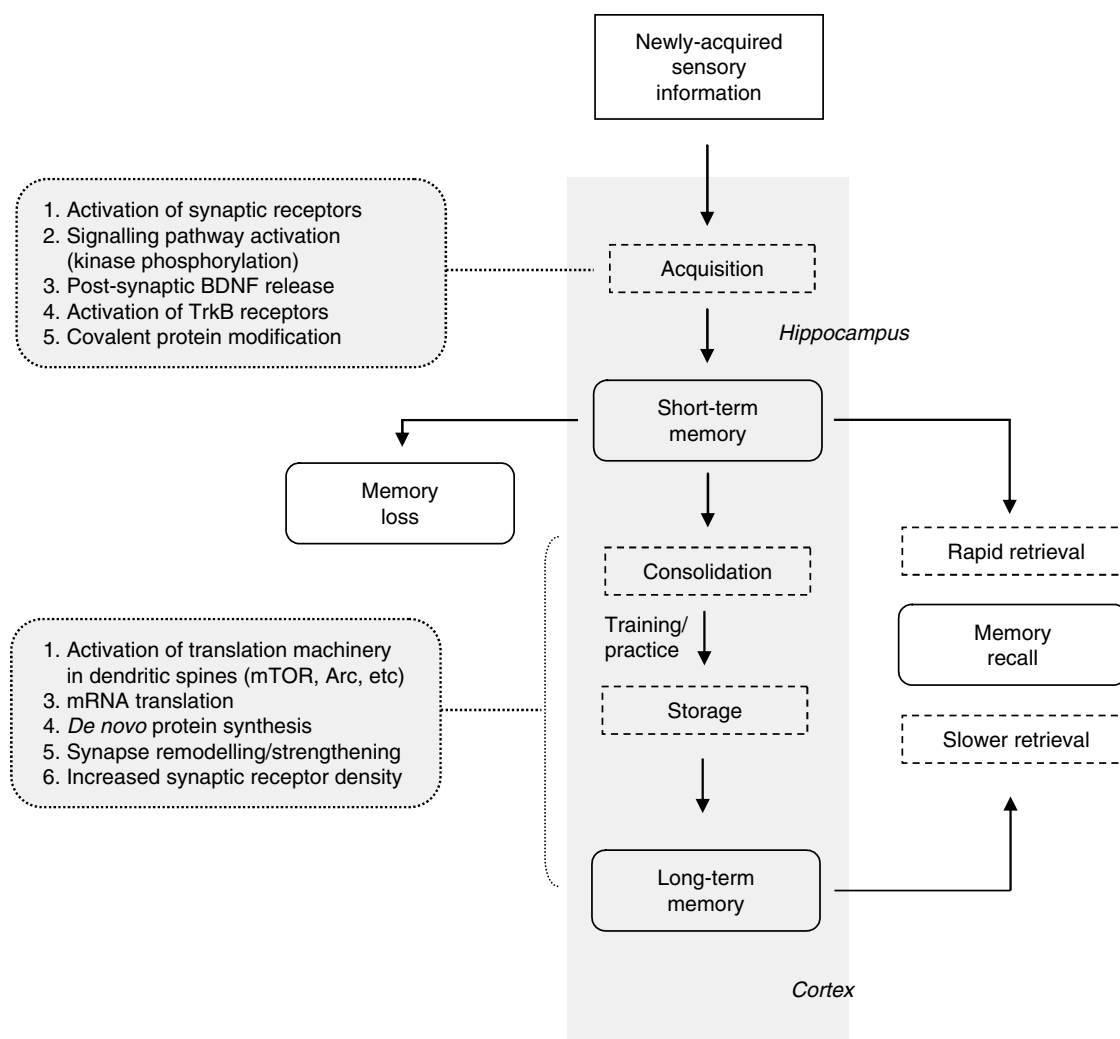


Fig. 2. The processes involved in memory acquisition, consolidation and storage in the brain. Acquisition and consolidation occur primarily in the hippocampus whilst storage is in the cortex or neocortex. BDNF, brain-derived neurotrophic factor; TrkB, tropomyosin receptor kinase B; mTOR, mammalian target of rapamycin; Arc, Arc/Arg3.1.

protein kinase (protein kinase A)⁽⁸⁵⁾; protein kinase B/Akt (Akt)⁽⁸⁶⁾; protein kinase C⁽⁸⁷⁾; Ca-calmodulin kinase⁽⁸⁸⁾; mitogen-activated protein kinase (MAPK)^(89,90). All five pathways converge to signal to the cAMP-response element-binding protein (CREB), a transcription factor that binds to the promoter regions of many genes associated with memory and synaptic plasticity^(91,92) (Fig. 3).

The importance of CREB activation in the induction of long-lasting changes in LTP and memory are highlighted by studies that show that disruption of CREB activity specifically blocks the formation of long-term memory⁽⁹³⁾, whereas agents that increase the amount or activity of CREB accelerate the process⁽⁹⁴⁾. Furthermore, robust CREB phosphorylation and cAMP-response element-reporter gene expression are detected in cortical neurons during developmental plasticity⁽⁹⁵⁾ and in hippocampal neurons in response to both LTP-inducing stimuli and memory-training tasks^(96,97). Furthermore, CREB is known to be a critical transcription factor linking the actions of

neurotrophins such as brain-derived neurotrophic factor (BDNF) to neuronal survival, differentiation and synaptic function^(98,99). Consequently, the central role of CREB in these processes has led to considerable interest in identifying safe effective agents that may enhance the activity of CREB in specific regions of the brain, as they may lead to an improvement in memory⁽⁹⁴⁾.

Do flavonoids access the brain?

In order to understand whether flavonoids and their metabolic derivatives are capable of acting as neuromodulators it is crucial to ascertain whether they are able to enter the central nervous system. In order for flavonoids to access the brain they must first cross the blood-brain barrier (BBB). The functions of the BBB include controlling the entry of xenobiotics into the brain and maintenance of the brain's microenvironment⁽¹⁰⁰⁾. *In vitro* and *in vivo* studies

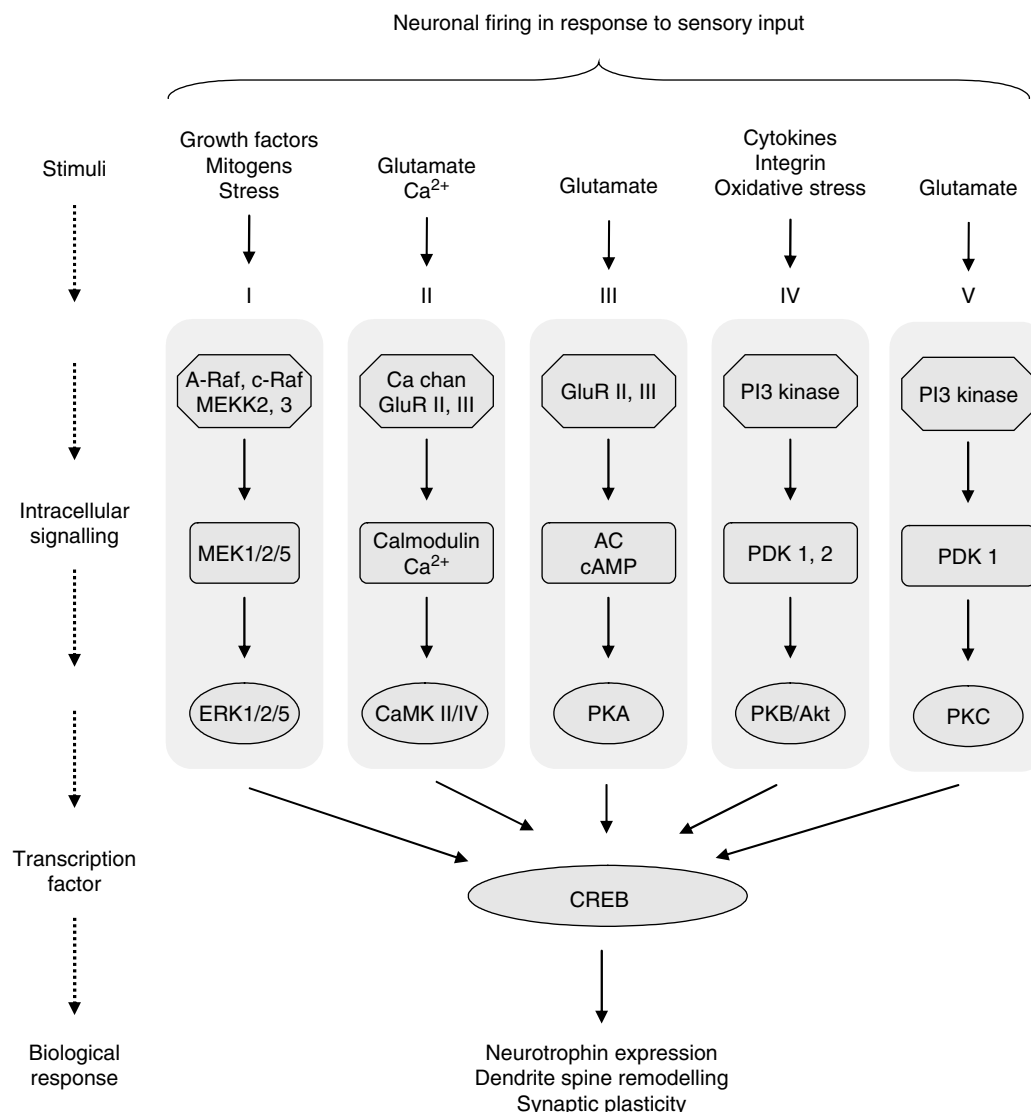


Fig. 3. Neuronal signalling pathways involved in long-term potentiation (LTP). Five distinct signalling pathways lead to the activation of the cAMP response element-binding protein (CREB), a transcription factor important in controlling LTP at neuronal synapses. Varying stimuli activate the mitogen-activated protein kinase (MAPK) pathway (I), the calcium–calmodulin kinase (CaMK) pathway (II), the protein kinase A (PKA) pathway (III), the protein kinase B (PKB)/Akt pathway (IV) and the protein kinase C (PKC) pathway (V) in response to synapse firing. Activation of these pathways results in the activation of CREB and a variety of downstream responses, including neurotrophin expression, enhanced *de novo* protein synthesis, dendritic spine remodelling and ultimately stable long-term LTP. MEK, MAPK kinase; MEKK, MEK kinase; ERK, extracellular signal-regulated protein kinase; Ca chan, calcium channel; GluR, glutamate receptor; AC, adenylyl cyclase; PI3 kinase, phosphoinositide 3-kinase; PDK, 3-phosphoinositide-dependent protein kinase.

have indicated that the flavanones hesperetin, naringenin and their relevant *in vivo* metabolites, as well as some dietary anthocyanins (cyanidin-3-rutinoside and pelargonidin-3-glucoside), are able to traverse the BBB^(101–103). Furthermore, it appears that the potential for flavonoid penetration is dependent on compound lipophilicity⁽¹⁰¹⁾. Accordingly, it is plausible that the uptake of the less-polar *O*-methylated metabolites, such as the *O*-methylated epicatechin metabolites (formed in the small intestine and liver), may be greater than the parent aglycone. For the same reason, the more-polar flavonoid glucuronidated

metabolites, which seem to have low BBB permeability values⁽¹⁰¹⁾, may not be able to access the brain. However, evidence exists to suggest that certain drug glucuronides may cross the BBB⁽¹⁰⁴⁾ and exert pharmacological effects^(105,106), suggesting that there may be a specific uptake mechanism for glucuronides *in vivo*. Apart from the flavonoids lipophilicity, their ability to enter the brain may also be influenced by their interactions with specific efflux transporters expressed in the BBB. One such transporter is P-glycoprotein, which plays an important role in drug absorption and brain uptake⁽¹⁰⁷⁾ and appears to

be responsible for the differences between naringenin and quercetin flux into the brain *in situ*⁽¹⁰³⁾.

Animal feeding studies also provide evidence that flavonoids may access the brain, with the tea flavanol epigallocatechin gallate being reported to access the brain after oral administration to mice⁽¹⁰⁸⁾. Furthermore, oral ingestion of pure epicatechin results in the detection of epicatechin glucuronide and 3'-O-methyl-epicatechin glucuronide in rat brain tissue⁽¹⁰⁹⁾. Anthocyanidins have also been detected in the brain after oral administration^(110,111), with several anthocyanidins being identified in different regions of the rat brain after animals were fed with blueberry⁽⁶⁴⁾. Such flavonoid localisation has been correlated with increased cognitive performance, suggesting a central neuroprotective role of these components. Despite their ability to access the brain, the concentrations of flavonoids and their metabolite forms accumulated *in vivo*⁽¹⁰⁹⁾ are lower (high nM–low μ M) than those recorded for small-molecule antioxidant nutrients such as ascorbic acid and α -tocopherol⁽¹¹²⁾. Consequently, the beneficial effects of flavonoid metabolites in the brain are unlikely to result from their ability to out-compete antioxidants such as ascorbate, which are present at higher concentrations (high μ M–mM). Instead, it appears that the cellular effects of flavonoids are likely to be mediated by their interactions with specific proteins central to neuronal intracellular signalling cascades⁽¹⁷⁾, such as the MAPK signalling pathway and the phosphoinositide 3-kinase (PI3K)/Akt signalling cascade.

How might flavonoids act to induce neuro-cognitive changes?

There are many ways in which dietary flavonoids may exert beneficial effects in the central nervous system. For example, they may protect neurons against oxidative stress-induced injury⁽¹¹³⁾, alleviate neuroinflammation⁽¹¹⁴⁾ and promote synaptic plasticity. As evidence supports the localisation of flavonoids within the brain, these phytochemicals may be regarded as potential neuroprotective agents or neuromodulators. It appears highly likely that such properties are mediated by their abilities to interact with both protein and lipid kinase signalling cascades^(16,115–120) rather than via their potential to act as classical antioxidants, and the concentrations of flavonoids in the brain are thought to be sufficiently high to exert pharmacological activity at receptors, kinases and transcription factors. Presently, the precise sites of action are unknown, although it is likely that their activity depends on their ability to: bind to ATP sites on enzymes and receptors; modulate the activity of kinases directly, i.e. MAPK kinase kinase, MAPK kinase or MAPK; affect the function of important phosphatases, which act in opposition to kinases; preserve Ca^{2+} homeostasis, thereby preventing Ca^{2+} -dependent activation of kinases in neurons; modulate signalling cascades lying downstream of kinases, i.e. transcription factor activation and binding to promoter sequences⁽¹²¹⁾.

Flavonoids have the potential to bind to the ATP-binding sites of a large number of proteins⁽¹²²⁾ including

mitochondrial ATPase⁽¹²³⁾, Ca plasma-membrane ATPase⁽¹²⁴⁾, protein kinase A⁽¹²⁵⁾, protein kinase C^(118,126–129) and topoisomerase⁽¹³⁰⁾. In addition, interactions with the benzodiazepine-binding sites of GABA_A receptors and with adenosine receptors^(131,132) have been reported. For example, the stilbene resveratrol and the citrus flavanones hesperetin and naringenin have been reported to have inhibitory activity at a number of protein kinases^(133–135). This inhibition is mediated via the binding of the polyphenols to the ATP-binding site, presumably causing three-dimensional structural changes in the kinase leading to its inactivity. They may also interact directly with mitochondria, for example by modulating the mitochondrial transition pore, which controls cytochrome c release during apoptosis^(136,137), or by modulating other mitochondrial-associated pro-apoptotic factors such as DIABLO/smac^(138,139). Potential interactions with the mitochondrial transition pore are especially interesting, as the transition pore possesses a benzodiazepine-binding site where flavonoids may bind^(131,132) and influence pore opening and cytochrome c release during apoptosis.

Interactions of flavonoids within the extracellular signal-regulated protein kinase/cAMP-response element-binding protein signalling pathway

Previous studies have suggested that phytochemicals, especially flavonoids, may exert cellular effects via direct modulation of protein and lipid kinase signalling pathways⁽¹⁵⁾. Interactions within the MAPK pathway are thought to be central to mediating the cellular effects of flavonoids such as those found in berries, tea and cocoa^(16,120). For example, the flavanol (–)-epicatechin induces both extracellular signal-regulated protein kinase (ERK) 1/2 and CREB activation in cortical neurons and subsequently increases CREB-regulated gene expression⁽¹⁴⁰⁾. Furthermore, another flavonoid, fisetin, has been shown to improve LTP and memory through a CREB/ERK mechanism⁽¹⁴¹⁾ and nanomolar concentrations of quercetin have also been observed to enhance CREB activation in neurons⁽¹⁶⁾. Thus, one potential mechanism of action of flavonoids in modulating neuronal function, LTP and synaptic plasticity may proceed via signalling through CREB. In support of this possibility, other flavonoids have also been shown to influence the ERK pathway, with the citrus flavanone hesperetin being capable of activating ERK1/2 signalling in cortical neurons⁽¹⁴²⁾ and flavanols such as epigallocatechin gallate restoring both protein kinase C and ERK1/2 activities in 6-hydroxydopamine-treated and serum-deprived neurons^(143,144). Furthermore, this ability to activate the ERK pathway is not restricted to neurons and has also been observed in fibroblasts exposed to low concentrations of epicatechin⁽¹⁴⁵⁾. However, although the majority of investigations have centred on the potential of flavonoids to modulate the phosphorylation state of ERK1/2^(16,17,120), it is more likely that their actions on this MAPK isoform result from effects on upstream kinases, such as MAPK kinases 1 and 2, and potentially membrane receptors⁽¹⁷⁾ (Fig. 3). This possibility appears likely as flavonoids have close structural homology to specific inhibitors of MAPK kinase 1, such as

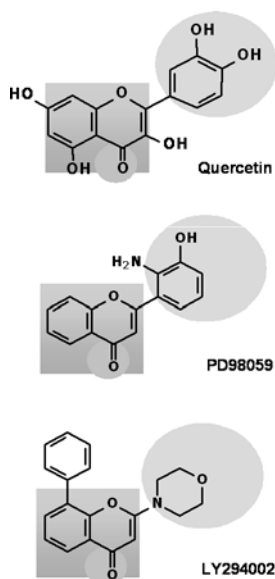


Fig. 4. The structure of mitogen-activated protein kinase kinase inhibitor PD98059 and the phosphoinositide 3-kinase inhibitor LY294002 have close structural homology to that of flavonoids. LY294002 and quercetin both fit into the ATP-binding pocket of the phosphoinositide 3-kinase, inhibiting its activity. It appears that the number and substitution of hydroxyl groups on the flavonoid ring B and the extent of unsaturation of the C-2–C-3 bond are important determinants of their activity. Such inhibitory actions have been proposed as potential mechanisms by which flavonoids act to modulate neuronal function.

PD98059 (2'-amino-3'-methoxyflavone; Fig. 4). Overall, their effects on the ERK pathway are likely to be related to their ability to exert high-affinity receptor-agonist-like actions at low concentrations and direct enzyme inhibition at higher concentrations^(117,146). Receptors reported to act as flavonoid-binding sites that are present in cortical neurons are adenosine⁽¹⁴⁷⁾ and GABA_A receptors^(148,149). However, a specific plasma-membrane binding site for polyphenols has recently been described in rat brain⁽¹⁵⁰⁾. In addition, monomeric and dimeric flavanols show nanomolar affinity and efficacy at testosterone receptors⁽¹⁵¹⁾ and resveratrol rapidly activates ERK signalling through α and β oestrogen receptors⁽¹⁵²⁾. Collectively, these findings raise the possibility that flavonoids may act on the ERK pathway by acting through steroid-like receptors in neurons to modulate ERK and CREB-mediated gene expression.

If flavonoids are able to promote the activation of neuronal CREB, they may be capable of enhancing the expression of a number of genes that contain cAMP-response element sequences in their promoter regions, including a number of neurotrophins⁽¹⁵³⁾. Particular emphasis has been given to the regulation of BDNF^(154,155), which has been implicated in synaptic plasticity and long-term memory⁽¹⁵⁶⁾ and is robustly induced in hippocampal neurons on synaptic stimulation⁽¹⁵⁷⁾. BDNF belongs to the neurotrophin family of growth factors and affects the survival and function of neurons in the central nervous system. Its secretion from neurons is under activity-dependent control

and is crucial for the formation of appropriate synaptic connections during development and for learning and memory in adults⁽¹⁵⁸⁾. Decreases in BDNF and pro-BDNF have been reported in Alzheimer's disease^(159,160) and the importance of pro-BDNF has been emphasised by the finding that a polymorphism that replaces valine for methionine at position 66 of the pro-domain is associated with memory defects and abnormal hippocampal function in human subjects⁽¹⁶¹⁾. In addition, genetic⁽¹⁶²⁾ as well as pharmacological inhibition⁽¹⁶³⁾ of BDNF or its receptor tropomyosin receptor kinase B⁽¹⁶⁴⁾ impairs learning and memory. On the other hand, agents that increase BDNF levels lead to improvements in spatial working memory, in part through the regulation of protein translation via the mammalian target of rapamycin (mTOR) signalling pathway⁽¹⁶⁵⁾ (Fig. 5). It has recently been shown that a 3–12-week supplementation of old rats with a 20 g blueberry/kg diet leads to improvement in spatial working memory, which is correlated with an activation of CREB and increases in both pro- and mature levels of BDNF in the hippocampus (CM Williams, MM Abd El Mohsen and JPE Spencer, unpublished results).

Interactions of flavonoids within the phosphoinositide 3-kinase/Akt signalling pathway

Flavonoids have long been known to inhibit PI3K and Akt via direct interactions with its ATP-binding site. Indeed, a number of studies have demonstrated that the structure of flavonoids determines whether or not they act as potent inhibitors of PI3K^(117,166). One of the most selective PI3K inhibitors available, LY294002 (Fig. 4), was modelled on the structure of quercetin^(115,116). LY294002 and quercetin fit into the ATP-binding pocket of the enzyme although with surprisingly different orientations⁽¹⁴⁶⁾. It appears that the number and substitution of hydroxyl groups on the B-ring and the extent of unsaturation of the C-2–C-3 bond are important determinants of this particular bioactivity. Interestingly, in this context quercetin and some of its *in vivo* metabolites inhibit pro-survival Akt signalling pathways⁽¹⁶⁾ by a mechanism of action consistent with quercetin and its metabolites acting at and inhibiting PI3K activity⁽¹¹⁵⁾. However, other flavonoids such as the citrus flavanone hesperetin cause the activation of Akt and the inhibition of pro-apoptotic proteins such as apoptosis signal-regulating kinase 1, Bad, caspase-9 and caspase-3 in cortical neurons⁽¹⁴²⁾. Thus, flavanones, and other flavonoids, may be capable of exerting beneficial effects in neurons via signalling through Akt and may also have the potential to activate CREB through activation of this pathway (Fig. 3).

At neuronal synapses flavonoid-induced activation of CREB and enhancement of BDNF expression in neurons would be expected to initiate the activation of the PI3K/Akt signalling pathway via the binding of BDNF to pre- or post-synaptic tropomyosin receptor kinase B receptors (Fig. 5). These events trigger the activation of the mTOR pathway and the increased translation of specific mRNA subpopulations⁽¹⁶⁷⁾, including the activity-regulated cytoskeletal-associated protein termed Arc/Arg3.1. Arc/Arg3.1 is known to be important in LTP and has been proposed to

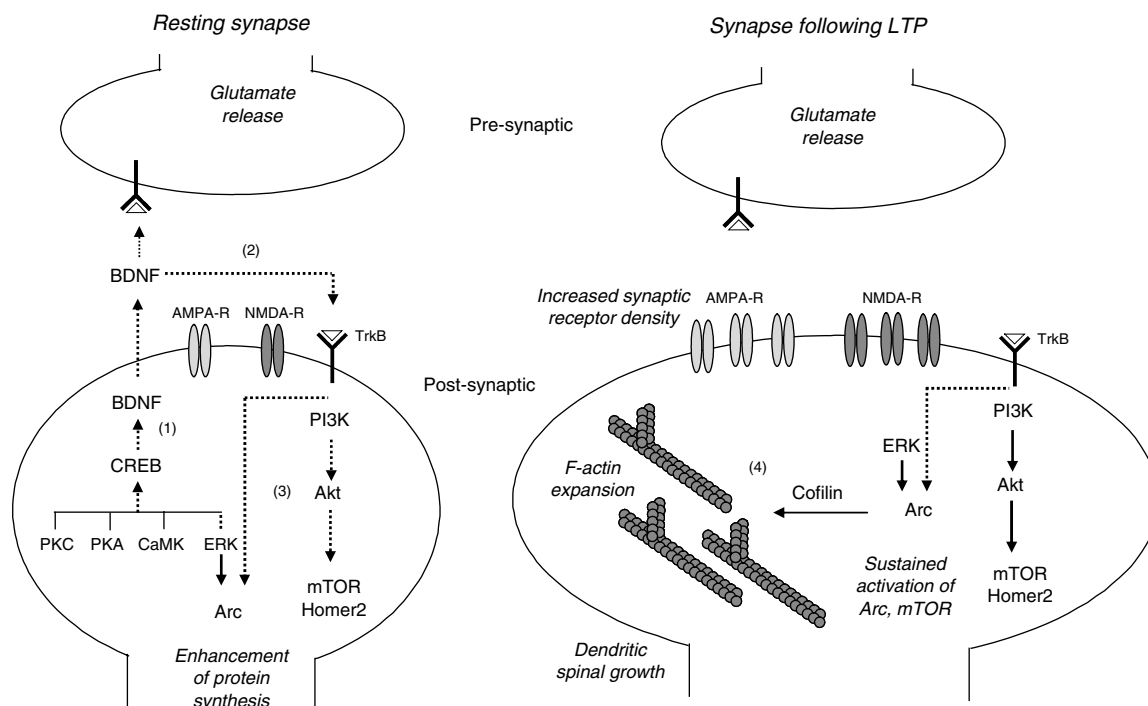


Fig. 5. Formation of stable long-term potentiation (LTP) at synapses. Increased expression and release of brain-derived neurotrophic factor (BDNF) from the synapse through enhanced cAMP-response element-binding protein (CREB) activation (1). BDNF binds to pre-and post-synaptic tropomyosin receptor kinase B (TrkB) receptors (2), triggering glutamate release and phosphoinositide 3-kinase (PI3K)/mammalian target of rapamycin (mTOR) signalling and Arc/Arg3.1 (Arc) synthesis (3). Sustained activation of mTOR leads to enhanced translational efficiency whilst Arc, in association with Cofilin, triggers F-actin expansion and synapse growth (mushroom synapse; 4). Akt, protein kinase B/Akt; PKC, PKA, protein kinase A and C respectively; CaMK, calcium-calmodulin kinase; ERK, extracellular signal-regulated protein kinase.

be under regulatory control of both BDNF⁽¹⁶⁸⁾ and the ERK signalling pathway⁽¹⁶⁹⁾ (Fig. 5). In addition to ERK and CREB activation, blueberry supplementation for 12 weeks has also been observed to lead to an activation of mTOR and the increased expression of hippocampal Arc/Arg3.1 (CM Williams, MM Abd El Mohsen and JPE Spencer, unpublished results). The sustained synthesis of Arc/Arg3.1 during a protracted time-window is necessary to consolidate LTP, whilst translation of pre-existing Arc/Arg3.1 mRNA contributes to early LTP expression and translation of new Arc/Arg3.1 mRNA mediates consolidation⁽¹⁷⁰⁾. Increased Arc/Arg3.1 expression may facilitate changes in synaptic strength and the induction of morphological changes such as that observed when small dendritic spines are converted into large mushroom-shaped spines through a mechanism dependent on actin polymerisation⁽¹⁷¹⁾ (Fig. 5). Whether flavonoids are capable of promoting changes in neuronal morphology *in vivo* is currently unclear, although studies have indicated that effects of flavonoid effects on neuronal morphology are possible⁽⁵⁶⁾ and that certain flavonoids can influence neuronal dendrite outgrowth *in vitro*⁽¹⁷²⁾. In addition, the known ability of flavonoids to activate signalling cascades upstream of mTOR and Arc/Arg3.1, notably ERK, CREB and BDNF, strengthens the concept that they are also capable of inducing changes in neuronal

morphology that underlie improvements in memory, learning and cognitive performance in mammalian species, including man.

Summary

Emerging evidence suggests that dietary phytochemicals, in particular flavonoids, may exert beneficial effects in the central nervous system by protecting neurons against stress-induced injury, by suppressing neuroinflammation and by promoting LTP and synaptic plasticity. Such effects, in particular the latter two, are likely to underpin their observed beneficial effects on human memory and neuro-cognitive performance. There is strong evidence that such beneficial properties are mediated by their ability to interact with a number of neuronal protein and lipid kinase signalling cascades known to be crucial in determining LTP and hence the acquisition, consolidation and storage of human memory. Such pathways include the MAPK signalling cascade, in particular the ERK1/2 pathway, the protein kinase A pathway and the Ca-calmodulin kinase cascade. The activation of these pathways along with the activation of the transcription factor CREB is known to be required during memory acquisition and consolidation, and agents capable of inducing pathways leading to CREB

activation have the potential to enhance both short-term and long-term memory.

In contrast to short-term memory, the storage of long-term memory requires the formation of stable LTP at synapses. This process is known to require an enhancement of synaptic mRNA translation and *de novo* synthesis of proteins such as F-actin and synaptic membrane receptors, which lead to an increase in dendritic spine density and membrane receptor density respectively. Flavonoids may trigger all these events via their ability to activate CREB and CREB-induced gene expression. In doing so they may increase the expression of neurotrophins, such as BDNF, which initiate the *de novo* synthesis of many proteins associated with LTP via the activation of mTOR signalling. Ultimately, these events lead to synapse growth, an increase in dendritic spine density and increased membrane receptor density, all factors known to be essential for efficient LTP, synaptic plasticity and ultimately the storage of long-term memory.

Acknowledgements

J.P.E.S. is funded by the Biotechnology and Biological Sciences Research Council (BB/C518222/1) and the Medical Research Council (G0400278/NI02).

References

1. Youdim KA & Joseph JA (2001) A possible emerging role of phytochemicals in improving age-related neurological dysfunctions: a multiplicity of effects. *Free Radic Biol Med* **30**, 583–594.
2. Youdim KA, Spencer JPE, Schroeter H & Rice-Evans C (2002) Dietary flavonoids as potential neuroprotectants. *Biol Chem* **383**, 503–519.
3. Galli RL, Shukitt-Hale B, Youdim KA & Joseph JA (2002) Fruit polyphenolics and brain aging: nutritional interventions targeting age-related neuronal and behavioral deficits. *Ann NY Acad Sci* **959**, 128–132.
4. Unno K, Takabayashi F, Kishido T & Oku N (2004) Suppressive effect of green tea catechins on morphologic and functional regression of the brain in aged mice with accelerated senescence (SAMP10). *Exp Gerontol* **39**, 1027–1034.
5. Haque AM, Hashimoto M, Katakura M, Tanabe Y, Hara Y & Shido O (2006) Long-term administration of green tea catechins improves spatial cognition learning ability in rats. *J Nutr* **136**, 1043–1047.
6. Kuriyama S, Hozawa A, Ohmori K, Shimazu T, Matsui T, Ebihara S, Awata S, Nagatomi R, Arai H & Tsuji I (2006) Green tea consumption and cognitive function: a cross-sectional study from the Tsurugaya Project 1. *Am J Clin Nutr* **83**, 355–361.
7. Wang Y, Wang L, Wu J & Cai J (2006) The in vivo synaptic plasticity mechanism of EGb 761-induced enhancement of spatial learning and memory in aged rats. *Br J Pharmacol* **148**, 147–153.
8. Inanami O, Watanabe Y, Syuto B, Nakano M, Tsuji M & Kuwabara M (1998) Oral administration of (-)-catechin protects against ischemia-reperfusion-induced neuronal death in the gerbil. *Free Radic Res* **29**, 359–365.
9. Luo Y, Smith JV, Paramasivam V, Burdick A, Curry KJ, Buford JP, Khan I, Netzer WJ, Xu H & Butko P (2002) Inhibition of amyloid-beta aggregation and caspase-3 activation by the Ginkgo biloba extract EGb761. *Proc Natl Acad Sci USA* **99**, 12197–12202.
10. Bastianetto S, Zheng WH & Quirion R (2000) The Ginkgo biloba extract (EGb 761) protects and rescues hippocampal cells against nitric oxide-induced toxicity: involvement of its flavonoid constituents and protein kinase C. *J Neurochem* **74**, 2268–2277.
11. Zimmermann M, Colciaghi F, Cattabeni F & Di Luca M (2002) Ginkgo biloba extract: from molecular mechanisms to the treatment of Alzheimer's disease. *Cell Mol Biol (Noisy-le-grand)* **48**, 613–623.
12. Datla KP, Christidou M, Widmer WW, Rooprai HK & Dexter DT (2001) Tissue distribution and neuroprotective effects of citrus flavonoid tangeretin in a rat model of Parkinson's disease. *Neuroreport* **12**, 3871–3875.
13. Rice-Evans CA, Miller NJ & Paganga G (1996) Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic Biol Med* **20**, 933–956.
14. Pollard SE, Kuhnle GG, Vauzour D, Vafeiadou K, Tzounis X, Whiteman M, Rice-Evans C & Spencer JPE (2006) The reaction of flavonoid metabolites with peroxynitrite. *Biochem Biophys Res Commun* **350**, 960–968.
15. Williams RJ, Spencer JPE & Rice-Evans C (2004) Flavonoids: antioxidants or signalling molecules? *Free Radic Biol Med* **36**, 838–849.
16. Spencer JPE, Rice-Evans C & Williams RJ (2003) Modulation of pro-survival Akt/PKB & ERK1/2 signalling cascades by quercetin and its in vivo metabolites underlie their action on neuronal viability. *J Biol Chem* **278**, 34783–34793.
17. Schroeter H, Boyd C, Spencer JPE, Williams RJ, Cadenas E & Rice-Evans C (2002) MAPK signaling in neurodegeneration: influences of flavonoids and of nitric oxide. *Neurobiol Aging* **23**, 861–880.
18. Manach C, Scalbert A, Morand C, Remesy C & Jimenez L (2004) Polyphenols: food sources and bioavailability. *Am J Clin Nutr* **79**, 727–747.
19. Rice-Evans C (2001) Flavonoid antioxidants. *Curr Med Chem* **8**, 797–807.
20. Rice-Evans C (1995) Plant polyphenols: free radical scavengers or chain-breaking antioxidants? *Biochem Soc Symp* **61**, 103–116.
21. Spencer JPE, Schroeter H, Rechner AR & Rice-Evans C (2001) Bioavailability of flavan-3-ols and procyanidins: gastrointestinal tract influences and their relevance to bioactive forms in vivo. *Antioxid Redox Signal* **3**, 1023–1039.
22. Spencer JPE, Abd El Mohsen MM, Minihaue AM & Mathers JC (2007) Biomarkers of the intake of dietary polyphenols: strengths, limitations and application in nutrition research. *Br J Nutr* **1**, 1–11.
23. Spencer JPE, Chowrimootoo G, Choudhury R, Debnam ES, Srai SK & Rice-Evans C (1999) The small intestine can both absorb and glucuronidate luminal flavonoids. *FEBS Lett* **458**, 224–230.
24. Spencer JPE (2003) Metabolism of tea flavonoids in the gastrointestinal tract. *J Nutr* **133**, 3255S–3261S.
25. Scheline RR (1999) Metabolism of oxygen heterocyclic compounds. *CRC Handbook of Mammalian Metabolism of Plant Compounds*, pp. 243–295 Boca Raton, FL: CRC Press Inc.
26. Spencer JPE, Kuhnle GG, Williams RJ & Rice-Evans C (2003) Intracellular metabolism and bioactivity of quercetin and its in vivo metabolites. *Biochem J* **372**, 173–181.
27. Spencer JPE, Schroeter H, Crossthwaithe AJ, Kuhnle G, Williams RJ & Rice-Evans C (2001) Contrasting influences

- of glucuronidation and O-methylation of epicatechin on hydrogen peroxide-induced cell death in neurons and fibroblasts. *Free Radic Biol Med* **31**, 1139–1146.
28. Miyake Y, Shimoi K, Kumazawa S, Yamamoto K, Kinae N & Osawa T (2000) Identification and antioxidant activity of flavonoid metabolites in plasma and urine of eriocitrin-treated rats. *J Agric Food Chem* **48**, 3217–3224.
 29. Terao J, Yamaguchi S, Shirai M, Miyoshi M, Moon JH, Oshima S, Inakuma T, Tsushida T & Kato Y (2001) Protection by quercetin and quercetin 3-O-beta-D-glucuronide of peroxynitrite-induced antioxidant consumption in human plasma low-density lipoprotein. *Free Radic Res* **35**, 925–931.
 30. Shirai M, Moon JH, Tsushida T & Terao J (2001) Inhibitory effect of a quercetin metabolite, quercetin 3-O-beta-D-glucuronide, on lipid peroxidation in liposomal membranes. *J Agric Food Chem* **49**, 5602–5608.
 31. Yamamoto N, Moon JH, Tsushida T, Nagao A & Terao J (1999) Inhibitory effect of quercetin metabolites and their related derivatives on copper ion-induced lipid peroxidation in human low-density lipoprotein. *Arch Biochem Biophys* **372**, 347–354.
 32. da Silva EL, Piskula MK, Yamamoto N, Moon JH & Terao J (1998) Quercetin metabolites inhibit copper ion-induced lipid peroxidation in rat plasma. *FEBS Lett* **430**, 405–408.
 33. Letenneur L, Proust-Lima C, Le Gouge A, Dartigues JF & Barberger-Gateau P (2007) Flavonoid intake and cognitive decline over a 10-year period. *Am J Epidemiol* **165**, 1364–1371.
 34. Ho SC, Chan AS, Ho YP, So EK, Sham A, Zee B & Woo JL (2007) Effects of soy isoflavone supplementation on cognitive function in Chinese postmenopausal women: a double-blind, randomized, controlled trial. *Menopause* **14**, 489–499.
 35. Lee YB, Lee HJ & Sohn HS (2005) Soy isoflavones and cognitive function. *J Nutr Biochem* **16**, 641–649.
 36. File SE, Jarrett N, Fluck E, Duffy R, Casey K & Wiseman H (2001) Eating soya improves human memory. *Psychopharmacology (Berl)* **157**, 430–436.
 37. Kim H, Xia H, Li L & Gewin J (2000) Attenuation of neurodegeneration-relevant modifications of brain proteins by dietary soy. *Biofactors* **12**, 243–250.
 38. Birge SJ (1996) Is there a role for estrogen replacement therapy in the prevention and treatment of dementia? *J Am Geriatr Soc* **44**, 865–870.
 39. Henderson VW (2006) Estrogen-containing hormone therapy and Alzheimer's disease risk: understanding discrepant inferences from observational and experimental research. *Neuroscience* **138**, 1031–1039.
 40. Casini ML, Marelli G, Papaleo E, Ferrari A, D'Ambrosio F & Unfer V (2006) Psychological assessment of the effects of treatment with phytoestrogens on postmenopausal women: a randomized, double-blind, crossover, placebo-controlled study. *Fertil Steril* **85**, 972–978.
 41. Kritz-Silverstein D, Von Mühlen D, Barrett-Connor E & Bressel MA (2003) Isoflavones and cognitive function in older women: the SOy and Postmenopausal Health In Aging (SOPHIA) Study. *Menopause* **10**, 196–202.
 42. File SE, Hartley DE, Elsbagh S, Duffy R & Wiseman H (2005) Cognitive improvement after 6 weeks of soy supplements in postmenopausal women is limited to frontal lobe function. *Menopause* **12**, 193–201.
 43. Zippel R, Balestrini M, Lomazzi M & Sturani E (2000) Calcium and calmodulin are essential for Ras-GRF1-mediated activation of the Ras pathway by lysophosphatidic acid. *Exp Cell Res* **258**, 403–408.
 44. Kreijkamp-Kaspers S, Kok L, Grobbee DE, de Haan EH, Aleman A, Lampe JW & van der Schouw YT (2004) Effect of soy protein containing isoflavones on cognitive function, bone mineral density, and plasma lipids in postmenopausal women: a randomized controlled trial. *JAMA* **292**, 65–74.
 45. Fournier LR, Ryan Borchers TA, Robison LM, Wiediger M, Park JS, Chew BP, McGuire MK, Sclar DA, Skaer TL & Beerman KA (2007) The effects of soy milk and isoflavone supplements on cognitive performance in healthy, postmenopausal women. *J Nutr Health Aging* **11**, 155–164.
 46. Kreijkamp-Kaspers S, Kok L, Grobbee DE, de Haan EH, Aleman A & van der Schouw YT (2007) Dietary phytoestrogen intake and cognitive function in older women. *J Gerontol A Biol Sci Med Sci* **62**, 556–562.
 47. Barnes S, Boersma B, Patel R, Kirk M, Darley-Usmar VM, Kim H & Xu J (2000) Isoflavonoids and chronic disease: mechanisms of action. *Biofactors* **12**, 209–215.
 48. Kim H, Peterson TG & Barnes S (1998) Mechanisms of action of the soy isoflavone genistein: emerging role for its effects via transforming growth factor beta signaling pathways. *Am J Clin Nutr* **68**, 1418S–1425S.
 49. Schroeter H, Heiss C, Balzer J, Kleinbongard P, Keen CL, Hollenberg NK, Sies H, Kwik-Urbe C, Schmitz HH & Kelm M (2006) (-)-Epicatechin mediates beneficial effects of flavanol-rich cocoa on vascular function in humans. *Proc Natl Acad Sci USA* **103**, 1024–1029.
 50. Francis ST, Head K, Morris PG & Macdonald IA (2006) The effect of flavanol-rich cocoa on the fMRI response to a cognitive task in healthy young people. *J Cardiovasc Pharmacol* **47**, Suppl. 2, S215–S220.
 51. Fisher ND, Sorond FA & Hollenberg NK (2006) Cocoa flavanols and brain perfusion. *J Cardiovasc Pharmacol* **47**, Suppl. 2, S210–S214.
 52. Dinges DF (2006) Cocoa flavanols, cerebral blood flow, cognition, and health: going forward. *J Cardiovasc Pharmacol* **47**, Suppl. 2, S221–S223.
 53. Gage FH (2000) Mammalian neural stem cells. *Science* **287**, 1433–1438.
 54. Palmer TD, Willhoite AR & Gage FH (2000) Vascular niche for adult hippocampal neurogenesis. *J Comp Neurol* **425**, 479–494.
 55. Harris KM & Kater SB (1994) Dendritic spines: cellular specializations imparting both stability and flexibility to synaptic function. *Annu Rev Neurosci* **17**, 341–371.
 56. van Praag H, Lucero MJ, Yeo GW *et al.* (2007) Plant-derived flavanol (-)epicatechin enhances angiogenesis and retention of spatial memory in mice. *J Neurosci* **27**, 5869–5878.
 57. Joseph JA, Shukitt-Hale B, Denisova NA, Prior RL, Cao G, Martin A, Taglialetela G & Bickford PC (1998) Long-term dietary strawberry, spinach, or vitamin E supplementation retards the onset of age-related neuronal signal-transduction and cognitive behavioral deficits. *J Neurosci* **18**, 8047–8055.
 58. Joseph JA, Shukitt-Hale B, Denisova NA, Bielinski D, Martin A, McEwen JJ & Bickford PC (1999) Reversals of age-related declines in neuronal signal transduction, cognitive, and motor behavioral deficits with blueberry, spinach, or strawberry dietary supplementation. *J Neurosci* **19**, 8114–8121.
 59. Casadesus G, Shukitt-Hale B, Stellwagen HM, Zhu X, Lee HG, Smith MA & Joseph JA (2004) Modulation of hippocampal plasticity and cognitive behavior by short-term blueberry supplementation in aged rats. *Nutr Neurosci* **7**, 309–316.

60. Shukitt-Hale B, Smith DE, Meydani M & Joseph JA (1999) The effects of dietary antioxidants on psychomotor performance in aged mice. *Exp Gerontol* **34**, 797–808.
61. Joseph JA, Shukitt-Hale B & Casadesus G (2005) Reversing the deleterious effects of aging on neuronal communication and behavior: beneficial properties of fruit polyphenolic compounds. *Am J Clin Nutr* **81**, 313S–316S.
62. Goyarzu P, Malin DH, Lau FC *et al.* (2004) Blueberry supplemented diet: effects on object recognition memory and nuclear factor-kappa B levels in aged rats. *Nutr Neurosci* **7**, 75–83.
63. Joseph JA, Denisova NA, Arendash G, Gordon M, Diamond D, Shukitt-Hale B & Morgan D (2003) Blueberry supplementation enhances signaling and prevents behavioral deficits in an Alzheimer disease model. *Nutr Neurosci* **6**, 153–162.
64. Andres-Lacueva C, Shukitt-Hale B, Galli RL, Jauregui O, Lamuela-Raventos RM & Joseph JA (2005) Anthocyanins in aged blueberry-fed rats are found centrally and may enhance memory. *Nutr Neurosci* **8**, 111–120.
65. Ramirez MR, Izquierdo I, do Carmo Bassols RM, Zuanazzi JA, Barros D & Henriques AT (2005) Effect of lyophilised Vaccinium berries on memory, anxiety and locomotion in adult rats. *Pharmacol Res* **52**, 457–462.
66. Chan YC, Hosoda K, Tsai CJ, Yamamoto S & Wang MF (2006) Favorable effects of tea on reducing the cognitive deficits and brain morphological changes in senescence-accelerated mice. *J Nutr Sci Vitaminol (Tokyo)* **52**, 266–273.
67. Shukitt-Hale B, Carey A, Simon L, Mark DA & Joseph JA (2006) Effects of Concord grape juice on cognitive and motor deficits in aging. *Nutrition* **22**, 295–302.
68. Singh A, Naidu PS & Kulkarni SK (2003) Reversal of aging and chronic ethanol-induced cognitive dysfunction by quercetin a bioflavonoid. *Free Radic Res* **37**, 1245–1252.
69. Patil CS, Singh VP, Satyanarayan PS, Jain NK, Singh A & Kulkarni SK (2003) Protective effect of flavonoids against aging- and lipopolysaccharide-induced cognitive impairment in mice. *Pharmacology* **69**, 59–67.
70. Wang H, Hu Y & Tsien JZ (2006) Molecular and systems mechanisms of memory consolidation and storage. *Prog Neurobiol* **79**, 123–135.
71. Squire LR & Zola-Morgan S (1991) The medial temporal lobe memory system. *Science* **253**, 1380–1386.
72. Squire LR (2004) Memory systems of the brain: a brief history and current perspective. *Neurobiol Learn Mem* **82**, 171–177.
73. Squire LR, Stark CE & Clark RE (2004) The medial temporal lobe. *Annu Rev Neurosci* **27**, 279–306.
74. Alvarez P & Squire LR (1994) Memory consolidation and the medial temporal lobe: a simple network model. *Proc Natl Acad Sci USA* **91**, 7041–7045.
75. Anagnostaras SG, Maren S & Fanselow MS (1999) Temporally graded retrograde amnesia of contextual fear after hippocampal damage in rats: within-subjects examination. *J Neurosci* **19**, 1106–1114.
76. Zola-Morgan SM & Squire LR (1990) The primate hippocampal formation: evidence for a time-limited role in memory storage. *Science* **250**, 288–290.
77. Squire LR & Alvarez P (1995) Retrograde amnesia and memory consolidation: a neurobiological perspective. *Curr Opin Neurobiol* **5**, 169–177.
78. Weeber EJ & Sweatt JD (2002) Molecular neurobiology of human cognition. *Neuron* **33**, 845–848.
79. Dash PK, Moore AN, Kobori N & Runyan JD (2007) Molecular activity underlying working memory. *Learn Mem* **14**, 554–563.
80. Tully T (1998) Toward a molecular biology of memory: the light's coming on! *Nat Neurosci* **1**, 543–545.
81. Carew TJ (1996) Molecular enhancement of memory formation. *Neuron* **16**, 5–8.
82. Bramham CR & Wells DG (2007) Dendritic mRNA: transport, translation and function. *Nat Rev Neurosci* **8**, 776–789.
83. Martin KC, Barad M & Kandel ER (2000) Local protein synthesis and its role in synapse-specific plasticity. *Curr Opin Neurobiol* **10**, 587–592.
84. Kelleher RJ III, Govindarajan A & Tonegawa S (2004) Translational regulatory mechanisms in persistent forms of synaptic plasticity. *Neuron* **44**, 59–73.
85. Arnsten AF, Ramos BP, Birnbaum SG & Taylor JR (2005) Protein kinase A as a therapeutic target for memory disorders: rationale and challenges. *Trends Mol Med* **11**, 121–128.
86. van der Heide LP, Ramakers GM & Smidt MP (2006) Insulin signaling in the central nervous system: learning to survive. *Prog Neurobiol* **79**, 205–221.
87. Alkon DL, Sun MK & Nelson TJ (2007) PKC signaling deficits: a mechanistic hypothesis for the origins of Alzheimer's disease. *Trends Pharmacol Sci* **28**, 51–60.
88. Wei F, Qiu CS, Liauw J, Robinson DA, Ho N, Chatila T & Zhuo M (2002) Calcium calmodulin-dependent protein kinase IV is required for fear memory. *Nat Neurosci* **5**, 573–579.
89. Sweatt JD (2001) The neuronal MAP kinase cascade: a biochemical signal integration system subserving synaptic plasticity and memory. *J Neurochem* **76**, 1–10.
90. Sweatt JD (2004) Mitogen-activated protein kinases in synaptic plasticity and memory. *Curr Opin Neurobiol* **14**, 311–317.
91. Impey S, McCorkle SR, Cha-Molstad H, Dwyer JM, Yochum GS, Boss JM, McWeeney S, Dunn JJ, Mandel G & Goodman RH (2004) Defining the CREB regulon: a genome-wide analysis of transcription factor regulatory regions. *Cell* **119**, 1041–1054.
92. Barco A, Bailey CH & Kandel ER (2006) Common molecular mechanisms in explicit and implicit memory. *J Neurochem* **97**, 1520–1533.
93. Bourtchuladze R, Frenguelli B, Blendy J, Cioffi D, Schutz G & Silva AJ (1994) Deficient long-term memory in mice with a targeted mutation of the cAMP-responsive element-binding protein. *Cell* **79**, 59–68.
94. Tully T, Bourtchouladze R, Scott R & Tallman J (2003) Targeting the CREB pathway for memory enhancers. *Nat Rev Drug Discov* **2**, 267–277.
95. Pham TA, Impey S, Storm DR & Stryker MP (1999) CRE-mediated gene transcription in neocortical neuronal plasticity during the developmental critical period. *Neuron* **22**, 63–72.
96. Impey S, Smith DM, Obrietan K, Donahue R, Wade C & Storm DR (1998) Stimulation of cAMP response element (CRE)-mediated transcription during contextual learning. *Nat Neurosci* **1**, 595–601.
97. Impey S, Mark M, Villacres EC, Poser S, Chavkin C & Storm DR (1996) Induction of CRE-mediated gene expression by stimuli that generate long-lasting LTP in area CA1 of the hippocampus. *Neuron* **16**, 973–982.
98. Finkbeiner S (2000) CREB couples neurotrophin signals to survival messages. *Neuron* **25**, 11–14.
99. Finkbeiner S, Tavazoie SF, Maloratsky A, Jacobs KM, Harris KM & Greenberg ME (1997) CREB: a major mediator of neuronal neurotrophin responses. *Neuron* **19**, 1031–1047.

100. Abbott NJ (2002) Astrocyte-endothelial interactions and blood-brain barrier permeability. *J Anat* **200**, 629–638.
101. Youdim KA, Dobbie MS, Kuhnle G, Prottogente AR, Abbott NJ & Rice-Evans C (2003) Interaction between flavonoids and the blood-brain barrier: in vitro studies. *J Neurochem* **85**, 180–192.
102. Youdim KA, Shukitt-Hale B & Joseph JA (2004) Flavonoids and the brain: interactions at the blood-brain barrier and their physiological effects on the central nervous system. *Free Radic Biol Med* **37**, 1683–1693.
103. Youdim KA, Qaiser MZ, Begley DJ, Rice-Evans CA & Abbott NJ (2004) Flavonoid permeability across an in situ model of the blood-brain barrier. *Free Radic Biol Med* **36**, 592–604.
104. Aasmundstad TA, Morland J & Paulsen RE (1995) Distribution of morphine 6-glucuronide and morphine across the blood-brain barrier in awake, freely moving rats investigated by in vivo microdialysis sampling. *J Pharmacol Exp Ther* **275**, 435–441.
105. Sperker B, Backman JT & Kroemer HK (1997) The role of beta-glucuronidase in drug disposition and drug targeting in humans. *Clin Pharmacokinet* **33**, 18–31.
106. Kroemer HK & Klotz U (1992) Glucuronidation of drugs. A re-evaluation of the pharmacological significance of the conjugates and modulating factors. *Clin Pharmacokinet* **23**, 292–310.
107. Lin JH & Yamazaki M (2003) Role of P-glycoprotein in pharmacokinetics: clinical implications. *Clin Pharmacokinet* **42**, 59–98.
108. Suganuma M, Okabe S, Oniyama M, Tada Y, Ito H & Fujiki H (1998) Wide distribution of [3H](–)-epigallocatechin gallate, a cancer preventive tea polyphenol, in mouse tissue. *Carcinogenesis* **19**, 1771–1776.
109. Abd El Mohsen MM, Kuhnle G, Rechner AR, Schroeter H, Rose S, Jenner P & Rice-Evans CA (2002) Uptake and metabolism of epicatechin and its access to the brain after oral ingestion. *Free Radic Biol Med* **33**, 1693–1702.
110. Talavera S, Felgines C, Texier O, Besson C, Gil-Izquierdo A, Lamaison JL & Remesy C (2005) Anthocyanin metabolism in rats and their distribution to digestive area, kidney, and brain. *J Agric Food Chem* **53**, 3902–3908.
111. Abd El Mohsen MM, Marks J, Kuhnle G, Moore K, Debnam E, Kaila SS, Rice-Evans C & Spencer JPE (2006) Absorption, tissue distribution and excretion of pelargonidin and its metabolites following oral administration to rats. *Br J Nutr* **95**, 51–58.
112. Halliwell B, Zhao K & Whiteman M (2000) The gastrointestinal tract: a major site of antioxidant action? *Free Radic Res* **33**, 819–830.
113. Vauzour D, Vafeiadou K, Corona G, Pollard SE, Tzounis X & Spencer JPE (2007) Champagne wine polyphenols protect primary cortical neurons against peroxynitrite-induced injury. *J Agric Food Chem* **55**, 2854–2860.
114. Vafeiadou K, Vauzour D & Spencer JPE (2007) Neuroinflammation and its modulation by flavonoids. *Endocr Metab Immune Disord Drug Targets* **7**, 211–224.
115. Matter WF, Brown RF & Vlahos CJ (1992) The inhibition of phosphatidylinositol 3-kinase by quercetin and analogs. *Biochem Biophys Res Commun* **186**, 624–631.
116. Vlahos CJ, Matter WF, Hui KY & Brown RF (1994) A specific inhibitor of phosphatidylinositol 3-kinase, 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one (LY294002). *J Biol Chem* **269**, 5241–5248.
117. Agullo G, Gamet-Payrastra L, Manenti S, Viala C, Remesy C, Chap H & Payrastra B (1997) Relationship between flavonoid structure and inhibition of phosphatidylinositol 3-kinase: a comparison with tyrosine kinase and protein kinase C inhibition. *Biochem Pharmacol* **53**, 1649–1657.
118. Gamet-Payrastra L, Manenti S, Gratacap MP, Tulliez J, Chap H & Payrastra B (1999) Flavonoids and the inhibition of PKC and PI 3-kinase. *Gen Pharmacol* **32**, 279–286.
119. Kong AN, Yu R, Chen C, Mandlekar S & Primiano T (2000) Signal transduction events elicited by natural products: role of MAPK and caspase pathways in homeostatic response and induction of apoptosis. *Arch Pharm Res* **23**, 1–16.
120. Schroeter H, Spencer JPE, Rice-Evans C & Williams RJ (2001) Flavonoids protect neurons from oxidized low-density-lipoprotein-induced apoptosis involving c-Jun N-terminal kinase (JNK), c-Jun and caspase-3. *Biochem J* **358**, 547–557.
121. Spencer JPE (2007) The interactions of flavonoids within neuronal signalling pathways. *Genes Nutr* **2**, 257–273.
122. Conseil G, Baubichon-Cortay H, Dayan G, Jault JM, Barron D & Di Pietro A (1998) Flavonoids: a class of modulators with bifunctional interactions at vicinal ATP- and steroid-binding sites on mouse P-glycoprotein. *Proc Natl Acad Sci USA* **95**, 9831–9836.
123. Di Pietro A, Godinot C, Bouillant ML & Gautheron DC (1975) Pig heart mitochondrial ATPase: properties of purified and membrane-bound enzyme. Effects of flavonoids. *Biochimie* **57**, 959–967.
124. Barzilai A & Rahamimoff H (1983) Inhibition of Ca²⁺-transport ATPase from synaptosomal vesicles by flavonoids. *Biochim Biophys Acta* **730**, 245–254.
125. Revuelta MP, Cantabrana B & Hidalgo A (1997) Depolarization-dependent effect of flavonoids in rat uterine smooth muscle contraction elicited by CaCl₂. *Gen Pharmacol* **29**, 847–857.
126. Lee SF & Lin JK (1997) Inhibitory effects of phytopolyphenols on TPA-induced transformation, PKC activation, and c-jun expression in mouse fibroblast cells. *Nutr Cancer* **28**, 177–183.
127. Ursini F, Maiorino M, Morazzoni P, Roveri A & Pifferi G (1994) A novel antioxidant flavonoid (IdB 1031) affecting molecular mechanisms of cellular activation. *Free Radic Biol Med* **16**, 547–553.
128. Kantengwa S & Polla BS (1991) Flavonoids, but not protein kinase C inhibitors, prevent stress protein synthesis during erythrophagocytosis. *Biochem Biophys Res Commun* **180**, 308–314.
129. Rosenblat M, Belinky P, Vaya J, Levy R, Hayek T, Coleman R, Merchav S & Aviram M (1999) Macrophage enrichment with the isoflavan glabridin inhibits NADPH oxidase-induced cell-mediated oxidation of low density lipoprotein. A possible role for protein kinase C. *J Biol Chem* **274**, 13790–13799.
130. Boege F, Straub T, Kehr A, Boesenberg C, Christiansen K, Andersen A, Jakob F & Kohrle J (1996) Selected novel flavones inhibit the DNA binding or the DNA religation step of eukaryotic topoisomerase I. *J Biol Chem* **271**, 2262–2270.
131. Medina JH, Viola H, Wolfman C, Marder M, Wasowski C, Calvo D & Paladini AC (1997) Overview – Flavonoids: a new family of benzodiazepine receptor ligands. *Neurochem Res* **22**, 419–425.
132. Dekermendjian K, Kahnberg P, Witt MR, Sterner O, Nielsen M & Liljefors T (1999) Structure-activity relationships and molecular modeling analysis of flavonoids binding to the benzodiazepine site of the rat brain GABA(A) receptor complex. *J Med Chem* **42**, 4343–4350.
133. Fischer PM & Lane DP (2000) Inhibitors of cyclin-dependent kinases as anti-cancer therapeutics. *Curr Med Chem* **7**, 1213–1245.

134. Huang YT, Hwang JJ, Lee PP, Ke FC, Huang JH, Huang CJ, Kandaswami C, Middleton E Jr & Lee MT (1999) Effects of luteolin and quercetin, inhibitors of tyrosine kinase, on cell growth and metastasis-associated properties in A431 cells overexpressing epidermal growth factor receptor. *Br J Pharmacol* **128**, 999–1010.
135. So FV, Guthrie N, Chambers AF, Moussa M & Carroll KK (1996) Inhibition of human breast cancer cell proliferation and delay of mammary tumorigenesis by flavonoids and citrus juices. *Nutr Cancer* **26**, 167–181.
136. Green DR & Reed JC (1998) Mitochondria and apoptosis. *Science* **281**, 1309–1312.
137. Tatton WG & Olanow CW (1999) Apoptosis in neurodegenerative diseases: the role of mitochondria. *Biochim Biophys Acta* **1410**, 195–213.
138. Goyal L (2001) Cell death inhibition: keeping caspases in check. *Cell* **104**, 805–808.
139. Srinivasula SM, Hegde R, Saleh A *et al.* (2001) A conserved XIAP-interaction motif in caspase-9 and Smac/DIABLO regulates caspase activity and apoptosis. *Nature* **410**, 112–116.
140. Schroeter H, Bahia P, Spencer JPE, Sheppard O, Rattray M, Rice-Evans C & Williams RJ (2007) (-)-Epicatechin stimulates ERK-dependent cyclic AMP response element activity and upregulates GLUR2 in cortical neurons. *J Neurochem* **101**, 1596–1606.
141. Maher P, Akaishi T & Abe K (2006) Flavonoid fisetin promotes ERK-dependent long-term potentiation and enhances memory. *Proc Natl Acad Sci USA* **103**, 16568–16573.
142. Vauzour D, Vafeiadou K, Rice-Evans C, Williams RJ & Spencer JPE (2007) Activation of pro-survival Akt and ERK1/2 signaling pathways underlie the anti-apoptotic effects of flavanones in cortical neurons. *J Neurochem* **103**, 1355–1367.
143. Levites Y, Amit T, Youdim MB & Mandel S (2002) Involvement of protein kinase C activation and cell survival/cell cycle genes in green tea polyphenol (-)-epigallocatechin 3-gallate neuroprotective action. *J Biol Chem* **277**, 30574–30580.
144. Reznichenko L, Amit T, Youdim MB & Mandel S (2005) Green tea polyphenol (-)-epigallocatechin-3-gallate induces neurorescue of long-term serum-deprived PC12 cells and promotes neurite outgrowth. *J Neurochem* **93**, 1157–1167.
145. Pollard SE, Whiteman M & Spencer JPE (2006) Modulation of peroxynitrite-induced fibroblast injury by hesperetin: a role for intracellular scavenging and modulation of ERK signalling. *Biochem Biophys Res Commun* **347**, 916–923.
146. Walker EH, Pacold ME, Perisic O, Stephens L, Hawkins PT, Wymann MP & Williams RL (2000) Structural determinants of phosphoinositide 3-kinase inhibition by wortmannin, LY294002, quercetin, myricetin, and staurosporine. *Mol Cell* **6**, 909–919.
147. Jacobson KA, Moro S, Manthey JA, West PL & Ji XD (2002) Interactions of flavones and other phytochemicals with adenosine receptors. *Adv Exp Med Biol* **505**, 163–171.
148. Johnston GA (2005) GABA(A) receptor channel pharmacology. *Curr Pharm Des* **11**, 1867–1885.
149. Adachi N, Tomonaga S, Tachibana T, Denbow DM & Furuse M (2006) (-)-Epigallocatechin gallate attenuates acute stress responses through GABAergic system in the brain. *Eur J Pharmacol* **531**, 171–175.
150. Han YS, Bastianetto S, Dumont Y & Quirion R (2006) Specific plasma membrane binding sites for polyphenols, including resveratrol, in the rat brain. *J Pharmacol Exp Ther* **318**, 238–245.
151. Nifli AP, Bosson-Kouame A, Papadopoulou N, Kogia C, Kampa M, Castagnino C, Stournaras C, Vercauteren J & Castanas E (2005) Monomeric and oligomeric flavanols are agonists of membrane androgen receptors. *Exp Cell Res* **309**, 329–339.
152. Klinge CM, Blankenship KA, Risinger KE, Bhatnagar S, Noisin EL, Sumanasekera WK, Zhao L, Brey DM & Keynton RS (2005) Resveratrol and estradiol rapidly activate MAPK signaling through estrogen receptors alpha and beta in endothelial cells. *J Biol Chem* **280**, 7460–7468.
153. Conkright MD, Guzman E, Flechner L, Su AI, Hogenesch JB & Montminy M (2003) Genome-wide analysis of CREB target genes reveals a core promoter requirement for cAMP responsiveness. *Mol Cell* **11**, 1101–1108.
154. Tao X, Finkbeiner S, Arnold DB, Shaywitz AJ & Greenberg ME (1998) Ca²⁺ influx regulates BDNF transcription by a CREB family transcription factor-dependent mechanism. *Neuron* **20**, 709–726.
155. Shieh PB, Hu SC, Bobb K, Timmusk T & Ghosh A (1998) Identification of a signaling pathway involved in calcium regulation of BDNF expression. *Neuron* **20**, 727–740.
156. Bramham CR & Messaoudi E (2005) BDNF function in adult synaptic plasticity: the synaptic consolidation hypothesis. *Prog Neurobiol* **76**, 99–125.
157. Patterson SL, Grover LM, Schwartzkroin PA & Bothwell M (1992) Neurotrophin expression in rat hippocampal slices: a stimulus paradigm inducing LTP in CA1 evokes increases in BDNF & NT-3 mRNAs. *Neuron* **9**, 1081–1088.
158. Thomas K & Davies A (2005) Neurotrophins: a ticket to ride for BDNF. *Curr Biol* **15**, R262–R264.
159. Peng S, Wu J, Mufson EJ & Fahnstock M (2005) Precursor form of brain-derived neurotrophic factor and mature brain-derived neurotrophic factor are decreased in the pre-clinical stages of Alzheimer's disease. *J Neurochem* **93**, 1412–1421.
160. Michalski B & Fahnstock M (2003) Pro-brain-derived neurotrophic factor is decreased in parietal cortex in Alzheimer's disease. *Brain Res Mol Brain Res* **111**, 148–154.
161. Egan MF, Kojima M, Callicott JH *et al.* (2003) The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell* **112**, 257–269.
162. Linnarsson S, Bjorklund A & Ernfors P (1997) Learning deficit in BDNF mutant mice. *Eur J Neurosci* **9**, 2581–2587.
163. Mu JS, Li WP, Yao ZB & Zhou XF (1999) Deprivation of endogenous brain-derived neurotrophic factor results in impairment of spatial learning and memory in adult rats. *Brain Res* **835**, 259–265.
164. Minichiello L, Korte M, Wolfner D, Kuhn R, Unsicker K, Cestari V, Rossi-Arnaud C, Lipp HP, Bonhoeffer T & Klein R (1999) Essential role for TrkB receptors in hippocampus-mediated learning. *Neuron* **24**, 401–414.
165. Wullschlegel S, Loewith R & Hall MN (2006) TOR signaling in growth and metabolism. *Cell* **124**, 471–484.
166. Ferriola PC, Cody V & Middleton E Jr (1989) Protein kinase C inhibition by plant flavonoids. Kinetic mechanisms and structure-activity relationships. *Biochem Pharmacol* **38**, 1617–1624.
167. Schrat GM, Nigh EA, Chen WG, Hu L & Greenberg ME (2004) BDNF regulates the translation of a select group of mRNAs by a mammalian target of rapamycin-phosphatidylinositol 3-kinase-dependent pathway during neuronal development. *J Neurosci* **24**, 7366–7377.
168. Yin Y, Edelman GM & Vanderklish PW (2002) The brain-derived neurotrophic factor enhances synthesis of Arc in synaptoneurosomes. *Proc Natl Acad Sci USA* **99**, 2368–2373.

169. Waltereit R, Dammermann B, Wulff P, Scafidi J, Staubli U, Kauselmann G, Bundman M & Kuhl D (2001) Arg3.1/ Arc mRNA induction by Ca²⁺ and cAMP requires protein kinase A and mitogen-activated protein kinase/extracellular regulated kinase activation. *J Neurosci* **21**, 5484–5493.
170. Soule J, Messaoudi E & Bramham CR (2006) Brain-derived neurotrophic factor and control of synaptic consolidation in the adult brain. *Biochem Soc Trans* **34**, 600–604.
171. Lyford GL, Yamagata K, Kaufmann WE, Barnes CA, Sanders LK, Copeland NG, Gilbert DJ, Jenkins NA, Lanahan AA & Worley PF (1995) Arc, a growth factor and activity-regulated gene, encodes a novel cytoskeleton-associated protein that is enriched in neuronal dendrites. *Neuron* **14**, 433–445.
172. Reznichenko L, Amit T, Youdim MB & Mandel S (2005) Green tea polyphenol (-)-epigallocatechin-3-gallate induces neurorescue of long-term serum-deprived PC12 cells and promotes neurite outgrowth. *J Neurochem* **93**, 1157–1167.