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

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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.

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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

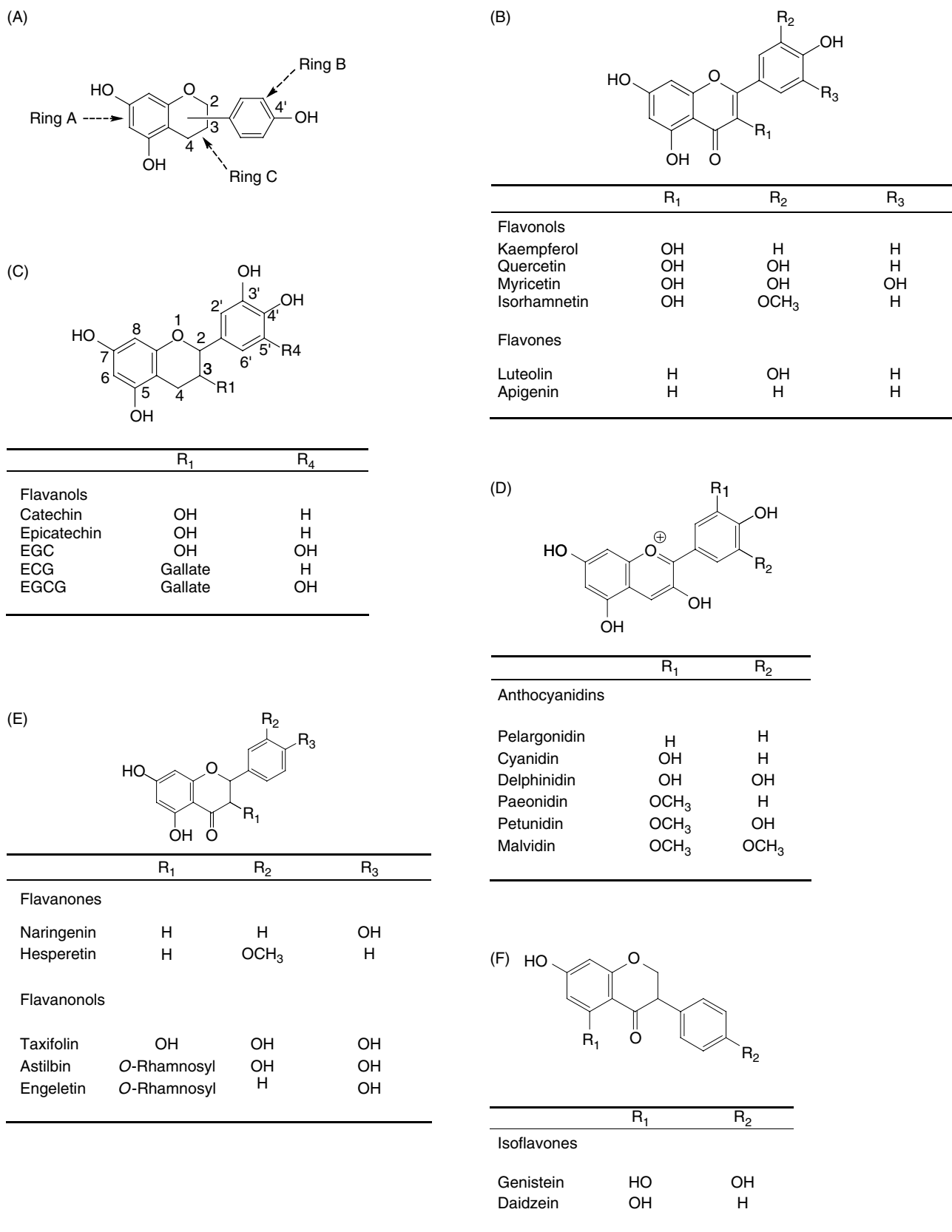


Fig. 1. The structures of the main classes of flavonoids. The major differences between the individual groups reside in the hydroxylation pattern of the ring-structure, the extent of saturation of the C-ring and the substitution of in the 3-position: (A) general structure of flavonoids, (B) structure 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

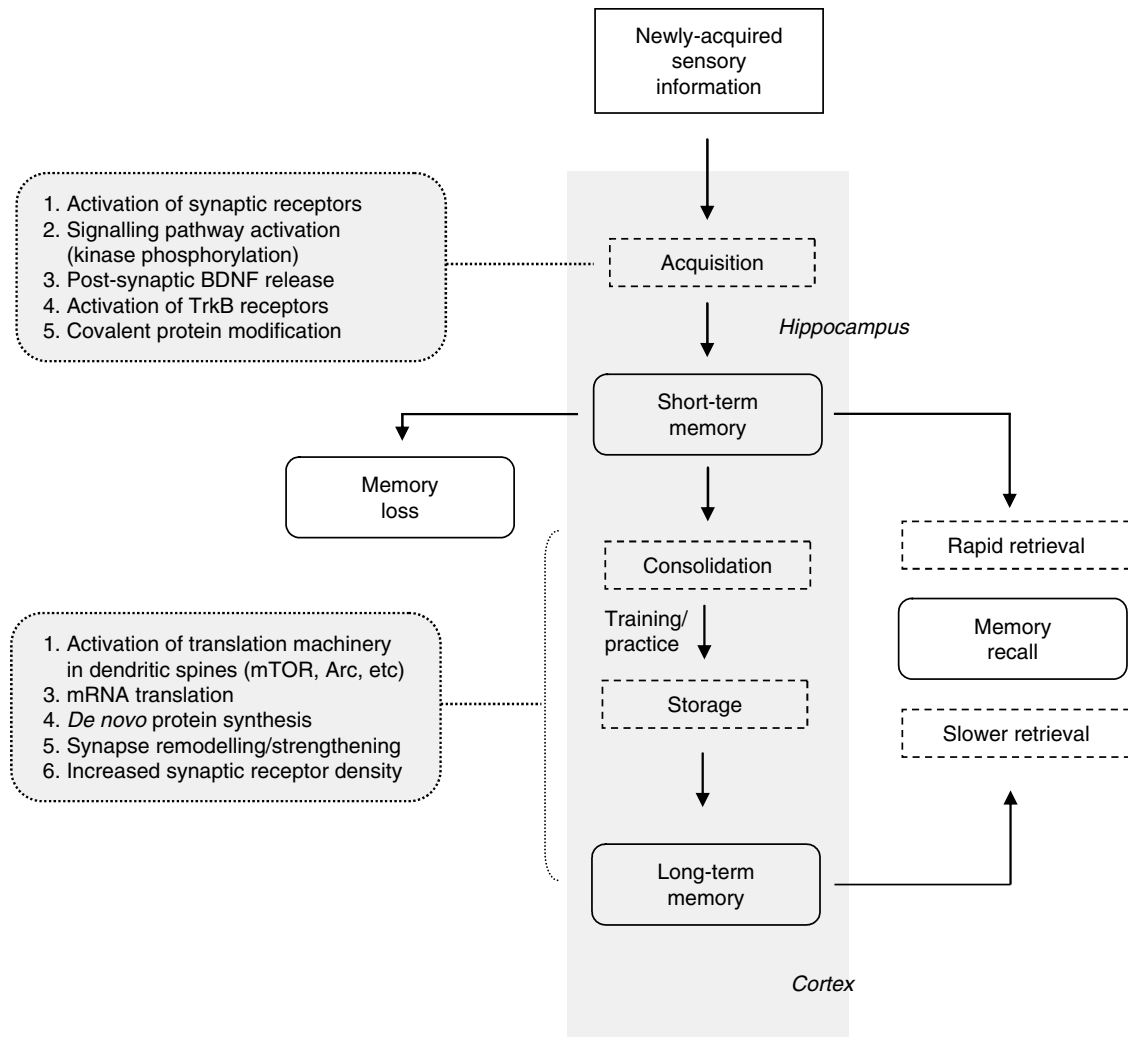


Fig. 2. The processes involved in memory acquisition, consolidation and storage in the brain. Acquisition and consolidation occur primarily in the hippocampus whilst storage is in the cortex or neocortex. BDNF, brain-derived neurotrophic factor; TrkB, tropomyosin receptor kinase B; mTOR, mammalian target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

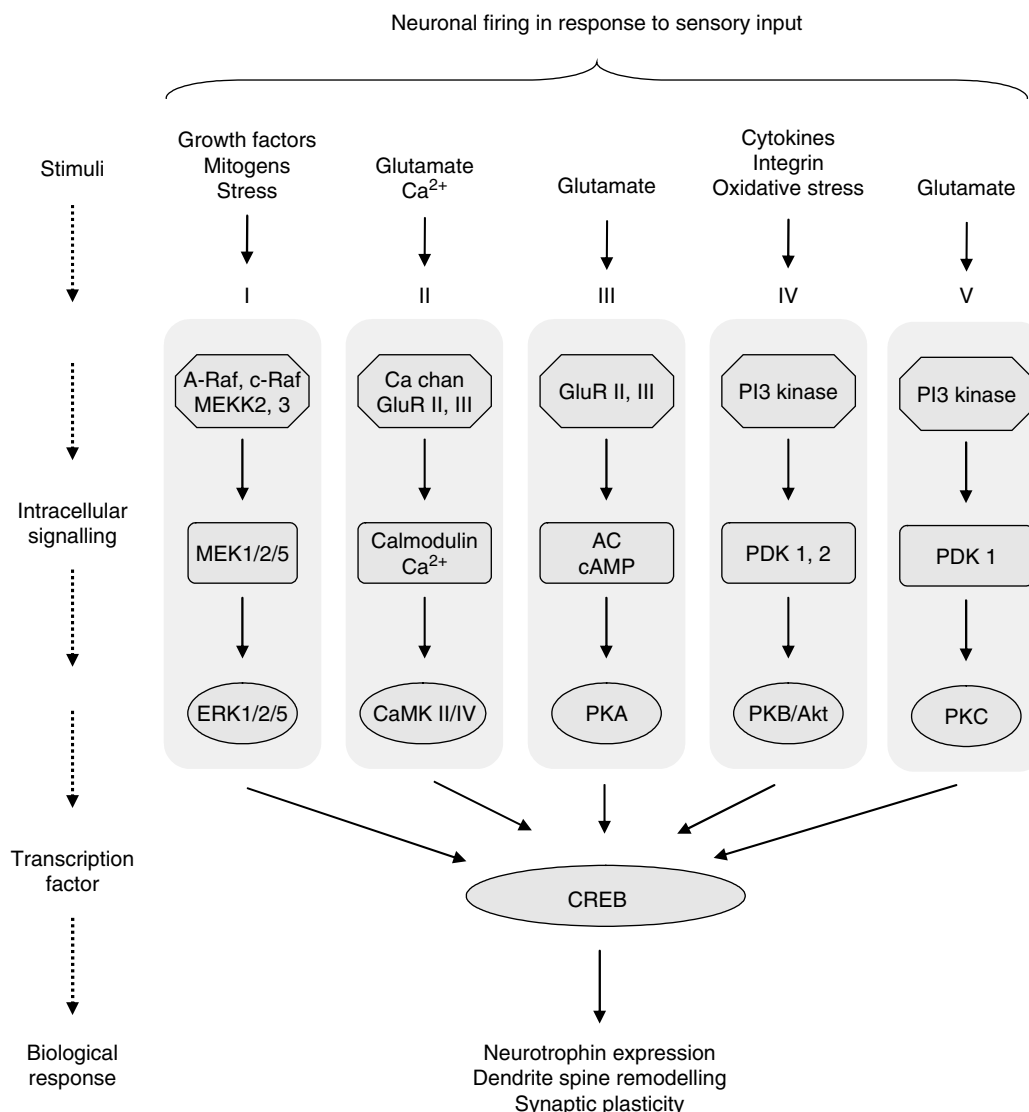


Fig. 3. Neuronal signalling pathways involved in long-term potentiation (LTP). Five distinct signalling pathways lead to the activation of the cAMP response element-binding protein (CREB), a transcription factor important in controlling LTP at neuronal synapses. Varying stimuli activate the mitogen-activated 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

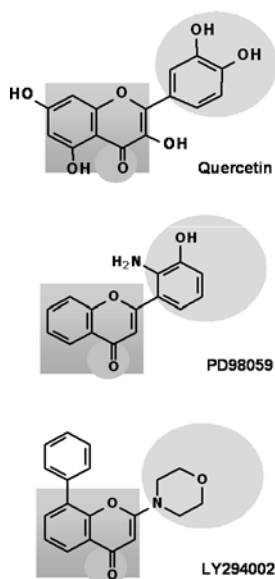


Fig. 4. The structure of mitogen-activated protein kinase kinase inhibitor PD98059 and the phosphoinositide 3-kinase inhibitor LY294002 have close structural homology to that of flavonoids. LY294002 and quercetin both fit into the ATP-binding pocket of the phosphoinositide 3-kinase, inhibiting its activity. 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

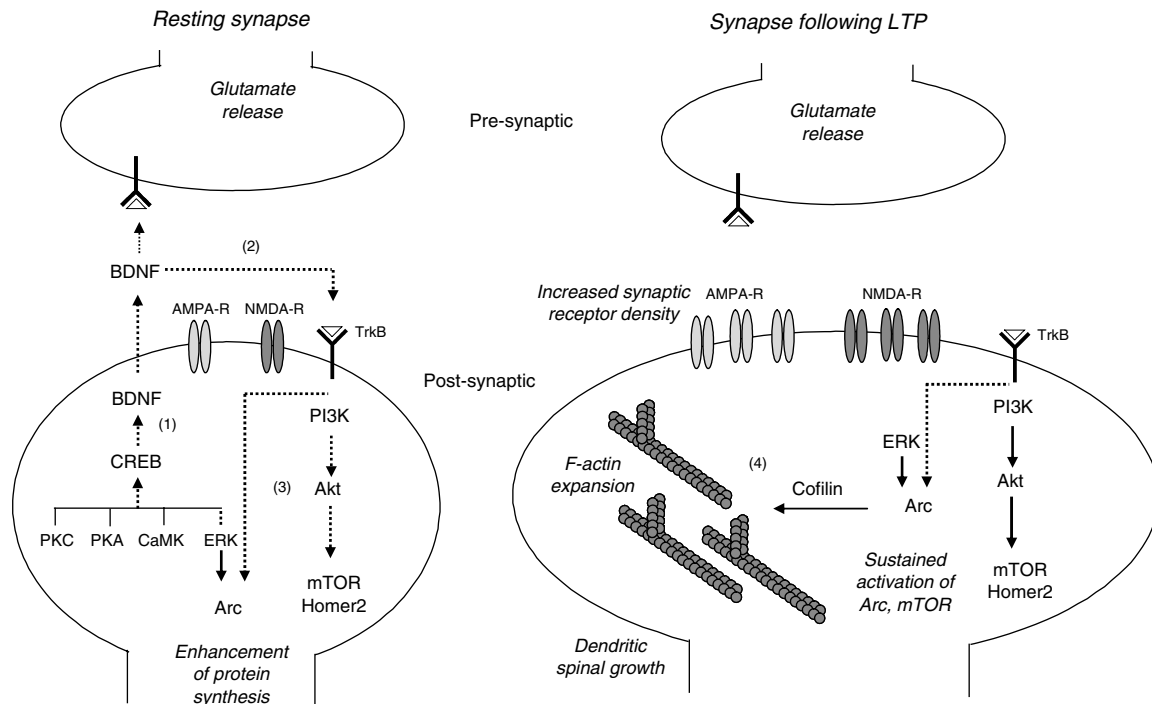


Fig. 5. Formation of stable long-term potentiation (LTP) at synapses. Increased expression and release of brain-derived neurotrophic factor (BDNF) from the synapse through enhanced cAMP-response element-binding protein (CREB) activation (1). BDNF binds to pre-and post-synaptic tropomyosin receptor kinase 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.

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