From Inflammation to Sickness and Cognitive Dysfunction: When the Immune System Subjugates the Brain

Rodney W. Johnson, PhD

Microglial cells, resident macrophages in the central nervous system (CNS), are relatively quiescent but can respond to signals from the peripheral immune system and induce neuroinflammation. In aging, microglia tend to transition to the M1 proinflammatory state and become hypersensitive to messages emerging from immune-to-brain signaling pathways. Thus, whereas in younger individuals in whom microglia respond to signals from the peripheral immune system and induce a well-controlled neuroinflammatory response that is adaptive (eg, when well controlled, fever and sickness behavior facilitate recovery from infection), in older individuals with an infection, microglia overreact and produce excessive levels of inflammatory cytokines, causing behavioral pathology including cognitive dysfunction. Importantly, recent studies indicate dietary flavonoids have anti-inflammatory properties and are capable of mitigating microglial cells in the brain of aged mice. Thus, dietary or supplemental flavonoids and other bioactives have the potential to restore the population of microglial cells in the old brain to a more quiescent state. The concept to constrain microglia through dietary intervention is significant because neuroinflammation and cognitive deficits are co-morbid factors in many chronic diseases. Controlling microglial cell reactivity has important consequences for preserving adult neurogenesis, neuronal structure and function, and cognition. This paper briefly describes the immune-to-brain signaling pathways, microglial cell activation, neuroinflammation, and, as one example, the potential of flavonoids to mitigate brain microglia and cognitive deficits induced by neuroinflammation.

Lines of Communication: How the Immune System Says "Hello" to the Brain

Neurological and cognitive effects associated with influenza infection have been reported throughout history (eg, following the 1918 "Spanish flu"), as well as during the recent novel influenza A H1N1 pandemic.[1-3] The simplest explanation for these neurocognitive effects is that influenza virus makes its way to the brain,

where it is detected by neurons. However, most influenza strains, including those responsible for pandemics, are considered non-neurotropic,[4-6] suggesting that neurological symptoms associated with influenza infection are not a result of direct viral invasion into the CNS. Moreover, neurons do not have receptors to detect viruses (or other pathogens) directly. Cells of the immune system do, however, as the immune system's primary responsibility is to recognize infectious pathogens and contend with them. For example, sentinel immune cells such as monocytes and macrophages are equipped with toll-like receptors (TLR) that recognize unique molecules associated with groups of pathogens (ie, pathogen-associated molecular patterns). Stimulation of TLRs that recognize viruses (TLR3 and TLR7) and bacteria (TLR4) on immune sentinel cells can have profound neurological and cognitive effects (Fig 1), suggesting the immune system conveys a message to the brain after detecting an infectious agent. This message is cytokine based.

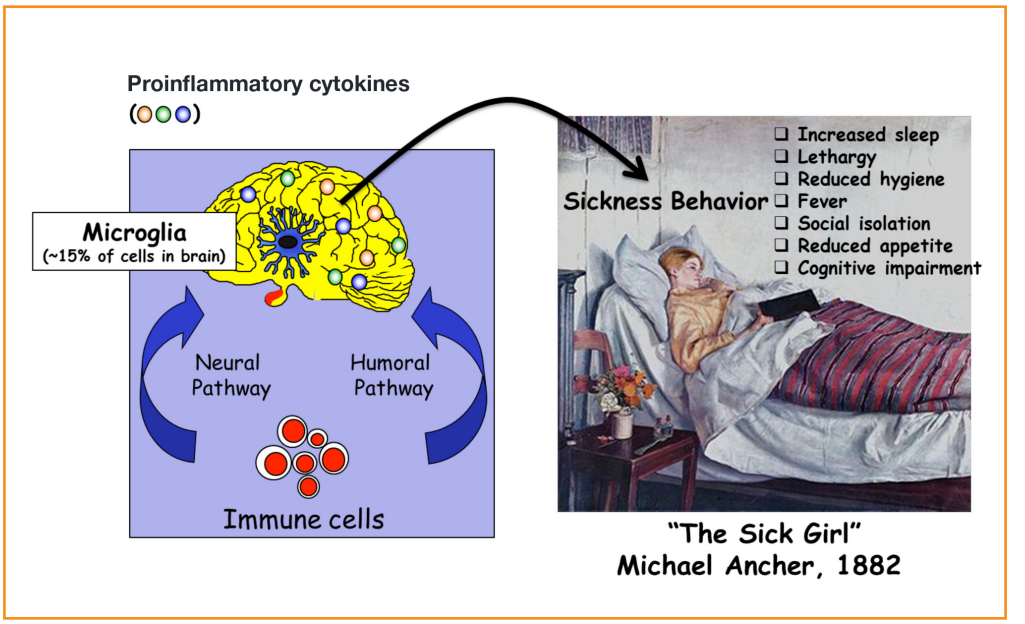


Fig 1. Immune-to-brain communication pathways stimulate brain microglia, which can induce neuroinflammation and neurocognitive deficits.

Macrophages and monocytes produce inflammatory cytokines (eg, interleukin [IL]-1β, IL-6, and tumor necrosis factor-α [TNF-α]) that facilitate communication between the periphery and brain. Early studies showing that infection-related neurocognitive changes could be induced in the absence of an infectious agent by injecting small quantities of recombinant IL-1β directly into a lateral cerebral ventricle indicated that inflammatory cytokines are essential to infection-related

behavior and that they somehow transcend the blood-brain barrier. Several cytokine-dependent pathways that enable the peripheral immune system to transcend the blood-brain barrier have been dissected.

First, there is good evidence that inflammatory cytokines present in blood can be actively transported into the brain.[7-11] Cytokines produced in the periphery need not enter the brain to elicit neurocognitive changes. This is because inflammatory stimuli in the periphery can induce microglial cells to produce a similar repertoire of inflammatory cytokines. Thus, brain microglia recapitulate the message from the peripheral immune system.[12,13] Hence, in a second pathway, inflammatory cytokines in the periphery can bind receptors on blood-brain barrier endothelial cells[14] and induce perivascular microglia or macrophages to express cytokines that are released into the brain parenchyma.[15,16] Furthermore, in a third pathway, cytokines in the periphery convey a message to the brain via the vagus nerve. After immune challenge, dendritic cells and macrophages that are closely associated with the abdominal vagus have been shown to express IL-1β protein[17]; IL-1 binding sites have been identified in several regions of the vagus as well.[18] When activated by cytokines, the vagus can activate specific neural pathways that are involved in neurocognitive behavior. However, activation of the vagus also stimulates microglia in the brain to produce cytokines via the central adrenergic system.[19-21] Finally, a fourth pathway provides a slower immune-to-brain signaling mechanism based on volume transmission.[22] In this method of immune-to-brain communication, production of IL-1β by the brain first occurs in the choroid plexus and circumventricular organs—brain areas devoid of an intact blood-brain barrier. The cytokines then slowly diffuse throughout the brain by volume transmission, along the way activating microglia, neurons, and neural pathways that induce sickness behavior and inhibit cognition.

What Are Microglia and What Do They Do?

A critical point is that the aforementioned communication pathways seem to have in common a need to activate microglial cells and induce neuroinflammation. An early definition of inflammation was based on four cardinal signs: dolor (pain), calor (heat), rubor (redness), and tumor (swelling); functio laesa (loss of function) was added later. While neuroinflammation can resemble its peripheral counterpart in circumstances such as viral and bacterial meningitis, head trauma, or autoimmune diseases of the CNS, the term neuroinflammation is increasingly used to identify a fundamentally different event that is exclusively driven by microglial cells and shows few if any of the cardinal signs described in the early definition.

From Inflammation to Sickness and Cognitive Dysfunction: When the Immune System Subjugates the Brain

Microglia account for 12%-15% of the cells in the brain. They originate from macrophages produced by primitive hematopoiesis in the yolk sac. The primitive macrophages migrate to the neural tube, where they give rise to microglia. Bone marrow-derived monocytes do not contribute to the mature microglia pool, suggesting microglia numbers are sustained by local progenitors. Under healthy conditions "resting" (quiescent) microglia are highly dynamic, randomly extending and contracting arms with filopodia-like protrusions to survey the microenvironment.[23] In response to insult, however, microglia become activated and proinflammatory (M1) (Fig 2). In this state, they can direct the movement of the protrusions toward the insult,[23] take on a de-ramified morphology that enables motility,[24] and/or express major histocompatibility complex class II (MHC class II) and other markers indicative of inflammation.[25]

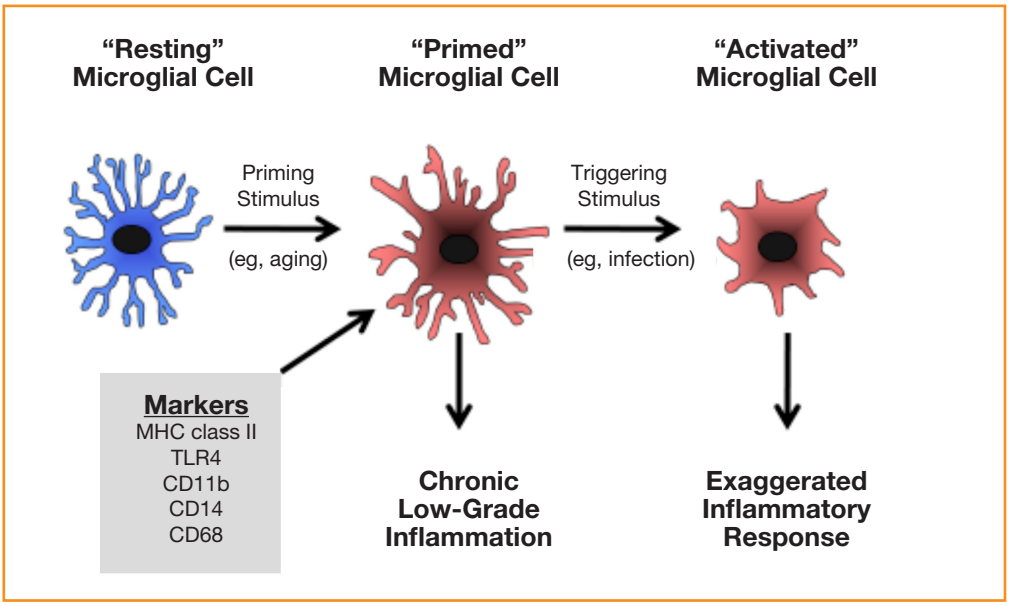


Fig 2. Priming of microglia (eg, with aging) and inflammatory response.

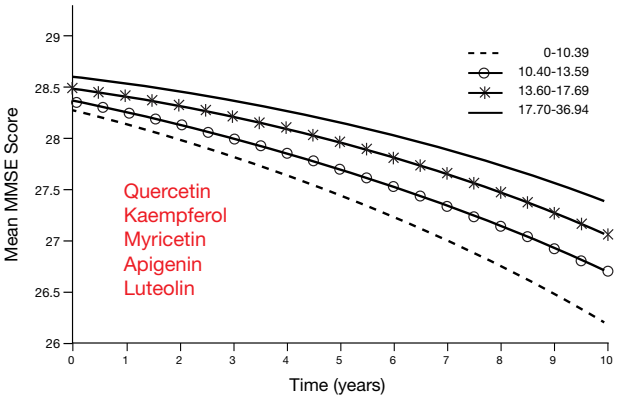
MHC class II=major histocompatibility complex class II, TLR=toll-like receptors, CD=cluster of differentiation

Notably, M1 microglia constitutively produce inflammatory cytokines and are hypersensitive to insults, including signals from the peripheral immune system.[26,27] Hence, microglial cell activation is frequently viewed to be tantamount to neuroinflammation. As neuroinflammation is deleterious to neurological function, mitigating microglial cell activity is vital for optimal brain health.

MHC class II frequently is used to identify activated microglia. During aging, the percentage of brain microglia that express MHC class II increases and signs of neuroinflammation emerge. For example, <3% of microglia isolated from the brain of healthy young adult mice stained positive for MHC class II.[28] This pales in comparison to the >25% of microglia from brains of old but otherwise healthy mice that were MHC class II-positive.[28] Most of the MHC class II-positive microglia from old mice were also IL-1β-positive.[28] This is consistent with a prior study in which the proportion of IL-6-positive microglia was markedly higher if the donor mouse was 22-24 months old compared to 6 months or 1 week old.[27] It is important to note that aging per se does not increase the number of microglial cells in the brain but rather it increases the proportion of resident microglia that are inflammatory and reactive to insults.[29] A recent study suggests that microglia from aged mice retain a prominent M1 profile and are less sensitive to the anti-inflammatory and M2-promoting effects of IL-4.[30] Reducing the proportion of microglia that are activated is a priority for reducing age-related neuroinflammation that may contribute to cognitive aging and be a predisposing factor for neurodegenerative disease.

Can Flavonoids Reduce Neuroinflammation and Inhibit Cognitive Aging?

Flavonoids are naturally occurring polyphenolic compounds present in plants. The major sources of flavonoids in the human diet include fruits, vegetables, tea, wine, and cocoa.[31] Significant evidence has emerged to indicate that consuming a diet rich in flavonoids may inhibit or reverse cognitive aging. For example, in a prospective study of individuals aged 65 years or older, dietary flavonoid intake (ie, mg/d of five flavonoids—apigenin, kaempferol, luteolin, myricetin, and quercetin) was associated with improved cognitive function over a 10-year period (Fig 3).[32]



- Subjects separated into quartiles based on flavonoid intake
- Higher flavonoid intake was associated with improved performance on the Mini-Mental State Examination
- Dietary flavonoid intake may protect against cognitive aging

Fig 3. Flavonoid intake (range 0-36.94 mg/d) and cognitive decline in people 65 years of age and older as shown by scores from the Mini-Mental State Examination (MMSE).[32]

Source: Letenneur L et al. *Am J Epidemiol.* 2007 Jun 15;165(12):1364-1371. Reprinted by permission of Oxford University Press.

Furthermore, analyses of data from the Chicago Health and Aging Project—a cohort study of 3790 older residents residing on the south side of Chicago—suggested that adherence to a Mediterranean dietary pattern reduced the rate of cognitive decline.[33] Numerous other studies have yielded consistent results with older rats or mice, which show improved cognitive function when fed a flavonoid-rich diet.[34-36]

Flavonoids may improve cognition in the aged through a number of physiological mechanisms, including scavenging of reactive oxygen and nitrogen species[37] and interactions with intracellular signaling pathways.[38] Through these physiological mechanisms, flavonoids also impart an anti-inflammatory effect that may improve cognition. This seems likely for the flavone luteolin, which is most prominent in parsley, celery, and green peppers. Whereas luteolin inhibits several transcription factors that mediate inflammatory genes (eg, nuclear factor kappa B [NF-κB][39] and activator protein 1 [AP-1][40]), it is a potent activator of nuclear factor erythroid 2-related factor 2 (Nrf2), which induces the expression of genes encoding antioxidant enzymes.[41] A recent study of old healthy mice found improved learning and memory and reduced expression of inflammatory genes in the hippocampus when luteolin was included in the diet.[34] Thus, dietary luteolin may improve cognitive function in the aged by reducing brain microglial cell activity. Indirect support for a microglia-dependent mechanism comes from a recent in vitro study in which luteolin stimulated the formation of filopodia and caused ramification of BV-2 cells (a microglia cell line) even when they were activated with *Escherichia*

coli lipopolysaccharide (LPS).[42] Furthermore, supernatants from LPS-stimulated BV-2 cells caused discernible cell death in Neuro.2a cells even if Neuro.2a cells were incubated with luteolin; however, treating BV-2 cells with luteolin prior to LPS reduced neuronal cell death caused by conditioned supernatants.[34] Hence, the flavonoid luteolin is a naturally occurring immune modulator that may be effective in reducing inflammatory microglia in the senescent brain.

Conclusion

In light of the recent evidence suggesting microglial cells become dysregulated due to aging and cause neuroinflammation, which can disrupt neural structure and function, it is an interesting prospect to think dietary flavonoids and other bioactives can be used to constrain microglia. But how can flavonoids impart this anti-inflammatory effect? Although in vitro studies clearly indicate that several flavonoids can act directly on microglial cells to restrict the inflammatory response, results from in vivo studies thus far do not prove that dietary flavonoids access the brain to interact with microglia in a meaningful way. This is a complicated question to dissect because flavonoids reduce inflammation in the periphery and microglia seem to act like an "immunostat," detecting and responding to signals emerging from immune-to-brain signaling pathways. Thus, whether dietary flavonoids enter the brain and impart an anti-inflammatory effect on microglia is an interesting question but one that is more theoretical than practical because what is most important is how the immunostat is adjusted, whether that is via a direct or indirect route. However, because flavonoids are detectable in the brain,[43-45] they most likely affect microglia both directly and by dampening immune-to-brain signaling.

References

1. González-Duarte A, Magaña Zamora L, Cantú Brito C, García-Ramos G. Hypothalamic abnormalities and Parkinsonism associated with H1N1 influenza infection. *J Neuroinflammation*. 2010 Aug 17;7:47.
2. Ravenholt RT, Foege WH. 1918 influenza, encephalitis lethargica, parkinsonism. *Lancet.* 1982 Oct 16;2(8303):860-864.
3. Studahl M. Influenza virus and CNS manifestations. *J Clin Virol*. 2003 Dec;28(3):225-232.
4. Kobasa D, Jones SM, Shinya K, et al. Aberrant innate immune response in lethal infection of macaques with the 1918 influenza virus. *Nature.* 2007 Jan 18;445(7125):319-323.
5. Schlesinger RW, Husak PJ, Bradshaw GL, Panayotov PP. Mechanisms involved in natural and experimental neuropathogenicity of influenza viruses: evidence and speculation. *Adv Virus Res.* 1998;50:289-379.
6. Wang GF, Li W, Li K. Acute encephalopathy and encephalitis caused by influenza virus infection. *Curr Opin Neurol.* 2010 Jun;23(3):305-311.
7. Gutierrez EG, Banks WA, Kastin AJ. Murine tumor necrosis factor alpha is transported from blood to brain in the mouse. *J Neuroimmunol.* 1993 Sep;47(2):169-176.
8. Banks WA, Kastin AJ. Blood to brain transport of interleukin links the immune and central nervous systems. *Life Sci.* 1991;48(25):PL117-121.
9. Banks WA, Kastin AJ, Broadwell RD. Passage of cytokines across the blood-brain barrier. *Neuroimmunomodulation.* 1995 Jul-Aug;2(4):241-248.
10. Banks WA, Kastin AJ, Ehrensing CA. Blood-borne interleukin-1 alpha is transported across the endothelial blood-spinal cord barrier of mice. *J Physiol*. 1994 Sep 1;479(Pt 2):257-264.
11. Banks WA, Kastin AJ, Gutierrez EG. Penetration of interleukin-6 across the murine blood-brain barrier. *Neurosci Lett.* 1994 Sep 26;179(1-2):53-56.
12. Ban E, Haour F, Lenstra R. Brain interleukin 1 gene expression induced by peripheral lipopolysaccharide administration. *Cytokine*. 1992 Jan;4(1):48-54.
13. Layé S, Parnet P, Goujon E, Dantzer R. Peripheral administration of lipopolysaccharide induces the expression of cytokine transcripts in the brain and pituitary of mice. *Brain Res Mol Brain Res*. 1994 Nov;27(1):157-162.
14. Ching S, Zhang H, Belevych N, et al. Endothelial-specific knockdown of interleukin-1 (IL-1) type 1 receptor differentially alters CNS responses to IL-1 depending on its route of administration. *J Neurosci.* 2007 Sep 26;27(39): 10476-10486.
15. Van Dam AM, Brouns M, Louisse S, Berkenbosch F. Appearance of interleukin-1 in macrophages and in ramified microglia in the brain of

endotoxin-treated rats: a pathway for the induction of non-specific symptoms of sickness? *Brain Res*. 1992 Aug 21;588(2):291-296.

16. Van Dam AM, Bauer J, Tilders FJ, Berkenbosch F. Endotoxin-induced appearance of immunoreactive interleukin-1 beta in ramified microglia in rat brain: a light and electron microscopic study. *Neuroscience*. 1995 Apr;65(3):815-826.
17. Goehler LE, Gaykema RP, Nguyen KT, et al. Interleukin-1β in immune cells of the abdominal vagus nerve: a link between the immune and nervous systems? *J Neurosci.* 1999 Apr 1;19(7):2799-2806.
18. Goehler LE, Relton JK, Dripps D, et al. Vagal paraganglia bind biotinylated interleukin-1 receptor antagonist: a possible mechanism for immune-to-brain communication. *Brain Res Bull*. 1997;43(3):357-364.
19. Bluthé RM, Michaud B, Kelley KW, Dantzer R. Vagotomy blocks behavioural effects of interleukin-1 injected via the intraperitoneal route but not via other systemic routes. *Neuroreport*. 1996 Nov 4;7(15-17):2823-2827.
20. Bluthé RM, Michaud B, Kelley KW, Dantzer R. Vagotomy attenuates behavioural effects of interleukin-1 injected peripherally but not centrally. *Neuroreport*. 1996 Jun 17;7(9):1485-1488.
21. Layé S, Bluthe RM, Kent S, et al. Subdiaphragmatic vagotomy blocks induction of IL-1 beta mRNA in mice brain in response to peripheral LPS. *Am J Physiol*. 1995 May;268(5 Pt 2):R1327-1331.
22. Konsman JP, Kelley K, Dantzer R. Temporal and spatial relationships between lipopolysaccharide-induced expression of Fos, interleukin-1beta and inducible nitric oxide synthase in rat brain. *Neuroscience*. 1999 May;89(2):535-548.
23. Nimmerjahn A, Kirchhoff F, Helmchen F. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. *Science*. 2005 May 27;308(5726):1314-1318.
24. Perry VH, Nicoll JA, Holmes C. Microglia in neurodegenerative disease. *Neurology*. 2010 Apr;6(4):193-201.
25. Varnum MM, Ikezu T. The classification of microglial activation phenotypes on neurodegeneration and regeneration in Alzheimer's disease brain. *Arch Immunol Ther Exp (Warsz).* 2012 Aug;60(4):251-266.
26. Frank MG, Barrientos RM, Watkins LR, Maier SF. Aging sensitizes rapidly isolated hippocampal microglia to LPS ex vivo. *J Neuroimmunol*. 2010 Sep 14;226(1-2):181-184.
27. Ye SM, Johnson RW. Increased interleukin-6 expression by microglia from brain of aged mice. *J Neuroimmunol.* 1999 Jan 1;93(1-2):139-148.
28. Henry CJ, Huang Y, Wynne AM, Godbout JP. Peripheral lipopolysaccharide (LPS) challenge promotes microglial hyperactivity in aged mice that is associated with exaggerated induction of both pro-inflammatory IL-1beta and anti-inflammatory IL-10 cytokines. *Brain Behav Immun*. 2009 Mar;23(3):309-317.

29. Norden DM, Godbout JP. Microglia of the aged brain: primed to be activated and resistant to regulation. *Neuropathol Appl Neurobiol*. Oct 5 2012.

30. Fenn AM, Henry CJ, Huang Y, Dugan A, Godbout JP. Lipopolysaccharide-induced interleukin (IL)-4 receptor-alpha expression and corresponding sensitivity to the M2 promoting effects of IL-4 are impaired in microglia of aged mice. *Brain Behav Immun*. 2012 Jul;26(5):766-777.

31. Harnly JM, Doherty RF, Beecher GR, et al. Flavonoid content of U.S. fruits, vegetables, and nuts. *J Agric Food Chem.* 2006 Dec 27;54(26):9966-9977.

32. Letenneur L, Proust-Lima C, Le Gouge A, Dartigues JF, Barberger-Gateau P. Flavonoid intake and cognitive decline over a 10-year period. *Am J Epidemiol*. 2007 Jun 15;165(12):1364-1371.

33. Tangney CC, Kwasny MJ, Li H, Wilson RS, Evans DA, Morris MC. Adherence to a Mediterranean-type dietary pattern and cognitive decline in a community population. *Am J Clin Nutr*. 2011 Mar;93(3):601-607.

34. Jang S, Dilger RN, Johnson RW. Luteolin inhibits microglia and alters hippocampal-dependent spatial working memory in aged mice. *J Nutr.* 2010 Oct;140(10):1892-1898.

35. Joseph JA, Shukitt-Hale B, Denisova NA, et al. Reversals of age-related declines in neuronal signal transduction, cognitive, and motor behavioral deficits with blueberry, spinach, or strawberry dietary supplementation. *J Neurosci.* 1999 Sep 15;19(18):8114-8121.

36. Williams CM, El Mohsen MA, Vauzour D, et al. Blueberry-induced changes in spatial working memory correlate with changes in hippocampal CREB phosphorylation and brain-derived neurotrophic factor (BDNF) levels. *Free Radic Biol Med.* 2008 Aug 1;45(3):295-305.

37. Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic Biol Med.* 1996;20(7):933-956.

38. Spencer JP, Vafeiadou K, Williams RJ, Vauzour D. Neuroinflammation: modulation by flavonoids and mechanisms of action. *Mol Aspects Med*. 2012 Feb;33(1):83-97.

39. Xagorari A, Papapetropoulos A, Mauromatis A, Economou M, Fotsis T, Roussos C. Luteolin inhibits an endotoxin-stimulated phosphorylation cascade and proinflammatory cytokine production in macrophages. *J Pharmacol Exp Ther.* 2001 Jan;296(1):181-187.

40. Jang S, Kelley KW, Johnson RW. Luteolin reduces IL-6 production in microglia by inhibiting JNK phosphorylation and activation of AP-1. *Proc Natl Acad Sci U S A*. 2008 May 27;105(21):7534-7539.

41. Lim JH, Park HS, Choi JK, Lee IS, Choi HJ. Isoorientin induces Nrf2 pathway-driven antioxidant response through phosphatidylinositol 3-kinase signaling. *Arch Pharm Res*. 2007 Dec;30(12):1590-1598.

42. Dirscherl K, Karlstetter M, Ebert S, et al. Luteolin triggers global changes in the microglial transcriptome leading to a unique anti-inflammatory and neuroprotective phenotype. *J Neuroinflammation*. 2010 Jan 14;7:3.

43. Youdim KA, Qaiser MZ, Begley DJ, Rice-Evans CA, Abbott NJ. Flavonoid permeability across an in situ model of the blood-brain barrier. *Free Radic Biol Med*. 2004 Mar 1;36(5):592-604.

44. de Boer VC, Dihal AA, van der Woude H, et al. Tissue distribution of quercetin in rats and pigs. *J Nutr.* 2005 Jul;135(7):1718-1725.

45. Paulke A, Schubert-Zsilavecz M, Wurglics M. Determination of St. John's wort flavonoid-metabolites in rat brain through high performance liquid chromatography coupled with fluorescence detection. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2006 Feb 17;832(1):109-113.