BRAIN AND COGNITIVE PROCESSES BENEFICIAL ROLES OF CITRUS

Executive Summary
Paul F. Cancalon

It is important to view the activity of citrus phytochemicals and vitamins in the light of recent scientific developments. Until the last few years, the accepted view of the mode of action of phytochemicals and vitamins was relatively simple. It was believed each compound had a single effect: Polyphenols were antioxidants, ascorbic acid was a vitamin acting mostly as a coenzyme to prevent scurvy. **In the last few years, this concept has been challenged and now many compounds are known to have multiple modes of action.** Many molecules have the ability to chemically scavenge free radicals and thus act as antioxidants in the test tube, but their main biological functions are by acting as hormones, moderators of enzyme activities and as structural components. In fact many "antioxidant" phytochemicals are present in the body in quantities much too small to have a significant 'direct' effect.

Beneficial activity of citrus of phytochemicals and vitamins

There are very few reports dealing with the influence of the whole fruit or juice on nervous system related problems. However, the beneficial effects of several citrus compounds on the nervous system have been extensively examined. Among those vitamins C, folic acid and flavonoids are the most active.

Anti-inflammation activity.

Citrus compounds act positively on inflammation-related ailments. This applies to the brain as well as to other organs. Most of the citrus health benefits that may improve the cardiovascular system will ultimately benefit the brain itself.

Access to the brain

To be effective, a chemical must have the properties that allow it to penetrate through 'protective barriers' in our body, e.g., the 'blood brain barrier.' The ability of certain citrus vitamins and phytochemicals to reach the brain can be seen as the most essential quality for being able to have a brain protection capacity. By contrast, vitamin E is a powerful antioxidant but cannot enter the brain and seems to have very limited effect on the brain cells.

Ascorbic acid (vitamin C)

Ascorbic acid plays a complex and multiple role in the brain biochemistry.

- Ascorbic acid acts as a vitamin (vitamin C).
- In the nervous system vitamin C is involved in the synthesis of 'neurotransmitters' – the chemicals that 'transmit the signal' from one nerve cell to the next.
- It is involved in the synthesis and modulation of these neurotransmitters.

- It protects the highly fatty or 'lipidic' brain tissues from oxidation.
- It plays a m ajor role in the in the interaction between neurons (nerve cells) and their supporting cells, the glial cells.
- It acts as an antioxidant neuroprotector and a neurotransmission modulator.

Polyphenols (Flavonoids) –

Polyphenols appear to have multiple modes of action as brain protecting compounds:

- They behave as direct or indirect antioxidants on the brain tissue.
- They are involved in cell signaling processes through interactions with genes ('modulation. ')
- Polyphenols also may act as direct anti-inflammation compounds.

Folic acid

The case of folate is more complex. Its role as protecting and promoting the synthesis of DNA appears well established and may contribute to protect brain cells.

Folic acid is necessary for the 'one-carbon cycle' to function and to allow many biochemical reactions to proceed. However the individual roles of each component of the cycle are still unclear. The specific roles of folate and vitamin B12 are still under investigation.

Most brain degenerative diseases are associated with low concentrations of folate and high levels of the by-product of the one-carbon cycle -- 'homocysteine'. However, these interactions have not been thoroughly evaluated and most evidence supporting these interactions is indirect --association or correlation.

Folate appears to have an important role in maintaining the proper functioning of the brain. Nevertheless, in some cases the exact influence of this vitamin, particularly in relation to observations associated with the measurement of homocysteine needs to be further clarified.

Long term beneficial effects of citrus compounds.

Many brain related diseases, including forms of decline in our cognitive processes, are slow developing problems that start early in life. For example, brain aging due to death of nerve cells appears to start in our thirties. Similarly, dementia begins as a mild cognitive impairment, long before becoming an incapacitating disease. Therefore the maximal benefits attributable to citrus compounds will likely be obtained if they are allowed to act during the entire life span. Under these conditions, direct or indirect antioxidant properties of citrus compounds, as well as their ability to modulate some neurotransmitter activities, may limit or prevent the development of age- or disease-related brain damage that occurs over decades, if recognized before the damaging effects reach an irreversible stage.

A point can be made than one should not wait until he is too old to take advantage of the protective properties of citrus. Older brains have been shown to have lower concentration of antioxidants and a more limited ability to neutralize oxidative species (the 'scavenging' of free radicals). Studies have shown that in older individuals the antioxidant uptake mechanisms become inefficient, particularly for ascorbic acid; the effectiveness of the mechanisms responsible for using these antioxidants to neutralize oxidative species declines. It has not yet been clearly demonstrated that increasing dietary intakes of antioxidants can reverse these declines.

Citrus compounds and established brain diseases

Studies have also shown that these citrus compounds' beneficial effects can also be recognized with most brain ailments including Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, multiple sclerosis, and aging. However, these diseases have genetic, autoimmune, and environmental influences among their origins, and develop through different mechanisms unrelated to those associated with the metabolism of citrus compounds. It is therefore unlikely that, in most cases, citrus phytochemicals and vitamins could reverse established diseases. However, citrus compounds, by reinforcing natural mechanisms and limiting cellular oxidation events, may have an influence in slowing the progression of these diseases.

It can be concluded that citrus compounds can access the brain and have several beneficial effects. However, since brain deterioration is usually a slow process starting early in life, a maximal protection would be obtained if citrus phytochemicals are allowed to interact with the brain during the entire life span.

Specific Considerations for the effects of citrus components and brain/cognitive functions

- Several citrus compounds: ascorbic acid, polyphenols (flavonoids), and folic acid appear to have a long term beneficial effect on the brain. These compounds have two main functions : 1) they act as antioxidants; and 2)act as regulators of molecules involved in the functioning of the brain (modulators of neurotransmitters).
- The prerequisite property of a compound to be active is to be able to reach its target. Compounds present in citrus (ascorbic acid, flavonoids and folic acid) have the ability to cross the barriers isolating the brain and reach the various nerve cells.
- The brain nerves cells are very vulnerable. They contain up to 80% fat and produce large quantities of oxidant molecules that can damage the lipids and the cells. Once injured, brain nerve cells (neurons) cannot regenerate and will die. Even in the absence of diseases, some brain degeneration starts occurring after 30 and keeps progressing during the rest of our lives, inducing brain aging and age-related 'senescent' loss of cognitive functions. Oxidative damage and the resulting inflammation are among the main causes of these problems. Citrus antioxidants may help limit cellular deterioration and protect brain activity, particularly against aging.
- Similarly, the major brain diseases, Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and multiple sclerosis appear also to start long before the appearance of clinical symptoms. Although each has specific causes, such as genetic or environmental influences, oxidation seems to play an active role in the progression of most of these diseases. Citrus antioxidants may possibly play a role in slowing down the progression of the major brain ailments.
- Citrus provide beneficial compounds that if consumed regularly may contribute to brain wellness. However the ability of the body to absorb and use these compounds decreases with age. It appears essential to start consuming citrus early in life to help maintain proper brain functioning particularly later in life.

- High levels of homocysteine have been shown to be associated with many brain ailments and loss of cognitive ability. Citrus contain significant amounts of folate which plays a role in limiting homocysteine accumulation. H owever, deficits and malfunctioning of several vitamins and enzymes, particularly vitamin B12 also contribute to the homocysteine problem and the exact role of folate remains to be clarified. The relationship between folate and homocysteine and the role of homocysteine in various diseases are areas that deserve further study.

Until recently the beneficial effects of phytochemicals and particularly citrus compounds was believed to be limited to a biochemical antioxidant effect. Newer studies indicate that the main effect of these compounds may be to regulate different physiological functions by interacting with enzymes. These results are particularly important for citrus. For example, hesperidin, the main orange flavonoid, has been unfavorably reported as a relatively poor chemical antioxidant and, consequently, it was perceived as a factor limiting the health benefits value of citrus. However, it has now been shown that hesperidin has multiple physiological targets. It may:

- Be absorbed more readily (be 'bioavailable') than other 'high antioxidant potential' compounds
- Enhance anti-oxidative enzymes and have a strong non-chemical antioxidant potential.
- Promote blood vessel health.
- Prevent post-menopausal and senescent osteoporosis.
- Promote brain health.

Studies of the physiological activities of phytochemicals have progressed beyond the traditional chemical antioxidant research and revealed several important health properties of citrus compounds. Citrus phytochemicals are of particular interest in the studies of brain and cognitive research.

A more detailed and referenced review of this topic follows:

BRAIN AND COGNITIVE PROCESSES

BENEFICIAL ROLE OF CITRUS

Full Technical Review

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I- The nervous system

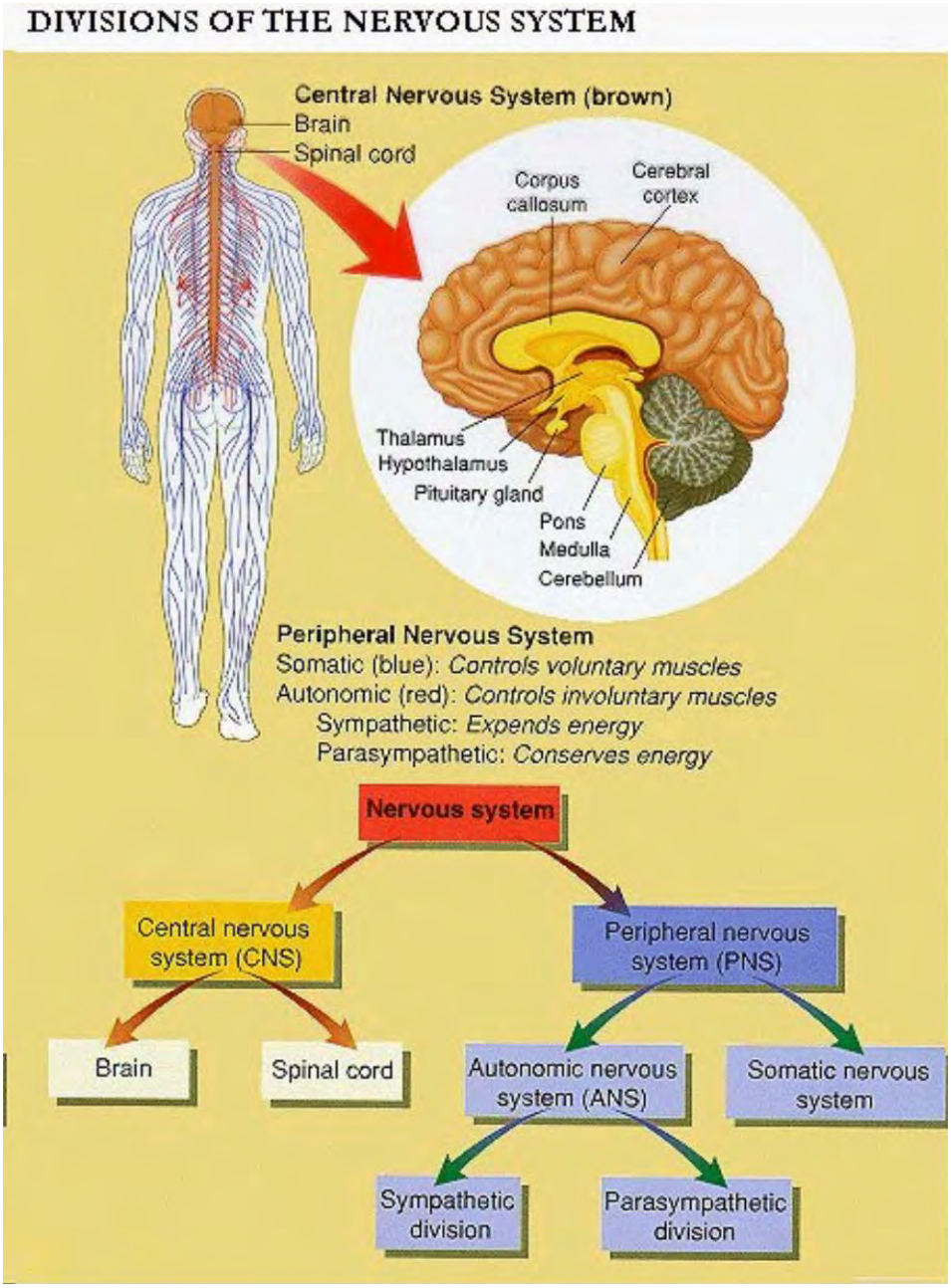


Fig.1: Diagram of the nervous system.

The Nervous System is the most complex and delicate of all the body systems. At the center of the nervous system is the brain. The brain sends and receives messages through a network of nerves.
The spinal cord is a thick bundle of nerves which runs down the center of the spine. Along the spinal cord smaller bunches of nerves branch out. From these bundles, smaller bundles of nerves branch out again. Finally, individual nerves branch out to every part of the body. This network of nerves allows the brain to communicate with every part of the body. Nerves transmit information as electrical impulses from one area of the body to another. Some nerves carry information to the brain. This allows us to see, hear, smell, taste and touch. Other nerves carry information from the brain to the muscles to control the body's movement (Oleksy 2001)

Communication is the main function of the nervous system. The nervous tissue is composed of nerve cells. Each nerve cell, or neuron, has a cell body, with thin, spider-like dendrites and one much longer, wire-like nerve fiber or axon. The axon's branched ends have button shaped axon bulbs, which almost touch other nerve cells, at junctions called synapses. Nerve signals travel along the axon at speeds of more than 100 meters per second and 'jump' from one nerve cell to another across a gap called synapse. The relay of the electrical signal from a neuron to another cell is insured by chemicals called neurotransmitters

The nervous system is divided into two sections with different functions and properties (Fig.1): The **central nervous system (CNS)** which is composed of the brain and the spinal cord, and the **peripheral nervous system (PNS),** (Afifi and Bergman, 2005). CNS and PNS have different physiological properties (Fig.2). This review will be mostly devoted to the CNS and particularly the brain.

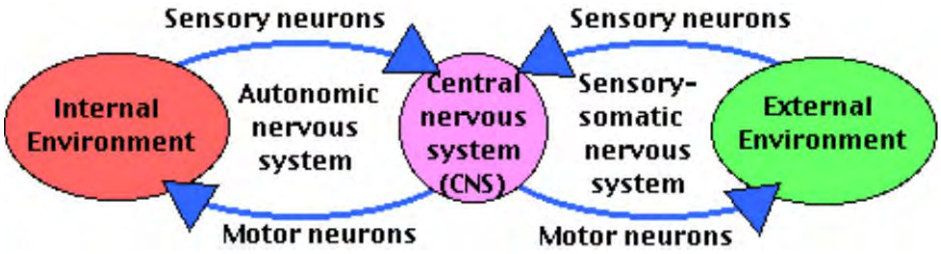


Fig.2: The nervous system

I-1 The peripheral nervous system

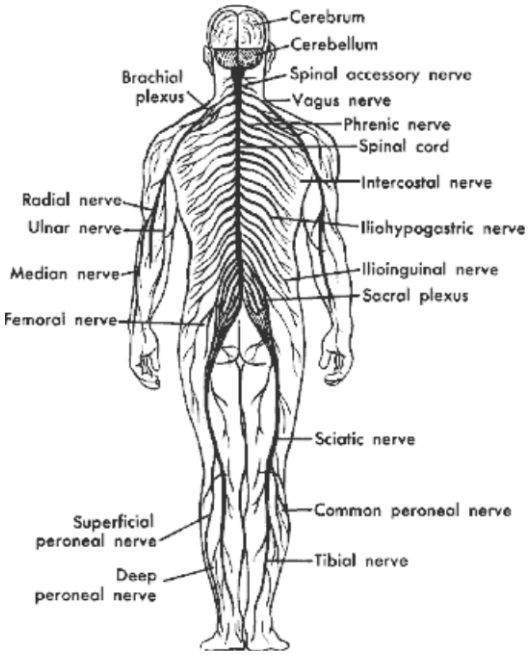


Fig. 3a : somatic PNS

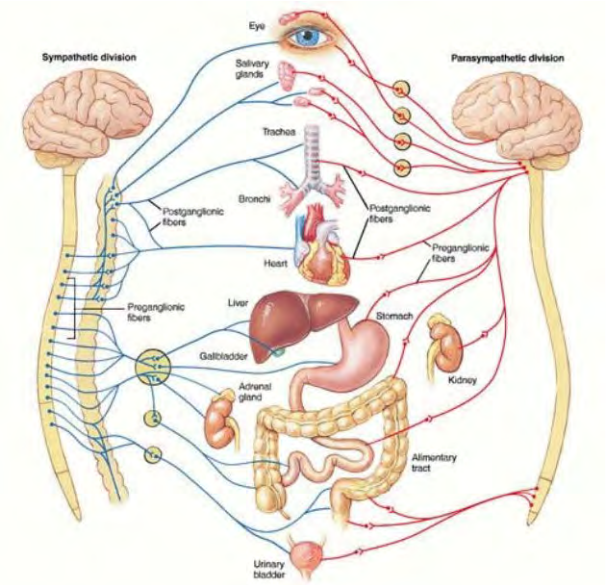


Fig. 3b: Autonomic PNS

The **PNS** will be quickly reviewed. It consists of sensory neurons running from stimulus receptors that inform the CNS of the stimuli. Motor neurons running from the CNS to the muscles and glands - called effectors - that take action.

The peripheral nervous system has been, in turn divided into:

-The somatic system which includes sensory neurons of the dorsal root and cranial ganglia that innervate the skin, muscles, and joints and provides sensory information to the central nervous system about muscle and limb position and about the environment (Fig.3a).

-The autonomic system which includes the motor system for the viscera, the smooth muscles and exocrine glands. It in turn consists of three segregated subdivisions: the sympathetic system which participates in the response of the body to stress. The parasympathetic system that acts to conserve de body's resources and restore homeostasis. The enteric nervous system which controls the function of smooth muscle of the gut (Fig.3b). The PNS neurons have the ability to regenerate their axon after an injury. If a nerve is cut for example in a finger, the nerve will eventually grow back and the feeling will reappear. (Wilson 1984, Cancalon, 1990)

I-2 The central nervous system

Although some very limited neuronal formation may exit in the brain, as a rule CNS neurons cannot regenerate and die once damaged. Because of that, spinal cord injuries lead to irreversible paralysis (Fig.4) and a large fraction of the brain cells die during our life time.(Cancalon, 1988.)

The central nervous system consists of six main regions:

I-2-1. The spinal cord

The spinal cord extends from the base of the skull through the first lumbar vertebra. The spinal cord receives sensory information from the skin, joints, and muscles of the trunk and limbs, and contains the motor neurons responsible for both voluntary and reflex movements. It also receives sensory information from the internal organs and controls many visceral functions. Within the spinal cord there is an orderly arrangement of sensory cell groups that receive input from the periphery and motor cell groups that control specific muscle groups. In addition, the spinal cord contains ascending pathways through which sensory information reaches the brain and descending pathways that relay motor command from the brain to motor neurons(Noback and Harting, 2003).

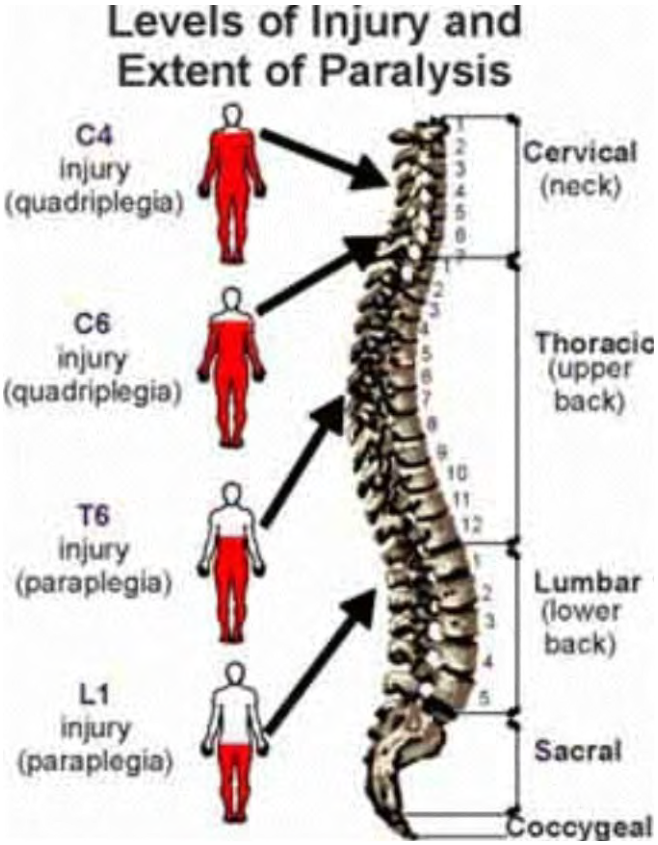


Fig.4: irreversible damage to the CNS

I-2-2. The brain

The **brain** is the higher, supervisory center of the nervous system. The human brain is a collection of 100 billion neurons, each linked with up to 25,000 others. This huge number of interconnecting neurons, often referred to as a neural ensemble, is what makes the brain able to analyze sensory signals, control the body, and think (Fig.5). (Edelman and Changeux , 2000)

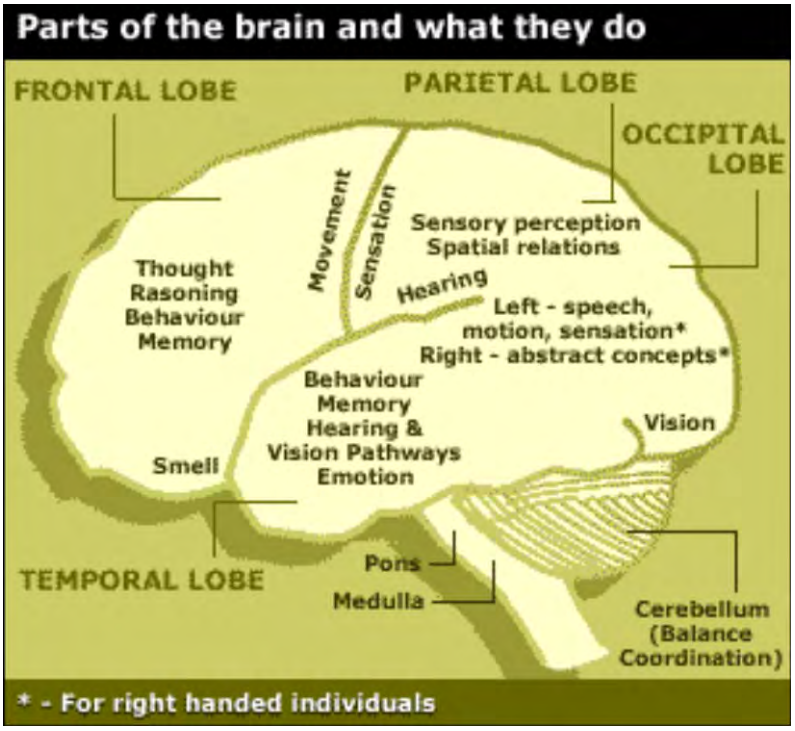


Fig. 5: The brain

I-2-2 -a The medulla

This structure is the direct rostral extension of the spinal cord. Together with the pons , it participates in regulating blood pressure and respiration. It resembles the spinal cord in both organization and function.

I-2-2-b The pons

It contains a large number of neurons that relay information from the cerebral hemispheres to the cerebellum. The cerebellum lays dorsal to the pons and medulla. It has a distinctive corrugated surface. The cerebellum receives somatosensory input from the spinal cord, motor information from the cerebral cortex and balance information from the vestibular organs of the inner ear. The cerebellum integrates this information and coordinates the planning, timing and patterning of skeletal muscle contractions during movement. The cerebellum plays a major role in the control of posture, head and eye movements.

I-2-2-c The midbrain

The midbrain contains essential relay nuclei of the auditory and visual system. Several regions of this structure play an important role in the direct control of eye movement, whereas others are involved in motor control of skeletal muscles.

I-2-2-d The Diencephalon

It consists of the thalamus and hypothalamus and lies between the cerebral hemispheres and the midbrain. The thalamus distributes almost all sensory and motor information going to the cerebral cortex. In addition, it is thought to regulate levels of awareness and some emotional aspects of sensory experiences. The hypothalamus lies ventral to the thalamus and regulates autonomic activity and the hormonal secretion by the pituitary gland.

I-2-2-e The cerebral hemispheres

It forms, in humans, the largest region of the brain. It consists of the cerebral cortex, the white matter under the cortex and three deeply located nuclei: the basal ganglia, the hyppocampal formation and the amygdala. The cerebral hemispheres are divided by the hemispheric fissure and are thought to be concerned with perception, cognition, emotion, memory and high motor functions.

I-3 Cells of the nervous system

The central nervous system consists of neurons and glial cells. Neurons constitute about half the volume of the CNS and glial cells make up the rest. (Brown, 2001)

I-3-1 Neurons

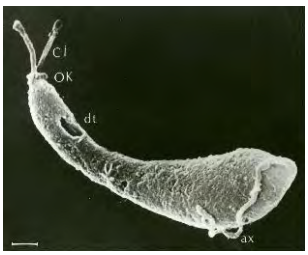


Fig.6a: Scanning electron micrograph of an olfactory neuron(Cancalon, 1978)

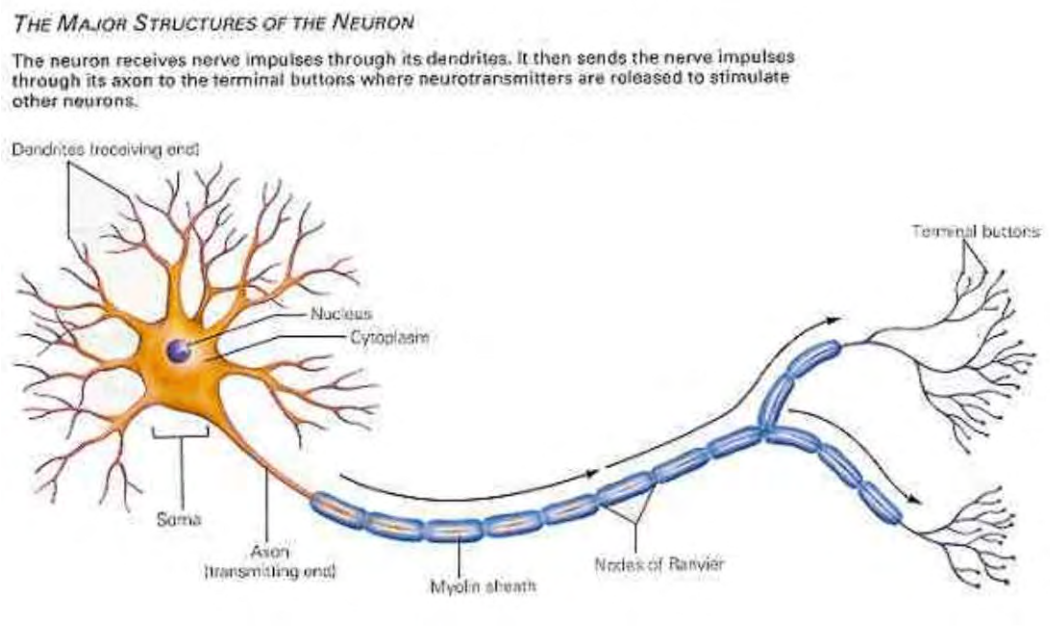


Fig.6b: Neuronal cell.

Nerves are made of individual nerve cells or neurons (Fig.6a ,b). Neurons are asymmetric in shape and consist of:

-The soma or cell-body, the relatively large central part of the cell.

- The axon a much finer, cable-like projection which may extend tens, hundreds, or even tens of thousands of times the diameter of the soma in length. This is the structure which carries nerve signals away from the neuron.

- The dendrite, a short, branching arbor of cellular extensions. Each neuron has many dendrites with profuse dendritic branches. These structures form the main information receiving network for the neuron.

Neurons are connected to each other by synapses A synapse is a small gap separating neurons (Fig.7). The synapse consists of:

A presynaptic ending that contains neurotransmitters, mitochondria and other cell organelles.
A postsynaptic ending that contains receptor sites for neurotransmitters.A synaptic cleft or space between the presynaptic and postsynaptic endings.

Synapses have a high level of plasticity and it is believed that memory is encoded into them. The most common explanation of memory is that it is stored by relative differences between individual synapses.

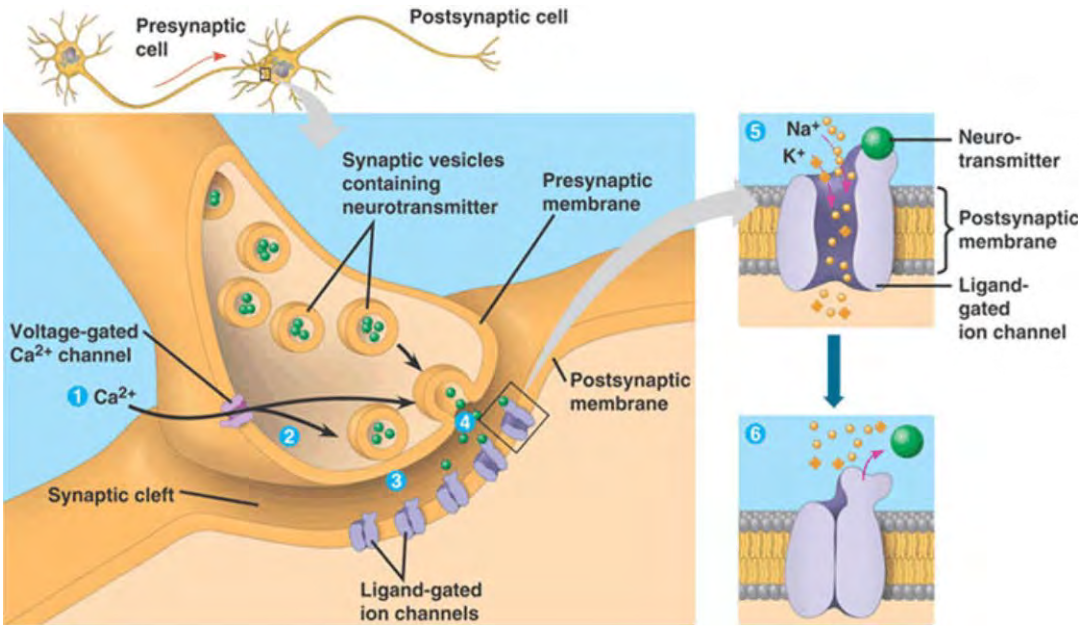


Fig.7: Synapse and synaptic cleft

I-3-2 Glial cells

Glial cells provide support and protection for neurons. They are thus known as the "supporting cells" of the nervous system. The four main functions of glial cells are: to surround neurons and hold them in place, to supply nutrients and oxygen to neurons, to insulate one neuron from another, and to destroy and remove the carcasses of dead neurons (clean up). The three types of CNS supporting cells are Astrocytes, Oligodendrocytes, and Microglia. The supporting cells of the PNS are known as Schwann Cells.

I-3-2-a Astrocytes

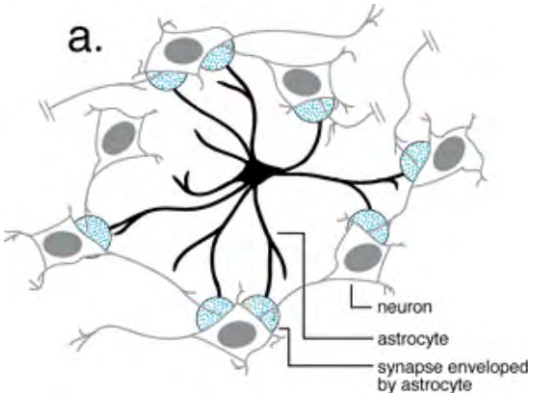


Fig.8

In order to provide physical support for neurons astrocytes (Fig.8) form a matrix that keep neurons in place. In addition, this matrix serves to isolate synapses. This limits the dispersion of transmitter substances released by terminal buttons, thus aiding in the smooth transmission of neural messages.

Astrocytes also perform a process known as phagocytosis. Phagocytosis occurs when an astrocyte contacts a piece of neural debris with its processes (arm of the astrocyte) and then pushes itself against the debris eventually engulfing and digesting it.

Astrocytes provide nourishment to neurons by

1) receiving glucose from capillaries
2) breaking the glucose down into lactate (the chemical produced during the first step of glucose metabolism)
3) releasing the lactate into the extra cellular fluid surrounding the neurons. The neurons receive the lactate from the extra cellular fluid and transport it to their mitochondria to use it for energy. In this process astrocytes store a small amount of glycogen, which stays on reserve for times when the metabolic rate of neurons in the area is especially high.

I-3-2-b Oligodendrocytes

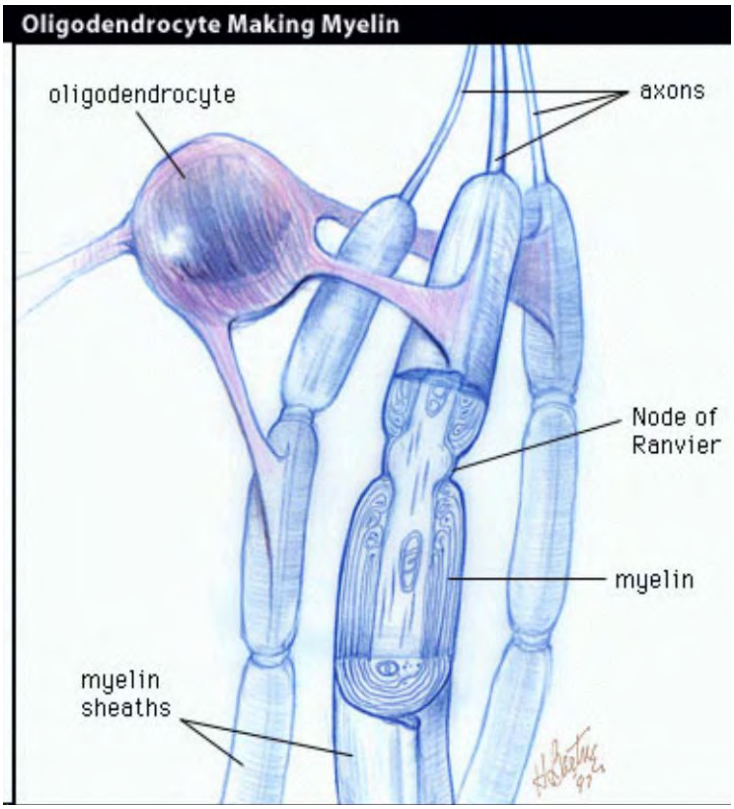


Fig.9

The principle function of oligodendrocytes (Fig.9) is to provide support to axons and to produce the myelin sheath, which insulates axons. Myelin is 80% lipid and 20% protein and allows for the efficient conduction of action potentials down the axon. Oligodendrocytes unlike Schwann cells of the PNS, form segments of myelin sheaths of numerous neurons at once. As can be seen in the above illustration, the processes of a given oligodendrocyte wrap themselves around portions of the surrounding axons. As each process wraps itself around, it forms layers of myelin. Each process thus becomes a segment of the axon's myelin sheath.

I-3-2-c Microglia

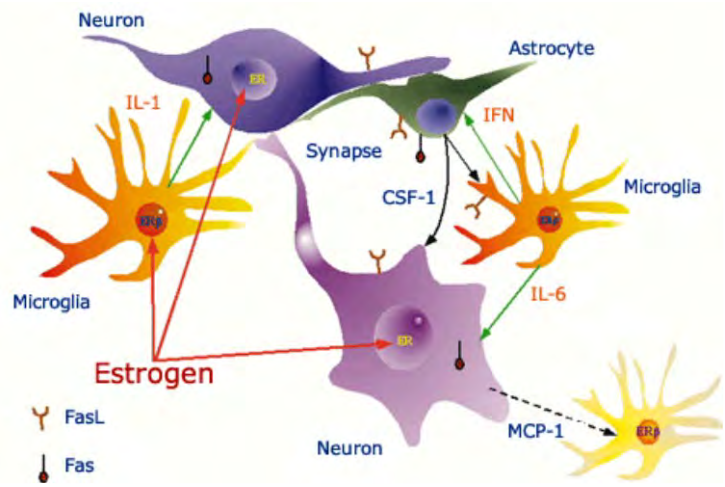


Fig.10

Microglia (Fig.10) are the smallest of the glial cells. Some act as phagocytes cleaning up CNS debris. Most serve as representatives of the immune system in the brain. *Microglia protect the brain from invading microorganisms and are thought to be similar in nature to microphages in the blood system. They play a major role in brain inflammation*

I-3-2-d Schwann cells

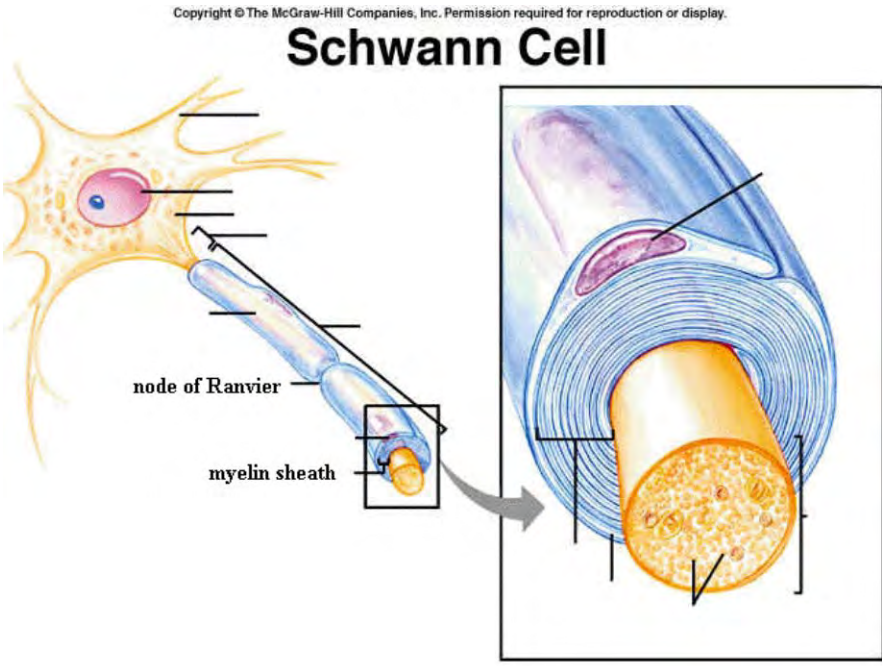


Fig.11

Schwann cells (Fig.11) are the supporting cells of the PNS. Like oligodendrocytes Schwann cells wrap themselves around nerve axons, but the difference is that a single Schwann cell makes up a single segment of an axon's myelin sheath. Oligodendrocytes on the other hand, wrap themselves around numerous axons at once. In addition to creating the myelin sheaths of PNS axons, Schwann cells also aid in cleaning up PNS debris and guide the regrowth of PNS axons. To do this Schwann cells arrange themselves in a series of cylinders that serves as a guide for sprouts of regenerating axons. If one of these sprouts encounters a cylinder the sprout will grow through the tube at the rate of 3-4 mm per day. Nonproductive sprouts simply wither away.

I-4 Blood brain barrier and cerebrospinal fluid barrier

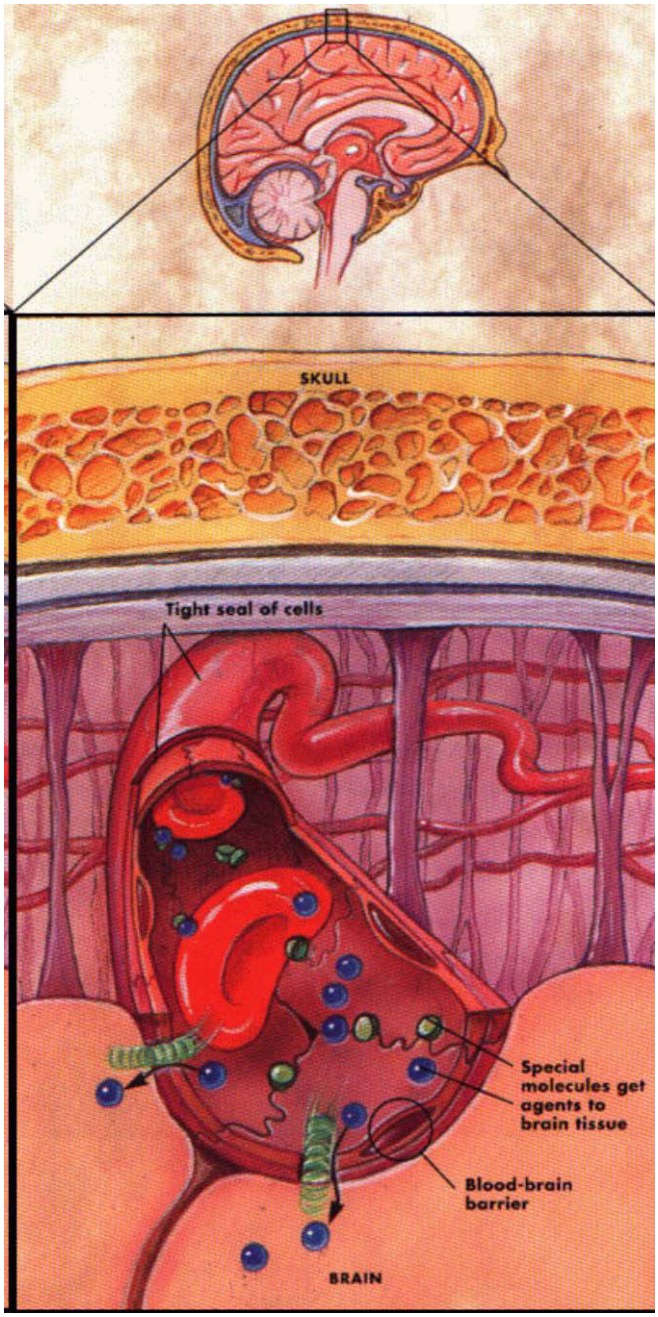
One major component of the CNS is the blood-brain barrier (BBB) (Hawkins and Davis, 2005) (Fig.12a). It is the specialized system of capillary endothelial cells that protects the brain from harmful substances present in the blood stream, while supplying the brain with the required nutrients for proper function (Pardridge, 1998). The BBB is created by the tight apposition of endothelial cells lining blood vessels in the brain, forming a barrier between the circulation and the brain parenchyma (astrocytes, microglias). Blood-borne immune cells such as lymphocytes, monocytes and neutrophils cannot penetrate this barrier. A thin basement membrane, comprising lamin, fibronectin and other proteins, surrounds the endothelial cells and associated pericytes, and provides both mechanical support and a barrier function. Thus, the BBB is crucial for preventing infiltration of pathogens and restricting antibody-mediated immune responses in the central nervous system. The BBB strictly limits transport into the brain through both physical (tight junctions) and metabolic (enzymes) barriers. Thus, the BBB is often the rate-limiting factor in determining permeation of therapeutic drugs into the brain. Additionally, BBB breakdown is theorized to be a key component in central nervous system (CNS) associated pathologies.

The BBB has several important functions:

1. Protect the brain from "foreign substances" in the blood that may injure the brain.
2. Protect the brain from hormones and neurotransmitters present in the rest of the body.
3. Maintain a constant environment for the brain.

The BBB creates a protected chemical environment for the brain wherein certain molecules can perform functions independent of the functions those molecules perform in the rest of the body.

While the largest interface between blood and brain is the BBB, this is also smaller less direct interface between blood and cerebrospinal fluid (CSF) (Fig.12b)(Johanson, 1998,2003). The *choroid plexus* and the *arachnoid membrane* act together as the barriers between the blood and CSF. Passage of substances from the blood through the arachnoid membrane is prevented by tight junctions (Fig.12c). T he arachnoid membrane is generally impermeable to hydrophilic substances, and its role is forming the Blood-CSF barrier is largely passive. While the capillaries of the choroid plexus are fenestrated, non-continuous and have gaps between the capillary endothelial cells allowing the free-movement of small molecules, the adjacent choroidal epithelial cells form tight junctions preventing most macromolecules from effectively passing into the CSF from the blood



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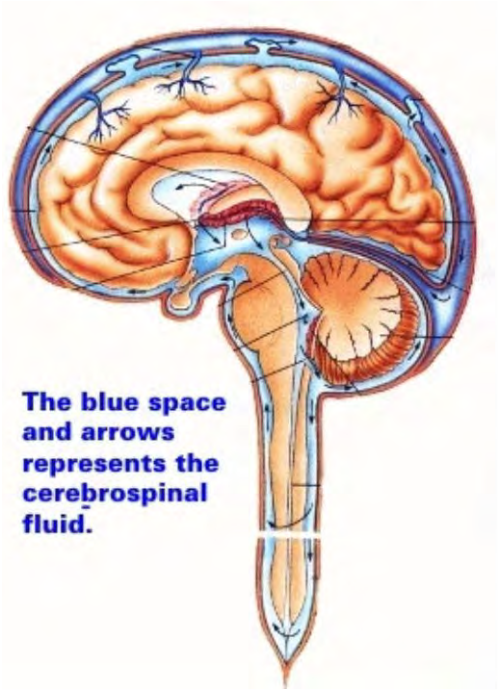
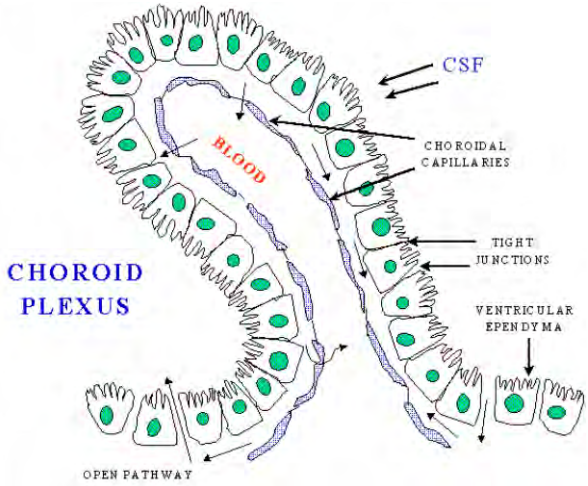


Fig. 12a: The blood brain barrier(from the Society for Neuroscience) Fig: 12c CSF barrier

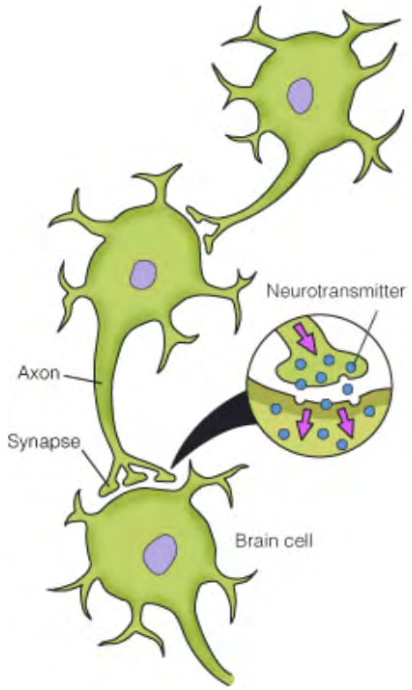


I-5 Nerve impulse and neurotransmission

I-5-1 Action potential

The role of nerves is to transmit and store information. T he propagation of information is done by electrical impulses moving from neuron (nerve cells) to neuron ((Fig.13a). N eurons have specialized projections called dendrites and axons. Dendrites bring information to the cell body and axons take information away from the cell body. The impulse called action potential (Fig.13b) is a wave of electrical discharge that travels along the membrane of a cell. Action potentials are the main means for the body to communicate fast internal messages between its tissues. They can be created by many types of body cells, but

are used most extensively by the nervous system to send messages between nerve cells and from nerve cells to other body tissues. Action potentials are caused by an exchange of ions across the neuron membrane. For information to flow from one neuron to another, the action potential must cross the gap separating neurons. (Johnson et al 2003)



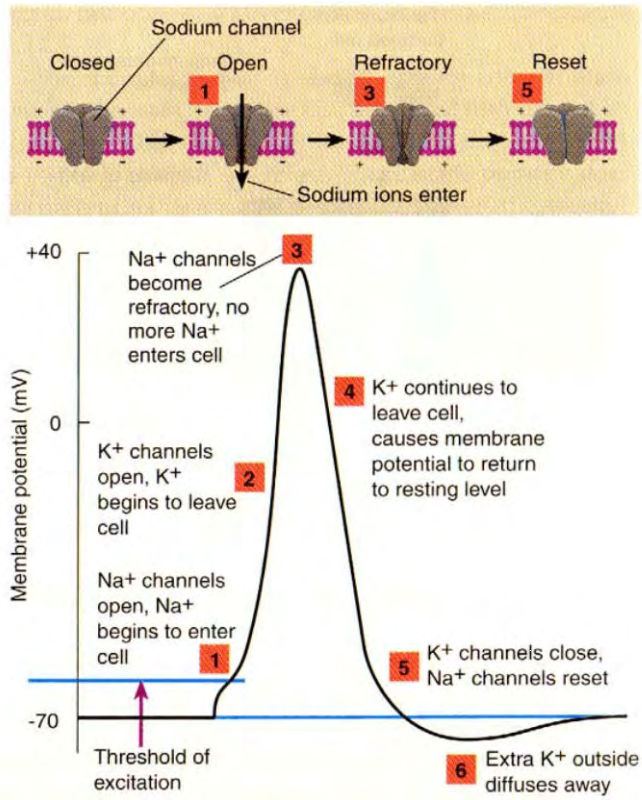


Fig. 13a:Communicating neurons

Fig.13b: Action potential

I-5-2 Neurotransmitters

A neurotransmitter is a chemical that is released from a nerve cell to transmit an impulse from a nerve cell to another nerve cell, a muscle, an organ, or other tissue. A neurotransmitter is a messenger of neurologic information from one cell to another. (Johnson et al 2003). A chemical can be classified as a neurotransmitter if it respects the following conditions:

1. It must be synthesized endogenously, within the presynaptic neuron.
2. It must be available in sufficient quantity in the presynaptic neuron to exert an effect on the postsynaptic neuron.

3. Externally administered, it must mimic the endogenously-released substance.

4. A biochemical mechanism for inactivation must be present.

The three major categories of substances that act as neurotransmitters are

1. amino acids (primarily glutamic acid, GABA, aspartic acid & glycine).

2.peptides (vasopressin, somatostatin, neurotensin).

3. monoamines (norepinephrine, dopamine & serotonin) plus acetylcholine

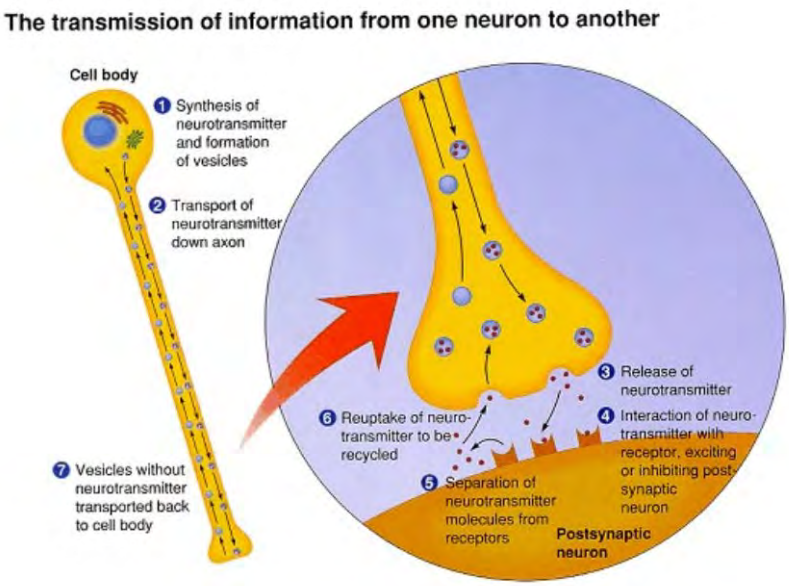


Fig.14: Neurotransmitter

II - Cognition

Although, many problems related to the PNS have been described, a l arge number of the ailments associated with the nervous system involve the CNS. Th is review will be mostly dealing with the central nervous system and particularly the brain.

II-1 Cognitive processes and functions

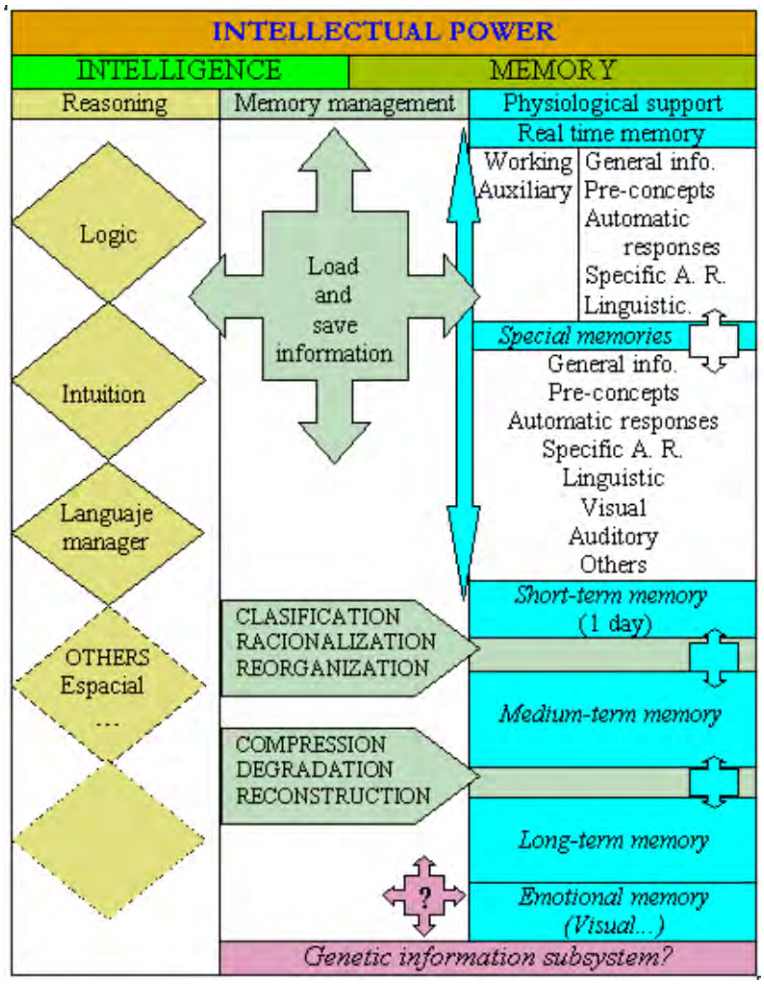


Fig.15: Cognitive processes

The mental processes described as cognition (Pick et al, 1992)or cognitive processes (Meyer and Schvaneveldt, 1975) apply to internal mental processes such as memory, attention, perception, action, memory, language, problem solving and mental imagery (Fig.15).
Traditionally emotion was not thought of as a cognitive process. This division is now regarded as largely artificial.
Various aspects of scientific research are devoted to the understanding of cognitive functions, among them:
Cognitive neuroscience (Gazzaniga, 1999) is a branch of neuroscience involving the study of the physiological and neurochemical mechanisms of cognition or "how the brain thinks."
Cognitive neuroscience overlaps with cognitive psychology, but whereas psychologists seek to understand the mind, cognitive neuroscience is concerned with understanding how the mental processes take place in the brain.

Cognitive neuropsychology (Sanford, 1986) is a branch of neuropsychology that aims to understand how the structure and function of the brain relate to specific psychological processes. It places a particular emphasis on studying the cognitive effects of brain injury or neurological illness with a view to inferring models of normal cognitive functioning.

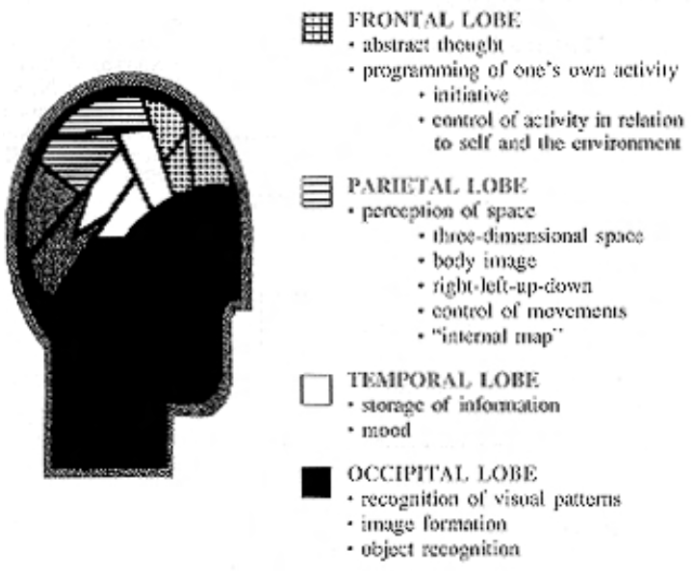


Fig. 16: cognitive processes and the brain

New brain imaging methods enable us to study what happens in the brain during learning and thinking and when carrying out various tasks (Fig.16). Indeed, brain research has in recent years shifted from studying the function of the resting brain to investigating the "actively working" brain. Cerebral electromagnetic phenomena are studied using *electro-* and *magnetoencephalographic* methods. These methods even allow the study of individual sensory systems, e.g., the optic tract and the processing of information in it. Cerebral metabolisms can be studied by *positron emission tomography (PET)* and by functional *magnetic resonance imaging (MRI).*

Modern brain imaging methods have shown, among other things, that speech reception, speech production, and analysis of speech contents each activate distinct areas of t he brain. The processing of visual information takes place in brain areas different from those involved in interpreting auditory information. Figure 1 is a simplified presentation of the main functions of the different brain areas. According to the present view, memory traces are stored throughout the brain and, thus, the functions of the brain cannot be considered in terms of "lobes." The frontal lobes of the cerebrum play an especially important role in higher brain functions such as learning and analyzing linguistic messages.

The cognitive processes of the brain translate intentions and decisions via motor programming into behavior. Behaviors are studied using methods of *cognitive psychology* and *neuropsychology.* Computer-assisted psychologic tests allow ever more detailed modeling of the cognitive processes of the brain.

The processing of information in the human brain can be roughly divided into *automatic* and *conscious processing.* Figure 2 is a schematic presentation of the current conception of the way the human brain handles information. Ultimately, both learning and recollection involve (1) reception of information by different sensory systems, (2) analysis and memorization of the information received, and (3) an ability to retrieve the stored memory contents. Correctly directed observation, attention, and recollection are prerequisites for thinking, the most complex form of information processing in the brain. The levels of observation and attention depend on factors such as emotions and motivation, as well as alertness.

A person's working memory, where the immediate analysis of incoming information takes place, can be divided into at least two sub-categories: a *visual* memory, based on seeing, and an *auditory* memory, based on hearing. Auditory memory deals with the coding of speech and loads the working memory in a different way than does visual memory. The processing of vision-based information has more "automated" features.

In the working memory, new information is actively related to previously absorbed information contents stored in long-term memory. Activity of the frontal lobes of the cerebrum is fundamental to the control of the brain's information processing systems. Individuals with frontal lobe lesions have an impaired ability to learn new things and are unable to manage large entities of information adequately

II-2 Deterioration of the cognitive processes

Diseases of the central nervous system (CNS) are among the most physically and mentally disabling conditions. It is estimated that as much as 5 percent of the adult population in the United States may live with non-dementing brain disorders (NIH Workshop, 2004). Dementing disorders cause global cognitive deficits and are generally thought to be progressive (Nussbaum and Ellis, 20003). Conversely, non-dementing disorders affect one or more targeted or focal cognitive functions while leaving others intact. Examples of non-dementing neurological conditions include degenerative conditions such as multiple sclerosis (MS), epilepsy, Parkinson disease, amyotrophic lateral sclerosis; congenital abnormalities such as cerebral palsy; and event-induced disorders such as strokes. Additional examples include migraine disorders, sleep disorders such as sleep apnea and insomnia, spinal cord injury, tic disorders such as Tourette syndrome, sensory disorders such as Meneire syndrome, ataxias such as Friedreich ataxia, and communication disorders such as expressive aphasia.

Among these ailments, memory loss can be linked to the aging of the brain and the deterioration of brain cells. At its peak performance, commonly occurring at the age of 30, the brain may have 10,000 connections for each of its 100 billion nerve cells, meaning there are as many as 1,000

trillion cell-to-cell connections. Since brain cells (neurons) are not replaced, in severe memory loss such as Alzheimer's, more than 90% of these connections can be lost (Bondareff et al, 1982, Whitehouse et al, 1982).

II-2-1 Mild Cognitive Impairment

Memory loss has long been recognized as a common accompaniment of aging. The inabilities to recall the name of a recent acquaintance or the contents of a short shopping list are familiar experiences for everyone, and this experience seems to become more common as we age.

In the past, memory loss in the elderly was viewed as inevitable accompaniments of aging, often referred to as "senility." More recently, it has become accepted that memory impairment of a certain degree is now considered pathological, and thus indicative of some kind of disease process affecting the brain(Kirchner, 2005). The threshold usually taken to make this judgment is that memory loss has progressed to such an extent that normal independent function is impossible. This degree of cognitive impairment has come to be referred to as dementia. Dementia has many potential causes, the most common of which is Alzheimer's disease.

However, many older individuals may complain of memory problems, but still manage to independently accomplish all their customary tasks. Usually, their ability to function well is based on compensation for these difficulties, such as increased reliance on a calendar or on reminder notes, lists, etc. In some cases, these memory difficulties are a sign that worsening memory loss. The characterization of this problem and its outcome is much better known than in the past. The syndrome of subjective memory problems has come to be commonly known as "Mild Cognitive Impairment" (MCI), although other terms have been used, including "Cognitive Impairment, Not Dementia" (CIND) (Kirchner, 2005; Schonknecht et al, 2005).

II-2-2 Symptoms of patients with MCI

The patient with MCI complains of difficulty with memory. Typically, the complaints include trouble remembering the names of people they met recently, trouble remembering the flow of a conversation, and an increased tendency to misplace things, or similar problems. In many cases, the individual will be quite aware of these difficulties and will compensate with increased reliance on notes and calendars. Most important, the diagnosis of MCI relies on the fact that the individual is able to perform all their usual activities successfully, without more assistance from others than they previously needed. (Nordlund et al, 2005). How do the memory difficulties in MCI differ from those of normal aging? This is a very difficult question to which there is, as yet, no definitive answer. (Kazui et al 2005) Several studies have examined the cognitive performance of patients with MCI. These have demonstrated that, in general, these patients perform relatively poorly on formal tests of memory, even when compared with other individuals in their age group. They also show mild difficulties in other areas of thinking, such as naming objects or people (coming up with the names of things) and complex planning tasks. These problems are similar, but less severe, than the findings associated with Alzheimer's disease.

Several studies have demonstrated that memory complaints in the elderly are associated with a higher-than-normal risk of developing dementia in the future. (Visser et al, 2005; Morris, 2005). Most commonly, the type of dementia that patients with MCI are at risk to develop is Alzheimer's disease, though other dementias, such as Vascular Dementia (VaD) or Frontotemporal Dementia (FTD) may occur as well. However, it is also clear that some patients with these complaints never develop dementia. This is called Cognitive Impairment, Non Demented, or CIND.

There are certain features are associated with a higher likelihood of progressive MCI. These include confirmation of memory difficulties by a knowledgeable informant (such as a spouse, child, or close friend), poor performance on objective memory testing, and any changes in the ability to perform daily tasks, such as hobbies or finances, handling emergencies, or attending to one's personal hygiene. Recent studies have shown homocysteine is a strong predictor of cognitive decline. As homocysteine levels increase, cognitive function steadily decreases. Elevated homocysteine has been identified as a treatable, independent risk factor for cognitive impairments (see below).

One factor that had to be controlled for in many of these studies was depression, as many patients with depression also complain about their memory. Several studies have suggested that certain measurements of atrophy (shrinkage) or decreased metabolism on images of the brain (PET or MRI scans) increase the chances of developing dementia in the future. Hippocampal atrophy seems to be the best structural predictor. Although these above factors increase the chances of going on to develop dementia, it is not possible currently to predict with certainty which patients with MCI will or will not go on to develop dementia. Not everyone with MCI will get dementia. Not everyone with dementia will get Alzheimer's disease. However, MCI is a risk state for the development of dementia of the Alzheimer type.

Though a significant body of research exists on dementing conditions like aphasia and Alzheimer disease, considerably less is known about milder conditions. It appears that there are cognitive changes that are common across non-dementing disorders including, but not limited to, aspects of memory function, language control, attentional control, fatigue, and pain. In order to understand disease-specific issues, as well as cognitive deficits across diseases, it is important to consider the heterogeneity of disease experiences. While a severe stroke can obviously strongly affect cognition, there has been little study of the effects of very small, "silent" strokes on cognitive ability. Stroke plays an important role in dementia, but it is now becoming clear that more subtle forms of vascular injury in the brain may play an equally important role in cognitive decline. Studies of large populations of elderly patients show that about one-third of those 65 and older have had at least one brain infarction, most of which were never recognized clinically. These subclinical infarcts can accumulate over time and they appear to cause cognitive deterioration. Another important element of these studies is understanding why silent strokes have a big negative impact on some people, while others don't seem to be much affected. The thought is that lots of brain activity makes the brain healthier through use, possibly by increasing alternative neural pathways that can be used if one pathway is damaged. (NIH Workshop, 2004).

Petersen et al (1999) found that patients with MCI progress to AD at a rate of 10% to 15% per year, while control subjects progress at a rate of 1% to 2% per year. About 40% of all patients who experience MCI will progress to AD within three years. (Smith, 2002)

II-3 Advance cognitive impairment and brain diseases

II-3-1 Brain Inflammation

As mentioned above, dementia is a general term for diseases involving nerve cell deterioration. Dementia is a slow, gradual process that may take months or even years to become noticeable. Symptoms vary depending on which areas of the brain are affected. Anyone or a combination of the following factors can cause age-associated cognitive dysfunctions:

- The damaging effects of chronic free radical exposure.
- The damaging effects of chronic inflammation causing injury to both cerebral blood vessels and neurons.
- Changes in lifestyle and diet leading to nutrient deficiencies.
- Decreases in oxygen available to brain cells because of impaired circulation due to pathology or lifetime habits (smoking, drinking, bad diet, or stress).
- Declining energy output of brain cells.
- Essential fatty acid deficiencies.

Inflammation is the body's response to a perceived threat. This involves a complex chain of events and may require the cooperation of a variety of specialized cells. Their activity is generally beneficial, but the goal is always the same: to rid the body of intruders and to dispose of damaged tissue so healing may take place (Cancalon, 2005). The inflammatory response is often worse than the stimulus that triggered it in the first place. Even when the original trigger is eliminated, inflammation may become self-perpetuating. This may be the case in neurodegenerative diseases such as Alzheimer's, Parkinson's, ALS and multiple sclerosis, which are characterized by a great deal of microglial activity. The presence of activated microglial cells is an indicator of chronic inflammation (Teimann et al, 2003; Pompl et al, 2003).

Because of its nature, the brain is very fragile and susceptible to various diseases. As seen previously many of the brain problems have an inflammation component (Cancalon, 2005). The brain uses large amounts of energy, with only 2% of the body weight it consumes 20% of the available glucose and oxygen. It should also be noted that this high metabolic activity generates radical oxidative species (ROS) that can damage neurons. Furthermore, the very high lipid content of the brain makes it a primary target for oxidation. Free-radical damage (oxidative stress) is a significant cause of biological aging. Neurons are extremely sensitive to attacks by destructive free radicals. The following evidence supports the hypothesis of free-radical damage being a central cause in Alzheimer's disease (Christen 2000). Brain neurons are particularly vulnerable to the effects of free radical oxidation because of their high-energy production. The

destructive effects of excess free radical activity have been implicated in many disease processes, including Alzheimer's disease and Parkinson's disease. Antioxidants neutralize free radicals and help prevent some of the damage associated with normal brain aging. (McGeer and McGeer, 1999)

The problem is heightened by the fact that the access to the brain is severely limited by the blood brain barrier (BBB). Substances in the blood that gain rapid entry into the brain include glucose, the main source of energy, certain ions that maintain a proper medium for electrical activity, and oxygen for cellular respiration. Small fat-soluble molecules, like ethanol, pass through the BBB. Therefore the brain needs a constant delivery of energy, if glucose and oxygen become unavailable the CNS neurons can die very rapidly. Cytokines circulating in the blood, affect CNS function through a variety of pa thways. One of these pathways is by being transported directly across the blood-brain barrier (BBB). Cytokine transport across the BBB, however, is complex (Aarli, 2003). Not all cytokines are transported and, for those which are, transport rates differ among cytokines, among brain regions, with physiological circumstances, and with disease (Youdim, 2004). In the brain, microglial cells, act as scavengers, in much the same fashion as macrophages. They engulf and eliminate dead neurons that have been damaged by injury or i llness. Unfortunately, they also secrete harmful neurotoxins and oxygen free radicals to neutralize foreign or undesirable substances. (Klegeris, 2002)

II-3-2 Alzheimer's disease

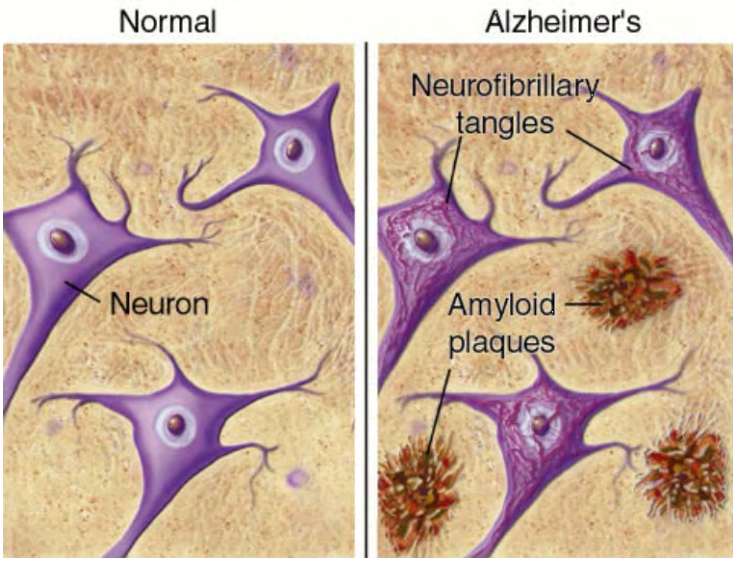


Fig. 17: Alzheimer’s disease damages to the brain

Alzheimer's disease (AD) begins slowly. At first, the only symptom may be mild forgetfulness, which can be confused with age-related memory change. Most people with mild forgetfulness do not have AD. In the early stage of AD, people may have trouble remembering recent events, activities, or the names of familiar people or things. However, as the disease goes on, symptoms are more easily noticed. Forgetfulness begins to interfere with daily activities. People in the middle stages of AD may forget how to do simple tasks like brushing their teeth or combing their hair. They can no longer think clearly. They can fail to recognize familiar people and places. They begin to have problems speaking, understanding, reading, or writing. Later on, people with AD may become anxious or aggressive, or wander away from home. Eventually, patients need total care.

II-3-2-a Alzheimer's disease and inflammation

The onset and progression of Alzheimer's disease have not been completely elucidated, but it seems that a peptide known as amyloid-B, triggers inflammation. (Hull and Stauss, 1996). The beta-amyloid peptide present in the core of senile plaques is a 42-amino acid chain produced by cleavage of a larger protein known as amyloid precursor protein (APP). In Alzheimer's disease, an inflammatory cascade begins in response to appearance of the beta-amyloid peptide. The inflammatory response involves cytosine and prostaglandin. Alzheimer's disease is also characterized by the development of intra neuronal neurofibrillary tangles (Fig.17). The senile plaques of beta-amyloid peptide, and the neurofibrillary tangles eventually lead to a loss of synapses, and ultimately neuronal. Beta-amyloid proteins initiate an inflammatory response, involving cytokines and prostaglandins. It has been postulated that inflammation is an underlying cause of Alzheimer's disease (Hull 1996; McGeer et al. 1999). The plaques are associated with reactive microglial cells, immune system proteins such as interleukin-6 (IL-6)and other. It should also be mentioned that the inflammation marker, C-reactive protein, is associated with Alzheimer's disease (Iwamoto et al. 1994). The presence of these markers is proof of a link with long-term inflammation(Scali et al, 2003).

A link seems to exist between interleukins and AD. Microglia, and astrocytes are distributed randomly in healthy brains. Amyloid Beta proteins provide an initial stimulus Microglia are then attracted to the stimulus by chemotaxis In turn, the microglia secrete a diffusible factor cytokine IL-1-Beta. Buildup of IL-1-Beta triggers astrocytes which produce the inflammatory chemical, IL-6, which is toxic to neurons. Neuronal death occurs when a fatal IL-6 concentration has has been reached (Iwamoto et al. 1994).

Alzheimer's disease generates a rise in the levels of the acetylcholinesterase, resulting in the depletion of the neurotransmitter acetylcholine. Uninterrupted, the inflammation gradually accelerates, killing nerve cells and causing a drastic decline in levels of a vital brain chemical, the neurotransmitter acetylcholine (McGeer et al. 1999). This contributes to loss of memory and loss of attentiveness. Decrease in the level of brain neurotransmitters by at least 50% impairs signal transduction between neurons and brain functions (Calderon-Garciduenas, 2004). Microglial cells, which accompany the neuritic plaques of Alzheimer's disease, are normally dormant. They are activated only in response to inflammation, thus their presence is a sure sign of brain inflammation. Although present in large numbers in the brains of patients with

degenerative neurological diseases, such as Huntington's (Sapp et al, 2001) and Alzheimer's diseases, the number of are also elevated in otherwise healthy elderly individuals. This implies that a certain degree of neuroinflammation is an ordinary result of nothing more than advanced age.(Teismann et al, 2003). It has been suggested that cognitive decline begins early in the aging process and is an inevitable result of advancing age (Jorm et al,1987). This downward spiral of neural degeneration commences with the induction of nearly undetectable inflammation, progresses to the erosion of memory, concentration and learning ability and ends with death.

Controlling inflammation, therefore, could presumably benefit anyone interested in preventing eventual memory loss and cognitive decline.

II-3-2-b Alzheimer's disease and oxidative stress

The damage begins when the beta-amyloid becomes concentrated in senile plaques and an inflammatory reaction with ongoing oxidative stress and free-radical damage starts. There is evidence to support the hypothesis that free-radical damage is a central cause in Alzheimer's disease (Christen 2000). The brain lesions present in the brains of Alzheimer's disease patients are typically associated with attacks by free radicals (for example, damage to DNA, protein oxidation, lipid peroxidation, and advanced glycosylation end products). Alzheimer's disease has been linked to mitochondrial anomalies affecting cytochrome c oxidase. These anomalies may contribute to the abnormal production of free radicals. Free radical scavengers (such as vitamin E, and C) have produced promising results in Alzheimer's disease.

The brain lesions present in the brains of Alzheimer's disease patients are typically associated with attacks by free radicals (for example, damage to DNA, protein oxidation, lipid peroxidation, and advanced glycosylation end products . Metals (such as iron, copper, zinc, and aluminum) are often present. These metals have a catalytic activity which produces free radicals. Beta-amyloid is aggregated and produces more free radicals in the presence of free radicals. Beta-amyloid toxicity is eliminated by free radical scavengers. Apolipoprotein E (ApoE) is subject to attacks by free radicals, and apolipoprotein E peroxidation has been correlated with Alzheimer's disease. In contrast, apolipoprotein E can act as a free radical scavenger.

Alzheimer's disease has been linked to mitochondrial anomalies affecting cytochrome c oxidase. These anomalies may contribute to the abnormal production of free radicals. Free radical scavengers have produced promising results in Alzheimer's disease (Christen 2000).

II-3-3 Amyotrophic Lateral Sclerosis (ALS)

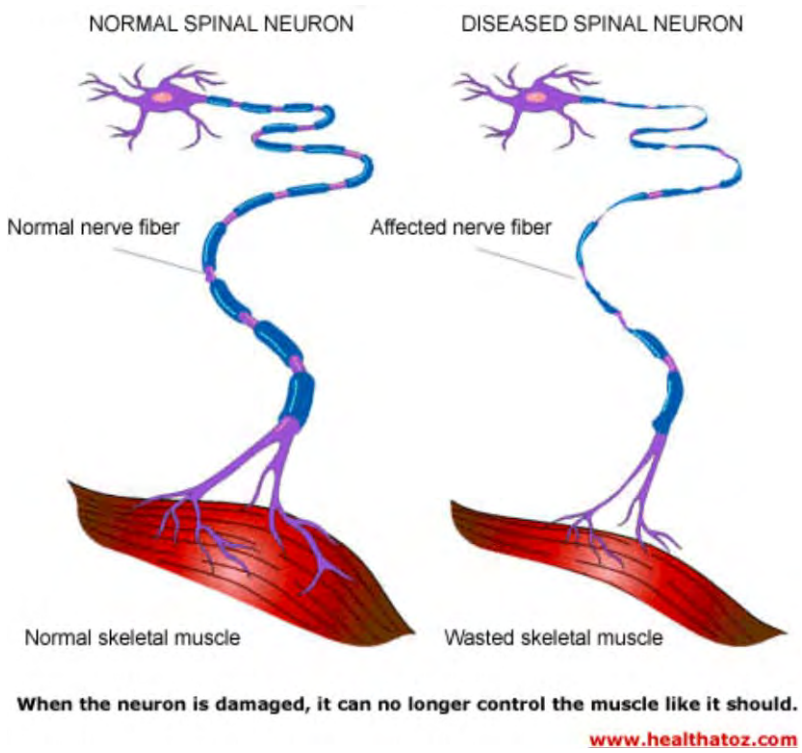


Fig. 18: ALS degeneration of the nerve muscle system.

The ALS is caused by a genetic defect in superoxide dismutase, an antioxidant enzyme that continuously removes superoxide free radical. A defective glutamate transport system has also been proposed as a mechanism for ALS . A study showed the plasma levels of glutamate in ALS patients to be as much as 70% higher as compared to controls. Defects in mitochondrial DNA have also been proposed as a causative mechanism in sporadic ALS (Beal 1998,1999).

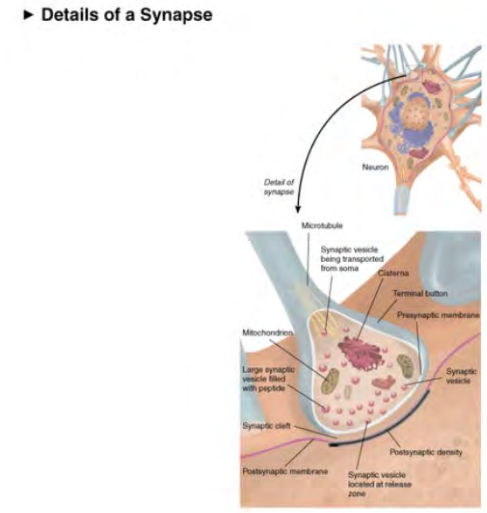
This explains only some cases of ALS but several other alternative theories exist:

The immune system may mistakenly attack and damage the neurons.

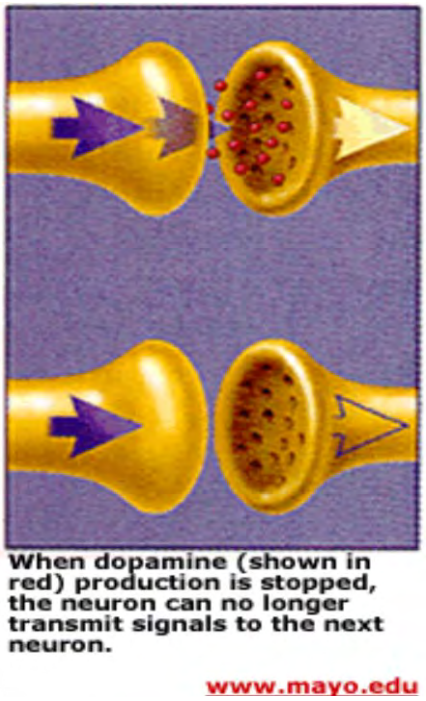
The structures that provide energy for the neurons may become damaged, harming the entire cell.

Neuronal support proteins, viruses, or bacteria may inflict the damage.

During ALS, motor axons die by Wallerian degeneration (Fig.18), and large motor neurons are affected to a greater extent than smaller ones. This process occurs as a result of the death of the anterior horn cell body, leading to degeneration of the associated motor axon. As the axon breaks down, surrounding Schwann cells catabolize the axon's myelin sheath and engulf the axon, breaking it into fragments. This forms small ovoid compartments containing axonal debris and surrounding myelin, termed myelin ovoids. Ovoids then are phagocytized by macrophages recruited into the area to clean up debris.



II-3-4 Parkinson's disease



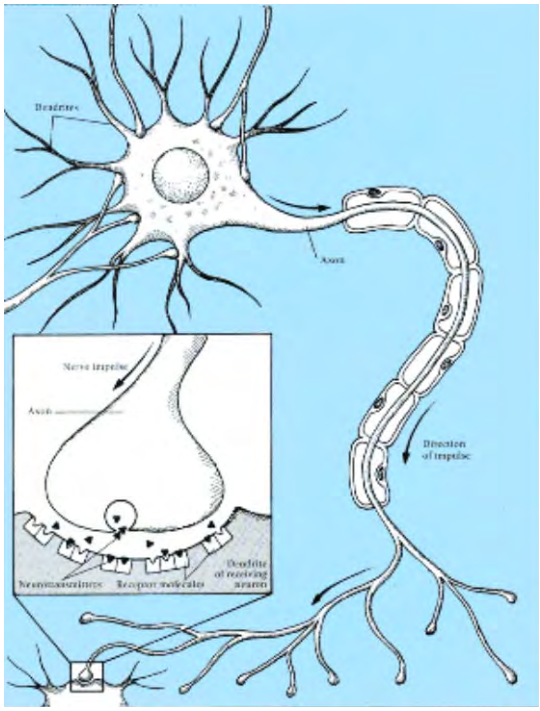
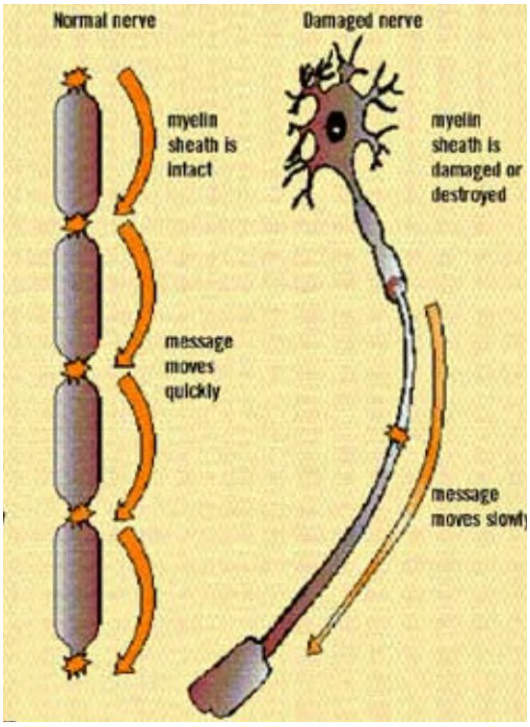


Fig. 19: Parkinson's disease and dopamine

PD is caused by a loss of neurons in a part of the brain called the substantia nigra (Fig.19). Normally, these neurons produce the chemical messenger dopamine, which helps direct muscle movement. When the neurons become damaged, dopamine production stops and the body loses full movement control. We do not know what damages the neurons. Some believe that free radicals, toxic particles normally deactivated in the body, are responsible. Others theorize that an

external toxin, such as a food or pesticide, harm the neurons; yet no research exists to verify this theory. Alternatively, damage to the cell structures that create energy or acceleration of the aging process could be involved. We continue to probe all of these theories

Parkinson's disease is a degenerative central nervous system disorder of unknown origin, affecting mainly older patients (Glanze 1996). However, the disease may occur in younger persons, particularly following inflammation of the brain (encephalitis) or from poisoning by carbon monoxide, metals, particularly aluminum (Cooper 1991; Farina et al. 1994). The condition results from several factors. One of the possible causes is the death of dopamine-producing neurons in the substantia nigra. Discoveries have led to the suggestion that Parkinson's disease may arise as a combined consequence of the ongoing aging process coupled with environmental exposures that accelerate the process of nigral cell death. Parkinson's disease has been observed in virtually all ethnic groups. This observation suggests that genetic factors may possibly have an important role in disease development. (Shults et al 1998, 1999)



II-3-5 Multiple Sclerosis

Fig. 20: Multiple sclerosis and myelin degeneration

Multiple Sclerosis (MS) is an autoimmune disease, whereby the body destroys its own myelin (protective coating surrounding the nerves in the central nervous system) (Fig 20). Because the myelin is damaged, messages moving along the nerve are transmitted more slowly or not at all (Lassmann 2005).

II-3-6 Aging and Free-Radical Damage

Many theories have attempted to explain aging. It is thought that human genes are programed for a life span of about 120 years (Ruiz-Torres and Beier , 2005). Chroni c inflammation is viewed as one of the main causes preventing us from reaching this optimal age. As we grow older, the ability to absorb and use antioxidants decreases, while at the same time our ability to

generate energy deteriorates and the production of oxidants increases. Although all organs are affected by aging, the brain is one of the main targets of the senescence process. Decrease in the efficiency of the energy handling mechanisms is believed to be a major cause of brain aging. The decline in respiratory processes and the increase of oxidation processes can be considered the root cause of brain aging and degeneration. I ncreasing respiratory processes would increase oxygen delivery to the neurons, improve neuronal glucose metabolism, and antioxidant defenses, and generally increase efficiency and functioning of the brain. (Youdim, 2004)

II-3-7 Brain and homocysteine

II-3-7-a One carbon metabolism and homcysteine toxicity.

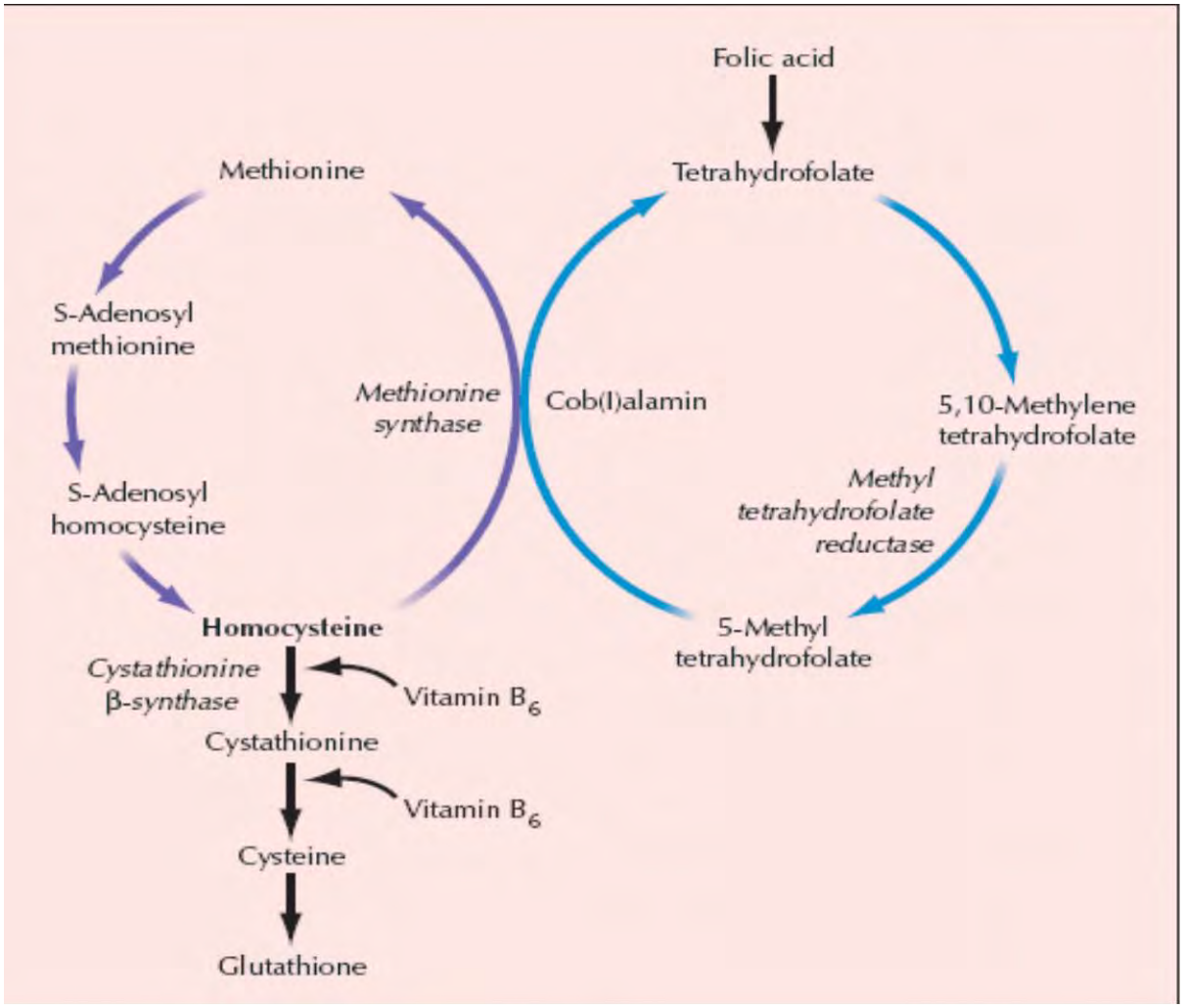


Fig.21: The one-carbon cycle

The distribution of a single carbon to various biochemical processes in very important for the maintenance of several physiological functions and particularly DNA synthesis and neurotransmitter synthesis. The essential amino acid methionine is the starting component of the one-carbon cycle (methyl cycle) (Fig.21) In this cycle methionine is transformed into S-adenosylmethionine (SAM) the one-carbon donor in numerous methylation reactions involving

proteins, phospholipids and biogenic amines. One of the main functions of SAM is to provide a pool of methyl groups for the production and repair of DNA. Upon transfer of its methyl group, SAM is converted to S-adenosylhomocysteine (SAH), which is subsequently hydrolyzed to homocysteine and adenosine(Pennypacker et al.,1992;. Parnetti et al. 1980). Then the vitamin B_{12}-dependent enzyme, methionine synthetase transfers a methyl group from methyl tetrahydrofolate to homocysteine to regenerate methionine.

The SAH hydrolysis is a reversible reaction that favors SAH synthesis. If homocysteine is allowed to accumulate, it will be rapidly metabolized to SAH, which is a strong inhibitor of all methylation reaction competing with SAM for the active site on the methyltransferase enzyme protein(Enk et al, 198; Bottiglien, 1997; Weir, 1988; Surtiss, 1991).

II-3-7-b Mechanisms of action of homocysteine in the brain

Many studies have shown that high levels of blood homocysteine predispose to arteriosclerosis and stroke (Lipton et al, 1997). It has been recently estimated that as many as 47% of patients with arterial occlusions manifest modest elevations in plasma homocysteine (Perry et al, 1997). Included among the many causes are genetic alterations in enzymes such as cystathionine beta-synthase, a defect found in 1-2% of the general population, and deficiencies in vitamins B6, B12, and folate whose intake was suboptimal in perhaps 40% of the population in 1977(Perry et al, 1997). The authors postulated that the strength of the association between homocysteine and cerebrovascular disease appears to be greater than that between homocysteine and coronary heart disease or peripheral vascular disease. During stroke or head trauma, disruption of the blood-brain barrier results in exposure of the brain to near plasma levels of amino acids, including homocysteine and glycine. Decreased remethylation of homocysteine to methionine and consequent slower production of SAM may impair the methylation reactions required for normal brain function. It is theorized that high homocysteine levels promote DNA damage and sensitize hippocampal neurons to oxidative stress induced by amyloid beta-peptide toxicity (Miller, 2003).

Vitamin B12 is the coenzyme that allows the methyl donation from 5 methyl tetrahydrofolate to homocysteine, necessary for methionine remethylation. During folate or vitamin B12 deficiency, the methionine synthetase reaction is severely impaired and can induce DNA strand breakage, oxidative stress and apoptosis(Martin et al.1988; Marathi et al, 2001 a.b.c, Malone, 2003; Oh and Brown, 2003, Shaw, 1993).

Homocysteine is metabolized through two different pathways: the methionine synthetase pathway that goes back to methionine and the cystathionine pathway producing glutathione (Fig.21). The effects of homocysteine in the brain are multiple but can be broadly divided into neurotoxic and vascular effects (Fig22).
The evidence for the neurotoxic effects comes largely from in vitro studies (Garcia and Zanibbi, 2004). It has been shown in vitro that homocysteine acts as an agonist on the glutamate binding site and as a partial antagonist on the glycine-binding site of the N methyl-D-aspartate (NMDA)

receptor. More precisely, it has been hypothesized that a pathway of oxidation of homocysteine to homocysteic acid is the potential explanation of the dangerous effect of homocysteine In fact, homocysteic acid is a mixed excitatory agonist acting preferentially at NMDA receptors. (Shaw, 1993). These receptors are well known in memory long-term potentiation system: hyper or abnormal activation of NMDA receptors results in a rise of intracellular calcium, consequent release of cellular proteases and eventual cell death (Lipton, 1997). Under conditions of elevated glycine concentrations, such as in stroke or head trauma, homocysteine at a concentration of 10 μ Mol/L had a neurotoxic effect through hyperstimulation of the NMDA receptor that resulted in an excess influx of calcium ions and production of reactive oxygen species (Garcia and Zanibbi, 2004).

Box 1: Effects of elevated homocysteine levels in the brain

Neurotoxic

- Hyperactivation of *N*-methyl-D-aspartate receptors
- Apoptosis

Vascular

- Increased proliferation of smooth muscle cells
- Increased platelet aggregation
- Increased number of strokes and white matter lesions

Fig.22: Brain and homocysteine

II-3-7-c Homocysteine and cognitive impairment

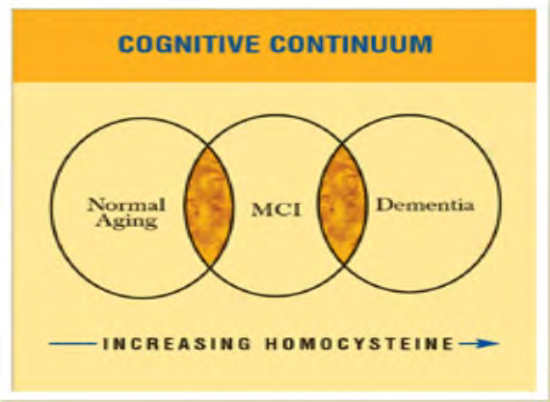


Fig.23: homocysteine and cognitive impairment.

Various studies have shown a link between homcysteine levels and the degree of cognitive impairment (Seshadri et al, 2002). Garcia and Zanibbi (2004) reviewed the literature linking homocysteine and cognitive functions (Tables I, II). Garcia and Zanibbi (2004) concluded that the cognitive continuum from normal to demented is associated with a similar rise in homocysteine levels (Fig. 22) and that increased serum homocysteine as well as age, years of education and are among the most relevant risk factors for dementia.

Table 1: Studies investigating relation between homocysteine and dementia

Study	Study population	Study design	Cognitive assessment/ diagnostic criteria	Results	Comment
Stewart, 2002[4]	238 African-Caribbean adults aged 55–75 yr	Cross-sectional	CERAD; WHO; MMSE	tHcy level > 13.85 µmol/L associated with cognitive impairment (OR 2.86, 95% CI 1.33–6.13)	OR adjusted for age, occupation, hypertension, diabetes, physical activity, and levels of cholesterol, triglycerides and fibrinogen
Clarke, 1998[5]	272 patients aged > 55 yr; control-matched comparison	Case–control	CERAD, NINDS	tHcy >14.0 µmol/l associated with AD (OR 2.0, 95% CI 1.1–3.4)	OR adjusted for age, sex, smoking, social class and apolipoprotein E
McCaddon, 1998[6]	60 patients with DSM-III-R criteria for AD	Case–control	CAMDEX	tHcy level inversely related to scores on cognitive testing	Results adjusted for age, sex, folate and cobalamin levels, smoking and hypertension. Effect of tHcy and cobalamin found to be interrelated
Miller, 2002[7]	80 patients with possible or probable AD	Case–control	NINCDS-ADRDA	tHcy level > 12 µmol/L associated with vascular disease (OR 1 0.0, 95% CI 1.2–82) but not with AD (OR 2.2, 95% CI 0.31–16)	OR adjusted for age, sex, and levels of red blood cell folate, plasma B_{12}, serum creatinine and serum thyroid stimulating hormone
Seshadri, 2002[8]	1092 dementia-free patients in Framingham cohort aged 68–97 yr	Prospective cohort (median follow-up 8 yr)	MMSE, DSM IV criteria, Clinical Dementia Rating Scale, NINCDS-ADRDA	tHcy level associated with increased risk of dementia and AD (RR 1.4, 95% CI 1.1–1.9 per 1 SD increase in log-transformed tHcy level)	RR adjusted for age, sex, apolipoprotein E genotype, education level, history of stroke, smoking, alcohol intake, diabetes, body mass index and systolic blood pressure
Vital Trial Collaborative Group, 2003[9]	149 patients with dementia or mild cognitive impairment	Intervention study of ASA and vitamin supplements on serum biomarkers of dementia	MMSE, TICS-M, DSM IV criteria	At baseline, tHcy level inversely associated with cognitive scores	Results adjusted for age

Note: AD = Alzheimer's disease, CAMDEX = Cambridge Examination for Mental Disorders of the Elderly, CERAD = Consortium to Establish a Registry for Alzheimer's Disease, CI = confidence interval, DSM = Diagnostic and Statistical Manual of Mental Disorders, MMSE = Mini-Mental Status Exam, NINCDS-ADRDA = National Institute of Neurological and Communication Disorders and Stroke/Alzheimer's Disease and Related Disorders Association, NINDS = National Institute of Neurological Disorders, OR = odds ratio, RR = relative risk, SD = standard deviation, tHcy = homocysteine, TICS-M = Telephone Interview of Cognitive Status, WHO = World Health Organization.

Table 2: Studies investigating relation between homocysteine and cognitive function scores

Study	Study population	Study design	Cognitive assessment	Results	Comment
Riggs et al, 1996[10]	70 people aged 54–81 yr in Normative Aging Study	Cross section of prospective cohort	CERAD, NES2	tHcy levels > 12.6 µmol/L associated with poorer spatial copying skills. No relation found between tHcy levels and perceptual speed, spatial reasoning or language abilities	Concluded that tHcy may have differential effects on cognitive abilities
Ravaglia et al, 2000[11]	54 people aged > 65 yr in Conselice Study	Cross section of prospective cohort	MMSE, clock-drawing test, prose memory test, Corsi block-tapping task, Mental Deterioration Battery	No association found between tHcy levels and cognitive test scores	Results adjusted for age, sex, education level, smoking status, alcohol or coffee consumption, and previous cardiovascular disease
Budge et al, 2002[12]	158 community-dwelling people aged 60–91 yr	Cross section of prospective cohort	CAMCOG, MMSE, GDS	Higher tHcy levels associated with lower memory scores per µmol/L (OR 1.15, 95% CI 1.10–1.27)	OR adjusted for age, sex, serum cystatin C level and systolic blood pressure
Duthie et al, 2002[13]	334 community-dwelling people who had participated in Scottish Mental Surveys of 1932 and 1947	Cross section	MMSE, NART, RPM, AVLT, WAIS	tHcy levels negatively associated with scores on RPM, WAIS in older cohort with higher tHcy levels (mean 10.9 µmol/L, 95% CI 10.1–11.5)	Results adjusted for childhood intelligence quotient
Prins et al, 2002[14]	1077 people aged 60–90 yr in Rotterdam Scan Study	Cross section of prospective cohort	Abbreviated Stroop test, Letter-Digit Substitution Task, Verbal fluency test, PPMST, Modified Rey's test	Patients with tHcy > 14 µmol/L had lower scores for global cognitive function (difference –0.20, 95% CI –0.30 to –0.11)	Results adjusted for age, sex, education level, depression, serum creatinine level
Miller et al, 2003[15]	1789 community-dwelling people aged ≥ 60 yr in Sacramento Area Latino Study on Aging	Cross section of prospective cohort	3MSE, verbal and visual memory tests, object naming, conceptualizatio n and attention span tests	Inverse relation between tHcy levels and scores on 3MSE ($p = 0.02$), picture association ($p = 0.05$), verbal attention span ($p = 0.04$) and pattern recognition tests ($p = 0.001$)	Multiple linear regression model inlcuded folate, cobalamin, creatinine, age, sex, education and acculturation

Table 2 continued

Study	Study population	Study design	Cognitive assessment	Results	Comment
Ravaglia et al, 2003[16]	650 community-dwelling people aged 65–91 yr (mean 73 yr) with normal cognitive function in Conselice Study	Population-based study	MMSE	Inverse relation between odds of tHcy level > 15 µmol/L and MMSE scores	Results adjusted for age, education level, income, serum creatinine level, serum vitamin B index, active lifestyle, and coffee and meat consumption
Garcia et al, 2004[17]	281 community-dwelling volunteers aged > 65 yr	Cross section	Stroop, Mattis DRS, CVLT	Subjects with elevated tHcy levels (> 13.9 µmol/L) had lower Stroop scores than those with normal tHcy levels in univariate analysis ($p < 0.05$)	Strongest association found between methylcitric acid and cognitive scores
Kalmijn et al, 1999[18]	702 community-dwelling people aged > 55 yr in Rotterdam Study	Prospective cohort; mean follow-up 2.7 yr	MMSE	No association between tHcy and cognitive impairment (highest v. lowest tertile, OR 0.91, 95% CI 0.52–1.58)	OR adjusted for age, sex, education level and baseline MMSE score
McCaddon et al, 2001[19]	32 community-dwelling people aged > 65 yr	Prospective cohort; 5-yr follow-up	MMSE, ADAS-Cog	Baseline tHcy predicted MMSE scores ($p = 0.001$) and ADAS-Cog scores ($p = 0.01$) at 5-yr follow-up	Results adjusted for age and education.
Dufouil et al, 2003[20]	1241 people aged > 60 yr in Epidemiology of Vascular Aging Study	Prospective cohort; 4-yr follow-up	MMSE, Trail Making Test Part B, Digit Symbol Substitution Test from the WAIS, Finger Tapping Test	Odds of cognitive decline 2.8 (95% CI 1.2–6.2) in patients with tHcy level ≥ 15 µmol/L	OR adjusted for age, sex, education level, baseline cognition, body mass index, alcohol consumption, smoking, hypertension, hypercholesterolemia, glycemix status, history of vascular disease, and folate and B_{12} levels
Teunissen et al, 2003[21]	144 normal aging adults (30–80 yr at baseline)	Prospective cohort; 6-yr follow-up	WLT, LDCT, Stroop	tHcy levels inversely correlated with performance on WLT at baseline but not at follow-up. No relation between tHcy levels and results of other cognitive tests	Results adjusted for age, sex and education level
Garcia et al (in press)[22]	180 normal community-dwelling people aged ≥ 65 yr	Prospective cohort; mean follow-up 2.3 yr	Stroop, Mattis DRS, CVLT	Rate of increase in tHcy levels correlated with rate of decline in Stroop scores	Results adjusted for age, education level, time interval between 2 visits, cobalamin and red blood cell folate levels, diabetes and hypertension

Note: ADAS-Cog = Alzheimer's Disease Assessment Scale, cognitive component, AVLT = Auditory Verbal Learning Test, CERAD = Consortium to Establish a Registry for Alzheimer's Disease, CAMCOG = Cambridge Examination for Mental Disorders of the Elderly, CVLT = California Verbal Learning Test, GDS = Geriatric Depression Scale, LDCT = Letter-Digit Coding Test, Mattis DRS = Mattis Dementia Rating Scale, MMSE = Mini-Mental Status Exam, NART = National Adult Reading Test, NES = Neurobehavioural Evaluation System, NINCDS-ADRDA = National Institute of Neurological and Communication Disorders and Stroke/Alzheimer's Disease and Related Disorders Association, PPMST = Paper and Pencil Memory Scanning Task, RPM = Raven's Progrssive Matrices, Stroop = Stroop Colour-Word Test, 3MSE = Modified Mini-Mental Status Exam, TICS-M = Telephone Interview of Cognitive Status, WAIS = Wechsler Adult Intelligence Scale, WLT = Word Learning Test.

From these data several conclusions could be drawn.

1. Too much homocysteine in the blood has been associated with increased risk in blood vessel disease, including brain blood vessels.
2. Elevated homocysteine levels may predict cognitive decline.
3. High levels of homocysteine are strongly associated with memory decline and may increase the chance of developing dementia.

4.Homocysteine >14 µmol/L nearly doubled a patient's risk of developing Alzheimer's disease (Seshadri et al, 2002).

II-3-7-d Homocysteine and mild cognitive impairment

Petersen (1999) found that patients with mild cognitive impairment (MCI) progress to Alzheimer's disease (AD) at a rate of 10% to 15% per year, while control subjects progress at a rate of 1% to 2% per year. About 40% of all patients who experience MCI will progress to AD within three years.

The syndrome of subjective memory problems has come to be commonly known as "Mild Cognitive Impairment" (MCI), although other terms have been used, including "Cognitive Impairment, Not Dementia" (CIND). What is meant by the term "Mild Cognitive Impairment? Memory loss has long been recognized as a common accompaniment of aging. The inabilities to recall the name of a recent acquaintance or the contents of a short shopping list are familiar experiences for everyone, and this experience seems to become more common as we age. Recent studies have shown that memory impairment of a certain degree can be considered pathological, and indicates some kind of disease process affecting the brain. The threshold most physicians use to make this judgment is that memory loss has progressed to such an extent that normal independent function is impossible. This degree of cognitive impairment has come to be referred to as dementia. Dementia has many potential causes, the most common of which is Alzheimer's disease.

Information has been emerging regarding a connection between homocysteine metabolism and cognitive function, from mild cognitive decline (age-related memory loss) to vascular dementia and Alzheimer's disease. Significant deficiencies in the homocysteine re-methylation cofactors cobalamin (B12) and folate, as well as the trans-sulfuration cofactor vitamin B6, are commonly seen in the elderly population, with a resultant increase in homocysteine with advancing age. Hyperhomocysteinemia has been shown to be an independent risk factor for cognitive dysfunction. Indirect and direct vascular damage can be caused by homocysteine, which has been implicated in vascular dementia, with an increased risk of multiple brain infarcts and dementia as homocysteine levels rise. A significant correlation has been found between risk of Alzheimer's disease and high plasma levels of homocysteine, as well as low levels of folic acid, and vitamins B6 and B12. All of these disease associations are thought to be interrelated via increased homocysteine and S-adenosylhomocysteine and subsequent hypomethylation of numerous substances, including DNA and proteins, that render vascular structures and neurons more susceptible to damage and apoptosis. (Miller 2003). The author proposed that an accumulation of homocysteine as a possible marker for the early detection of cognitive impairment in the elderly (Miller at al, 2003). Several other studies have found elevated homocysteine levels in Alzheimer's patients (Gottfries et al. 1998; McCaddon et al. 1998; 2001a,b). Tucker et al (2005) showed that most elevations in homocysteine result from inadequate folate, vitamin B-12, or vitamin B-6 intake. The authors reported that a high

homocysteine concentration was associated with a decline in recall memory. Low B vitamin and high homocysteine concentrations predict cognitive decline in aging men and may appear before the onset of cognitive impairment.
Elevated homocysteine has even been associated with depression. Chen at al (2005) examined the association of homocysteine levels or methylenetetrahydrofolate reductase (MTHFR C677T) genotype and late-onset major depressive disorder (MDD) and assess whether this may be affected by brain magnetic resonance imaging (MRI)signal hyperintensities. Thirty-nine elderly patients with MDD with first episode occurring after age 50 and 20 comparison subjects were recruited and total plasma homocysteine levels, MTHFR genotype, and brain MRIs were assessed. Plasma total homocysteine levels were higher in elderly patients with late-onset MDD versus comparison subjects. The association did not change after controlling for MRI hyperintensities, and the distribution of MTHFR C677T genotype was not different between the groups. The authors concluded that elevated homocysteine levels were associated with late-onset MDD, and the association did not appear to be mediated by vascular pathology as identified by brain MRI.

II-3-7-e Homocysteine and aging

How do the memory difficulties in MCI differ from those of normal aging? Several studies have examined the cognitive performance of patients with MCI. These have demonstrated that, in general, these patients perform relatively poorly on formal tests of memory, even when compared with other individuals in their age group(Solfrizzi et al, 2004; Nordlund et al, 2005). They also show mild difficulties in other areas of thinking, such as naming objects or people (coming up with the names of things) and complex planning tasks. These problems are similar, but less severe, than the findings associated with Alzheimer's disease.
The relation between homocysteine, aging and cognitive performance was examined by Elias et al (2005) in the Framingham Offspring Study. Using data collected between 1991 and 2002, the authors investigated the associations between tHcy and multiple measures of cognitive performance in 2,096 dementia- and stroke-tree participants who were stratified into three age groups (40-49 years, 50-59 years, 60-82 years), after findings of statistically significant tHcy-by-age interactions for multiple cognitive measures. Regardless of statistical adjustment for age, sex, gender, the vitamin cofactors, and cardiovascular risk factors, statistically significant inverse associations between tHcy and multiple cognitive domains were observed for individuals aged 60 or more years; no such associations were observed for participants aged less than 60 years. The authors concluded that plasma total homocysteine (tHcy) concentrations were associated with deficits in cognitive performance in persons free from dementia and that early preventive interventions to prevent rises in homocysteine may be important.
Seshadri.et al, 2002 showed that elevated homocysteine levels were associated with poor cognition and dementia and may predict cognitive decline. The authors examined a total of 1092 subjects without dementia (667 women and 425 men; mean age, 76 years) from the Framingham Study. They compared the plasma total homocysteine level measured at base line and that measured eight years earlier to assess the risk of newly diagnosed dementia on follow-up. The relative risk of Alzheimer's disease was 1.8 (95 percent confidence interval, 1.3 to 2.5) per

increase of 1 SD at base line and 1.6 (95 percent confidence interval, 1.2 to 2.1) per increase of 1 SD eight years before base line. With a plasma homocysteine level greater than 14 umol per liter, the risk of Alzheimer's disease nearly doubled. Seshadri et al concluded that increased plasma homocysteine level is a strong, independent risk factor for the development of dementia and Alzheimer's disease.
These studies have revealed that high plasma homocysteine may predispose to deficits in cognitive performance in aging persons long before the onset of dementia and that controlling homocysteine levels may be important in slowing or preventing the onset of degenerative mental diseases.

These studies have shown homocysteine is a strong predictor of cognitive decline. As homocysteine levels increase, cognitive function steadily decreases. Most risk factors for cognitive impairment are non-modifiable; however, elevated homocysteine has been shown to be a treatable, risk factor for cognitive impairment.

II-3-7-f Homocysteine and Alzheimer's disease

As just seen, memory complaints in the elderly are associated with a higher-than-normal risk of developing dementia in the future. Most commonly, the type of dementia that patients with MCI are at risk to develop is Alzheimer's disease, though other dementias, such as Vascular Dementia (VaD) or Frontotemporal Dementia (FTD) may occur as well. Morris (2003) reviewed the link between homocysteine and Alzheimer's disease and found that epidemiological studies show associations between hyperhomocysteinaemia and both histologically confirmed AD and disease progression. The data also revealed that dementia in AD was associated with evidence of brain infarcts on autopsy. Thus, hyperhomocysteinaemia and AD could be linked by stroke or microvascular disease. However, given known relations between B-group-vitamin deficiency and both hyperhomocysteinaemia and neurological dysfunction, direct causal mechanisms are also plausible. Serum homocysteine levels were found to be significantly higher and serum folate and vitamin B12 levels were lower in patients with Alzheimer's disease as compared with control (Clarke et al. 1991). Similar results were obtained by Joosten et al (1997) and Miller (1999). Vitamin B12 deficiency is common in the elderly and may be a risk factor for Alzheimer's disease (Regland et al. 1991).

Plasma and serum biochemical markers proposed for Alzheimer disease (AD) are based on pathophysiologic processes such as amyloid plaque formation [amyloid β-protein (Aβ), Aβ autoantibodies, platelet amyloid precursor protein (APP) isoforms], inflammation (cytokines), oxidative stress (vitamin E, isoprostanes), lipid metabolism (apolipoprotein E, 24S-hydroxycholesterol), and vascular disease [homocysteine, lipoprotein (a)]. Most proteins or metabolites evaluated in plasma or serum thus far are, at best, biological correlates of AD: levels are statistically different in AD versus controls in some cohorts, but they lack sensitivity or specificity for diagnosis or for tracking response to therapy. Approaches combining panels of existing biomarkers or surveying the range of proteins in plasma (proteomics) show promise for discovering biomarker profiles that are characteristic of AD, yet distinct from nondemented patients or patients with other forms of dementia (Irizarry, 2004)

III Beneficial effects of citrus on the nervous system

The beneficial effects of citrus compounds on the body, particularly with regard to inflammation were reviewed recently (Cancalon, 2005). As already pointed out only a few studies have been performed on whole citrus fruits or juices but citrus contain a large array of health promoting compounds. Among those, ascorbic acid, folic acid, carotenes, flavonoids and limonoids are the most active. Published results appear to give conflicting results but it should be kept in mind that little if anything is known of the dose-effect relationship of these compounds. Furthermore little is still known of the formation of citrus compounds metabolites in the body, of the distribution of these metabolites in various organs, and finally of their respective pharmacological activity.

Studies have shown that free recall, recognition, and vocabulary were related to levels of antioxidant vitamins and phytochemicals. The levels of these antioxidants were found to be significant predictors of cognitive function (Perrig et al. 1997). A study determined that low folate levels were not only associated with cognitive deficits, but that patients treated with folic acid for 60 days showed a significant improvement in both memory and attention efficiency (Fioravanti et al. 1997). Higher intakes of vitamins A, C, E, and B complex were shown to be significantly related to better performance on abstraction and visio-spatial tests (La Rue et al. 1997). Studies showed that vitamin B6 and folate taken at high dosages reduced blood levels of homocysteine (McCaddon et al., 1998,1002a,b; Miller, 1999). One study revealed that less-than-optimal levels of vitamin B6, B12, and folic acid lead to a deficiency of S-adenosylmethionine (SAMe). SAMe deficiency can cause depression, dementia, or demyelinating myelopathy (a degeneration of the nerves) (Abou-Saleh et al. 1986).

As already mentioned, the brain is a functionally and chemically distinct organ. Fifty percent of brain dry weight is lipids (in contrast to 6-20% for other organs). Brain membranes are more than 80% lipids, mostly unsaturated and are therefore easily oxidizable (Youdim et al ,2000). The brain uses large amounts of energy. With only 2% of the body weight it consumes 20% of the available glucose and oxygen, this high metabolic activity generates radical oxidative species (ROS) that can damage neurons. Contrary to the muscles or the liver, the brain stores very little energy (glycogen). Furthermore, the access to the brain by blood molecules is severely limited by the blood brain barrier (BBB). Substances in the blood that gain rapid entry into the brain include glucose, the main source of energy, certain ions that maintain a proper medium for electrical activity, and oxygen for cellular respiration. Small fat-soluble molecules, like ethanol, pass through the BBB. Therefore the access to the brain is limited but it needs a constant delivery of energy. If glucose and oxygen become unavailable CNS neurons can die very rapidly. As a result, inflammatory markers can easily accumulate in the brain (Aarli, 2003).

III-1 Determination of compounds beneficial to physiological activity

Gilgun-Sherki et al (2001) reviewed the importance of antioxidants for proper brain activity. The authors pointed out that oxidative stress (OS) has been implicated in the pathophysiology of many neurodegenerative diseases and that OS can cause cellular damage and subsequent cell death because the reactive oxygen species (ROS) oxidize vital cellular components such as lipids, proteins, and DNA. They also showed that the brain is exposed throughout life to excitatory amino acids (such as glutamate), whose metabolism produces ROS, thereby promoting excitotoxicity. Antioxidant defense mechanisms include removal of O(2), scavenging of reactive oxygen/nitrogen species or their precursors, inhibition of ROS formation, binding of metal ions needed for the catalysis of ROS generation and up-regulation of endogenous antioxidant defenses. However, the authors reported that since our endogenous antioxidant defenses are not always completely effective, and since exposure to damaging environmental factors is increasing, exogenous antioxidants could be very effective in diminishing the cumulative effects of oxidative damage. Antioxidants of widely varying chemical structures have been investigated as potential therapeutic agents. However, Gilgun-Sherki et al (2001) questioned the therapeutic use of most of these compounds and their metabolites since they do not cross the blood brain barrier (BBB). *The authors concluded that any antioxidant molecules designed as potential neuroprotective treatment in acute or chronic neurological disorders should have the mandatory prerequisite that they can cross the BBB after administration*.

Even at present, this prerequisite has been largely ignored when assessing the beneficial activity of a food and most of the studies have been done in vitro. This is particularly the case for antioxidant potentials, which are determined by chemical analyses like the ORAC method. They provide calculated values without consideration for their physiological properties. An extensive review of polyphenols bioavailabilty was published by Manach et al (2005). Results showed that the biological properties of the metabolites, and specific accumulation in target tissues are the most important factors when the health benefits of phytochemicals are examined. *The authors concluded that the most abundant polyphenols in our diet are not necessarily those that have the best bioavailability profile*. **Reports have shown that the major citrus phytochemicals and vitamins including naringin, hesperidin, tangeretin and particularly ascorbic acid and folate can cross the brain barrier.**

III-2 Reassessment of the mode of action of phytochemicals and vitamins.

Until recently, the accepted view of the mode of action of phytochemicals and vitamins was relatively simple, each compound had a single effect: Polyphenols were antioxidants, ascorbic acid was a vitamin acting mostly as a coenzyme to prevent scurvy. In the last few years, this concept has been challenged and now many compounds are known to have multiple modes of action.

In fact, in a few cases, the original mode of action of some phytochemicals is now believed to be of secondary importance

III-2-1 Polyphenols

Polyphenols have long been known to act as antioxidants and radical scavengers (Chenier, 2005). However the average concentration of these compounds in plasma is very low (1 mM or less) and much lower than those of the endogenous antioxidant uric acid(150–450 mm) (Chewier, 2005; Scalbert et al, 2005). It is difficult to explain that direct scavenging of free radicals by polyphenols is the key mechanism explaining their effects on oxidative stress biomarkers and other risk factors. It is now hypothesized that other properties of these compounds are responsible for a significant proportion of their beneficial effects. More specifically it has been proposed that polyphenols act on various gene modulation. Waddington et al (2004) suggested that the ability of red wine polyphenols to prevent atherosclerosis is independent of any antioxidant action and that they act by limiting arterial lipid deposition inhibition. The authors showed that in mice, red wine polyphenols had no effect on markers of lipid peroxidation or monocyte chemoattractant protein 1 concentrations, but that lipid deposition in the aorta was significantly less in the aorta of mice given red wine than in those not given red wine. This could occur through modulation of smooth muscle cell proliferation or regulation of adhesion molecules.
Similarly, Maniac et al (2005) showed that a major effect of polyphenols in limiting atherosclerosis may be due to the renewal and preservation of blood vessels endothelial cells through mechanisms involved in cell mediated modulation of the redox status such as action on the modulation of redox signaling protein. More generally they proposed that polyphenols act mainly by various gene modulation processes. Mackenzie et al (2004) reported that flavanols can regulate the immune response in part by modulating the oxidant-responsive transcription factor NF-κB.

III-2-2 Vitamin E

Vitamin E is a well known antioxidant but recently Zingg and Azzi (2004) reviewed the non-antioxidant activities of vitamin E. They pointed out that many molecules have the ability to chemically scavenge free radicals and thus act in the test tube as antioxidant, but their main biological function is by acting as hormones, ligands for transcription factors, modulators of enzymatic activities or as structural components. In fact, oxidation of these molecules may impair their biological function, and cellular defense systems exist which protect these molecules from oxidation. Vitamin E is present in plants in 8 different forms with more or less equal antioxidant potential (alpha-, beta-, gamma-, delta-tocopherol/tocotrienols); nevertheless, in higher organisms only alpha-tocopherol is preferentially retained suggesting a specific mechanism for the uptake for this analogue. In the last 20 years, cellular functions of alpha-tocopherol that are independent of its antioxidant/radical scavenging ability have been characterized. At the posttranslational level, alpha-tocopherol inhibits protein kinase C, 5-

lipoxygenase and phospholipase A2 and activates protein phosphatase 2A and diacylglycerol kinase. Some genes (scavenger receptors, alpha-TTP, alpha-tropomyosin, matrix metalloproteinase-19 and collagenase) are modulated by alpha-tocopherol at the transcriptional level. Alpha-tocopherol also inhibits cell proliferation, platelet aggregation and monocyte adhesion. These effects are unrelated to the antioxidant activity of vitamin E, and possibly reflect specific interactions of alpha-tocopherol with enzymes, structural proteins, lipids and transcription factors. Azzi et al (2004) concluded that it seems likely that the non-antioxidant activities of tocopherols represent the main biological reason for the selective retention of alpha-tocopherol in the body, or vice versa, for the metabolic conversion and consequent elimination of the other tocopherols.

III-2-3 Ascorbic acid

In the past, the role of vitamin C was largely limited to an anti scurvy function. It is still this role that dictates the minimal amount of ascorbic acid necessary for human survival. Today, however, ascorbic acid appears to be one of the vitamins with the largest number of modes of action

1) Ascorbic acid acts as a vitamin (vitamin C). Vitamin C is a coenzyme for various enzymes and is required for at least eight intracellular enzymatic reactions. In the nervous system vitamin C is involved in the synthesis of neurotransmitters such as catecholamines (norepinephrine, epinephrine and dopamine) and in the tyrosine metabolism (Cancalon, 2005).

2) A somewhat different role of ascorbic acid is that of a chemical antioxidant. The antioxidant properties of ascorbic acid on the body have been reviewed recently (Cancalon, 2005). Ascorbic acid is an effective antioxidant because it can be oxidized more easily than other molecules. It can donate a hydrogen atom to a free radical molecule, neutralizing the free radical while becoming an ascorbate radical which can be regenerated from its dehydroascorbic acid form. The role of ascorbic acid is particularly important during the oxidative processes. Once a superoxide radical is formed, the enzyme superoxide dismutase catalyzes its conversion to O_2 and H_2O_2 (Galley and Thiollet, 1993). Ascorbate, non-enzymatically, also converts superoxide to H_2O_2 but is oxidized in the process to the ascorbate free radical and dehydroascorbate. As seen previously, the ascorbate free radical and the dehydroascorbate are reduced back to ascorbate either by NADH (catalyzed by semidehydroascorbate reductase and forming NAD) or reduced glutathione (GSH) (catalyzed by dehydroascorbate reductase and forming oxidized glutathione (GSSG)) . Some of the peroxide can be converted to oxygen and water by catalase but most will be destroyed by a glutathione-requiring enzyme system. GSH (catalyzed by glutathione peroxidase) reduces the peroxide to water but in the process is oxidized to GSSG. The resulting GSSG is reduced by NAD(P)H (catalyzed by glutathione reductase).

3) These are also indications that in the brain, ascorbate simultaneously acts as an antioxidant and a modulator of some neurotransmitter activities. Rebec et al (2005) examined the role of ascorbic acid in neurotransmission. The extracellular ascorbate, which may increase by several hundred micromoles in striatum during behavioral activation, directly alters glutamate transmission. The authors monitored strata glutamate transients evoked by electrical stimulation of the cerebral cortex in anesthetized rats tested with varying concentrations of ascorbate (0, 50, 200, and 500 uM). Ascorbate elevated striatal glutamate in a concentration-dependent fashion. Addition of 500 uM ascorbate not only more than doubled basal glutamate levels relative to the ascorbate-free condition, but significantly increased both the magnitude of the electrically evoked glutamate response as well as its subsequent return to baseline. The authors concluded that the level of extracellular ascorbate plays a critical role in regulating corticostriatal glutamate transmission. The authors (Rebec and Pierce, 1994) further reviewed the activities of ascorbate in the brain. Ascorbate accumulates in the brain from the blood supply and remains at a relatively high concentration under widely varying conditions. Although neurons are known to use this vitamin in many different chemical and enzymatic reactions, recent evidence suggests a role for ascorbate in inter neuronal communication. Ascorbate is released from glutamatergic neurons as part of the glutamate reuptake process, in which the high-affinity glutamate transporter exchanges ascorbate for glutamate. This exchange process, which also may occur in glial cells, ensures a relatively high level of extracellular ascorbate in many forebrain regions. Ascorbate release is regulated, at least in part, by dopaminergic mechanisms. Ascorbate also may alter the redox state of the N-methyl-D-aspartate (NMDA) glutamate receptor thus block NMDA-gated channel function. Similarly Grunewald (1993) showed that ascorbic acid acts as an intracellular antioxidant, and as such is neuroprotective. The author showed that a unique role of ascorbate might be as an antioxidant in the brain extracellular microenvironment, where its concentration is modulated by glutamate-ascorbate heteroexchange at glutamate uptake sites. Rice (1999), examined the role played by ascorbate in the interaction between neurons and glia. They showed that on the average the intracellular concentration of ascorbic acid is 10 mM in neurons and 1 mM in glial cells. By contrast the low molecular weight antioxidant, glutathione is somewhat more concentrated in glia than in neurons. The author concluded that data is evidence for the roles of ascorbate as a neuroprotective antioxidant and as a neuromodulator in the CNS. Although in the adrenal medulla, ascorbate is co-released with catecholamines, the release of ascorbate from brain cells is associated principally with the activity of glutamatergic neurons. The author concluded that this phenomenon indicates a possible role of ascorbate as a neuromodulator and a neuroprotective agent in the brain. *These results show intertwined roles of ascorbic acid in the brain as an antioxidant neuroprotector and a modulator of glutamate neurotransmission*.

III-3 Beneficial effects of ascorbic acid on the CNS

III-3-1 Brain uptake of ascorbic acid

Ascorbic acid is highly concentrated in the brain is found at millimolar levels in neurons, but ascorbate does not to cross the blood-brain barrier but instead enters the CNS through the

cerebrospinal fluid (CSF) barrier. In the CSF it can be found in concentrations proportional to dietary intake (Spector, R. and Eells, 1997, Brown et al, 1996). Transport of ascorbic acid across the blood-CSF barrier is mediated by the Na(+)-dependent transporter SVCT2 (Angelow et al ,2003). Lam and Daniel (1986) showed that ascorbic acid passes through the capillaries by a carrier-mediated process but also by simple diffusion. By comparison vitamin E, a fat-soluble vitamin and a powerful antioxidant, does not readily cross the blood-brain barrier nor does it accumulate in the cerebrospinal fluid (Pappert, et al., 1886). Consequently reports have show limited influence of vitamin E on brain degeneration (Fahn,1991,1992)

III-3-2 Ascorbic acid and cognitive functions

Cognitive impairment is a major component of age-related dementing diseases and it has been suggested that it could share the same pathological pathways with neurodegenerative processes and cerebrovascular lesions. The free radical theory of aging could be one of these pathways. Berr (2002) reported that the published relationships between cognitive impairment and antioxidant status argue for a protective effect of antioxidants on cognitive performance. Martin et al (2002) reported that diets rich in fruits and vegetables have been shown to improve human well-being and to significantly delay the development of pathologic processes, including neurodegenerative disorders. They also showed that human trials of varying doses of vitamins E and C, including low, supplemental, and pharmacologic, have revealed that these nutrients may improve immunity, vascular function, and brain performance (Martin et al, 2002). Schmitt-Schillig (2005) pointed out that the brain contains the highest concentrations of ascorbic acid of all body tissues and may exert protective effects on cognitive functions in humans. The role of vitamins C and E on the cognitive function of young and aged mice were examined by Arzi et al (2004). The results indicated a significant improvement in the cognitive function of aged mice while there were no significant changes in young animals.

III-3-3 Ascorbic acid and the elderly

The beneficial effects of vitamin C supplementation may be evenstronger in older people than in the general population since with age some of the ability to absorb ascorbic acid is lost. Salerno-Kennedy and Cashman, (2005) showed that various dietetic factors play a role in cognitive processes. A deficiency of antioxidants such as vitamins C and E, and beta-carotene, as well as nutrition-related disorders like hypercholesterolemia, hypertension, and diabetes, may also have some role in cognitive impairment. *The authors emphasized that these factors can be present for a long time before cognitive impairment becomes evident, and that they could be detected and corrected in a timely manner to prevent or minimize the loss of brain functions.*

III-3-3-a Ascorbic acid bioavailability in the elderly

Reports have shown that vitamin C levels appear to decline in the elderly due mainly to a decline in bioavailibility. The mechanism responsible for vitamin C transport in the gut and all

other tissues is a sodium-dependent vitamin C transporter (SVCT). Two isoforms of this protein, SVCT1 and SVCT2, are known to exist in every mammal, including humans. It has been hypothesized, that the decline in age-related vitamin C levels is due to a loss of vitamin C transport, primarily due to changes in SVCT activity. (Selhub 2000; Fillenbaum et al.2005). *If the low levels of ascorbic acid in the elderly are solely due to transport failure, increasing dietary intakes may not completely resolve the problem.*

III-3-3-b Ascorbic acid and aging brain

Swanson (1993) investigated the effect of aging on extracellular ascorbate concentration in the rat brain. Recordings from the nucleus accumbens in 3-, 6- and 18-month-old Sprague-Dawley rats revealed an age-related decrease in basal extracellular ascorbic acid concentration. The mean ascorbic acid current measured in 18-month-old rats was less than 10% of the current measured in 3-month-old *rats. These results suggest that in the aging brain ascorbic acid is either present in much lower quantities than in younger brains or that the available brain ascorbate is not used properly*.

Muthuvel et al, (2000) revealed a general reduction in the level of ascorbic acid with age leading to an impairment in the quenching of free radicals by antioxidants. This in turn increases the risk of age-associated disorders. Elderly, healthy volunteers of both sexes at different age groups were subjected to vitamin C supplementation. From these observations, the authors concluded that ascorbic acid normalizes lipid peroxidation and partially restores the antioxidant status of the brain.

III-3-4 Ascorbic acid and Alzheimer's disease

Alzheimer's disease (AD) is an irreversible, progressive disorder in which brain cells (neurons) deteriorate, resulting in the loss of cognitive functions, primarily memory, judgment and reasoning, movement coordination, and pattern recognition. In advanced stages of the disease, all memory and mental functioning may be lost. The condition predominantly affects the cerebral cortex and hippocampus, which lose mass and shrink (atrophy) as the disease advances.

Plaques and Tangles

The two most significant physical findings in the cells of brains affected by Alzheimer's disease are neuritic plaques and neurofibrillary tangles. Another significant factor in AD is the greatly reduced presence of acetylcholine in the cerebral cortex. Acetylcholine is necessary for cognitive function.

While some **neuritic plaques**, or patches, are commonly found in brains of elderly people, they appear in excessive numbers in the cerebral cortex of Alzheimer's disease patients. A protein called beta amyloid occupies the center of these plaques. Surrounding the protein are fragments of deteriorating neurons, especially those that produce **acetylcholine (ACh)**, a neurotransmitter essential for processing memory and learning. Neurotransmitters are chemicals that transport information or signals between neurons.

Neurofibrillary tangles (NFTs) are twisted remnants of a protein called tau, which is found inside brain cells and is essential for m aintaining proper cell structure and function. An abnormality in the tau protein disrupts normal cell activity.

There are conflicting reports about the potential role of vitamin antioxidants in preventing and/or slowing the progression of various forms of cognitive impairment including AD. Although several studies show the importance of oxidative stress in the pathogenesis of AD, there are few evidences of the role of free radicals in Mild Cognitive Impairment (MCI). Mecocci (2005) showed that MCI is associated with oxidative stress and of an early occurrence of oxidative processes in the development of dementing disorders of the Alzheimer type. Th e author concluded that since MCI represents a c ondition of increased risk for AD, use of antioxidants could be of importance for prevention cognitive impairment and possibly to the onset of AD.
Maxwell et al (2005) examined data from the Canadian Study of Health and Aging, a population-based, prospective 5-year investigation of the epidemiology of dementia among Canadians more than 65 years old. The primary objective was to examine the association between supplemental use of antioxidant vitamins and subsequent risk of significant cognitive decline among subjects with no evidence of dementia at baseline . The authores explored the relationship between vitamin supplement use and dementia. Subjects reporting a co mbined use of vitamin E an d C supplements and/or multivitamin consumption at baseline were significantly less likely to experience significant cognitive decline during a 5-year follow-up period. The results suggest a possible protective effect for antioxidant vitamins in relation to cognitive decline.

Alzheimer s disease (AD), according to the free radical hypothesis, affects brain regions where free radical damage occurs. Antioxidant nutrients may help to protect these brain regions. Charlton et al (2004) investigated whether plasma vitamin C and E levels are lower in subjects aged 65 a nd older with AD and dementia. Cognitive function was measured using the Mini Mental State Examination
(MMSE) and nutritional status assessed using the Mini Nutritional Assessment (MNA) tool. The control group had significantly higher scores for the MNA, MMSE and Activities of Daily Living, compared with the dementia group. Controls had a significantly higher plasma vitamin C concentration than dementia patients. The dementia groups were more likely to have sub-optimal plasma vitamin C levels despite having similar dietary vitamin C intakes. Plasma vitamin C was positively associated with MMSE score. No difference was found between the groups for either plasma or dietary vitamin E. The authors concluded that plasma vitamin C levels were lower in subjects with dementia compared to controls, which was not explained by their dietary vitamin C intakes. This data supports the free radical theory of oxi dative neuronal damage. Further investigations of whether supplementation with this vitamin may prevent or delay the progression of cognitive decline in patients with AD and senile dementia appears warranted. It should be pointed out however that Fillenbaum et al (2005) showed that vitamin C and E do not slow the onset of Alzheimer disease.
Ascorbic acid seems to slow cognitive impairment that may evolve into AD but it appears that low levels of vitamin C in demented patients may not be improved by diet.

III-3-5 Ascorbic acid and Parkinson's disease

Parkinson's disease is a chronic, progressive neurodegenerative movement disorder. Tremors, rigidity, slow movement (bradykinesia), poor balance, and difficulty walking are characteristic of Parkinson's disease. It results from the degeneration of dopamine-producing nerve cells in the brain, specifically in the substantia nigra and the locus caeruleus. Dopamine is a neurotransmitter that stimulates motor neurons, those nerve cells that control the muscles. When dopamine production is depleted, the motor system nerves are unable to control movement and coordination. Parkinson's disease patients have lost 80% or more of their dopamine-producing cells by the time symptoms appear.

As early as 1991 Fahn showed that antioxidants slow down the progression of Parkinson's disease. In patients who were given large doses of oral vitamin-C and vitamin-E (3000 mg and 3200 IU daily respectively) the progression of the disease was delayed to the point where they needed l-dopa 2.5 years later than a group of patients who were not taking supplements(Fahn,1991,1992). Later research showed that vitamin E in itself does not retard the progression of Parkinson's disease(Parkinson study group,1993). Thus it is likely that it is vitamin C by itself or its combination with vitamin E that was the active component. *As seen previously the beneficial effects of ascorbic acid as compared to vitamin E, is most likely due to the fact that vitamin C is hydrophilic and able to reach the brain while the hydrophobic vitamin E cannot (Pappert et al, 1996). It can be hypothesized that ascorbic acid quenches hydroxyl radicals which have been implicated in dopamine-cell destruction and that vitamin C may be a protector against Parkinson's disease and may help in slowing down the progression of the disease (Brown et al, 1996).*

III-3-6 Ascorbic acid and Huntington's disease

Huntington's disease (HD) is a fatal hereditary disease that destroys neurons in areas of the brain striatum involved in the emotions, intellect, and movement. The course of Huntington's is characterized by jerking uncontrollable movement of the limbs, trunk, and face (chorea); progressive loss of mental abilities; and the development of psychiatric problems. HD is a genetic disease. The genetic mutation that occurs in gene IT-15, located on chromosome 4, alters the Huntington protein. How the mutation of gene IT-15 alters the function of the protein is not well understood.

Rebec et al (2002) studied the R6/2 mouse line which expresses the human gene for Huntington disease. HD mice were less active than controls and showed a relatively restricted range of spontaneous movements. Both the ascorbate and behavioral deficits were evident in 6-week-old HD mice and persisted in all subsequent test sessions through 10 weeks of age. The results are consistent with inadequate antioxidant protection in the HD striatum a forebrain target of HD. The authors further reported (Rebec et al, 2003) that in the striatum of R6/2 mice there is a behavior-related deficit in extracellular ascorbate. Regular injections of ascorbate (300 mg/kg/day, four days/week), beginning at symptom onset, restored the behavior-related release of ascorbate in striatum and also improved behavioral responding. Compared to control, ascorbate treatment significantly attenuated the neurological motor signs of HD without altering overall motor activity. *Ascorbate accumulation in the forebrain and its regulation of striatal*

function may play an active role in limiting or preventing HD but it is a genetic disease and it cannot be cured by dietary supplements

III-3-7 Ascorbic acid and amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS), also called Lou Gehrig's disease, is a progressive neuromuscular disease that causes the destruction of nerve cells in the brain and spinal cord. The principle characteristic in the pathology of amyotrophic lateral sclerosis(ALS) is loss of motor nerve cells in the anterior horns of the spinal cord and in the motor nuclei of the brain stem weakens and eventually destroys motor neurons (components of the nervous system that connect the brain with the skeletal muscles). Skeletal muscles are involved with voluntary movements, such as walking and talking. The motor neurons transmit the command to move from the brain to the skeletal muscles, which respond by contracting.
Proposed mechanisms for amyotrophic lateral sclerosis include altered superoxide dismutase and neurofilament proteins, autoimmune attack, and hyperglutamatergic activity. Kok (1997) proposed that an age-dependent decline in ascorbate availability may trigger the disease. The author reviewed the relationship between ascorbate and ALS and suggested several types of interactions.
1) Superoxide radicals are common substrates for superoxide dismutase and ascorbate.
2) A steady decline in ascorbate plasma levels and cellular availability with age.
3) An association of ascorbate release with motor activity in central nervous system regions.
4) The coupling of brain-cell ascorbate release with glutamate uptake.
5) A role of ascorbate in modulating the N-methyl-D-aspartate receptor activity.
6) The ability of ascorbate to prevent peroxynitrite anion formation.
7) A competition between superoxide dismutase and ascorbate may be a mechanism responsible for the development of ALS and low level of ascorbate may play a part in AMS development

III-3-8 Ascorbic acid and multiple sclerosis

Multiple sclerosis or MS, is a chronic, slowly progressing disease of the brain, the spinal cord, and the optic nerves. MS is an immune system disease, it causes inflammation and, ultimately, destruction of the insulating material, called myelin, which is wrapped around the nerve fibers of the brain and spinal cord. Without myelin, signals transmitted throughout the central nervous system are not sent properly and symptoms develop. Nerve fibers are damaged as well. The most common initial symptom is the sudden loss of vision in one eye and/or a tingling or feeling of numbness in an arm or leg. Weakness in the limb can cause fumbling or an unsteady gait. Other symptoms include mental changes, slurred speech.
lgun-Sherki et al (2004) showed that accumulating data indicate that oxidative stress (OS) plays a major role in the pathogenesis of multiple sclerosis (MS). Reactive oxygen species (ROS), leading to OS, generated in excess primarily by macrophages, have been implicated as mediators of demyelination and axonal damage in both MS and experimental autoimmune encephalomyelitis (EAE), its animal model. ROS cause damages to cellular components such as lipids, proteins and nucleic acids (RNA, DNA), resulting in cell death by necrosis or apoptosis.

In addition, weakened cellular antioxidant defense systems in the central nervous system (CNS) in MS, and its vulnerability to ROS effects may increase damage. Thus, treatment with antioxidants might theoretically prevent propagation of tissue damage and improve both survival and neurological outcomes. Indeed, several experimental studies have been performed to see whether a dietary intake of several antioxidants prevents or reduces the progression of EAE. Although a few antioxidants showed some efficacy in these studies, little information is available on the effect of treatments with such compounds in patients with MS. Well-designed clinical studies using antioxidant intakes, as well as investigations based on larger cohorts studied over a longer period of time, are needed in order to assess whether antioxidant intakes together with other conventional treatments, might be beneficial in treating MS.

Again ascorbic acid appears important in promoting brain health and limiting the development of several neurological diseases. However it seems less likely that it can significantly reverse major ailments once established

III-3-9 Conclusion

Ascorbic acid reaches the brain and is very concentrated in neuronal tissue.

Studies have shown that ascorbic acid has three distinctive functions in the brain:

1) It acts as a vitamin (vitamin C). In the nervous system vitamin C is involved in the synthesis of neurotransmitters such as catecholamines
2) It is involved in the modulation of neurotransmitters
3) It protects the highly lipidic brain tissue from oxidation. This antioxidant activity is of major importance in limiting a large number of brain ailments including.

-Aging: brain deterioration and neuronal death appear very early in life and lead to progressive cognitive impairment. It seems that it is at this level that ascorbic acid is most beneficial.
-Oxidative damages play a role in the development of the major degenerative brain disorders including Alzheimer's, Parkinson's, Huntington's diseases, amyotrophic lateral sclerosis and multiple sclerosis. Although, these diseases have other causes, ascorbic acid was active in limiting their progression but it is unlikely that by itself it can reverse these degenerative precesses.

-It should also be pointed out that an increase in the ascorbate intake may not always be enough to reverse ascorbic acid deficits in the brain since the problem may lie with a deterioration of the transport mechanisms, as seen particularly during aging.

-Any beneficial effects of ascorbic acid on the cardiovascular system in preventing atherosclerosis, strokes, embolisms, high blood pressure will have a beneficial effect on the brain (Cancalon, 2005, Valim and Barros, 2005)

III-4 Neuroprotective effect of flavonoids and polyphenols

In the last few years great attention has been given to health benefits of polyphenols (Scalbert. et al, 2005) The present state of knowledge in that area was recently summarized in a book "Flavonoids in health and disease" by Rice-Evans and Packer, (2003). From these reviews, several points can be made:

1) What is active is not the compound in an ingested food, but the modified metabolites that reach a given target. Most often research has failed to include the biologically relevant metabolites of the flavonoids known to enter the circulation.
The main steps of the bioavailibility process of flavonoids are:

- The modification of the food compounds in the intestine by local enzymes microorganisms (Rice-Evans, 2000; Kuhnle et al, 2000ab; Nemeth et al, 2003, Manach et al, 2003; Gonthier et al.2003; Spencer et al 2003a; Spencer 2003b).
- The absorption of these original and modified chemicals in the upper intestine or the colon.
- Further modifications mainly in the liver (Spencer et al, 2003d)
- Absorption of the final metabolites by the target organ. In the case of the brain ,compounds must be able to cross the blood brain barrier or the cerebrospinal fluid barrier (Johanson, 1998; Abd El Mohsen et al, 2002, Spencer et al, 2004; Hawkins and Davis, 2005).

2) The mode of action of flavonoids is far more complex than originally thought

- The original concept that polyphenols act as simple electron provider is being challenged.

At least part of the antioxidant potential of polyphenols could be due to an indirect activation of specific enzymes (Williams et al, 2004). More specifically, there is an emerging view that *flavonoids, and their in vivo metabolites, do not act as conventional hydrogen-donating antioxidants but exert modulatory actions* at the level of protein kinase and lipid kinase signaling pathways (Schroeter et al, 2001; Schroeter et al, 2003; Spencer 2003bc; Youdim, et al, 2002; Waddington et al, 2004; Dajas et al, 2005)

-In the CNS polyphenols may also act at the neurotransmitter level (Williams, et al 2004 Spencer, 2004)

3) As seen for ascorbic acid, any beneficial effects of polyphenols on the cardiovascular system in preventing atherosclerosis, strokes, embolisms, high blood pressure will have a beneficial effect on the brain (Cancalon, 2005, Valim and Barros, 2005).

III-4-1 Brain bioavailability of flavonoids

As already mentioned, for any compound to have an effect on an organ it must be able to reach it. The first step is for chemicals to cross from the intestine into the blood stream. The biovailibility of polyphenols has been examined by several groups.

Manach et al (2003) studied the nature of the circulating metabolites, the plasma kinetics and the urinary excretion patterns of the flavanones, hesperidin and narirutin of a commercial orange juice providing 444 mg/l hesperidin and 96.4 mg/l narirutin, together with a polyphenol-free breakfast. Flavanones metabolites appeared in plasma three h after juice ingestion, reached a peak between five and seven h, then returned to baseline at 24 h. The peak plasma concentration of hesperetin was 0.46. uMol/l and 1.28 uMol/l after 0.5 and one l intake, respectively. It was lower for naringenin: 0.20 uMol/l after the one l dose. The circulating forms of hesperetin were glucuronides (87%) and sulphoglucuronides (13%). For both flavanones, the urinary excretion was nearly complete 24 h after the orange juice ingestion. The relative urinary excretion was similar for hesperetin and naringenin and did not depend on the dose: values ranged from 4.1 to 7.9% of the intake. *The authors concluded that moderate or high consumption of orange juice flavanones may represent an important part of the pool of total polyphenols present in the blood plasma.*

Once in the blood stream compounds have to be able to reach the brain. Youdim et al (2003) examined the ability of dietary flavonoids, and their metabolites, to enter the brain endothelium and cross the blood-brain barrier (BBB) using in vitro models, brain endothelial cell lines and ECV304 monolayers co-cultured with C6 glioma cells. The authors reported that the citrus flavonoids, hesperetin, naringenin and their in vivo metabolites, as well as the dietary anthocyanins are taken up by two brain endothelial cell lines from mouse (b.END5) and rat (RBE4). In both cell types, uptake of hesperetin and naringenin was greatest, increasing significantly with time and as a function of concentration. They showed a high apparent permeability of the citrus flavonoids, hesperetin and naringenin, and their relevant in vivo metabolites across the in vitro BBB model (apical to basolateral) relative to their more polar glucuronidated conjugates. The results demonstrate that flavonoids and some metabolites are able to traverse the BBB and that the potential for permeation is consistent with compound lipophilicity. (Youdim, 2003). The potential for BBB permeation is dependent on the compound's lipophilicity and the activity of efflux transporters, such as P-glycoprotein (Pgp) in the BBB (Youdim et al, 2004). Inhibitory and stimulatory effects of quercetin on Pgp activity have been found in vitro and in vivo, recently (Mitsunaga, 2000; Weber et al, 2004). The exact mechanism, however, by which flavonoids modulate P-gp activity, is still unclear. Nonetheless, the ability of flavonoids to enter the brain has been confirmed by their identification in brain materials. Abd El Mohsen et al. (2002) reported the presence of picomolar concentrations of epicatechin glucuronide and 3. -O-methyl epicatechin glucuronide in the brain of rats after oral supplementation with epicatechin (100 mg/kg body weight) for 1, 5 or 10 days. Similarly, naringenin and hesperetin have been detected in brain tissue after intravenous administration (Peng et al, 1998; Tsai et al 2000). Following chronic oral administration, significant levels of

tangeretin were detected in the hypothalamus, striatum and hippocampus of 6-hydroxydopamine lesion rat models (3.88, 2.36 a nd 2.00 ng/mg, respectively). (Juergenliemk, 2003. Dakta et al, 2001)
It can be concluded that most citrus flavonoids or a large number of their metabolites can cross the BBB and are able to reach the CNS.

III-4- 2 Mode of action of polyphenols on the CNS

As already seen, it is generally believed that oxidative stress is a major cause of neuronal loss in neurodegenerative diseases and during age-associated cognitive decline (Smith et al, 1999. Floyd , 1999 Schroeter et al, 2001). Flavonoids have been proposed to play a useful role in protecting the central nervous system against oxidative and excitotoxic stress. Recent studies highlight a role with respect to their potential neuroprotective actions, in particular toward deficits commonly observed with aging, such as reduced performance of cognitive, memory and learning tasks. These neurological functions can be influenced by supplementation of dietary flavonoids. Oxidative stress has been associated with neuronal loss in neurodegenerative diseases and during age-associated cognitive decline (Williams et al, 2004). Using oxidized low-density lipoprotein (oxLDL) as the oxidative insult, Schroeter et al (2001) investigated the mechanism of neurotoxicity and attempted to identify possible sites of action of epicatechin and kaempferol, in cultured primary neurons. A major in vivo metabolite of epicatechin, 3'-O-methyl-epicatechin was as effective as epicatechin in protecting neurons. *The authors concluded that dietary flavonoids might have potential as protective agents against neuronal apoptosis through selective actions within stress-activated cellular responses.*
The most familiar mode of action of polyphenols is the conventional electron transfer antioxidant as shown by a large number of in vitro studies of food antioxidant potential (Huang et al, 2005). Scalbert et al (2005) showed that so far the effects of polyphenols on bio markers of oxidative stress, lipoma (benign tumor of fatty tissues) and inflammation appear inconclusive. They postulated that a direct scavenging of free radicals by polyphenols may not be the key mechanism explaining their effects on oxidative stress biomarkers and cardiovascular risk factors. As mentioned previously they pointed out that polyphenol concentrations in plasma are low (1 mM or less)and much lower than those of uric acid, an endogenous antioxidant (150–450 mM). The extensive conjugation of polyphenols to glucuronic acid and sulfate groups in the tissues may markedly decrease their antioxidant potential. According to the authors, a major effect of polyphenols in limiting atherosclerosis is due to the renewal and preservation of blood vessels endothelial cells and mechanisms involved in cell mediated modulation of the redox status such as action on the modulation of redox signaling protein. *More generally they propose that polyphenols act on various gene modulation processes*
Youdim et al (2002) also pointed out the importance of studying the biologically relevant flavonoid metabolites since they are the most likely to be bioavailable to cells and tissues. *They suggested that the mechanisms of action of flavonoids and their bioavailable metabolites in cytoprotection against oxidative stress, which may be independent of conventional antioxidant reducing activities, and that the in vivo metabolites, do not act as conventional antioxidants but may exert modulatory actions in cells*. Flavonoids and their metabolites, have been reported to

act at phosphoinositide 3-kinase (PI 3-kinase),Akt/protein kinase B (Akt/PKB), tyrosine kinases, protein kinase C (PKC), and mitogen activateprotein kinase (MAP kinase) signaling cascades. Inhibitory or stimulatory actions at these pathways are likely to affect cellular function profoundly by altering the phosphorylation state of target molecules and by modulating gene expression. *Williams et al (2004) concluded that the understanding of the mechanisms of action of flavonoids, either as antioxidants or modulators of cell signaling, is key to the evaluation of these biomolecules as inhibitors of neurodegeneration*.

III-4-3 Polyphenols and the aging brain

III-4-3-a The aging brain

As the elderly population increases, the prevalence of aging-related brain diseases is likely to increase. Even though the cause of neurodegenerative diseases is not completely clarified, oxidative stress seems to have an important contribution to brain aging and neurodegenerative diseases. The brain is the most susceptible organ to oxidative damage due to its high oxygen consumption and high lipid content. High oxygen consumption is linked to leakage of electrons along the respiratory chain with subsequent radical formation. A second reason is the high amount of polyunsaturated fatty acids (PUFAs) present in neuronal membranes (Floyd, 1999)10). These PUFAs are especially prone to undergo lipid peroxidation reactions resulting in the formation of cytotoxic aldehydes such as malondialdehyde (MDA) and 4-hydroxynonenal (HNE) (Smith et al 1999). Furthermore, the insufficient supply of nutrients, caused by an impaired cerebromicrovascular function, contributes to the diminished neuronal capacity in the aging brain (Zarchin et al 2002). Oxidative stress, deprivation of essential nutrients as well as age related and oxidative-stress induced damage hampers the activity of mitochondria, the key organelles for ATP production. Reduced energy supply will lead to reduced neuronal and finally brain function including impaired cognition. Elevated oxygen consumption may lead to oxidative stress as reactive oxygen (ROS) and nitrogen species (RNS). Oxidative stress arises from an imbalance between cellular ROS/RNS production and the ability of cells to defend themselves against this stress. ROS and RNS cause cellular damage by oxidizing proteins, membrane lipids, and DNA. Normal cells have various mechanisms to protect themselves against oxidative and nitrosative attacks. Beside glutathione (GSH), vitamins C and E, the major cellular defense consists of antioxidative enzymes and bioactive molecules such as flavonoids. (Finch, 2003, Rutten et al 2003)
Galli et al (2002) concluded that increasing dietary intake of fruits and vegetables high in antioxidant activity may be an important component of a healthy living strategy designed to maximize neuronal and cognitive functioning into old age.

III-4-3-b Neuroprotection of flavonoids

Some of the neuroprotective effects of flavonoids were reviewed by Schmitt-Schillig et al (2005) (Tables I,II). They can be divided in several categories that will be examined:

Table 1: Neuroprotective effects of flavonoids and flavonoid-rich extracts

Source	Treatment	Beneficial properties
Strawberry extract, Spinach extracts, Vitamin E	9.5 g/kg 6.4 g/kg 500 IU/kg	Reduction of striatal ROS level in 6 month old rats after two weeks of supplementation
Crataegus flavonoid extract	0.5 mg/mL 2.5mg/mL	Protection against ischemia/reperfusion injury-caused delayed cell death in gerbils after 15 days of extract application through drinking water
Grape polyphenol extract	5 mg/dl	Prevention of ethanol-induced dopamine uptake activity decrease in young rats after grape polyphenol supplementation for 2 month
Catechin	3.4 µM 34 µM 340 µM	Reduction of lipid peroxidation in rat embryonic mesencephalic cell cultures after 6-OHDA-induced neuronal cell death
Resveratrol	30 mg/kg BW	Reduction of kainic acid-induced cell death in adult rat brains after i.p. administration for 5 days
(-)-epigallocatechine gallate	10 µM 25 or 50 m/kg i.p.	Attenuation of MDA formation in rat embryonic hippocampal neurons Reduction of postischemic brain edema in gerbils

(from Schmitt-Schillig et al 2005)

Direct antioxidant activities

Flavonoids possess a highly reactive hydroxyl group that gets oxidized by electron-donation, thus stabilizing a radical to a less reactive molecule this attenuates the deleterious effect of free radicals, reactive oxygen species (ROS) and reactive nitrogen species (RNS. (Cancalon, 2005). This was demonstrated in PC12 cells, these cells are derived from chromaffin cells of the suprarenal medulla and can differentiate into sympathetic neurons in the presence of nerve growth factor (NGF). Quercetin and some structurally related flavonoids, were shown by Dajas et al (2003) to have a cytoprotective effect on PC12 cells exposed to H_2O_2 . In these cells, the

addition of flavonoids or flavonoid-rich extracts suppressed the hydroperoxide induced increase in ROS, thus improving cell survival (Sasaki et al 2003, Horakova 2003). Matsuoka et al. (1995) showed that catechins protect cultured newborn-mouse cerebral nerve cells from ROS-induced cell death after addition of glucose oxidase. The authors suggested that catechins decrease ROS induced cytotoxicity through intracellular scavenging. Another beneficial effect resulting of this scavenging activity is to decrease oxidation of membrane lipids. For example, gossypin dramatically inhibited lipid peroxidation initiated by Fe^{2+} and ascorbic acid in rat brain homogenates (Abd El Mohsen, 2002).

Antioxidant effects of flavonoids have also been detected in vivo. The volume of the lipid peroxidation marker lipofuscin was significantly reduced in the brain of adult rats chronically exposed to ethanol (da Silva 2004). These findings are supported by data from Zhang et al. (2004) who reported a decrease of ROS production in brain homogenates in a Mongolian gerbil stroke model after Crataegus flavonoid supplementation. Also significant ROS reduction in striatum of aged rats was found by Joseph et al.(1999) after eight weeks of supplementing diet with either blueberry, strawberry or spinach extracts.
Neuroprotective effects of a natural antioxidant tangeretin, a citrus flavonoid, were elucidated in the 6-hydroxydopamine (6-OHDA) lesion rat model of Parkinson's disease (PD), after bioavailability studies. Following the chronic oral administration (10 mg/kg/day for 28 days), significant levels of tangeretin were detected in the hypothalamus, striatum and hippocampus (3.88, 2.36 and 2.00 ng/mg, respectively). The levels in the liver and plasma were 0.59 ng/mg and 0.11 ng/ml respectively. Unilateral infusion of the dopaminergic neurotoxin, 6-hydroxydopamine (6-OHDA; 8 ug), onto medial forebrain bundle significantly reduced the number of tyrosine hydroxylase positive (TH+) cells in the substantia nigra and decreased striatal dopamine content in the vehicle treated rats. Sub-chronic treatment of the rats with high doses of tangeretin (20 mg/kg/day for four days; p.o.) before 6-OHDA lesioning markedly reduced the loss of both TH+ cells and striatal dopamine content. These studies, for the first time, give evidence that tangeretin crosses the blood-brain barrier. The significant protection of striato-nigral integrity and functionality by tangeretin suggests its potential use as a neuroprotective agent. (Datla et al 2001). Zbarsky et (2005) confirmed the neuroprotective properties of the naringenin in a 6-OHDA model of Parkinson's disease.

Indirect antioxidant activities

Hodnick et al. (1994), compared the ability of flavonoids to limit NADH-oxidase (a key enzyme in mitochondrial respiration) inhibition. The order of potency was robinetin, rhamnetin, eupatorin, baicalein, 7,8- dihydroxyflavone and norwogonin. Cos et al. (1998) reported that flavones have higher xanthine oxidase inhibitory activity than flavonoles. The authors further showed that hydroxyl groups at C-3 are essential for high superoxide scavenging. Recently, Suzuki et al. (2004) administered catechin extract to rats for five days before und during middle cerebral artery occlusion and reperfusion to examine their protective effects on various deteriorative processes following stroke. Catechins significantly limit neurological deficits observed after reperfusion by the inhibition of inducible nitric oxide synthetase (iNOS)

expression and infiltration of neutrophiles. In addition, the formation of peroxynitrite was found to be decreased due to the potent radical scavenging properties of catechins.

Anti-inflammatory activities

Many flavonoids are known to possess anti-inflammatory properties (Cancalon, 2005). Several mechanisms for the anti-inflammatory effect of flavonoids in the brain have been proposed. Lee et al (2003) showed that the flavonoid Wogonin inhibited microglial inflammatory activity and reduced their cytotoxicity. Wogonin diminished lipopolysaccharide-induced tumor necrosis factor-alpha (TNF-alpha), interleukin-1beta, and nitric oxide (NO) production in vitro. In animal models, Wogonin conferred neuroprotection by attenuating the death of hippocampal neurons and the neuroprotective effect was associated with inhibition of the inflammatory activation of microglia. The flavonoid Baicalein, showed anti-inflammatory properties on dopaminergic neurons by limiting lipopolysaccharide-induced damage to dopaminergic neurons (Li et al, 2004). Youdim et al (2002b) showed that TNFalpha induced damage to human microvascular endothelial cells. Polyphenols from both berries were able to localize intoendothelial cells subsequently reducing endothelial cells vulnerability to increased oxidative stress at both the membrane and cytosol level. Furthermore, berry polyphenols also reduced TNFalpha induced up-regulation of various inflammatory mediators (IL-8, MCP-1 and ICAM-1) involved in the recruitment of leukocytes to sites of damage or inflammation along the endothelium. They concluded that, polyphenols isolated from both blueberry and cranberry were able to afford protection to endothelial cells against stressor induced up-regulation of oxidative and inflammatory insults. Quercetin, catechin, chrysin, puerarin, naringenin, and genestein protected mesencephalic cultures from injury by 1-methyl-4-phenylpyridinium (MPP(+)) which has a selective toxicity to dopaminergic neurons. Similarly, catechin reduced cellular injury induced by hydrogen peroxide (Mercer et al, 2005). The flavonoid silymarin. was also shown to have anti-inflammatory properties. It exerts neuroprotective effects against lipopolysaccharide-induced neurotoxicity in mesencephalic mixed neuron-glia cultures (Wang et al 2002).

Protection against b-amyloid peptide neurotoxicity

The neuronal pathology of Alzheimer's disease is characterized by intracellular neurofilament tangles and the extracellular deposition of b-amyloid peptide (Ab) as senile plaques (Fig. 17) (Selkoe, 2001). The pathological effects of Ab have been characterized by their oxidative stress-inducing properties (Varadarajan, 2000). Wang et al. (2001) investigated in cortical neurons the effect of several flavonoids on Ab-induced cytotoxicity. Pre-incubation with 50 μM of kaempferol, apigenin, luteolin, and quercetin significantly lowered the Ab-mediated increase in ROS production. Furthermore, apigenin and kaempferol - but not quercetin decreased Ab-induced neuronal death by alleviating the release of cytochrome c and the activation of the caspase cascade. Moreover, quercetin potentized the protective effect of apigenin. Neuroprotective effects of green tea and soy flavonoids due to their ability to scavenge Ab-induced ROS have also been shown by Choi et al. (2001) and Zeng et al. (2004), respectively. An additional explanation for the neuroprotective activity of flavonoids has been presented,

recently. Myricetin, morin, and quercetin dose-dependently not only inhibited the formation and extension of Ab fibrils, but also destabilized preformed Ab fibrils (Ono et al 2003). Bastianetto et al., showed that flavonoids-rich Ginkgo biloba and red wine extracts are able to significantly protect hippocampal cells against Ab peptides- and/or oxidative stress-induced toxicities (Bastianetto and Quirion, 2004).

Improvement of cognition in aging and dementia

Table 2: Cognition improvement properties of flavonoids and flavonoid-rich extracts

Source	Treatment	Beneficial properties
Apigenin-7-glucoside Quercetin	5-20 mg/kg i.p. 25-100 mg/kg i.p.	Reduction of aging-induced impairment of Passive Avoidance Performance in aged mice after chronic administration for 7 days i.p.
DHA and Catechins	1.5 % 0,5 %	Enhancement of Maze behaviour in adult and aged mice after 3.5 month intake of DHA and catechin supplemented 5 % lard diet
Green tea catechins	0,02 % in tab water	Suppressive effect on cognitive dysfunction markers (passive avoidance and Y maze test) in accelerated senescence mice
Strawberry extract Spinach extract Blueberry extract	14.8 g/kg 9.1 g/kg 18.6 g/kg	Reversal of age-related declines in several cognitive and motor behavioural deficits in aged male rats after 8 weeks of extract intake
Quercetin	10-50 mg/kg	Amelioration of cognitive performance in aged mice after chronic quercetin intake for 24 days measured as retention performance in passive avoidance and elevated plus-maze test

(from Schmitt-Schillig et al 2005)

A substantial number of studies about the effect of flavonoids or flavonoids-rich extracts on cognitive function in aged animals have been published (Table II). Patil et al. (2003) reported for aged, but not young mice, significant effects of quercetin supplementation on the step-through and transfer latency in the passive avoidance maze test and elevated plus-maze task. Similarly, the learning time of aged mice supplied with catechin-enriched tap water was improved (Unno et al 2004). Latter results are supported by Shirai and Suzuki (2004) who fed adult and old mice

with diets containing catechin. Time needed and distance traveled to reach the maze exit and the number of times strayed into blind alleys were significantly improved in the treated group. An extract of purple sweet potato anthocyanin markedly enhanced cognitive performance, assessed by passive avoidance test in ethanol-treated mice (Cho et al, 2003).
There are few studies showing significant effects of flavonoids on cognitive deficits in aged humans or demented patients. One of the few reports (Table III, from Schmitt-Schillig et al 2005) indicates that the development of dementia might be slowed down by flavonoid rich diet. The Paquid Study was done in 1991-1996 in south-west France. The authors concluded that the relative risk of incident dementia was inversely related to the mean antioxidant flavonoid intake during the last five years as determined by a questionnaire. After adjustment to age, gender, education, weight and vitamin C intake, the relative risk of dementia for the highest flavonoid intake percentile was 0.49.

Table 3: Effects of flavonoid intake on Dementia (Paquid Study, France) (1)

Study design	Flavonoid consumption (mg/day)	Age-adjusted model		
1367 subjects		RR	95 % CI	*p*-value
	< 11.5 mg	1.00	-	-
	11.5, 16.2	0.50	0.27 – 0.94	0.03
	> 16.2 mg	0.59	0.33 – 1.06	0.08

Authors investigated whether flavonoid intake could be associated with a lower incidence of dementia in a cohort study of over 1300 subjects above 65 years of age. A questionnaire was used to evaluate their intake of flavonoids and subjects were followed-up for 5 years. Relative risk (RR) of dementia according to tertiles of flavonoid intake was estimated using a Cox model.
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It can be concluded that flavonoids seem to be able to slow cognitive impairment by a variety of mechanisms, including direct antioxidant activity, indirect antioxidant activity through enzyme modulation. Flavonoids also show anti-inflammatory activity by limiting the reaction of microglias. In the case of Alzheimer's disease flavonoids may slow b-amyloid peptide and fibril formation. In some cases direct effect of flavonoid on memory and cognition was shown in animals and humans.

III-5 Beneficial effects of Folic acid

Molloy and Scott (2001) summarized recent findings on the beneficial effects of folate. Studies in the past decade have demonstrated that low or inadequate folate status may contribute to congenital malformations and the development of chronic disease in later life. Th ere is conclusive evidence that maternal periconceptional supplementation with folic acid prevents the majority of neural tube defects, probably by overcoming one or more genetically inherited metabolic blocks in folate dependent enzymes and mostly by promoting DNA synthesis

There is also evidence indicating that elevated plasma homocysteine may be an independent risk factor for heart disease and stroke. The contribution of folate to cancer risk is not well defined although there is reasonable evidence to implicate low folate status in the specific case of colorectal cancer. Various publications have also shown that many mental disorders including Alzheimer's Disease can be associated with low folate levels or elevated plasma homocysteine. While a correlation appears to exist, it is hard to determine if there is a cause effect relationship.

III-5-1 Brain bioavailability of folate

As mentioned previously a drug or chemical can only affect an organ if it is able to reach it. The transport mechanisms for folate in mammalian cells--their molecular identities and organization, tissue expression, regulation, structures, and their kinetic and thermodynamic properties were examined by Matherly and Goldman (2003). Best characterized is the reduced folate carrier, a member of the SLC19 family of facilitative carriers. But other facilitative organic anion carriers (SLC21), largely expressed in epithelial tissues, transport folate as well. In addition to these bi-directional carrier systems are the membrane-localized folate receptors alpha and beta, that mediate folate uptake unidirectionally into cells via an endocytotic process. There are also several transporters, typified by the family of multidrug resistance-associated proteins, that unidirectionally export folate from cells.

The brain is relatively resistant to folic acid deficiency, indicating specialized transport systems may exist for this vitamin localized within the blood-brain barrier (BBB). Wu and Pardridge (1999) studied the BBB transport of [3 H]-methyltetrahydrofolic acid (MTFA) in vivo and in isolated human brain capillaries in vitro. A time- and temperature- dependent uptake of [3 H]-MTFA in human brain capillaries was observed. The uptake of [3H]-MAFA by either rat brain in vivo or by human brain capillaries in vitro was equally inhibited by 10 uM concentrations of either unlabeled MAFA or unlabeled folic acid. The authors concluded that a saturable transport system exists at the BBB for folic acid derivatives and since this transport is equally inhibited by either folic acid or MTFA, it is inferred that this transport system is the folic acid receptor.

Botez and Bachevalier (1981) examined the rise in cerebrospinal fluid (CSF) folate in patients with low CSF folate levels. A significant increase in CSF folate values was reported in patients with folate-responsive neurological disorders, after replacement therapy, was higher as compared to control. The authors concluded that there is no blood-brain barrier for folate because the lower the CSF folate level was, the more rapid and spectacular the increase in CSF folate after replacement therapy.
These data confirm that folate can reach the brain through the BB and CSF barriers.

III-5-2 Brain activity and folate

Many studies have been performed on the effects of folate on the brain, many of them however examined simultaneously folate and B12 and in some cases it is difficult to assess the specific influence of each compound. The central nervous system requires a constant supply of B vitamins that participate in the one-carbon metabolism (folate, vitamin B12, and vitamin B6).

Deficiency of or congenital defects in the enzymes involved in these pathways are associated with severe impairment of brain function. Folate and vitamin B12 are required both in the methylation of homcysteine to methionine and in the synthesis of S-adenosylmethionine (SAM). SAM is involved in numerous methylation reactions involving proteins, phospholipids, DNA, and neurotransmitter metabolism. It has been proposed that a defect in methylation processes is central to the biochemical basis of the neuropsychiatric manifestations of these vitamin deficiencies. As a consequence both folate and vitamin B12 deficiency may cause similar neurological depression (Coppen and Bolander-Gouaille, 2005; Williams et al, 2005)dementia (Lipton et al, 1997; Weir et al 1998), and a demyelinating myelopathy.(Surtees et al1991). Although severe vitamin deficiencies and congenital defects are rare, milder subclinical vitamin deficiencies are common in the elderly. (Slhuh et al, 2000; Nilsson-Ehle et al 11998; Bottiglien et al (2001); Wang et al.2001)

Since these studies examining various compounds simultaneously, several questions need to be clarified, particularly concerning the specific role of folate and vitamin B12 and homcysteine on the nervous system.

1. What is the role of folate, vitamin B12, and homocysteine in neurological practice?
2. What is the intrinsic relationship between folate and vitamin B12?
3. Is vitamin B12 alone a sufficient, causative factor for the onset of neuropsychiatric symptoms?
4. What is the independent role of folate?
5. What is the isolated role of homocysteine?

Moretti et al, 2004 reviewed the literature and summarized the evidence relating to these questions. Relevant articles were identified from a search of the Cochrane Dementia and Cognitive Improvement Group's Specialized Register. What emerges from their analysis is that vitamin B12 and folate can cause neuropsychiatric disturbances by:

1) Directly, through two functions: nucleic acid synthesis and the methylation reactions.
2) Indirectly, due to their deficiency which causes SAM-mediated methylation reactions' inhibition by its product S-adenosylhomocysteine (SAH)
3) Also indirectly, through the related toxic effects of homcysteine which causes direct damage to the vascular endothelium and inhibition of N-methyl-D-aspartate receptors .

Quadri et al (2004,2005) showed that patients with mild cognitive impairment and dementia had significantly lower serum folate levels than control groups. They further showed that patients whose folate levels were in the lowest third of all subjects had three times the risk of mild cognitive impairment than those whose levels were in the top third. Subjects with dementia also had higher homocysteine levels, while no difference was found for vitamin B12 levels. Elevated homocysteine levels were found to exist in a greater proportion of subjects with increased disease severity, being found in 31 percent of the controls, 42 percent of those mild cognitive impairment, and 56 percent of those with dementia. The results may suggest that low folate levels rather than B12 may be an early risk factor for mild cognitive impairment which in turn is a strong risk factor for the development of dementia or Alzheimer's disease.

D'Anci and Rosenberg (2004) reviewed recent studies on the link between folate and brain

function in the elderly and reported that subclinical levels of folate inadequacy can have significant negative impacts on health in older individuals. Serum and red blood cell folate levels are associated with depression in younger individuals, but the relationship is less clear in older people. However, folate status does predict response to antidepressant treatment in older individuals. Cognitive decline and some forms of dementia, including Alzheimer's disease, are associated with lower folate levels. The authors suggested supplementation with folic acid may provide some cognitive benefits.

More recently, degrees of folate inadequacy, not severe enough to produce anaemia, have been found to be associated with high blood levels of homocysteine. A study of 126 patients, including 30 with Alzheimer's disease, found that the levels of folate in the cerebral spinal fluid (CSF) were lowest in late-onset Alzheimer's disease patients (Serot et al. 2001).
Whalley et al (2003) studied 82 non-demented old people and, using MRI, derived measures of grey and white matter and intracranial volumes. Controlling for sex and intracranial volume, they related grey and white matter volumes to plasma concentrations of vitamins C, B12, folate, homocysteine, cholesterol, triglycerides, high density and low density lipoproteins. The results showed that lower grey matter volume was associated with lower plasma vitamin C and higher homocysteine, cholesterol and LDL. These data are consistent with the putative benefits of dietary vitamin C and folate intakes and the role of cholesterol in age related neurodegeneration. Similarly, thirty patients with mild cognitive impairment and an increased homocysteine level in serum were examined by Lehmann et al (2003). They were supplemented orally with a high dose of a vitamin B12-B6-folate combination for 270 days. When treated with a vitamin B12-B6-folate combination, patients with mild cognitive impairment and hyperhomocysteinaemia appear to improve their blood-brain barrier function. They may also have stabilized their cognitive status.

Coppen and Bolander-Gouaille (2005) reviewed the findings in major depression of a low plasma and red cell folate, but also of low vitamin B12 status. Low plasma or serum folate has also been found in patients with recurrent mood disorders treated by lithium. A link between depression and low folate has similarly been found in patients with alcoholism. However, in populations with diets rich in folate, patients with major depression, have high serum folate concentrations. Low folate levels are furthermore linked to a poor response to antidepressants, and treatment with folic acid is shown to improve response to antidepressants. Increased homocysteine levels are found in depressive patients. In a large population study from Norway increased plasma homocysteine was associated with increased risk of depression but not anxiety. There is now substantial evidence of a common decrease in serum/red blood cell folate, serum vitamin B12 and an increase in plasma homocysteine in depression. The authors suggested that oral doses of both folic acid (800 ug daily) and vitamin B12 (1 mg daily) should be tried to improve treatment outcomes of depression.

Negative reports were also published by Malouf et al (2003). The authors found no beneficial effect of 750 ug of folic acid per day on measures of cognition or mood in older healthy women. In patients with mild to moderate cognitive decline and different forms of dementia there was no benefit from folic acid on measures of cognition or mood. However, folic acid plus vitamin B12

were effective in reducing the serum homocysteine concentrations. Williams et al (2005) examined the veracity of reports suggesting that low folate status may be detrimental to mood and associated with depleted cerebrospinal fluid levels of the neurotransmitter serotonin (5-hydroxytryptamine; 5-HT). A placebo-controlled trial was carried out to determine the effect of folic acid supplementation (100 ug for six weeks followed by 200 ug for a further six weeks) upon subjective mood (Positive and Negative Affect Schedule) and biochemical markers of mood (5-HT) in healthy males. Blood samples were obtained at baseline (week 0) and during the intervention at week six and week 12. Subjective mood assessments were obtained at week zero and week 12. The results showed an increase in serum and erythrocyte folate concentrations and a corresponding decrease in plasma homocysteine in response to the folic acid intervention. Neither subjective mood nor 5-HT levels, however, were significantly altered in response to the change in folate status. Folic acid given at physiological doses did not appear to improve the mood of healthy folate-replete individuals over a 12-week period

Conversely, several studies suggest that SAMe may have an anti-depressant effect, and further studies are indicated. SAMe may exert a beneficial effect selectively on endogenous rather than neurotic depression. SAMe crosses the blood-brain barrier (Carney et al.1987). SAMe is involved in several central enzyme pathways relating to transmethylation and folate and monoamine metabolism as well as in membrane function and neuro-transmission. The neuropharmacology of SAMe's effect on mood and the switch mechanism has yet to be fully explored. The actions of SAMe on the dopaminergic system are as yet unclear. SAMe is a physiologic substance that is non-toxic and relatively free of severe side effects (with the exception of mania, which may be a manifestation of the basic mood disorder.

It can be concluded that folate is involved in the one-carbon cycle which plays an essential role in many physiological processes by providing carbon units to biochemical reactions. Besides folate, vitamins B12, B6 and several enzymes are part of the carbon transfer processes. It has been somewhat difficult to differentiate the individual effects of each of these components. Genetic diseases can affect some of the enzymes. The detrimental effects of low levels of B12 and folate are related and difficult to separate. Furthermore, they can be due to a lack of dietary intake or to poor absorption.

Besides a lack of proper biochemical methylation of many reactions, misfunction of the cycle will result in the accumulation of homocysteine. This amino acid is by itself cytotoxic and can have negative effects on many organs. High levels of homocyteine have been shown to be associated with many brain disorders, but it has not conclusively been proven that the amino acid is a major cause of brain degenerative aliments.

However despite the lack of absolute proof, studies appear to show that folate may have a beneficial effect in slowing the progress of mild cognitive impairment leading to AD.

III-6 Beneficial effects of carotenoids

Carotenoids are natural pigments which are synthesized by plants and are responsible for the colors of various fruits and vegetables. Beta-carotene and others carotenoids have antioxidant properties in vitro and in animal models. Mixtures of carotenoids or associations with others antioxidants (vitamin E) can increase their activity against free radicals. Epidemiologic studies have shown an inverse relationship between presence of various cancers and dietary carotenoids or blood carotenoid levels (Cancalon, 2005). It appears that carotenoids (including beta-carotene) can promote health when took at dietary levels, but may have adverse effects when taken in high doses by subjects who smoke or who have been exposed to asbestos (Pavia and Russell, 1999).

The carotenoids that have been most studied are beta-carotene, lycopene, lutein, and zeaxanthin. In part, the beneficial effects of carotenoids are thought to be due to their role as antioxidants. Beta-carotene may have added benefits due its ability to be converted to vitamin A. Furthermore, lutein and zeaxanthin may be protective in eye disease because they absorb damaging blue light that enters the eye. (Johnson 2002)

III-6-1 Carotenoid and the nervous system

Studies on the uptake of carotenoids by the brain have been very limited. Recently, Craft et al (2004) examined the presence of the major carotenoids, tocopherols, and retinol in frontal and occipital regions of human brain. Ten samples of brain tissue from frontal lobe cortex and occipital cortex of five cadavers were examined. Sections were dissected into gray and white matter, extracted with organic solvents, and analyzed by HPLC. The authors found that at least 16 carotenoids, three tocopherols, and retinol were present in human brain. Major carotenoids were identified as lutein, zeaxanthin, anhydrolutein, alpha- cryptoxanthin, beta- cryptoxanthin, alpha-carotene, cis- and trans-betacarotene, and cis- and trans-lycopene. Xanthophylls (oxygenated carotenoids) accounted for 66-77% of total carotenoids in all brain regions examined. Similar to neural retina, the ratio of zeaxanthin to lutein was high and these two xanthophylls were significantly correlated. The tocopherol isomers occurred in the brain over a wider range of mean concentrations (0.11-17.9 nMol/g) than either retinol (87.8 - 163.3 pMol/g) or the identified carotenoids (1.8-23.0 pMol/g). They concluded that the frontal cortex, generally vulnerable in Alzheimer's disease, had higher concentrations of all analytes than the occipital cortex which is generally unaffected. Moreover, frontal lobes, but not occipital lobes, exhibited an age-related decline in retinol, total tocopherols, total xanthophylls and total carotenoids. The importance of these differences and the roles of these antioxidants in the brain remains to be determined. Other studies (FAHA, 1991,1992) show however, that vitamin E does not penetrate the brain and does not improve Parkinson’s disease.

Brain microglia are phagocytic cells that are the major inflammatory response cells of the central nervous system and widely held to play important pathophysiologic roles in Alzheimer's disease (AD) in both potentially neurotoxic responses and potentially beneficial phagocytic responses.

Hsiao et al (2004) investigated the antioxidant effects of lycopene, on the free radical-scavenging activity and lipid peroxidation in rat brain homogenates as well as nitric oxide (NO) formation in cultured microglia stimulated by lipopolysaccharide. The authors also examined the therapeutic effect of lycopene in attenuating ischemia/reperfusion brain injury induced by middle cerebral artery (MCA) occlusion in rats. Lycopene (1, 2 and 5 uM) increased inhibition of iron-

catalyzed lipid peroxidation (TBARS formation) in rat brain homogenates in concentration-dependent manners. Furthermore, lycopene (5 and 10 uM) significantly inhibited nitrite production by about 31% and 61% in microglia stimulated by LPS, respectively. Rats which received lycopene at a dosage of 4 mg/kg showed significant infarct size reductions compared with control. The lycopene was shown to have a protective effect on ischemic brain injury in vivo. Lycopene, through its antioxidative property, mediates at least a portion of free radical-scavenging activity and inhibits microglia activation, resulting in a reduction in infarct volume in ischemia/reperfusion brain injury.

Burri et al (2003) used reversed-phase HPLC with diode array detection to simultaneously measure the major isoforms of vitamins A, E, and the carotenoids in serum from 55 healthy people with spinal cord injuries. Typically, the method measured retinol (vitamin A), alpha-tocopherol (vitamin E) and beta-carotene, alpha-carotene, lutein, lycopene, and cryptoxanthin (carotenoids). gamma-Tocopherol (vitamin E), 25 -hydroxycalciferol (vitamin D), and the carotenoid zeaxanthin could also be measured when they were present in high concentrations. Healthy people with spinal cord injuries were more likely than similar people without injuries to have low concentrations of alpha-tocopherol, and to a lesser extent retinol and beta-carotene.

In order to assess peripheral levels and activities of a broad spectrum of non-enzymatic and enzymatic antioxidants in elderly subjects with (MCI) and Alzheimer's disease (AD), plasma levels of water-soluble (Vitamin C and uric acid) and of lipophilic (Vitamin A, Vitamin E and carotenoids including lutein, zeaxanthin, beta-cryptoxanthin, lycopene, alpha-and beta-carotene) antioxidant micronutrients as well as activities of plasma and red blood cell (RBC) superoxide dismutase (SOD) and of plasma glutathione peroxidase (GPx) were measured by
Rinaldi et al (2003) measured peripheral levels and activities of antioxidants [water-soluble (Vitamin C and uric acid) and of lipophilic (Vitamin A, Vitamin E and carotenoids including lutein, zeaxanthin, beta-cryptoxanthin, lycopene, alpha-and beta-carotene)] in 25 patients with mild cognitive impairment, 63 with AD and 53 controls. Values were low in MCI and AD patients as compared to controls. An increased intake of antioxidants in patients with MCI could be helpful in lowering the risk of conversion to dementia.

The effect of carotenoids on the brain appears somewhat controversial. It is not clear to which extent they can cross the BBB and if so, they could have an antioxidant activity.

III-7 Beneficial effects of limonoids

Limonoids have been shown to have antioxidant properties. This activity is maximal in limonoid aglycone when the fruits are unripe (Paul, 1995,2000; Berhow,2000). But the limonoid glucosides which form when the fruit matures have also been shown to possess antioxidant activity but not as high as the aglycones.

There are about 40 limonoids in citrus, with limonin and nomilin being the principal ones. These compounds, which occur in high concentrations in grapefruit and orange juice, partly provide the bitter taste in citrus. Limonoids possess the ability to inhibit tumor formation by stimulating the

enzyme glutathione S-transferase (GST) (28). GST is a detoxifying enzyme that catalyzes the reaction of glutathione with dangerous electrophiles to form less toxic and more water-soluble compounds that can be easily excreted from the body.
Poulose et al (2004) examined the superoxide radical quenching activity and cytotoxic action of limonoids against undifferentiated human SH-SY5Y neuroblastoma cells in culture. When added to the medium of SH-SY5Y cells in culture, micromolar amounts of LG and OG, compared with untreated controls, caused a cessation of cell growth and rapid cell death.
Little has been done on the absorption of limonoids by the brain. Most of the beneficial effects of these compounds appear to be associated with cancer protection (Seltzer and Seltzer, 2003, Miller et al, 2004).

IV-Summary

IV-1 Summary of the properties of the nervous system.

IV-1-1 The nervous system

The nervous system has properties significantly different from those of other organs.

The nervous system is divided into two sections with different functions and properties:

The *central nervous system (CNS)* which is composed of the *brain and the spinal cord*, and the *peripheral nervous system (PNS)* made up the various nerves and neurons going to and from each organ.

This network of nerves allows the brain to communicate with every part of the body**.** *Nerves transmit information as electrical impulses* from one area of the body to another. Some nerves carry information to the brain, and others carry information from the brain to the muscles to control the body's movement. *Nerves are composed of cells called neurons*. Neurons have a long extension, the axon. The axon's branched ends almost touch other nerve cells, at junctions called synapses. *Nerve signals travel along the axon* at speeds of more than 100 meters per second *and 'jump' from one nerve cell to another across a gap called synapse. The relay of the electrical signal from a neuron to another cell is insured by chemicals called neurotransmitters.* However, neurons could not function without the help of the glial cells which provide support and protection for neurons**.** They are known as the "supporting cells" of the nervous system. The main functions of glial cells are: to surround neurons and hold them in place, to supply nutrients and oxygen to neurons, to insulate one neuron from another. *Some of them (microglia) remove the dead neurons and are similar to microphages in the rest of the body*.

CNS and PNS have different physiological properties.

The PNS neurons have the ability to regenerate their axon after an injury. If a nerve is cut for example in a finger, the nerve will eventually grow back and the feeling will reappear.

*As a rule CNS neurons cannot regenerate and die once damaged***.** As a result, spinal cord injuries lead to irreversible paralysis and damage to the brain, for example from a stroke cannot be repaired. Furthermore, a large fraction of the brain cells die during our life time. The brain is the higher, supervisory center of the nervous system. The human brain is a collection of 100 billion neurons, and each linked with up to 25,000 others. This huge number of interconnecting neurons, is what makes the brain able to analyze sensory signals, control the body, and *think. The brain has very unique properties. It is isolated from the rest of the body by the blood-brain barrier (BBB) and the cerebrospinal fluid (CSF) barrier*.

IV-1-2 Role of the nervous system

The cognitive processes of the brain translate intentions and decisions via motor programming into behavior.

The processing of information in the human brain can be divided into *automatic* and conscious processing, both involve:
(*1) Reception of information by different sensory systems.*
(2) Analysis and memorization of the information received.
(3) Retrieval of stored memory contents.

IV-1-3 Deterioration of the brain and of the cognitive processes

IV-1-3-a Deterioration of the cognitive process

Cognitive deterioration has been shown to be a progressive and slow process. The inabilities to recall the name of a recent acquaintance or the contents of a short shopping list are familiar experiences for everyone, and this experience seems to become more common with aging. Recent studies have shown that memory impairment of a certain degree can be considered pathological, and that it indicates some kind of disease process affecting the brain. It has been referred as "Mild Cognitive Impairment. *Cognitive decline may progress to a degree referred to as dementia*, characterized by such a memory loss that normal independent function is impossible. Dementia can be caused by several degenerative processes, the most common of which is Alzheimer's disease.

At its peak performance (occurring around the age of 30) the brain may contain as many as 1,000 trillion cell-to-cell connections. However, since neurons are not replaced, in severe memory loss such as Alzheimer's, more than 90% of these connections can be lost. The problem starts with mild cognitive impairment and may progress to severe dementia. About 40% of all patients who experience MCI will progress to AD within three years.

IV-1-3-b Causes of brain deterioration

Because of its composition and activity, the brain is very fragile. It uses large amounts of energy. With only 2% of t he body weight it consumes 20% of t he available glucose and oxygen. Furthermore, the very high lipid content of the brain makes it a primary target for oxidation (50 % of dry brain weight are lipids, in contrast to 6-20% for other organs). Most of the brain lipids are located in membranes which are made of up to 80% lipids. Furthermore, the BBB limits the exchanges with the rest of t he body. *Therefore, the high energy production, that generates a large number of radical oxidative species, combined with a very high lipid content, makes neurons extremely sensitive to attacks by destructive free radicals. It has been hypothesized that free-radical damage is a central cause of brain aging and also of many neurodegenrative diseases such as of Alzheimer's disease*.

Cognitive impairment may b e linked with the level of blood homocysteine. The cognitive continuum from normal to demented is associated with a similar rise in homocysteine levels. High homocysteine and age are among the most relevant risk factors for dementia.

IV-2 Beneficial effects of citrus on brain and brain functions

Citrus compounds have indirect beneficial effects on the brain. Any beneficial effects of t he cardiovascular system in preventing atherosclerosis, strokes, embolisms, high blood pressure will in turn have a positive action on the brain (for review see, Cancalon 2005).

IV-2-1 Bioavailibility

What is important is not the compound in an ingested food, but the modified metabolites that reach a given target.
The main steps of the bioavailibility process are:

- The modification of the food compounds in the intestine.
- The absorption of these original and modified chemicals in the upper intestine or the colon.
- Further modifications mainly in the liver.
- Absorption of the final metabolites by the target organ. In the case of the brain compounds must be able to cross the blood brain barrier or the cerebrospinal fluid barrier.

IV-2-1-a Ascorbic acid

Ascorbic acid is highly concentrated in the brain and is found at high concentrations in neurons. Vitamin C, while not crossing the blood-brain barrier, does enter the cerebrospinal fluid and can be found in the CNS at concentrations proportional to dietary intakes. By comparison, vitamin E, a fat-soluble vitamin, does not readily cross the blood-brain barrier nor does it accumulate in the cerebrospinal fluid.

IV-2-1-b Polyphenols

Polyphenols go through a long process of transformation and transport before reaching their target organ**.** *However, most citrus flavonoids or some of their metabolites can cross the BBB and are able to reach the CNS.*

IV-2-1-c Folic acid

The presence of specific CNS folate transporters has been demonstrated.. This confirms that *folate can reach the brain through the BB and CSF barriers.*

It can be concluded that the main advantage of citrus in protecting the brain is the ability of most of the citrus vitamins and phytochemicals to reach the brain through the BBB or the CSF barrier.

IV-2-2 Beneficial effects of citrus phytochemicals and vitamins on the nervous system

IV-2-2-a Ascorbic acid

The brain is the organ with the highest concentration of vitamin C. It is present in the CNS at concentrations proportional to dietary intakes .

Ascorbic acid has now been shown to have several types of activities in the body besides its well-known role as vitamin C.

1. Ascorbic acid acts as a vitamin (vitamin C). Vitamin C is a coenzyme for various enzymes and is required for at least eight intracellular enzymatic reactions In the nervous system vitamin C is involved in the synthesis of catecholamine neurotransmitters (norepinephrine, epinephrine and dopamine). The availability of neurotransmitters is essential for the proper functioning of the nervous system.

2. Ascorbic acid is an effective antioxidant because it can be oxidized more easily than other molecules. Ascorbic acid can donate a hydrogen atom to a free radical molecule. In the brain ascorbic acid is compartmentalized between neurons and glia with an average intracellular concentration of 10 mM in neurons and 1 mM in glial cells. By contrast, the other antioxidant,

glutathione, used to regenerate oxidized ascorbic acid is more concentrated in glia than in neurons. The balance between the antioxidant concentrations in those two types of cells appears essential in maintaining the brain oxidative homeostasis.

3. The antioxidant activity of ascorbate in the brain has been shown to be intertwined with neurotransmitter modulation activities. These are indications that ascorbate may modulate glutamatergic transmission. The release of ascorbate from brain cells is associated mainly with the exchange of glutamate and ascorbate across the cell membranes of neurons and glias. It appears, therefore, that the level of extracellular ascorbate plays a critical role in regulating corticostriatal glutamate transmission.

From these results it can be concluded that ascorbic acid has two main functions in the brain.

1. It is involved in the synthesis and modulation of neurotransmitters.

2. It protects the highly lipidic brain tissue from oxidation. This antioxidant activity is of major importance in limiting a large number of brain ailments including.

Aging: brain deterioration and neuronal deaths appear very early in life and lead to progressive cognitive impairment.

The major degenerative brain disorders Alzheimer's, Parkinson's, Huntington's diseases and amyotrophic lateral sclerosis. Although cellular oxidation certainly plays a role in the onset of these diseases, the exact role of ascorbate is not absolutely clear.

It should also be pointed out that an increase in the ascorbate intake may not always be enough to reverse ascorbic acid deficits in some organs since the problem may lie with a deterioration of the transport mechanisms, seen particularly during aging. *However it appears likely that the proper ascorbic acid intake during the entire life should have a beneficial effect in limiting the oxidant related brain deteriorations that may become irreversible late in life***.**

IV-2-2-b Polyphenols

Polyphenols have been shown to have multiple beneficial effects on the nervous system.

1.Direct antioxidant activity. The most well known mode of action of polyphenols is the conventional electron transfer antioxidant process, as shown by a large number of in vitro studies of food antioxidant potential.

2. Indirect antioxidant activity. Flavonoids and their bioavailable metabolites have been shown to act not only as conventional hydrogen-donating antioxidants but also by modulating some antioxidative enzymes.

3. In the CNS polyphenols may also act at the neurotransmitter level.

4. Polyphenols act as anti-inflammatory agents by limiting the reaction of microglias which act in the brain in a manner similar to macrophages in the rest of the body. In the case of Alzheimer's disease flavonoids may slow b-amyloid peptide and fibril formation.

5. In some cases, direct in vivo effect of flavonoids on memory and cognition was shown in animals and humans.

In conclusion, citrus polyphenols and their metabolites can access the brain. In the last few years, the role of polyphenols in the body and particularly the brain has been expanding from a simple chemical antioxidant activity to a complex role as enzyme modulators. It is more likely that the beneficial effects of polyphenols are most effective through a life long protective activity, that limit progressive deterioration leading to severe damages.

IV-2-2-c Folic acid

The distribution of a single carbon to various biochemical processes is very important for the maintenance of several physiological functions and particularly DNA and neurotransmitter synthesis. Th e essential amino acid methionine is transformed into S-adenosylmethionine (SAM) the one-carbon donor i n numerous methylation reactions involving proteins, phospholipids, biogenic amines and repair of DNA. SAM is converted to homocysteine. *Then the vitamin B_{12}-dependent enzyme, methionine synthetase transfers a methyl group from methyl tetrahydrofolate to homocysteine to regenerate methionine*.
Many of the effects of folate have been well documented. Low or inadequate folate levels may contribute to congenital malformations and the development of chronic disease in later life. Maternal periconceptional supplementation with folic acid prevents the majority of neural tube defects, probably by overcoming one or more genetically inherited metabolic blocks in folate dependent enzymes. Substantial evidence indicates that elevated plasma homocysteine may be an independent risk factor for heart disease and stroke. Plasma homocysteine levels can be reduced by folic acid supplements. Various publications have also shown that many mental disorders including Alzheimer's Disease are associated with low folate levels and elevated plasma homocysteine. While a correlation appears to exist, it is hard to determine if this is a cause effect relationship.
Some of the main correlations between homocysteine and diseases are:

1. Too much homocysteine in the blood has been associated with increased risk in blood vessel disease, including brain blood vessels.

2. Elevated homocysteine levels may predict cognitive decline and a continuum appears to exist between cognitive impairment and homocysteine levels.

3. High levels of homocysteine are strongly associated with memory decline and may increase the chance of developing dementia.

4. Homocysteine >14 μmol/L nearly doubled a patient's risk of developing Alzheimer's disease.

Conclusion: Folate has been shown to have a beneficial effect in reducing homocysteine by boosting the one-carbon cycle. However several enzymes and vitamins particularly B6 and B12 are also involved in the cycle. To this day, the specific role of folate and B12 has not been completely elucidated, and the two vitamins have many similar or even identical effects. Therefore, the rise in homocysteine levels can have many causes.

Cognitive impairment and dementia appear to be associated with high homocysteine levels. Indeed homocysteine and particularly S-adenosyl homocysteine have been shown to be cytotoxic, however, if an association exists, a clear cause-effect relationship has not been completely demonstrated.

As part of the one-carbon cycle, folate plays an important part in many biochemical processes particularly in the brain, but the vitamin acts as part of a complex enzymatic mechanisms with several important components. The role of folate in DNA protection appears the strongest but the overall interaction with homocysteine may need further studies.

V Conclusions

V-1 Mode of action of phytochemicals and vitamins

It is important to view the activity of phytochemicals and vitamins in the light of recent scientific developments. Until the last few years, the accepted view of the mode of action of phytochemicals and vitamins was relatively simple. It was believed each compound had a single effect: Polyphenols were antioxidants, ascorbic acid was a vitamin acting mostly as a coenzyme to prevent scurvy. **In the last few years, this concept has been challenged and now many compounds are known to have multiple modes of action.** Many molecules have the ability to chemically scavenge free radicals and thus act as antioxidants in the test tube, but their main biological functions are by acting as hormones, ligands for transcription factors, modulators of enzymatic activities and as structural components. In fact many “antioxidant” phytochemicals are present in the body in quantities much too small to have a significant effect as chemical electron donors.

V-2 Beneficial activity of citrus of phytochemicals and vitamins

There are very few reports dealing with the influence of the whole fruit or juice on nervous system related problems. However, the beneficial effects of several citrus compounds on the

nervous system have been extensively examined. Among those vitamins C, folic acid and flavonoids are the most active.

V-2-1 Anti-inflammation activity.

As seen previously citrus compounds act positively on inflammation related ailments. This applies to the brain as well as to other organs. Most of the citrus health benefits that may improve the cardiovascular system will ultimately benefit the brain itself.

V-2-2 Access to the brain

The ability of most citrus vitamins and phytochemicals to reach the brain through the BBB or the CSF barrier can be seen as the most essential quality for being able to have a brain protection capacity. By contrast, vitamin E is a powerful antioxidant but cannot enter the brain and seems to have very limited effect on the brain cells.

V-2-3 Ascorbic acid

Studies have shown that ascorbic acid plays a complex and multiple role in the brain biochemistry.

1. Ascorbic acid acts as a vitamin (vitamin C). In the nervous system vitamin C is involved in the synthesis of neurotransmitters.
2. It is involved in the synthesis and modulation of neurotransmitters.
3. It protects the highly lipidic brain tissue from oxidation.
4. Ascorbic acid plays a major role in the in the interaction between neurons and their supporting cells, the glias. It act as an antioxidant neuroprotector and a neurotransmittion modulator.

V-2-4 Polyphenols (Flavonoids)

Polyphenols appear to have multiple mode of action as brain protecting compounds:

1. They behave as direct or indirect antioxidants on the brain tissue.
2. Polyphenols are involved in cell signaling processes through gene modulation.
3. Polyphenols also may act as direct anti-inflammation compounds.

V-2-5 Folic acid

The case of folate is more complex. Its role as protecting and promoting the synthesis of DNA appears well established and may contribute to protect brain cells.

Folic acid is necessary for the one-carbon cycle to function and to allow many biochemical reactions to proceed. However the individual roles of each component of the cycle are still unclear. The specific roles of folate and B12 should be elucidated.

Most brain degenerative diseases are associated with high levels of the one-carbon cycle by-product homocysteine and low folate concentrations. However, these interactions have not been elucidated and are qualified of association or correlation.

Folate appears to have an important role in maintaining the proper functioning of the brain. Nevertheless, in some cases the exact influence of this vitamin, particularly in relation with homocysteine needs to be further clarified.

V-2-6 Long term beneficial effects of citrus compounds.

Many brain related diseases including decline in cognitive processes are slow developing problems that start early in life. For example brain aging due to neuronal death appears to start in the thirties. Similarly, dementia begins as a mild cognitive impairment, long before becoming an incapacitating disease. **Therefore the maximal benefits of citrus compounds will be obtained if they are allowed to act during the entire life**. Under these conditions, direct or indirect antioxidant properties of citrus compounds, as well as, their ability to modulate some neurotransmitter activities, may limit or prevent the development of age or disease related brain damages that occur over decades, before they reach an irreversible stage.
A point can be made than one should not wait until he is too old to take advantage of the protective properties of citrus. Older brains have been shown to have a low concentration of antioxidants and limited ability to neutralize oxidative species. Studies have shown that in older individuals the antioxidant uptake mechanisms become inefficient, particularly for ascorbic acid, and that the effectiveness of the mechanisms responsible for using these antioxidants to neutralize oxidative species declines. It has not been demonstrated that increasing dietary intakes can reverse these shortcomings.

V-2-7 Citrus compounds and established brain diseases

Studies have also shown that these citrus compounds' beneficial effects can also be active on most brain ailments including Alzheimer's disease, Parkinson's disease, Huntington's disease, Amyotrophic lateral sclerosis, multiple sclerosis, and aging. However, these diseases have various origins such as genetic, autoimmune, environmental and develop through different mechanisms unrelated to those followed by citrus compounds. It is therefore unlikely that, in most cases, citrus phytochemicals and vitamins could reverse established diseases. **However, citrus compounds, by reinforcing natural mechanisms and limiting oxidation, may have an influence in slowing the progression of these diseases**.

It can be concluded that citrus compounds can access the brain and have several beneficial effects. However, since brain deterioration is usually a slow process starting early in life, a maximal protection would be obtained if they are allowed to interact with the brain during the entire life span.

VI- Considerations

VI-1 Several citrus compounds: Ascorbic acid, polyphenols (flavonoids), and folic acid appear to have a long term beneficial effect on the brain. These compounds have two main functions. **Ascorbic acid, flavonoids, and folic act as antioxidants and as regulators of molecules involved in the functioning of the brain (modulators of neurotransmitters).**

VI-2 The prerequisite property of a compound to be active is to be able to reach its target. **Compounds present in citrus (ascorbic acid, flavonoids and folic acid) have the ability to cross the barriers isolating the brain and reach the various nerve cells.**

VI-3 The brain nerves cells are very vulnerable. They contain up to 80% fat and produce large quantities of oxidant molecules that can damage the lipids and the cells. Once injured, brain nerve cells (neurons) cannot regenerate and will die.
Even in the absence of diseases, some brain degeneration starts occurring after 30 and keeps progressing during the rest of the life, inducing brain aging and senescent loss of cognitive functions. Oxidative damage and the resulting inflammation are among the main causes of these problems**. Citrus antioxidants may help limit cellular deterioration and protect brain activity, particularly against aging.**

VI-4 Similarly, the major brain diseases, Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and multiple sclerosis appear also to start long before the appearance of clinical symptoms. Although, each has specific causes, such as genetic or environmental, oxidation seems to play an active role in the progression of most of these diseases. **Citrus antioxidants may possibly play a role in slowing down the progression of the major brain ailments**.

VI-5 Citrus provide beneficial compounds that if consumed regularly may contribute to brain wellness. However the ability of the body to absorb and use these compounds decreases with age. **It appears essential to start consuming citrus early in life to help maintain proper brain functioning particularly later in life.**

VI-6 High levels of homocysteine have been shown to be associated with many brain ailments and loss of cognitive ability. Citrus contain significant amounts of folate which plays a role in limiting homocysteine accumulation. However, deficits and malfunctioning of several vitamins and enzymes, particularly B12 also contribute to the homocysteine problem and the exact role of folate remains to be clarified. **The relationship between folate and homocysteine and the role of homocysteine in various diseases are areas that deserve further study.**

VII-7 Until recently the beneficial effects of phytochemicals and particularly citrus compounds was believed to be limited to a chemical antioxidant effect.
Newer studies indicate that the main effect of these compounds may be to regulate many physiological functions by interacting with enzymes.
These results are particularly important for citrus. For example, hesperidin, the main orange flavonoid had been reported in the past as being a poor chemical antioxidant, consequently, it was perceived as a factor limiting the health value of citrus.
However, it has now been shown that hesperidin has multiple physiological targets. It may:

-Enhance antioxidative enzymes and have a strong non chemical antioxidant potential.
-Promote blood vessel health.
- Prevent post-menopausal and senescent osteoporosis.
- Promote brain health.

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