Minireview

Changes of the Brain Synapses During Aging. New Aspects

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The process of brain aging is an interaction of age-related losses and compensatory mechanisms. This review is focused on the changes of the synaptic number and structure, their functional implications, regarding neurotransmission, as well as the electrical activity of neuronal circuits. Moreover, the importance of calcium homeostasis is strongly emphasized. It is also suggested that many neuronal properties are preserved, as a result of adaptive mechanisms, and that a series of interdependent factors regulate brain aging. The "new frontier" in research is the challenge of understanding the effects of aging, both to prevent degenerative diseases and reduce their consequences. New aspects are considered a) the role of nitric oxide, b) free radicals and apoptosis, c) impaired cerebral microcirculation, d) metabolic features of aging brain, e) the possible neuroprotective role of insulin-like growth factor-1 (IGF-1) and ovarian steroids and e) stress and aging. These numerous multifactorial approaches are essential to understand the process of aging. The more we learn about it, the more we realize how to achieve "successful" aging.

Introduction

Aging leads to a progressive decline in brain and body function. It is characterized by heterogeneity, because some individuals may show less deficits and/or possess better adaptive mechanisms that compensate for losses and preserve key-functions.

A probable definition of aging could be: "an irreversible process that begins at maturity and is characterized by increasing deviations as compared to the ideal functional state" (Siegel *et al.*, 1989).

With aging, both "normal" senescent age-related changes (ARCs) and late-onset diseases affect the brain, produce declines in performance. The brain, as a postmitotic structure, is particularly vulnerable to ARCS and senescence is by far the most powerful risk factor for neurological diseases of the elderly such as sporadic Alzheimer's disease. Both individual and species differences in longevity illustrate the variable effects of time. Human life expectancy has been extended by prevention and treatment of specific diseases and life span can be altered by modifying the processes producing ARCs (Drachman, 1997).

The advent and implementation of new design-based stereological techniques allow the quantification of cell number without the assumptions required when obtaining areal densities. This newly emerging view of retained cell number during aging has a major impact on biogerontology, prompting re-evaluation of the long-standing hypothesis of age-related cell loss as a cause of age-related functional impairments in brain (Long *et al.*, 2000).

Aging is determined by the following features: a) It is the last period of one's life, b) it is met in every kind of species, c) it is not defined by the environment, d) it is evolutionary and e) it causes degenerative lesions and dysfunctions of CNS (Leclercq, 1992).

Synaptic number and structure

Neuronal loss seems to exist in aging animals, confined to specific brain areas whereas synaptic junctional areas are remodelled through lifespan. In the elderly, the number of contacts and the total surface area of the synapses decrease significantly and their average synaptic size increases at a dif-

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ferent extent according to the brain area taken into account. This is probably due to a compensatory phenomenon, counteracting the synaptic reduction in number (Bertoni-Freddari *et al.*, 1996).

Synaptic structure shows a variety of developmental changes. Pre- and postsynaptic elements thicken with age, while vesicle size decreases. Additionally, a small increase in synaptic element length is observed early in the development but no change in cleft width or in the number of synaptic vesicles (Markus *et al.*, 1987).The role of synaptic vesicles in neuronal development and plasticity is of great interest, since alterations in synaptic vesicles have been associated with a number of functional states (Pysh and Wiley, 1972; Vrensen and DeGroot, 1974; Gully, 1978; Reinecke and Walther, 1981; De Voogt *et al.*, 1985; Riccio and Matthews, 1985; Markus, 1987).

Although synaptic transmission is an important means of communication between neurons, neurons themselves and neurons and glia also communicate by extrasynaptic "volume" transmission, which is mediated by diffusion in the extracellular space (ECS). The ECS of the central nervous system (CNS) is the microenvironment of neurons and glial cells. The composition and size of ECS change dynamically during neuronal activity as well as during pathological states. Following their release, a number of neuroactive substances, including ions, mediators, metabolites and neurotransmitters diffuse via the ECS to targets located a long distance apart from their release sites (Sykova and Chvatal, 2000).

Synaptic vesicle size and number depend on the status of the nerve terminal, prior to and during fixation, varying with the nature of the fixative and the fixative-induced release, where the observed changes in vesicles are due to development (Markus *et al.*, 1987).

Neurochemical changes

The aged brain presents a failure in almost every transmission system and at almost every step of the chemical transmission process (Agnati *et al.*, 1990). It may be suggested that a general decrease of transmitter content, receptor density and transduction mechanism efficacy can cause a reduction of the set-point of synaptic feedback mechanisms (Fuxe *et al.*, 1986), and more generally, a decreased functional reserve of synaptic transmission (Table I). Moreover, chemical signals should be secreted in relatively high amounts to reach distant high-affinity receptors at sufficient concentrations.

Table I. Age-related changes in pre- and postsynaptic markers of the striatal dopamine-synapse (Agnati *et al.*, 1990).

Presynaptic markers	
TH-IR	D
TH activity	D
DA levels	D/NC
DA turnover	NC/I
Cold stress-induced DA turnover increase	D
Reserpine-induced DA turnover increase	D
DA turnover increase induced by training in reaction time test	I
Postsynaptic markers	
D_1 receptor levels	D/NC
D_2 receptor levels	D
D_1 receptor turnover	D
D_2 receptor turnover	D
Adenylate cyclase actiivty	D
cAMP-induced phosphorylation	D
DARPP-32 IR	D
DA/CCK receptor interaction	D
D_2 denervation supersensitivity	NC

D = decrease, I = increase, NC = no change, IR = immunoreactivity, DA = dopamine, TH = tyrosine hydroxylase, CCK = cholecystokinin, DARPP-32 = chemically identified cell bodies in the rat striatum.

Furthermore, changes of the proteolytic enzyme concentrations in the neuronal membranes facing the extracellular fluid, or in the chemical composition of the extracellular matrix, may play a role in aging (Agnati *et al.*, 1990). The observed increase of the brain protease activity during aging may also include alterations in the enzyme substrate specificity and/or in its endogenous substrates (susceptibility to breakdown) (Benuck *et al.*, 1996).

The failure in integrating neuronal and hormonal signals has also been reported during aging (Angelucci *et al.*, 1983). It has been proved that the proper spatio-temporal convergence of neurotransmitters, neuropeptides and peripheral hormones onto neuronal networks, undergoes an age-related alteration, which probably explains the impairment of some intellectual performances in aged subjects.

Additionally, adenosine receptors show age-related changes. A statistically significant decrease is observed in the density of A_1 and A_2 adenosine

receptors in the aged rats (24 months old), as adenosine inhibits the release of several neurotransmitters, such as acetylcholine (ACh), dopamine (DA), noradrenaline (NA), serotonin (5-HT), glutamate and gamma-aminobutyric acid (GABA) (Fredholm and Dunwiddie, 1988). An overall reduction of this inhibitory mechanism could, in part, explain the well known "release" phenomena, such as involuntary movements, sudden unrelated emotions and, more generally, psychological instability, often observed with the elderly.

Moreover, it has been suggested that adenosine may have protective effects (Dragunow and Faull, 1988), possibly regulating blood flow (mainly via A_2 vascular receptors) and reducing oxygen (O_2) demand (mainly via A_1 central receptors). These responses are triggered when a marked reduction in energy storage is required and a pronounced ATP hydrolysis, following an increase in the central nervous system (CNS) metabolic demands (e.g. after ischemia, hypoxia, etc). Thus, adenosine may play a crucial role in balancing energy expenses with energy storage in the brain. A reduction in this control system may lead to sudden failures of the "basic biochemical machinery", which is the basis of any plastic response. Increasing evidence (Agnati *et al.*, 1988) indicates that an important step to ensure neuronal survival, after mechanical and toxic lesions, may be the reduction of neural electrical activity which accounts for about 60% of the entire neuronal energy expenditure.

A previous study (Agnati *et al.*, 1986) reports that the intracytoplasmic part of chemical transmission is less effective during aging. In fact, both cAMP and calcium-induced protein phosphorylation appear to be reduced in the striatal and cortical regions. An impaired phosphorylating activity could also contribute to alterations in the response of receptors to neurotransmitters in the aging brain. In this view, the modulation of protein phosphorylation may restore some functional changes of the aging brain, from short term events (e.g. ion channel activity), to long-lasting processes, (e.g. the potentiation of synaptic efficacy). In addition, it is believed that drug administration, as well as the exposition to harmful substances, may interfere with neurotransmission mechanisms. For example, excessive alcohol consumption has been shown to alter biochemical and homeostatic events, from receptor adaptive phenomena to transducing systems (Magnoni *et al.*, 1990).

At membrane level, there is a change in the interaction with chemical transmissions (Agnati *et al.*, 1984; Fuxe and Agnati, 1985 and 1987). This phenomenon can be explained as a reduction in the miniaturization of the computing circuits of the brain at molecular level, resulting not only in an impaired transmission of information, but also in a decrease in the membrane capability to integrate the chemical signals (Agnati *et al.*, 1986). These membrane phenomena suggest that the morphofunctional changes observed in the aged brain can explain, at least in part, the decreased capability of the aging nervous system to store and retrieve information.

The most important functional consequence of the above alterations is the inability of the "aged" synapse to give prompt and effective responses, to allow interactions between different chemical transmissions and thus, to be a part of an effective optimal control system.

Neurotransmitters and the aging brain

Aging affects mostly the cholinergic and catecholaminergic system (Xafenias and Hampatzis, 1995).

a) *Catecholaminergic system:* i) DA-neurons in the *substantia nigra* of the human brain are selectively vulnerable and their number declines about 5–10% per decade of aging (Naoi and Maruyama, 2000). As a result, there is a decline in DA function (Volkow *et al.*, 1998), which is also related to the decreased activity of the enzymes tyrosine hydroxylase and L-dopa decarboxylase. In addition, aging of the human brain is associated with a decline in dopamine (DA) function, generally interpreted as DA cell loss. Positron emission tomography studies revealed that in healthy individuals, the age-related losses in DA transporters (presynaptic marker) were associated with losses in D2 receptors (postynaptic marker) rather than with increases as is known to occur with DA cell loss. This association was specific for DA synaptic markers, because they were not correlated with striatal metabolism. Furthermore, their association was independent of age, suggesting that a common mechanism regulates the expression of receptors and transporters irrespective of age (Volkow *et al.*, 1998).

ii) NA appears to be reduced, mainly in the hindbrain, because of the reduction of its receptor density (Xafenias and Hampatzis, 1995).

iii) Distributional changes of serotonergic fibers associated with aging were demonstrated immunohistochemically. Old rat brains were morphologically characterized by the presence of peculiar features of serotonergic fibers not found in the young adult brain. In 24-month-old rats, these aberrant serotonergic fibers were subdivided into two groups according to morphological alterations: type 1 fibers consisting of thin fibers with moderately enlarged varicosities and type 2 fibers consisting of much thicker fibers that have even larger varicosities and a tortuous course. These two types of fibers were distributed differentially in the forebrain. Type 1 fibers were found mainly in the striatum and frontoparietal cortex, whereas type 2 fibers were found in the posterior cingulate cortex and dentate gyrus of the hippocampal formation. Both types of aberrant fibers were seen in amygdala, frontoparietal cortex, hypothalamus and thalamus. In 36-month-old rats, more highly degenerating arborizations were detected, and these aberrant ramifications were classified as follows based on shape as: type 3 fibers consisting of highly arborized thin fibers with a larger number or larger varicosities, and type 4 fibers consisting of thick fibers with abundant larger varicosities. Distributional difference indicated that type 1 fibers develop into type 3 fibers, and type 2 into type 4 fibers. These findings suggest the possibility that one set of pathological fibers emanate from the dorsal raphe nucleus and the other from the median raphe. Moreover, both two sets of serotonergic fibers show age-related aberrations in their morphology over same time course (Nishimura *et al.*, 1999).

iv) Monoaminoxidase (MAO) activity is reported to increase remarkably. As a consequence of this finding, the acetylenic selective monoamine oxidase (MAO) type B suicide inhibitor selegiline ([R]-[-]-N, a-Dimethyl-N-[2-propynyl] phenethylamine) (previously called L-deprenyl) has proved to be useful adjuvant to levodopa (L-Dopa) therapy and monotherapy of Parkinson's disease (PD). The drug binds to brain regions with a high MAO-B content, such as the thalamus, the striatum, the cortex and the brainstem. It is rapidly metabolized in humans, mainly in the liver, to form desmethylselegiline and methamphetamine, which are further metabolized to amphetamine (phenylisopropylamine). Although not all features of its anti-PD action area known, brain studies have shown that selective inhibition of MAO-B, with the concomitant increase of phenylethylamine and dopamine (DA) but not of serotonin or noradrenaline, in the basal ganglia may be regarded as its mode of action. These protective effects afforded by selegiline in PD, resulting in a delayed need for levodopa therapy, have been variously interpreted in terms of the involvement of an endogenous neurotoxin or an oxygen free radical mechanism (oxidative stress) in the development of PD (Gerlach *et al.*, 1996).

b) *Cholinergic system:* Muscarinic receptor density is decreased with aging (Buyukuysal *et al.*, 1998). Choline acetyltransferase (ChAT), an enzyme implicated in the biosynthesis of ACh, shows a decreased activity. Moreover, acetylcholinesterase (AChE), has a decreased activity (Xafenias and Hampatzis, 1995). Age-related alterations of presynaptic functions were studied in terms of acetylcholine (ACh) synthesis and release using synaptosomes isolated from mouse brain cortices. The following three findings were obtained: 1) Choline acetyltransferase activity and ACh production rate remained constant throughout all ages tested. This observation, obtained with synaptosomes, was not consistent with data reported for brain slices. Various conditions, such as low glucose or membrane depolarization, modulated ACh synthesis to similar extents in young and aged synaptosomes. 2) Depolarization-induced release of ACh from synaptosomes significantly decreased in the senescent stage. The fraction of ACh released from aged synaptosomes was less than that released from young synaptosomes, although the ACh contents in the synaptosomes did not change with age (Tanaka *et al.*, 1996).

c) *GABA:* This neurotransmitter is the "executor" of virtually all age-related neurodegenerative processes. With advancing age, the declining mitochondrial ATP synthesis unleashes GABA synthesis, leading to dystrophy of axon terminals and blocking the transport of the neurotrophins. As a result, "starvation" and death of neuronal somata are often observed (Marczynski, 1998).

d) *Neuropeptides:* Little is known about their participation in the aging process. With advancing age (in healthy individuals), neuropeptide Y

(NPY) and corticotropin-releasing factor (CRF), seem to be decreased in gyrus cinguli (Arranz *et al.*, 1996).

In general, decreased transmitter release may be inconsequential during basal activity, but may be inadequate during periods of peak activity. Since neurotransmitter systems act in concert, it is possible that deficits in one transmitter may be compensated for by changes in another, but a decline in two interdependent transmitter systems may produce more than an additive decline. Current data suggest that age-related alterations need to be examined regionally, as well as according to cell type and transmitter system (Marczynski, 1998).

The role of calcium ion homeostasis in brain aging

The regulation of Ca^{2+} influx and its compartmentalization is essential to presynaptic, as well as postsynaptic events. Calcium influx induced by depolarization was lower in the synaptosomal preparations from aged mice than in those from young mice. A strong positive correlation was observed between the amounts of ACh released and increased calcium levels when the data for all preparations, both from young and aged mice, were plotted. This indicates that diminished calcium influx may cause the reduced ACh release by aged synapses (Tanaka *et al.*, 1996).

Additionally, calcium ion homeostasis in different brain compartments (e.g. pre- and postsynaptic) may respond to aging in a different way. Cytosolic free Ca^{2+}, which is the active pool of Ca^{2+}, is only 1/10,000th of total neuronal Ca^{2+}. Thus, changes in the intracellular/extracellular Ca^{2+} ratio could produce harmful alterations in cell function. Consequently, the increase of calcium levels in the cytosol is the common factor of all the neurodegenerative processes affecting the nervous system (Ramirez-Exposito and Martinez-Martos, 1998). A number of components are also influenced by Ca^{2+} homeostasis, including receptor mobility, Ca^{2+} transport systems, Ca^{2+} binding proteins and Ca^{2+}-activated proteases.

Furthermore, in the hippocampal slice preparations found that synaptic plasticity was impaired with aging, apparently due to excess Ca influx. In subsequent analyses it was found that the Ca-dependent afterhyperpolarization, the Ca action potential and voltage-activated Ca currents were all increased in aged CA1 neurons. This was not due to impaired inactivation processes. Multiple types of Ca channels appear to be affected by aging. A long Ca tail current was also found in these studies, which seems to represent an unrecognized and significant Ca entry pathway at resting potential. In primary cell cultures, Ca currents and single Ca channels increase steadily over the life cycle of the cultured neurons and are correlated with cell death. Single L-type Ca channels were also studied in brain neurons of an aged mammal (rat), using the partially dissociated ("zipper") hippocampal slice preparation. A substantial increase in the density of functionally available Ca channels was present in CA1 neurons of aged rats, similar to the increase seen in cultured neurons. Thus, a gradual increase in the density of Ca channels appears to be a consistent property of hippocampal neuronal aging and might well be a factor in the vulnerability of aged neurons to Alzheimer's disease and other neurodegenerative/traumatic conditions (Landfield, 1996).

Synaptic plasticity during aging

Structural plasticity accompanying the aging process is not yet well understood. It is noteworthy, however, that the elimination of the synapses is highly selective during aging (Barnes, 1988; Flood and Coleman, 1988). According to recent data, there is a dramatic decline in the levels of synaptic proteins involved in the plasticity of axons and dendrites (Hatanpaa *et al.*, 1999). As far as neuronal dendrites are concerned, it has been observed that there is a decrease of the dendritic branches and their endings, their total dendritic length and their cell soma size during aging process (Anderson and Rutledge, 1996). The impaired synaptic plasticity in the elderly may contribute to cognitive dysfunctions. Thus, reactive synaptogenesis appears to be very important to the aging brain, where numerous neuronal losses and degenerative processes take place. An other study showed that enriched rearing conditions restored the age-related decrease or synaptophysin contents. This might be due to increased numerical synaptic density or enhanced packing density of synaptic vesicles in synapses. The results of this study support the latter explanation; that is, synaptic vesicle contents were increased without

changes in synaptic density. Synaptic plasticity induced by environmental stimulation is shown to relate with synaptic strengthening, but not with the formation of new synapses (Nakamura *et al.*, 1999).

In aged animals, the innervation process is delayed. The decreased rate of synaptic replacement may be due to the rate at which degenerative debris is cleared from the neuropil. In aged rats, a delay in the initiation of clearance of degenerative debris was also observed, as a consequence of a slower innervation response (Siegel, 1989).

Moroever, microglia also seems to play an important role in the clearance of degenerative debris from the brain (Vijayan and Cotman, 1987). In normal aging, reactive gliosis has been described in specific areas of the limbic system and neocortex that undergo selective neuronal or synaptic degeneration in nondemented elderly persons (Unger, 1999).

New aspects of brain aging

a) *Free radicals and apoptosis:* Recent data in cell cultures has shown that brain neurons are particularly vulnerable to degeneration by apoptosis (programmed cell death). The main inducers of this phenomenon are oxidative damage and low energy metabolism (Cotman and Su, 1996). In fact, free radicals derived from oxygen may attack nerve terminals and peroxidize the membrane. When aged neuronal membranes are subjected to hyperoxia, their permeability to sucrose increases, while the synaptic plasma membrane fluidity is decreased. The cholesterol/phospholipids (C/P) ratio of the membranes significantly increases with age, and even more in the oxygen-exposed rats. These results suggest that free radicals lead to the deterioration of function of brain synapse, and that susceptibility of synapse to oxidative stress is remarkably increased with age (Urano *et al.*, 1998).

In a very recent study (Tsakiris, 2001a), it was shown that the inhibitory effect of L-phenylalanine on rat brain AChE and its stimulatory effect on rat brain Na^+,K^+-ATPase were decreased with age, as a consequence of aging factors, such as free radicals (Tsakiris *et al.*, 2000) and/or reduced density of a- and β-adrenergic receptors in the tissue (Tsakiris, 2001b).

Furthermore, the activities of antioxidant enzymes display an age-dependent decline in the old rats, contributing to the serious oxidative protein damage seen in the elderly. For example, catalase and cytochrome C oxidase activities appear to be decreased during aging (Tian *et al.*, 1999).

On the other hand, previous reports have indicated that cholesterol (CHO) accumulates in neuronal membranes and alters their structural and signal transduction properties during aging. Such an accumulation may serve to protect neuronal tissue from oxidative damage. Actually, CHO seems to retard or enhance the harmful effects of oxidative stress in an age-dependent manner (Joseph *et al.*, 1997).

Another neuroprotective factor against oxidative damage, is N-acetylcysteine (NAC). It has recently been found to prevent apoptotic death in neuronal cells and protect synaptic mitochondria proteins from oxidative stress in aged mice. Thus, the administration of NAC may be beneficial for elderly subjects (Martinez *et al.*, 2000).

b) *The role of nitric oxide (NO) in brain aging:* NO is considered to have a cytotoxic action on neurons (Nomura, 1996). According to the "NO hypothesis of aging" (McCann *et al.*, 1999), NO produces oxidant damage to the aging brain, and the effects of NO-synthase are mediated mainly by the free radical oxidant properties of this soluble gas. Antioxidants, such as melatonin, vitamin C and vitamin E (α-tocopherol), probably play an important acute and chronic role in reducing or eliminating the harmful effects of NO. Furthermore, there is evidence that nitric oxide (NO) plays an important role as a diffusible messenger in learning and memory, and was also examined the role played by NO in the effect of aging on spatial memory in rats. The performance of aged rats (30 months old) in a radial-arm maze task (a complicated action) was significantly impaired as compared to that of adult rats (3 months old). The number of neurons containing NADPH-diaphorase (NADPH-d) reactivity in the cerebral cortex and striatum of aged rats was significantly less than that in the adult rats. The daily administration of NG-nitro-L-arginine methyl ester (L-NAME; 10–60 mg/kg, i.p.) resulted in a dose-dependent impairment of acquisition in the radial-arm maze task, while it failed to affect previously acquired performance, i.e., retention, in the adult rats. The content of 5-hydroxyindoleacetic acid in the hippocampus and of 3,4-dihydroxyphenyla-

cetic acid in the striatum was significantly decreased and increased, respectively, in the L-NAME (60 mg/kg/day)-treated adult rats compared with that in controls. These findings demonstrated that NO production in the brain may be decreased in aged rats, suggesting that this alteration may be involved in memory processes, especially in the acquisition, but not in the retention, of spatial learning in rats, and further, that endogenous NO may be involved in the regulation of dopamine and 5-hydroxytryptamine metabolism (*Noda et al.*, 1997).

c) *Cerebral vessels and normal aging:* The integrity of the cerebral vasculature is crucial to the maintenance of cognitive functions during aging. Prevailing evidence suggests that cerebrovascular functions decline with age. A number of subtle alterations in the intracranial vessels and capillaries are apparent. Thus, the neuronal populations in certain brain regions could become vulnerable, because of the hypoperfusion (Kalaria, 1996). The aging of the cerebral microcirculation also affects the blood-brain barrier (BBB), which becomes more susceptible to disruption by external factors and drugs. However, it is not yet known whether changes in the BBB contribute to the degeneration of the aged brain or are merely epiphenomena of aging (Shah and Mooradian, 1997).

d) *Metabolic features of the aging brain:* The existing literature on chronic food restriction in rodents shows the delay of some processes of aging in the nervous system and generally supports interactions of peripheral metabolism with brain aging (Finch and Cohen, 1997). Caloric restriction has been the most popular method of extending life span in the laboratory animal. It causes a reduction of brain 5HT-levels, prevents age-related losses in striatal DA-receptors, and improves the learning performance in aged rats (Siegel, 1989). Moreover, according to a recent study, adenosine-5′-triphosphatase (ATPase) activity in the brain cytosol of 50-week-old rats was significantly decreased, as compared to that of 5-week-old rats (Hanahisa and Yamaguchi, 1999).

e) *The possible neuroprotective role of Insulin-like Growth Factor-I (IGF-I) and ovarian steroids:* IGF-I is a protein that acts on many tissues; it contributes to the maintenance of the integrity and homeostasis of the nervous system. The widespread distribution of its receptor, allows IGF-I to affect the survival of numerous populations of neuronal and glial cells during aging and it seems that IGF-I can reduce or slow down neuronal losses (Drachman, 1997).

On the other hand, ovarian steroids affect brain areas such as the basal forebrain, hippocampus, midbrain raphe and brainstem locus coeruleus. Estrogens and progestins regulate synaptogenesis in the CA_1 region of the hippocampus. Formation of new exitatory synapses is induced by estradiol and involves N-methyl-D-aspartate (NMDA) receptors, whereas synaptic down-regulation involves intracellular progestin receptors. Furthermore, ovarian steroids affect the brainstem and midbrain catecholaminergic neurons, midbrain 5-HT pathways and the basal forebrain cholinergic system, producing cognitive effects during aging (McEwen *et al.*, 1997). Additionally, the neuroprotective efficacy of estrogens has been well described. It has been reported that the estrogens activate the kinase pathway, regulate the cAMP signal transduction pathways, modulate intracellular calcium homeostasis and show direct antioxidant activity (Green and Simpkins, 2000).

f) *Stress and aging:* A loss of endocrine and neurotransmitter system intractions may underlie the age-related deficits in the hypothalamic-pituitary-adrenal axis, including adapting to stress (Maines *et al.*, 1998). Because of this bad adjustment, chronic stress may accelerate age-related damage, as it has been found in the hippocampus. Adrenal glucocorticoids are thought to be responsible for this damage, because of their ability to compromise energy metabolism and make neurons more vulnerable to glutamate excitotoxicity. Stress has been found to decrease brain-derived neurotrophic factor (BDNF) mRNA in the hippocampus, whereas nerve growth factor (NGF) and neurotrophin-3 (NT-3) are increased, perhaps as a compensatory mechanism to stress-induced-damage (Smith, 1996).

Agnati L. F., Fuxe K., Benfenati F., Battistini N., Zini I., Camurri M. and Hökfelt T. (1984), Postsynaptic effects of neuropeptide comodulators at central monoamine synapses. In: Catecholamines: Neuropharmacology and Central Nervous System-Theoretical Aspects (E. Usdin, A. Carlsson, A. Dahlström, and J. Engel, ed.). Alan R. Liss, New York, pp. 191–198.

Agnati L. F., Fuxe K., Zoli M., Merlo Pich E., Benfenati F., Zini I. and Goldstein M. (1986), Aspects on the information handling by the central nervous system: focus on cotransmission in the aged rat brain. In: Coexistence of Neuroactive Substances. A New Principle in Synaptic Transmission, Prog. Brain Res. Vol. **68** (T. Hökfelt, K. Fuxe and B. Pernow, ed.). Elsevier, Amsterdam, pp. 291–301.

Agnati L. F., Zini I., Zoli M., Fuxe K., Merlo Pich E., Grimaldi R., Toffano G. and Goldstein M. (1988), Regeneration in the central nervous system: concepts and facts. In: Advances and Technical Standards in Neurosurgery (Vol. **16**) (L. Symon, ed.). Springer-Publ., Vienna, pp. 4–50.

Agnati L. F., Zoli M., Grimaldi R., Fuxe K., Toffano G. and Zini I. (1990), Cellular and synaptic alterations in the aging brain. Aging **2**, 5–25.

Anderson B. and Rutledge V. (1996), Age and hemisphere effects on dendritic structure. Brain **119 (Pt 6)**, 1983–1990.

Angelucci L., Patacchioli F. R., Samuel D., Algeri S., Gershon S., Grimm V. S. and Toffano G. (1983), Integrative neurohumoral mechanisms: relevance to the aging brain. In: Aging of the Brain. Raven Press, New York, pp. 373–380.

Arranz B., Blennow K., Ekman R., Eriksson A., Mansson J. E. and Marcusson J. (1996), Brain monoaminergic and neuropeptidergic variations in human aging. J. Neural Transm. **103**, 101–115.

Barnes C. A. (1988), Selectivity of neurological and mnemonic deficits in aged rats. In: Neural Plasticity. A Lifespan Approach (T. L. Petit and O. I. Gwen, ed.). Alan R. Liss, New York, pp. 235–264.

Benuck M., Banay-Schwartz M., DeGurman T. and Lajtha A. (1996), Changes in brain protease activity in aging. J. Neurochem. **67**, 2019–2029.

Bertoni-Freddari C., Fattoretti P., Paoloni R., Caselli U., Galearzi L. and Meier-Ruge W. (1996), Synaptic structural dynamics and aging. Gerontology **42**, 170–180.

Buyukuysal R. L., Ulus I. H. and Kiran, B. K. (1998), Age-related alterations in pre-synaptic and receptor-mediated cholinergic functions in rat brain. Neurochem. Res. **23**, 719–726.

Cotman C. W. and Su J. H. (1996), Mechanisms of neuronal death in Alzheimer's disease. Brain Pathol. **6,** 493–506.

De Voogd T. J., Nixdorf B. and Nottebohm F. (1985), Synaptogenesis and changes in synaptic morphology related to acquisition of a new behavior. Brain Res. **329**, 304–308.

Drachman D. A. (1997), Aging and the brain: a new frontier. Ann. Neurol. **42**, 819–828.

Dragunow M. and Faull R. L. M. (1988), Neuroprotective effects of adenosine. Trends Pharmacol. Sci. **9**, 193–194.

Finch C. E. and Cohen D. M. (1997), Aging, metabolism and Alzheimer disease: review and hypotheses. Exp. Neurol. **143**, 82–102.

Flood D. G. and Coleman P. D. (1988), Cell type heterogeneity of changes in dendritic extent in the hippocampal region of the adult brain in normal aging and in Alzheimer's disease. In: Neural Plasticity: A Lifespan Approach (T. L. Petiti and O. I. Gwen, ed.). Alan R. Liss, New York, pp. 265–281.

Fredholm B. B. and Dunwiddie T. V. (1988), How does adenosine inhibit transmitter release? Trends Pharmacol. Sci. **9**, 130–134.

Fuxe K. and Agnati L. F. (1985), Receptor-receptor interactions in the central nervous system. A new integrative mechanism in synapses. Med. Res. Rev. **5**, 441–481.

Fuxe K., Agnati L. F., Härfstrand A., Janson A. M., Neumeyer A., Andersson K., Ruggeri M., Zoli M. and Goldstein M. (1986), Morphofunctional studies on the neuropeptide Y/adrenaline costoring terminal systems in the dorsal cardiovascular region of the medulla oblongata. Focus on receptor-receptor interactions in cotransmission. In: Coexistence of Neuronal Messengers, Progr. Brain Res., Vol. **68** (T. Hökfelt, K. Fuxe and B. Pernow, ed.). Elsevier, Amsterdam, pp. 303–320.

Fuxe K. and Agnati L. F. (1987), Receptor-Receptor Interactions. A New Intramembrane Integrative Mechanism. Macmillan, London.

Gerlach M., Youdim M. B. and Riederer P. (1996), Pharmacology of selegiline. Neurology **47** (6 suppl. 3), S137–145.

Green P. S. and Simpkins J. W. (2000), Neuroprotective effects of estrogens: potential mechanisms of action. Int. J. Dev. Neurosci. **18**, 347–358.

Gully R. L. (1978), Changes in the presynaptic membrane of the synapses of the anteroventral cochlear nucleus with different levels of acoustic stimulation. Brain Res. **146**, 373–379.

Hanahisa Y. and Yamaguchi M. (1999), Effect of calcium-binding protein on adenosine 5′-triphosphatase activity in the brain cytosol of rats of different ages: the inhibitory role of regucalcin. Biol. Pharm. Bull. **22**, 313–316.

Hatanpaa K., Isaacs K. R., Shirao T., Brady D. R. and Rapoport S. I. (1999), Loss of proteins regulating synaptic plasticity in normal aging of the human brain and in Alzheimer disease. J. Neuropathol. Exp. Neurol. **58**, 637–643.

Joseph J. A., Villalobos-Molinas R., Denisova N. A., Erat S. and Strain J., (1997), Cholesterol: a two-edged sword in brain aging. Free Radic. Biol. Med. **22**, 455–462.

Kalaria R. N. (1996), Cerebral vessels in ageing and Alzheimer's disease. Pharmacol. Ther. **72**, 193–214.

Landfield P. W. (1996), Aging-related increase in hippocampal calcium channels. Life Sci. **59**, 399–404.

Leclercq V. (1992), Successful aging. UCB Pharma, Bruxelles, Belgium, pp 180–185.

Long J. M., Mouton P. R., Jucker M. and Ingram D. K. (2000), What counts in brain aging? Design-based stereological analysis of cell number. J. Gerontol. A Biol. Sci. Med. Sci. **54**, B407–417.

Magnoni M. S., Govoni S., Battaini F. and Trabucchi M. (1990), The aging brain: protein phosphorylation as a target of changes in neuronal function. Life Sci. **48**, 373–385.

Maines L. W., Keck B. J., Dugar A. and Lakoski J. M. (1998), Age-dependent loss of corticosterone modulation of central serotonin 5-HT1A receptor binding sites. J. Neurosci. Res. **53**, 86–98.

Marczynski T. J. (1998), GABAergic deafferentation hypothesis of brain aging and Alzheimer's disease revisited. Brain Res. Bull. **45**, 341–379.

Markus E. J., Petit T. L. and LeBoutillier J. C. (1987), Synaptic structural changes during development and aging. Dev. Brain Res. **35**, 239–248.

Martinez M., Hernandez A. I. and Martinez N. (2000), N-Acetyl-cysteine delays age-associated memory impairment in mice: role in synaptic mitochondria. Brain Res. **855**, 100–106.

McEwen B. S., Alves S. E., Bulloch K. and Weiland N. G. (1997), Ovarian steroids and the brain: implications for cognition and aging. Neurology **48**, S8-S15.

Nakamura H., Kobayashi S., Ohashi Y. and Ando S. (1999), Age-changes of brain synapses and synaptic plasticity in response to an enriched environment. J. Neurosci. Res. **56**, 307–315.

Naoi M. and Maruyama W. (1999), Cell death of dopamine neurons in aging and Parkinson's disease. Mech. Ageing Dev. **111**, 175–188.

Nishimura A., Ueda S., Takeuchi Y., Matsushita H., Sawada T. and Kawata M. (1998), Vulnerability to aging in the rat serotonergic system. Acta Neuropathol. Berl. **96**, 581–595.

Noda Y., Yamada K. and Nabeshima T. (1997), Role of nitric oxide in the effect of aging on spatial memory in rats. Behav. Brain Res. **83**, 153–158.

Nomura Y. (1996), Neurochemical aspect of brain aging, neuronal death and decreased synaptic functions. Hokkaido Igaku Zasshi **71**, 309–314.

Petit T. L. and Markus E. J. (1987), The cellular basis of learning and memory: the anatomical sequel to neuronal use. In: Neuroplasticity, Learning and Memory (N. W. Milgram, C. M. McLeod and T. L. Petit, ed.). Alan R. Liss, New York.

Pysh J. J. and Wiley R. G. (1972), Morphologic alterations of synapses in electrically stimulated superior cervical ganglia of the cat. Science **176**, 191–193.

Ramirez-Exposito M. J. and Martinez-Martos J. M. (1998), The molecular basis of neurodegenerative processes in the central nervous system. Rev. Neurol. **26**, 91–100.

Reinecke M. and Walther C. (1981), Ultrastructural changes with high activity and subsequent recovery at local motor nerve terminals. A stereological analysis. Neuroscience **6**, 489–503.

Riccio R. V. and Matthews M. A. (1985), The postnatal development of the rat primary visual cortex during optic nerve impulse blockade by intraocular tetrodotoxin: a quantitative electron microscopic analysis. Dev. Brain Res. **20**, 55–68.

Shah G. N. and Mooradian A. D. (1997), Age-related changes in the blood-brain barrier. Exp. Gerontol. **32**, 501–519.

Siegel G. J. (1989), Basic neurochemistry. In: Molecular, Cellular and Medical Aspects, 4th edition (B. W. Agranoff, R. W. Albers and P. B. Malinoff, ed.). Raven Press Ltd, New York, pp. 479–540.

Smith M. A. (1996), Hippocampal vulnerability to stress and aging: possible role of neurotrophic factors. Behav. Brain Res. **78**, 25–36.

Sykova E. and Chvatal A. (2000), Glial cells and volume transmission in the CNS. Neurochem. Int. **36**, 397–409.

Tanaka Y., Hasegawa A. and Ando S. (1996), Impaired synaptic functions with aging as characterized by decreased calcium influx and acetylcholine release. J. Neurosci. Res. **43**, 63–76.

Tian L., Cai Q. and Wei H. (1999), Alterations of antioxidant enzymes and oxidative damage to macromolecules in different organs of rats during aging. Free Radic. Biol. Med. **24**, 1477–1484.

Tsakiris S., Angelogianni P., Schulpis K. H. and Stavridis J. C. (2000), Protective effect of L-phenylalanine on rat brain acetylcholinesterase inhibition induced by free radicals. Clin. Biochem. **33**, 103–106.

Tsakiris S. (2001a), Effects of L-phenylalanine on acetylcholinesterase and Na^+,K^+-ATPase activities in adult and aged rat brain. Mech. Ageing Dev. **122**, 491–501.

Tsakiris S. (2001b), Effects of L-phenylalanine on acetylcholinesterase, (Na^+,K^+)-ATPase and Mg^{2+}-ATPase activities in adult rat whole brain and frontal cortex. Z. Naturforsch. **56c**, 132–137.

Unger J. W. (1999), Glial reaction in aging and Alzheimer's disease. Microsc. Res. Tech. **43**, 24–28.

Urano S., Sato Y., Otonari T., Makabe S., Suzuki S., Ogata M. and Endo T. (1998), Aging and oxidative stress in neurodegeneration. Biofactors **7**, 103–112.

Vijayan V. J. and Cotman C. W. (1987), Hydrocortisone administration alters glial reaction to entorhinal lesion in the rat dentate gyrus. Exp. Neurol. **96**, 307–320.

Volkow N. D., Wang G. J., Fowler J. S., Ding Y. S., Gur R. C., Gatley J., Logan J., Moberg P. J., Hitzemann R., Smith G. and Pappas N. (1998), Parallel loss of presynaptic and postsynaptic dopamine markers in normal aging. Ann. Neurol. **44**, 143–147.

Vrensen G. and De Groot D. (1974), The effect of dark rearing and its recovery on synaptic terminals in the visual cortex of rabbits. A quantitative electron microscopic study. Brain Res. **78**, 263–278.

Xafenias D. A. and Hampatzis P. S. (1995), Proceedings of the 21st Annual Greek Medical Symposium, Athens, pp. 27–35.