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The Principal Inferior Olivary Nucleus in Aging and Alzheimer's Disease

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ABSTRACT

Neuronal degeneration is a commanding event in Alzheimer's disease (AD). Neuronal loss is one of the major hallmarks and is frequently reported in regions with presence of neuritic plaques (NPs) and neurofibrillary tangles (NFTs). The causative role of β-amyloid (Aβ) and tau protein in neurofibrillary pathology is controversial, and the main trigger for neurodegeneration in AD is still unknown. Thus we investigated neuronal and glial changes in the principal inferior olivary nucleus (PO) in normal aging and AD. This region is known not to develop NPs or NFTs in sporadic AD, although a few diffuse Aβ accumulations can be found. To obtain a precise number of cells an unbiased quantitative methodology should be applied. In the first study using a stereological method, optical fractionator, we estimated the total number of neurons in PO, where we reported a significant neuronal loss of up to 34% in AD group compared to age-matched controls. It is well established that neurons are in very close contact and cohabitation with glial cells. Whether neurons in PO die solely or other cells are involved was not known. In the second study we expanded a quantitative analysis and estimated a total number of oligodendrocytes and astrocytes in PO in AD and control brains. The total number of astrocytes remained unchanged, but oligodendroglial cell number was diminished by 46% indicating interdependent degeneration among neurons and oligodendrocytes. We could conclude that neurodegeneration takes place in AD brains, even in absence of neurofibrillary pathology. The patomechanisms behind the neuronal loss are still unknown. In the third study we applied immunohistochemistry with unbiased quantification and ELISA method to analyse astroglial markers (S100B, GFAP and vimentin) known to be involved in neurodegeneration. A 2-fold significant increase of S100B was found by immunohistochemistry and by ELISA in AD *vs.* age-matched controls. Due to the fact that S100B can have both beneficial and toxic effects, depending on its concentration, we performed additional immunostaining for iNOS and NT-3, as downstream markers for oxidative stress and neurotoxicity. In AD brains remarkably stronger and widespread staining pattern was noted both in neurons and glial cells compared to controls indicating predominantly neurotoxic events. These results strongly pointed toward oxidative damage to be involved in neuronal and glial degeneration. In the last study we investigated the presence of $[Ca^{2+}]_i$ related neuronal markers, calcium binding proteins (calbindin-CB, calretinin-CR, parvalbumin-PV) as pivotal molecules in understanding Ca-dependent neurodegeneration. We observed a significant reduction of CB and CR up to 65% in AD brains. Thus loss of the calcium binding proteins can play an additional role in neuronal and glial degeneration in PO.

In summary, using a quantitative approach based on stereology we were able to demonstrate that neurodegeneration with glial changes occurs also in the brain region that is not affected by neurofibrillary pathology. Furthermore, the changes occurring in PO can be used as a model to investigate diverse pathomechanisms involved in neuronal and glial pathology.

List of publications

I. **Lasn H,** Winblad B and Bogdanovic N. (2001). The Number of Neurons in the Inferior Olivary Nucleus in Alzheimer's Disease and Normal Aging: A Stereological Study Using the Optical Fractionator. Journal of Alzheimer Disease 3: 159-168

II. **Lasn H**, Winblad B and Bogdanovic N. (2006). Neuroglia in the inferior olivary nucleus during normal aging and Alzheimer's disease. Journal of Cellular and Molecular Medicine 10(1): 145-156

III. **Lasn H**, Cedazo-Minguez A, Winblad B, Bogdanovic N. (2006) Astroglial activity in the human olivary nucleus in aging and Alzheimer's Disease. *Submitted.*

IV. **Lasn H,** Winblad B, Schober R and Bogdanovic N**.** Loss of calcium binding proteins (CBPs) in principal inferior olivary nucleus in Alzheimer's disease. *Submitted.*

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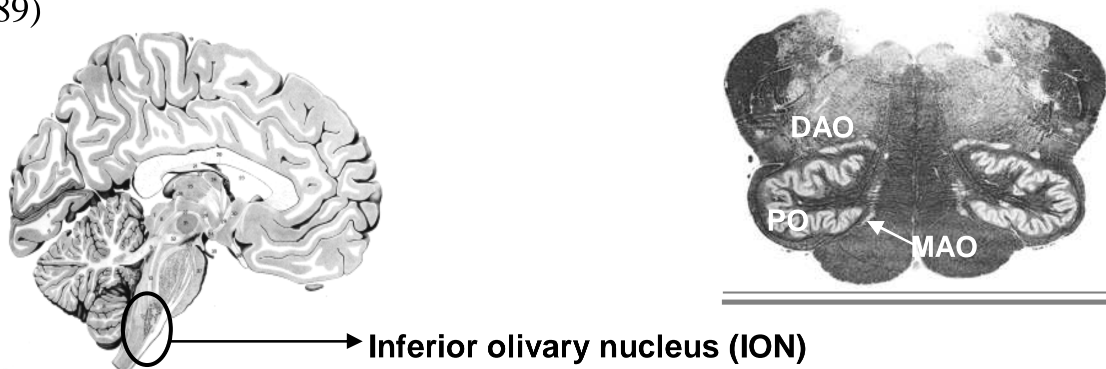
List of abbreviations

Ach	acetylcholine
AD	Alzheimer's disease
AMPA	α-**a**mino-5-hydroxy-3-**m**ethyl-4-isoxazole **p**ropionic **a**cid
ApoD	apolipoprotein D
APP	amyloid precursor protein
ATP	adenosine triphosphate
Aβ	β-amyloid
BDNF	brain derived neurotrophic factor
BV	biological variability
Ca	calcium
Ca^{2+}	calcium ion
$[Ca^{2+}]_i$	intracellular calcium
CBPs	calcium binding proteins
CB	calbindin
CE	coefficient of error
CFs	climbing fibres
CF-reflex	climbing fiber reflex
CNS	central nervous system
CO	old control group
CR	calretinin
CRF	corticotrophin releasing factor
CV	coefficient of variance
CY	young control group
DA	dopamine
DAO	dorsal accessory olive
DLB	Diffuse Lewy Body disease
DNA	deoxyribonucleic acid
ELISA	Enzyme Linked Immuno-Sorbent Assay
EPT	essential palatal tremor
ET	essential tremor
(f)MRI	functional magnet resonance imaging
$GABA_A$	gamma aminobutric acid receptor subtype A
GFAP	glial fibrillary acidic protein
Glu	glutamate
His	histamine
IGF-I	insulin growth factor-I
IL-1	interleukin-1
ION	inferior olivary nucleus
iNOS	inducible nitric oxide synthetase
Lbs	Lewy bodies
LC	locus ceruleus
LTD	long term depression
LTP	long term potentiation
MAO	medial accessory olive
MAO-inhibitor	monoamino oxidase inhibitor
MCI	mild cognitive impairment
MK	mitochondria
NA	noradrenaline
NO·	nitric oxide radical
NT-3	nitrotyrosine
PCs	Purkinje cells
PD	Parkinson's disease
PET	positron emission tomography
PO	principal inferior olivary nucleus
PV	parvalbumin
RNA	ribonuclein acid
ROS	reactive oxygen species
SD	standard deviation
SEM	standard error of mean
SSRIs	selective serotonin reuptake inhibitors
SPT	symptomatic palatal tremor
SSs	simple spikes
S100b	glial calcium-binding protein
TGFβ	transforming growth factor
2D	two-dimensional
3D	three-dimensional
5HT	serotonin
$5HT_{1c}$, $5HT_2$, $5HT_3$	serotonin receptor subtypes

1. INTRODUCTION

1.1. Anatomy and evolutional development of human PO

In the human brain the inferior olivary nucleus (ION) is located in the medulla oblongata and consists of primarily (85%) of the principal olivary nucleus (PO) which is accompanied by the dorsal accessory olive (DAO) and the medial accessory olive (MAO) (reviewed in Brodal, 1940, Azizi, 1987, De Zeeuw et al., 1998). While in monkey and rat the MAO is largest (Robertson and Stoler, 1974). During evolution the PO has increased in size in proportion to the increase of neocortex and neocerebellum. Thus, it is likely that the PO is morphologically and functionally the most developed subnucleus of inferior olivary complex in humans and primates (Ramon y Cajal, 1909, Kooy, 1917, Escobar et al., 1968, Azizi, 1987, Nelson et al., 1989)



1.2. Morphology of human PO

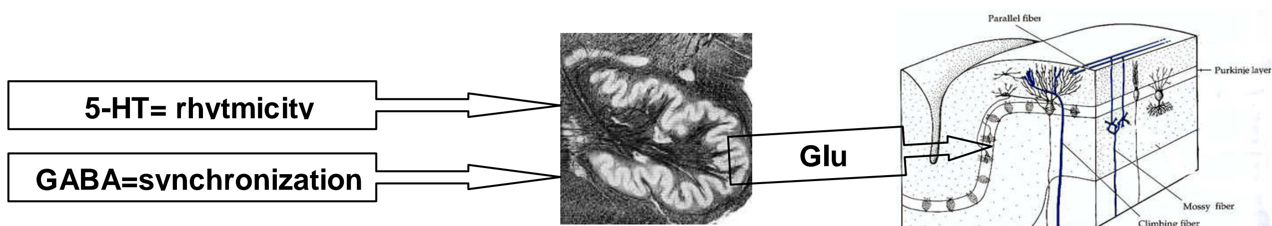
Two types of neurons in the PO can be distinguished, large multipolar and small interneurons. Large multipolar neurons make up about 90% of all the neurons. They are about 25 μm in diameter with centrally located large spherical nuclei, and positive for aspartate and glutamate (Szteyn, 1988, Fredette et al., 1992, Sadowski Pine et al., 1997). Human olivary projection neurons are also positive for corticotrophin-releasing factor (CRF) (Powers et al., 1987) and for insulin growth factor-I (IGF-I), (Bondy, 1991) which serve as modulatory transmitters and trophic factors for PCs in olivo-cerebellar system. The second population (5-10%) of neurons in the PO are pear-shaped (rounded) GABA-positive interneurons (about 7-10 μm) in the PO. These small interneurons terminate within the nucleus (Szteyn, 1988, Fredette et al., 1992) and provide inhibitory modulation within

olivocerebellar system (Lang et al., 1996, Ruigrok, 1997, Wegiel et al., 1999). The large multipolar neurons are a source of efferent projections called climbing fibers (CFs) target the contralateral cerebellar cortex, mainly dendrites of PCs (Powers, 1987), but also basket and Golgi cells (Scheibel & Scheibel, 1954, Powers et al., 1987) and dentate nucleus (Szteyn, 1988, Rub et al, 1998,). This projection from olivary nucleus is a part of the cerebello-dentato-rubro-olivary loop also called *Guillan-Mollaret triangle* described in 1931 (Trelles, 1943, Amstrong, 1974, Lapresle, 1979, Gudovic, 1996, DeZeeuw et al., 1997).

Olivary neurons fire rhythmically and synchronously (Benardo and Foster, 1986) due to electrotonic coupling via gap junctions (Llinas & Yarom, 1981). Gap junctions are dendro-dendritic connections via spines of several olivary units and they form glia-covered glomeruli (King, 1976, DeZeeuw et al., 1990). Electrical coupling between olivary neurons mediates the CF-reflex (Llinas & Sasaki, 1989) and this can occur without chemical synaptic transmission (Llinas & Yarom, 1981, Lang, 2001). The neurons of ION have a spontaneous low firing rate (Lang, 2001), similarily to PCs and dentate neurons (Raman & Bean, 1999, Lang, 2001). Each single neurons is directly coupled to 50 neighbouring neurons (Devor & Yarom, 2002). The ION has the highest density of electrical synapses in the adult brain (Llinás et al., 1974). Electrical synapses make it possible for signals become highly distributed throughout the neuronal network before being spread to cerebellum, each ION neuron makes 200 synapses with single PCs. The dendritic arbors in the PO are one of the most complex in whole human brain (Amstrong, 1974). During embryogenesis the survival of ION neurons is independent of the presence of PCs, but immediately after birth they become critically dependent for some time, while with aging the target dependence deminishes (Chu et al., 2000, Hess et al., 2003). PO neurons are known to be strongly positive for calbindin (CB) and parvalbumin (PV) as early as gestation week 11 (Nag, 2004). There are no developmental reports about calretinin (CR) positivity in human PO. We have shown for the first time that the neurons in PO are positive for CR immediately after birth and throughout the lifespan (Lasn et al., *paper IV*). Adult neurons remain CB and CR positive until late senescence (Nag, 2004, Lasn et al., 2006, *paper IV*) while PV positivity is lost after midlife in normal brains (Nag, 2004, Lasn et al., 2006, *paperIV*).

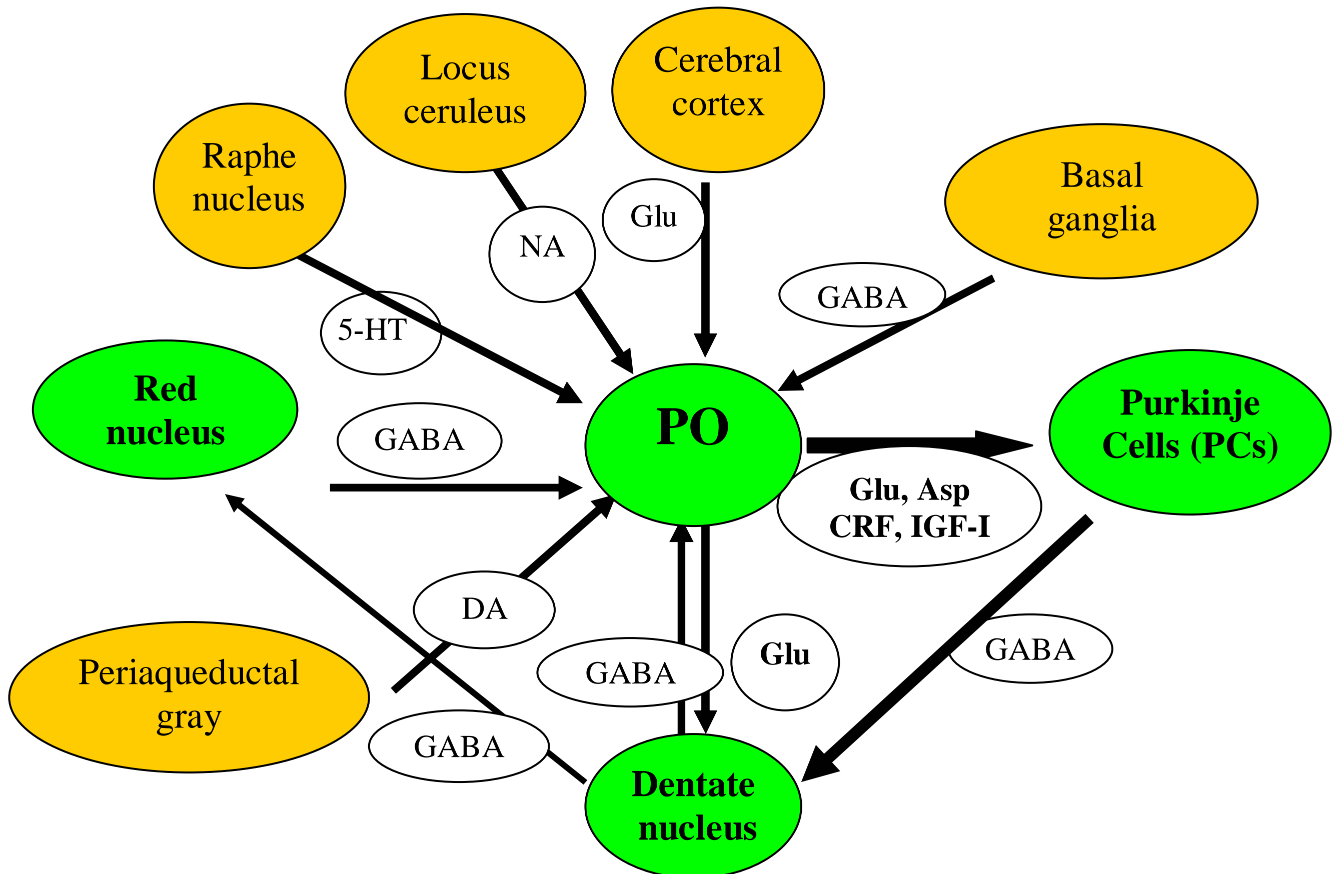
1.3. Afferent and efferent PO connections

The main inhibitory (GABAergic) **afferents** to the PO emerge from the parvocellular part of the red nucleus (*ruber*), basal ganglia and dentate nucleus (*dentatus*) (Bishop et al., 1976, Brodal, 1981, Szteyn, 1988, Ten Donkleaar, 1988, Schwarz and Schmitz, 1997). Additional glutaminergic afferents from ipsi- and contralateral motor cortex (Amstrong, 1974, Giaquinta et al., 1999). Serotonergic (5HT) afferents are from nucleus raphe (Weiss & Pellet, 1982) and dopaminergic input from periaquaeductal gray (Brodal 1981) and noradrenergic input from the medulla but not from the locus ceruleus (LC) (Powers et al., 1990). The strongest control over PO synchronization is controlled by input from dentate nucleus (Giaquinta et al., 1999, Bengtsson et al., 2006). In turn the output of dentate neurons depends on the balance between excitation (glutamate from PO) and inhibition (GABA from Purkinje cells) of these cells, and thereby influences the motor coordination (Sastry et al., 1997). Thus the PO is involved in a multiple transmitter system, which all are essential to regulate the physiological firing pattern of PO, where the 5HT is most strongly related to PO rhytmicity (Headley et al., 1976, Serrano-Dueñas, 2002) and GABA to synchronization (Giaquinta et al., 1999). The parvocellular part of red nucleus which terminates in ipsilateral PO connects the olivo-cerebellar loop with motor and premotor cortices (Ulfig, 2001). These structures play an important role in language learning – word finding (cognitive function) and word expressing (motor) form a language learning loop (Wegiel, 1999).



The PO provides the strongest input to cerebellum, by sending glutamatergic **efferent** climbing fibres (CFs) to Purkinje cells (PCs). This projection is one of the most unique in the CNS due to its 1:1 (each PC receives one CF) topographical relationship (Eccles et al., 1966, Sugihara, 2006). Whereas one climbing fiber gives off 7-10 collaterals by contacting 7-10 PCs (1:7-1:10) (Kandel et al., 2002, Sugihara, 2006). The CF endings wrap around PC cell bodies and proximal dendrites making numerous synaptic contacts (Kandel, et al., 2002). Climbing fibres induce long term

depression (LTD), which is important in motor learning (Kandel, 2002). In different experimental models, it has been shown that the CFs have high potential for regeneration and plasticity, partly related to the high levels and sensitivity to IGF-I (Nieto-Bona et al., 1995, Fernandez et al., 1998).



1.4. The physiology and functional importance of the PO.

It has been proposed that the olivocerebellar system functions as a timing device for motor coordination in which PO acts as a "pacemaker", coordinating cerebellar rythmicity and organizing movements in time and space (Benardo and Foster, 1986, Llinas & Welsh, 1993, Welsh et al., 1995, Lang, 2001). Olivocerebellar control over movements is mediated by populations of olivary neurons operating as a distributed system whose collective activity is rhythmic and temporally related to specific parameters of movement (Welsh, 1995). PO output into PCs generates complex spikes (CSs) which are the bursts of action potentials in PCs triggered by a single CF (Eccles et al., 1966, Welsh et al., 1995). This generates a prolonged voltage-gated calcium conductance in the soma and dendrites of

postsynaptic PC (Kandel, 2002). In contrast, mossy fibers (MFs) produce a brief excitatory postsynaptic potential that generates a single action potential ie simple spikes (SSs) (Kandel, 2002). Although the ION input to PCs is excitatory, the long-term effect is to suppress their spontaneous activity, thus providing tonic effect on PCs via climbing fibers (Colin et al., 1980, Demer et al., 1985, Bengtsson et al., 2006) and influencing PC responsivess to parallel fiber input (Ito, 1989, Bengtsson, 2005, thesis). When the PCs are deprived of CF input, rapid increase in tonic firing will occur (Colin et al., 1980, Demer et al., 1985, Cerminara and Rawson, 2004). The important role of inferior olive in motor control is demonstrated in studies of the behavioral effects of tremogenic drugs (harmaline) (DeMontagny & Lamarre, 1974, Headley et al., 1976) and in extracellular electrophysical studies (Llinás and Volkind, 1973, deMontigny and Lamarre, 1973).

Functionally the PO is involved in synchronization and timing of motor movements and learning, cognitive planning, practice-related learning, judging time intervals and the velocity of moving stimuli, cognitive operations in three dimensional space (Wegiel, 1999). Since the *Guillan-Mollaret triangle* is a closed loop, of one part of the intact circuit will lead to imbalance in the whole circuit. (Colin et al., 1980, Demer et al., 1985, Cerminara and Rawson, 2004, Bengtsson et al., 2006). CFs are known to transmit unconditioned stimuli (Bengtsson et al., 2006) while MFs mediate conditioned stimuli in learning process (Hesslow et al., 1999).

In pathological situation, PO is considered to be a place that generate the hyperrhythmic components of tremor and myoclonus (Welsh et al., 1998, Lang, 2001, Serrano-Dueñas, 2002). Experimental lesions at the level of PO produce ataxic and dysmetric movements (Welsh et al., 1995). ION neurons have the rhythmical property of 0,5-12 Hz (Serrano-Dueñas, 2002). Moreover, without serotonin the neurons of inferior olive are predisposed to oscillate continuously, which leads to tremor at 10Hz, which in turn generate hyper-rythmic conditions to thalamus and cortex (Welsh et al., 2002, Serrano-Dueñas, 2002). In patients treated with fluoxetin the most frequent side-effect is tremor, which disappeared after the treatment was discontinued or by Closapine, via 5HT1c, 5HT2 and 5HT3 receptors (Serrano-Dueñas, 2002).

1.5. Clinical symptoms and other disorders associated with PO pathology.

In brief, the physiological and pathological roles of ION have been investigated in detail for several decades, and there is substantial evidences that support ION role in generating different clinical symptoms such as essential tremor, palatal myoclonus and cerebellar ataxia (Welsh et al., 1998) but few studies show that the ION may not be the only keyplayer to cause these symptoms. However it has been shown that thalamo-cortical projections are also involved (Hua et al., 1998, Elble, 1998).

1.5.1. Clinical symptoms related to ION pathology

Essential or action tremor (ET), is one of the most representative tremor disorders in humans (Elble, 1998, Deuchl & Elble, 2000, Martin, 2004). ET affects ca 5% of elderly people over 65 years old and is characterized by 4-12Hz postural and kinetic tremor involving the arms (95%), and less commonly head (34%), lower limbs (20%) , trunk (5%) and voice (5-12%) (Elble, 2000, Ondo et al., 2006). The olivocerebellar system is shown to play a key role of generating ET both in harmaline induced animal models (DeMontigny & Lamarre, 1973,1974, Batini et al., 1979, 1981) and in humans (Deuchl & Elble, 2000, Louis et al., 2002, Pinto et al., 2003). Experimental studies have demonstrated that harmaline, the most often used tremogenic drug, selectively affects neuronal rhythmic activity in ION, modulating rhythm-generating ionic currents, thus facilitating rhythm-generation resulting in generalized tremor (DeMontigny & Lamarre, 1974, Llinás Y & Yarom, 1986) inducing selective PCs death possibly due to excitotoxic effect via CFs (O'Hearn & Molliver, 1993, Miwa et al., 2006). A similar tremogenic effect in humans is found to be mediated by serotonin (5-HT), via 5-HT2A receptor (Sugihara et al., 1995, Welsh et al., 1998) which is in agreement with the tremogenic effect of SSRIs (Serrano Dueñas, 2002) also supported by animal studies (Sugihara et al., 1995). However 5HT and harmaline appear to have independent mechanisms, since the action of harmaline cannot be blocked by 5HT antagonist (Sugihara et al., 1995). Also alcohol and antiepileptic medications such as barbiturates and benzodiazepines, levetiracetam (Bushara et al., 2002) as well as toprimate and propranolol (Ondo et al., 2006) are shown to inhibit inferior olivary

rhythmic oscillations and reduce essential tremor (ET). Toprimate via $GABA_A$ and AMPA/kinate type receptors, known to play crucial role in IO rhytmicity and synchronization (Ondo et a., 2006). Autopsy studies reveal no gross or microscopic abnormalities in the brains of ET patients (Deuschl & Elble, 2000).

Myoclonus ie. palatal tremor is a prevalent and debilitating neurological disorder that is characterized by sudden and shock-like involuntary movements (Welsh et al., 1998). Palatal tremor is divided into essential (EPT) which cause is unknown and symptomatic (SPT) occurs after olivo-cerebellar damage e.g. in *Guillain-Mollaret triangle* (Deuschl et al., 1994). Myoclonus is often rhythmic and persists through sleep, anesthesia and coma. It is generally accepted that decreased serotoninergic transmission leads to hyperexcitability of motor system in these patients (Pranzatelli, 1994, Welsh et al., 1998). Most of the SPT occurs after vascular event in dentate-olivary tract, causing (pseudo)hypertrophy and transsynaptic degeneration of ION (Deuschl et al., 1994, Pierot et al., 1992, Goyal et al., 2000, Deuschl & Wilms, 2002), leading to disturbances in electrotonic coupling between olivary neurons (Deuschl et al., 1994). Olivary hyperactivity with or without hypertrophy has been shown in SPTpatients in PET and MRI studies (Welsh et al., 2002, Samuel et al., 2004). In contrast, there were no pathological autopsy changes in the brains of EPT patients (Goyal et al., 2000). Palatal myoclonus has been reported in very few cases of progressive supranuclear palsy (PSP) and in AD, but ION hypertrophy is uncommon (Fukutani et al., 1999, Katsuse & Dickson, 2004). The presence of myoclonus in AD patients is related to pathology in neuronal volume in dentate nucleus, with no neuronal loss or astrocytosis (Fukutani et al., 1999). Increasing evidence indicates a key role for 5HT on the level of ION via $5HT_2$ receptor by reducing low-threshold calcium conductance thus regulating the rhythm of olivary neurons (Welsh et al., 2002). However the final pathomechanisms are still controversial (Zadikoff et al., 2006).

Cerebellar ataxia, although reversible has been demonstrated in rodents after damage of inferior olive (Fernandez et al., 1998). The mechanism involved is related to significant role of IGF-I produced by ION, which palys key role in survival and physiology of PCs. Moreover, IGF-I is involved in regulating CB levels in PCs, leading to impaired motor learning when ION was abolished (Nieto-Bona, 1995). Ataxia developed in subgroup of patients with symptomatic palatal tremor (SPT) and high signals in inferior olive

were present all patients with myoclonus with or without ataxia in an MRI study (Samuel et al., 2004).

1.5.2 Other diseases and syndromes where ION pathology is shown to be involved.

There is a wide range of different pathological conditions where important role of ION is postulated, sush as: progressive supranuclear palsy (PSP) (Katsuse & Dickson, 2004), multiple system atrophy (MSA) (Braak et al., 2003) and its subtype olivo-ponto-cerebellar atrophy (OPCA) (Ozawa et al.,2004), sudden infant death syndrome (SIDS) (Kinney et al., 2002), spino-cerebellar ataxia (Koeppen, 2005, Rüb etal., 2005), dyslexia (Finch et al., 2002) and autism (Bauman & Kemper, 2005, Welsh et al., 2005).

1.6. Normal aging brain

… old men have grey beards… their faces are wrinkled, their eyes purging, thick amber and plumtree gum, and they have a plentiful lack of wit, together with most weak hams… (Shakespeare, Hamlet)

Aging people show a decreased ability to make use of and retain both new and existing knowledge (Verkhratsky & Toescu, 1998). During aging several unfavorable physiological events take place, such as increased oxidative stress, impaired energy metabolism, perturbed cellular and Ca^{2+} homeostasis (Verkhratsky & Toescu, 1998, Mattson, 2004). These changes make the aged neurons more vulnerable to several factors what contribute to the development of neurodegeneration if trigger should appear. Interestingly, 5HT has been shown to suppress age-related oxidative stress (Mattson et al., 2004).

Memory impairment during aging is related to neuronal dysfunction, impairment of LTP and a decrease in synaptic efficacy and plasticity with no concomitant neuronal loss (Geinisman et al., 1995, Barnes, 1997, Kelly et al., 2006), and these events are Ca^{2+}-dependent (Hof, 2004, Kelly et al., 2006). The mechanisms behind increased $[Ca^{2+}]_i$ most likely occur at all levels: greater influx and less extrusion as well as more release of Ca^{2+} from intracellular stores together with a decrease of Ca-buffers (Verkhratsky & Toescu, 1998, Geula, 2003). Altered $[Ca^{2+}]_i$ signalling in neurons leads to

decrease in activity-dependent signalling, energy metabolism and biosynthesis, which will further result in downregulation of synaptic plasticity and axonal regression (Kelly et al., 2006). Glial cells, especially astrocytes rely strongly on $[Ca^{2+}]_i$ signaling, which influences their responsiveness to neurotransmitters, glia-glial and neuron-glial interactions (Verkhratsky & Kettenmann, 1996, Perea & Araque, 2006). Via altered glial metabolism, astrocytic hypertrophy and changes in extracellular matrix, impaired cognition and neuronal vulnerability are augmented (Blalock et al., 2003). Mitochondrial (MK) dysfunction is known to occur in aging (Toescu et al., 2000). Mitochondrias are the major source (ca 90%) of the ATP required by for normal neuronal function. The defective MK function alters APP metabolism by increasing Aβ levels with concomitant ATP deletion leading to impairment of $[Ca^{2+}]_i$ –buffering and results in of oxidative stress (Toescu et al., 2000, Mattson, 2004).

1.7. Alzheimer's Disease (AD) and motor symptoms in AD

Alois Alzheimer described (1906) a neuropsychiatric disorder affecting the elderly, which today is known as Alzheimer's disease. AD is the most common type of dementia and Parkinson's disease (PD) the most frequent movement disorder which are morphologically characterized by progressive neuronal degeneration (Jellinger & Stadelmann, 2003). Clinically, AD is characterized by progressive memory loss, intellectual and emotional dysfunction as well as changes in neuroendocrine and autonomic functions. Nearly 2% of the population in industrialized countries suffer from AD. The risk increases dramatically when seventy years of the lifespan have been reached, and it is predicted that in next fifty years the number of people suffering from AD triple (Mattson, 2004). In the preclinical phase the brain regions responsible for memory and cognitive abilities become affected – such as hippocampus and temporal lobe, where progressive dysfunction and neuronal death occur (Ball, 1976, Braak & Braak, 1991). AD is a disease, in which multi-transmitter-systems are affected, neurons that produce Glu, GABA, Ach, 5HT, DA, NA, somatostatin, neuropeptide Y, CRF and substance P are affected in AD (Selkoe, 1997, Mattson, 2004). AD is so called "triple protein disorder" where at least three different building block proteins (tau, alpha-synuclein and Aβ) fibrillize and aggregate into pathological deposits within neurons (NFTs, Lbs) and in extracellular space (NPs) neurons (Trojanowski & Mattson, 2003). Synergistic interactions between these three proteins are suggested in

different neurodegenerative disorders (Giasson et al., 2003), and AD is known to overlap with tauopathies and a-synucleopathies (Trojanowski & Mattson, 2003). The etiopathology behind AD is multifactorial and involves numerous intra- and extracellular cascades. The most common etiopathological basis to develop AD hallmarks has been proposed to be Aβ accumulation according to "amyloid hypothesis" (Selkoe 1991,1994, Hardy & Selkoe, 2004). Since 1984 when Aβ was discovered, the amyloid as a pathogenic factor has been central point of research. But today there is increasing evidence that Aβ deposition is only one of several major factors causing the disease although it may certainly contribute to the mechanisms underlying the pathophysiology of AD (Moccini et al., 2001, Jellinger & Stadelmann, 2003). Aβ is continously secreted by neurons and is also found in CSF and blood (Haass et al., 1992, Seubert et al., 1992). Excessive Aβ accumulation in brain parenchyma in early stages of AD induces oxidative stress and release of reactive oxidative species (ROS) (Hardy & Selkoe, 2002), while in late AD fibrillary Aβ accumulation impair membrane functions by inducing lipid oxidation (LOX) and leads to cell death (Mattson and Chan, 2003). An interesting fact is that neuronal dysfunction does not correlate with Aβ deposition in experimental animals (Craft et al., 2005) or in humans (Giannakopoulos et al., 2003, Lee et al., 2004) which supports the idea that neuropathological changes occur long before the NPs do.

Another most interesting "non-amyloid hypothesis" are proposed to be disturbances in glucose metabolism including insulin resistance, Ca^{2+}-dyshomeostasis and defective mitochondrial functions. Oxidative stress is one of the earliest features in AD brain, which suggests that mitochondrial dysfunction lies at the heart of increased oxidative stress in AD (Zhu et al., 2006, De la Monte et al., 2006). In addition, DNA fragmentation during aging increases cell vulnerability in AD (Jellinger & Stadelmann, 2003). In hippocampus the number of neurons and glial cells which show DNA fragmentation and apoptotic signs increase 3 to 6 fold in relation to NPs and NFTs (Jellinger & Stadelmann, 2003). The underlying pathomechanisms in AD are believed to be similar to several other neurodegenerative disordes. The mechanisms leading to protein misfolding and aggregation, are believed to be following: intracellular oxidative stress as central event, which is triggered either by excitotoxcity, failure of normal antioxidative mechanisms, impairment of chapreones or impairment of protein refolding mechanisms (Trojanowski & Mattson, 2003) leading to profound cell death in many brain regions. The presence of numerous apoptotic cells is a feature of AD (Su et al., 1994, Cotman & Su, 1996,), which is linked to multiple factors such as cytokines, NFT, Aβ and immunological reactions (Behl,

2000). These similar, but not completely established mechanisms lead to diverse pathology in different brain regions and similar drugs may be advantageous for different neurodegenerative disorders. (Trojanowski & Mattson, 2003). In AD the levels of both BDNF and 5HT are furthermore decreased compared to age-matched controls, abnormalities of BDNF and 5HT signalling probably contribute the detrimental effects of uncontrollable stress on the brain, thus contributing to the pathogenesis of AD (Mattson, 2004).

Motor disturbances are common in AD and progress rapidly during its course (Scarmeas et al., 2004) along with cognitive and learning impairment. Less severe locomotor disturbances have been noted earlier in the disease process but traditionally interpreted as a result of decreased cognitive capacity (Reisberg, 1983). Moreover about 20-50% of patients who suffer from AD develop some extrapyramidal symptom in later stage (Clark et al., 1997). Often these symptoms are related to poorer prognosis (Wegiel et al., 1999, Scarmeas et al., 2005). Rigidity and bradykinesia, hypomimia (facial mask), difficulty in talking, postural instability, abnormal gait are the most common symptoms found in late AD patients (Clark et al., 1997, Tsolaki et al., 2001, Scarmeas et al., 2004, Petersson, 2005) while tremor is less frequent (Scarmeas et al., 2004). Futhermore, recent studies conclude that motor functions are impaired in very early AD, but not in MCI (Pettersson et al., 2005). Mild parkinsonian signs, especially rigidity (Louis et al., 2005) and essential tremor (Benito-Leon et al., 2006) are associated with the presence of dementia, but the underlying pathology has not been investigated. Majority of the symptoms are explained by nigral pathology or use of neuroleptics in AD patients (Tsolaki et al., 2001, Burns et al., 2006,). However, 92% neuroleptic-free AD patients showed such symptoms during their first two years of illness (Tsolaki et al., 2001). Although it is still not clear which part of the motor axis is most involved. It has been proposed that the degeneration of motor system starts in higher cortical structures with the involvment of executive cortical regions and neuronal population, followed by a progression of the disease, in which subcortical and brainstem structures become affected (Braak & Braak, 1996). The central role of olivary nucleus in maintaining the synchonization and rhythmicity of executive function in the brain suggests that degeneration of PO may have important consequence for the motor function.

1.8. Differences in normal aging and AD brains.

1.8.1. Neurons in aging and AD

The above mentioned early changes (see part 1.6.) are not sufficient to cause neuronal death during aging according to numerous studies of different brain regions (Hof 2004), including entorhinal cortex or subiculum (Korbo et al., 2004), which are the regions first affected in AD (Braak and Braak, 1991, 1995). In contrast, substantial neuronal loss is found in MCI and very mild AD (Hof, 2004). A common finding after the second decade is that there are few NFTs or NPs, but the distribution and proportions differ from AD (Hof, 2004). Thus AD is not simply an acceleration or natural continuation of aging (Hof et al., 2004, Mattson, 2004). In AD profound neuronal loss is mainly reported in following brain regions: entorhinal cortex (layerII), hippocampus (CA1), frontal, parietal and temporal cortices (West et al., 1994, Simic, 1997), basal forebrain (Arendt et al., 1983, Rogers et al., 1985), nucleus raphe (Lyness et al., 2003), locus ceruleus (Tomlinson et al., 1981), amygdala (Scott, 1993, Vereecken et al., 1994,) and substantia nigra (Lyness et al., 2003). There are several hypothesis about what the pre-requisites for neuronal damage and loss in AD might be e.g.: 1) neurons with long and sparsely myelinated axons are more susceptible to dyshomeostasis and toxic events (Braak and Braak, 1996), 2) the downregulation of genes in those neurons that either promote or prevent cellular death of including Glu-transporters, CBPs, and neurtrophic factors (Mattson, 2004). 3) Neuronal survival has been shown to be dependent of normal IGF-I levels both in cortical (Rivera et al., 2005) and olivo-cerebellar circuits (Nieto-Bona et al., 1995, Fernandez et al., 1998). In AD, there are numerous intraneuronal NFTs in cortical as well as in different subcortical regions, but not all neurons that die develop tangles and often the neuronal loss exceeds the amount of tangle-bearing neurons in AD (Gomez-Isla et al., 1997). DNA fragmentation is human brains is too frequent to account for the continuous loss in these progressive diseases. DNA fragmentation may actually be a sign of inability to repair DNA and does not necessarily lead to apoptosis (Jellinger & Stadelmann, 2003). DNA fragmentation may make the cells more vulnerable to hypoxia, oxidative stress and other pathogenic factors (Jellinger & Stadelmann, 2003). It is proposed that the neurons which have developed NFT, express apoptotic markers, such as immunochemically detectable caspases, while dying neurons without neurofibrillary tangles are

often negative for caspases (Rohn et al., 2001, 2005, Hyman et al., 2006). This may be explained by caspase-mediated cleavage of tau in AD (Rissman, et al., 2004).

In addition to NPs and NFTs in AD, neuronal loss (Selkoe, 1991, Giannakopoulos et al., 1998, Jellinger 2001), reactive astrocytosis (Selkoe, 1991, Brun et al., 1995; Liu et al., 1996; Overmyer et al., 1999; Unger 1998), activated microglia (Selkoe, 1991, Unger 1998) and oligodendroglial loss (Kobayashi et al., 2004, Lasn et al., 2006), are found in AD brains. Most studied in AD have focused on areas where the neuronal loss is accompanied by the presence of NFTs and NPs in neocortex (West et al., 1994, Gomez-Isla et al., 1997) and hippocampus (Ball, 1976, West, 1994), while fewer have studies the regions without classical hallmarks as dentate gyrus (Simic and Bogdanovic, 1997), cerebellar cortex and cerebellar nuclei (Fukutani et al., 1997, Wegiel et al., 1999). The classical NFTs and NPs do not occur in the olivary complex although diffuse amyloid precipitation can be found in definite sporadic AD cases (Lasn et al., 2006). The fate of olivary neurons in AD was not known until our first report where we found significant neuronal loss up to 34% (Lasn et al., 2001).

1.8.2. Oligodendroglia in aging and AD

The protracted development of cortical regions is driven by oligodendrocytes, which continue to differentiate into myelin-producing cells until fifth decade of life (Bartzokis, 2004). The high metabolic demands such as myelin sheet formation, trophic factor and cholesterol supply, high iron content, low levels of reduced glutathione (GSH) and glutathione peroxidase make the oligodendrocytes especially susceptible to different insults during aging and in pathological conditions (Bratzokis, 2004). The cholesterol produced by oligodendrocytes play an important role in continual functional plasticity, influencing synaptogenesis and dendritic outgrowth in gray matter (Bartzokis, 2004). In normal brain three types of oligodendrocytes are present: 1) progenitor cells (9%), with light pale nucleus, have rapid mitotic activity, 2) medium-shade (35%) with smaller nucleus than progenitors, have reduced mitotic activity 3) dark cells (56%) with dense nucleus are mitotically inactive mature oligodendrocytes (Berry et al., 2002). Oligodendrocytes are strikingly vulnerable to oxidative stress, inflammatory cytokines and excitotoxic neurotransmitters (Noble, 2004), especially the medium-shade type (Gard et al., 2001, Back et al., 2002, rev. Whitman & Cotman, 2004). Both mature and progenitor oligodendrocytes expresses receptors for Glu and GABA which induce changes in $[Ca^{2+}]_i$

regulation of oligodendrocyte differentiaion (Berry et al., 2002), but may also lead to $[Ca^{2+}]_i$ overload and cellular death (Salter & Fern, 2005). During aging iron levels in oligodendrocytes increase, which contributes to increased intracellular oxidation, which is necessary for triggering oligodendrocyte precursors to differentiate, thus inadequate iron levels result in poor myelination (Bratzokis, 2004). Both mature and progenitor oligodendrocytes are important providers of trophic factors for neurons but at present we know nothing about the fate of progenitors in gray matter of AD brains (Noble, 2004, Whitman & Cotman, 2004). Despite of these alterations in aging, the number of oligodendroglial cells remains unchanged in cortex (Pakkenberg, 2003) and inferior olivary nucleus (Lasn et al., 2006). However the total length of myelinated axons in human cortex is reduced by 46% in old age (Tang et al., 1997, Pakkenberg et al., 2003), primarily small calibre fibers which are more susceptible to AD pathology. (Braak, 1996, 2004, Bartzokis, 2004).

In AD there only very few reports focusing on oligodendroglial fate and number (Pelvig et al., 2003, Kobyashi et al., 2002, 2004). Using stereological approach Pelvig and collegues found no changes in cortical oligodendroglial, neuronal or astroglial number in AD (Pelvig 2003). Although no significant oligodendroglial loss has been reported in cortical regions, there is increasing evidence that oligodendrocytes degenerate, possibly via apoptosis (Kobayashi et al., 2004) or necrosis (Mitrovic, 1995). Pathological events such as Aβ accumulation, oxidative stress etc. occurring in AD are toxic for oligodendroglial cells (Gard et al., 2001, Xu et al., 2001, Lee et al., 2004, rev. Whitman & Cotman, 2004). It is also possible that degeneration of oligodendrocytes contributes to neuronal degeneration by iron release which accelerates oxidative stress (Mitrovic, 1995, Whitman & Cotman, 2004).

1.8.3. Astrocytes in aging and AD

Normal astrocytes constitute nearly 40% of the total CNS cell population. Astrocytes function as communication partners for neighbouring neurons by taking part in synaptic transmission (Navarro, 1994, Perea & Araque, 2002), participating actively in glutamate metabolism (Hafidi, 1994), metabolic exchange (Martinez et al., 1998, Unger, 1998, Ventura et el.1999, Bacci, 1999, Dringen 2000, Kirchhoff et al., 2001) and providing diverse growth factors (Tacconi, 1998, Du and Dreyfus, 2002), providing energy storage for glycogen and catabolising various toxins (Tacconi, 1998). The phenotype of astrocytes is especially dependent and monitored by

neighbouring neurons (Graeber et al., 2002). Two types of astrocytes can be distinguished according to their morphological and biochemical characteristics: a) *Fibrous astrocytes (type 1)* are found predominantly in white matter among myelinated axons they have longer processes (Walz, 2000). These astrocytes are immunoreactive for GFAP, but negative for ApoD (Navarro, 2004). b) *Protoplasmatic astrocytes (type 2)* have few laminar or membranous filaments with mossy appearance, are mingled with neurons. These astrocytes interact extensively with neurons in gray matter and influence their activity. (Walz, 2000). Usually the GFAP expression in gray matter astrocytes is very low (Navarro, 2004), but they start to co-express GFAP and ApoD when they are activated (Navarro, 2004). Astrocytes express wide variety of receptors for many neurotransmitters including Glu, NA, GABA, His, ATP, Ach etc. (rev. Perea & Araque, 2005). Traditionally astrocytes were believed to be non-excitable cells, because their membrane potential remains relatively stable after increase of $[Ca^{2+}]_i$ (Perea & Araque, 2002, 2006). Today, however, astrocytes are considered to be excitable cells but the increase of $[Ca^{2+}]_i$ in astrocytes is not transmitter-specific, but is selectively induced by specific synaptic pathways (Perea & Araque, 2002, 2006).

Moderate astocytosis has been reported in aging brains, but the data are still contradictory. During lifetime and normal aging the density of astrocytes has been shown to remain stable, but they proliferate slowly in normal adult, there must be a continuous turnover of these cells. Classical reactive astrocytosis is characterised by hypertrophy, hyperplasia, overproduction GFAP (Monzon-Mayor et al., 2000, Wilhelmsson et al., 2004) and re-expression of vimentin (Ridet, 1997, Menet et al., 2000, 2001, Wilhelmsson et al., 2004). Although GFAP expression is shown to increase progressively during normal aging, both in humans and rodents, the number of astrocytes remains stable, but GFAP increase per cell (Finch, 2003). Several studies indicate that not only oligodendrocytes but also reactive astrocytes inhibit regeneration of CNS (Menet et al., 2003, Wilhelmsson et al., 2004). In aging the levels of S100β protein are shown to be upregulated although a lesser extent than in AD (Sheng et al., 1996, 2000, Griffin et al., 1998, Royston et al., 1999, Mrak and Griffin, 2001) which may thus favor the onset of plaque pathology (Mrak et al., 2004) and trigger pathological iNOS activity (Hu et al., 1996, 1997, Esposito et al., 2006).

Neurodegeneration is known to trigger astrocytosis. (Ridet, 1997). In AD brains (cortical regions) the level of astrocytes have been shown to increase secondary to the degeneration of neurons and synapses (Brun et al., 1995, Liu et al., 1996)and to the presence of NPs (Mrak et

al.,1996, Monzón-Mayor et al., 2000), or both NFTs and NPs (Van Eldik and Griffin, 1994, Overmyer et al., 1999). In cortical areas, hippocampus and pons S100β is overexpressed up to 2-4 times measured by ELISA (Van Eldik & Griffin, 1994). In PO, histologically qualitative gliosis is reported (Sjöbeck, 1999), with no quantification of total number of astrocytes. However, there is also increasing evidence that astrocytes degenerate in AD (Smale et al., 1995, Kobayashi et al., 2004). Apoptotic astrocytes show an increase in lysosomal activity and decreased GFAP expression (Kobayashi et al., 2002). Astrocyte degeneration is related to Aβ, but on the other hand, Aβ alone is not believed to be exclusively responsible for apoptosis of astrocytes (Kobayashi et al., 2004). The caspases cleave GFAP and this may contribute to astrocyte degeneration (Mouser et al., 2006). The density of apoptotic astrocytes has not found to correlate with apoptotic neurons or NFTs. (Kobayashi et al., 2004). In general, heterogenous and controversial data about astrocytes in aging and degeneration could be due to the fact that astrocytes exhibit region-specific features and they do not respond in stereotypic manner to all forms of CNS insults (Ridet et al., 1997).

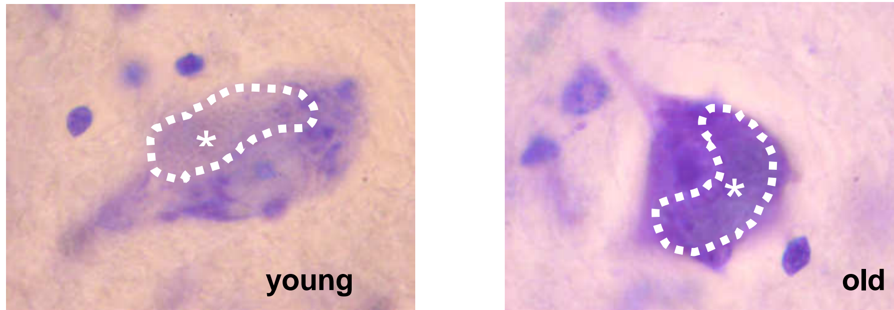
1.8.4 Neuron-glia morphofunctional network in general and in PO

A normal functional unit requires a properly functioning network of neurons, surrounding astrocytes, oligodendrocytes and microglia. Since the neuron-glial interactions play an important role in information processing in the CNS, pathology or dysfunction of one cell group interfers the whole morpho-functional network. In CNS gray matter the oligodendroglial cells are localized in close vicinity to neurons. They are also called “perineuronal satellite oligodendrocytes” which provide support to neurons *via* trophic factors such as BDNF, neurotrophin 3, TGFβ and IGF-1 (Hardy & Reynolds, 1993, Barres et al. 1993, Pratt & McPherson, 1997, Ludwin, 1997, Berry et al, 2002, Du & Dreifus, 2002) and regulate the microenvironment around neurons, play important role in axon growth and maintainance (Ludwin, 1997). A close interrelationship and cohabitation of oligo-dendroglia and neurons is essential for the existence of both cell types (Barres et al., 1993, Ludwin, 1997, Berry et al., 2002). Oligodendrocytes are connected to each other *via* tight junctions and to astrocytes *via* gap junctions. The presence and normal function of neurons and astrocytes is necessary for oligodendrocyte mitosis and maintainance (Hardy & Reynolds, 1993, Barres, 1993).

Astrocytes and oligodendrocytes are funcionally and electrically coupled with neurons (Alvarez-Meubecin et al., 2000, Lang, 2001) supporting rhythmic and synchronous neuronal firing (Alvarez-Maubecin et al, 2000, Du and Dreyfus, 2002). The astrocyte-neuron structural connection is called glomerul in which a thin layer of astrocytic cytoplasm separates and ensheats neuronal elements (Amstrong 1974). Neurons constantly require new carbon skeletons of glutamate (*de novo* synthesis from glycose) (Hertz et al.,1999). When these supplies are withdrawn, neurons are unable to generate aminoacid transmitters and oxidative metabolism becomes impaired (Hertz et al., 1999). As a consequence, depletion of 5HT in neurons triggers an overexpression of S100B and iNOS in astrocytes, which lead to activation of neurotoxic cascades (Ramos et al., 2002), see part 6. Astrocytes contain high levels of glutamate transporters (GluT) to take up and metabolise glutamate, thus normal astrocyte function is necessary for preventing an excess of glutamate with subsequent excitotoxicity (Hansson & Rönnbäck, 2003). Astrocytes play a key role in Glu uptake and providing lactate – an valuable metabolic fuel for neurons similarily to glucose (Hertz et al.,1999). Astrocytes respond to neuronal activity, modulate neuronal excitability and synaptic transmission (Perea & Araque, 2006) by responding to neuronal signals and releasing gliotransmitters such as Glu, D-serine, TNFα, or ATP (Perea & Araque, 2002, 2005). Astrocyte-neuron interaction can be pertubed by reactive astrocytes which secrete several proteases and protease inhibitors, what leads to degradation of matrix and therefore contributes to neurodegeneration (Ridet et al., 1997).

1.9. Inferior olivary nuclus during aging and AD

A characteristic feature in the olivary nucleus during aging is a progressive accumulation of lipofuscin, “an aging pigment”. Lipofuscin is considered to be one of the most consistent cytological alterations in the aging mammalian brain (Mann et al., 1978, Drach et al., 1998,). There is increasing evidence that the accumulation of lipofuscin has numerous negative impacts on cellular homeostasis, and it is a marker of brain vulnerability, stress and aging (Riga et al., 2006). Lipofuscin content in ION is comparable in control and AD brains, while lower in DLB, and is modified by drugs and diet (Dracht et al., 1998).



There is few reports investigating morphological changes in human PO during aging, most related to neuronal number. Studies that have estimated total neuronal number in inferior olive, are in agreement that there is no neuronal loss during aging (Moatamed, 1966,Escobar, 1968, Monagle, 1974, Lasn et al., 2001) despite differences in quantitative design. On the other hand, studies estimating neuronal density report non-specific decrease in PO neurons in aging (Grandi, 1992, Sjöbeck et al., 1999). Our first study (*paper I*) was the first to apply stereological approach for estimating the total number of neurons in human PO during aging and AD. At the cerebellar level there is no global PCs loss in aging, only slight decrease in anterior lobe, the part of cerebellum which regulates motor contol over movements (Andersen et al., 2003), while in AD PCs are shown to degenerate (Wegiel et al., 1999, Sjöbeck et al., 2001). In cerebellar dentate nucleus no neuronal loss occurred in AD, while changes in neuronal volume were noted (Fukutani, 1996). There have been no studies about oligodendroglia in human PO either in aging or AD.

2. AIMS

The aims of these studies are:

1) To test the working hypothesis whether human inferior principal olivary nucleus (PO) undergoes neuronal loss in AD or not by applying a novel quantitative unbiased stereological approach – An optical fractionator method. (*paper I*)

2) To estimate the total astroglial and oligodendroglial cell number in PO in normal aging and definite AD cases by optical fractionator method. (*paperII*)

3) To use immunohistochemistry and an ELISA method to characterize the astrocytes in aging and in relation to neurodegeneration in AD, by applying glia-related markers (GFAP, S100β, vimentin, iNOS). (*Paper III*)

4) To analyse calcium binding proteins (CBPs) as markers for neuronal vitality and degeneration in aging and AD brains, by examining the presence or absence of CBPs and possible relation to degeneration. (*Paper IV*)

5) To quantitate neurons and glial cells by applying 3D stereology (total number of cells) compared to 2D stereology-based principles (cell density) on sections used in routine neuropathology (3D *vs* 2D). (*paper III and IV*)

These results should provide a basic knowledge about morphological changes in human PO during normal aging and AD. These studies can be used as the templates for future investigations.

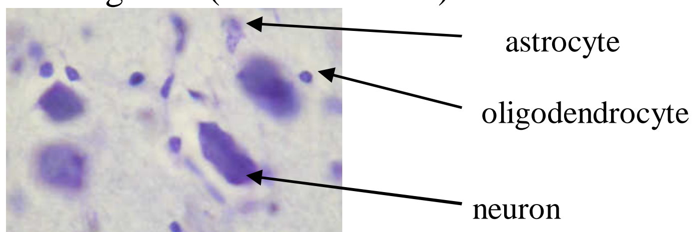
3. MATERIALS AND METHODS

3.1. Information about the brains used in all studies

All brains (controls and AD) used in these four studies were obtained from routine autopsies at the Huddinge Brain Bank in accordance with the laws and the permission of the ethical committee. Brains with Parkinson disease were received through European Brain Bank Network program (BNE) from Department of Neuropathology, University of Leipzig, Germany. The sporadic AD group included brains of patients who had had clinically and pathologically confirmed AD (range 79-90 years). Clinical diagnosis was based on a combination of DSM-III-R (American Psychiatric Association, 1987) and NINCDS-ADRDA (McKhann, 1984) criteria. The neuropathological diagnosis of AD was determined by using CERAD criteria (Mirra, 1993; Bogdanovic and Morris, 1995). Brains of AD patients with clinical Parkinson-like symptomatology and brains with other major neuropathological co-findings, e.g. multi-infarcts or presence of Lewy bodies were excluded from the analysis. All the brains were kept at +4ºC and fixed within 48 hours of death.

3.2. Histology and Nissl staining

Neurons, oligodendrocytes and astrocytes contain Nissl substance (extranuclear RNA) which is primarily composed of rough endoplasmic reticulum (with ribosomes). This RNA content is very basophilic, it is very sharply stained with basic aniline dyes. Nissl bodies are involved in protein synthesis and metabolism; their condition varies with physiological and pathological conditions (F.Nissl, 1894). We used Nissl staining to visualize the morphology of neurons and glial cells, the soma, nucleus and nucleolus. This stain enables us easily and clearly distinguish neurons, oligodendroglia and astrocytes in human PO providing reliable cellular morphology for the investigation (see foto below).



***Nissl staining technique*:** Cut paraffin sections and allow to dry overnight at +37ºC.

1. Deparaffinaze and hydrate in xylen and alcohol (xylen 2x10.min, 100% alcohol 2x10.min, 95% alcohol 10.min, 70% alcohol 10.min, water 10.min).
2. 0,5% Cresyl violet 2.min
3. rinsed in destilled water for 5.min
4. wash in 70% alcohol 3.min,
5. wash in 70% alcohol with few (2-4) drops 25% acetic acid for 2.min
6. wash in 70% alcohol for 10.min
7. wash in 95% alcohol for 10.min
8. wash in 100% alcohol for 5.min
9. xylen for 2x5.min
10. mount the slides with mounting media and cover with coverslip

Staining solution 0,5% Cresyl Echt Violet

0,5% Cresyl Echt Violet Acetate 0,5g

Destilled water 100ml

3.3. Immunohistochemistry

Amyloid plaques and abnormal *tau* detection

The presence of amyloid plaques and amyloid angiopathy was detected with polyclonal antibody for Aβx- 42 and Aβx-40 (each 1:1000, kindly provided by J. Näslund, Sweden). For detecting abnormal *tau* formation we used monoclonal antibody for AT8 (1:200, Innugenetics, Belgium) and Gallyas silver iodide stain (according to Braak and Braak, 1991).

Astrocyte markers

We used polyclonal anti-rabbit S100B antibody (1:10, Serotec, UK), monoclonal anti-mouse GFAP (1:1000, DAKO, Danmark) and monoclonal anti-mouse vimetin antibodies (1:100, DAKO, Danmark), monoclonal anti-mouse iNOS (1:40, R&D systems, UK)

Neuronal markers

Primary antibodies for calbindin (CB) (1:200, polyclonal anti-rabbit, Sigma), calretinin (CR) (1:250, polyclonal, anti-rabbit, Sigma), parvalbumin (PV) (1:200, monoclonal, anti-mouse, NovoCastra), and nitrotyrosine (NT-3) (1:500, polyclonal anti-rabbit, Chemicon) were used.

Immunostaining technique

After deparaffinizing sections chosen for CB, CR, PV, GFAP and vimentin staining (*paper II and IV*) were pre-treated in microwave for 10.min at 80ºC in recovery buffer (10mM citrate acid monohydrate, pH=6,0) to enhance antigene reactivity and achieve a more distinct staining. Sections for Aβ staining were pretreated with formic acid for 3 minutes (*paper II*), and sections for iNOS and NT-3 were incubated for 10 min at +37ºC with 1 part trypsin and 3 parts trypsin buffer. Then all sections were bocked for non-specific sites with Dako protein block (X0909), (Dako Cytomation, Denmark) for 30 min before being incubated with primary antibodies: The slides with primary antibody were incubated overnight or two nights at +4ºC, depending on the antibody and thickness of the section. For all washing steps 0,5M TRIS buffer, pH =7,6 was used. Both anti-mouse and anti-rabbit secondary antibodies were diluted 1:300 (DAKO), and visualized with the avidin-biotin-peroxidase complex (ABC) kit (Vector, Burlingame, Calif.) and thereafter with 3,3'-diaminobenzidine-4HCl/H2O2 (DAB, Sigma, St.Louis, Mo.). All immunostained sections were counterstained with 1:4 hematoxylin solution . In negative controls primary antibodies were omitted.

3.4. Quantification

3.4.1. Optical fractinator method (3D)

To estimate the total number of neurons, astrocytes or oligodendrocytes in the PO, we applied a stereological approach - a uniform systematic random sampling and the unbiased "optical fractionator" method (West et al., 1991), in *paper I, II, and III*. The method involves counting objects (cells) which constitute a known fraction of the volume from the region of interest, using an optical dissector probe in a uniform systematic manner (West et al., 1991). The method is independent of tissue shinkage and the particles of interest have the same probability of being presented regardless of size, shape or orientation in tissue (Gundersen, 1986, West et al., 1991). The counting equipment consists of a BH-2 Olympus microscope with a high-numerical-aperture (NA=1.4) x100 oil-immersion objective, a motorized stage, and an electronic microcator with digital readout for measuring movements in the Z-direction with 0.5 µm precision as well as computer program CAST-GRID v2.0 (Olympus, Denmark). Briefly, the area of interest, in our case the human PO was delineated in low magnification (x 2.5) using the computer program's cursor. Due to the clear anatomical

borders, it is not difficult to distinguish the human PO from its surrounding regions. A meander sampling function of the GRID v2.0 program was used for stepping through the delineated area with a chosen counting frame. The counting frame was defined prior every study, according to the principle that at least two full size counting particles (either neurons or glial cells, dependent on study) could fit into the frame. Then, a 100-x oil-immersion objective was moved into place and the appropriate counting frame superimposed on the screen. The desired horizontal and vertical step lengths, assisted by highly precise servo-controlled motorized microscopy stage were dimensioned for the appropriate distance between the counting frames on x- and y-axe. The cells in space were counted by an optical dissector probe (z-axis) with a height of 15 micrometers of the total thickness of the section (26-29 μm) in each study where the total number of cells was estimated (paper I, II and III). The thickness of every section was measured at three randomly chosen spots to get the mean value of section thickness. According to the rules of stereology we counted between 150-200 cells per whole structure (Gundersen et al., 1999). Finally, the total number of cells per region ie. whole PO (neurons, oligodendrocytes or astrocytes) was estimated according to the following equation:

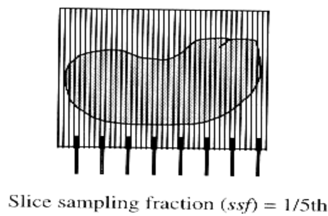
$$\mathbf{N_{total} = \sum Q^{-} \times 1/tsf \times 1/asf \times 1/ssf}$$

∑Q- = the number of cells in a known fraction of the volume of the structure, i.e., the known fraction of the total number of cells

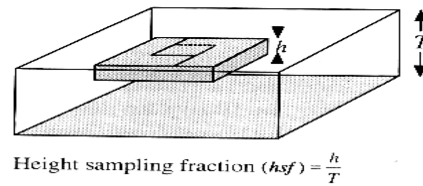
tsf = thickness sampling fraction , h/T (height of dissector/section thickness)

asf = area (frame)/area (Δ*x* x Δ*y* step) is an area -sampling fraction, i.e., the area of counting frame relative to the area associated with each step of the stepping motors.

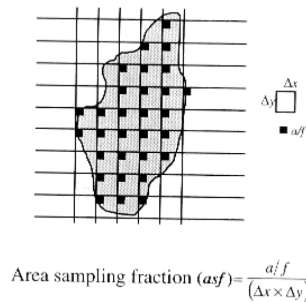
ssf = section sampling fraction, (1/20) section sampled by a known fraction, yielding finally 5-10 sections per brain to be analysed.



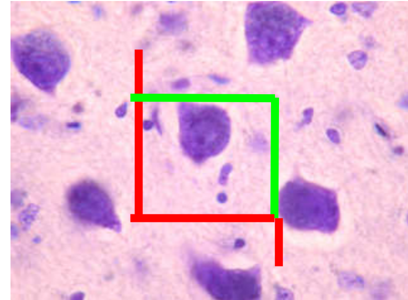
Slice sampling fraction (ssf) = 1/5th



Height sampling fraction (hsf) = $\frac{h}{T}$



Area sampling fraction (asf) = $\frac{a/f}{(\Delta x \times \Delta y)}$

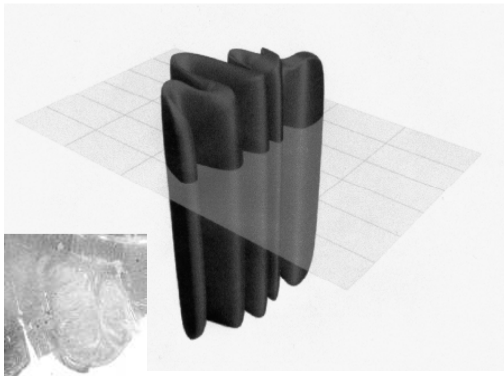


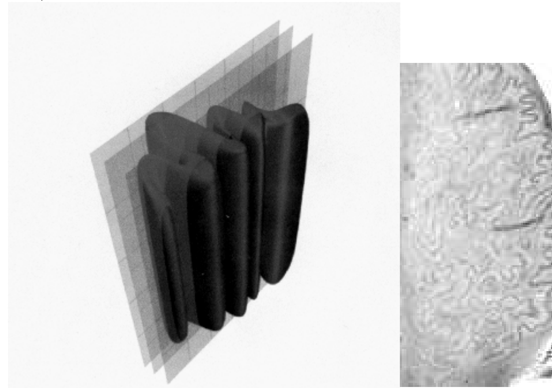
From Howard & Reed, 1998

3.4.2. Two-dimensional (2D) numerical density

Commonly used approach in morphometry is to estimate rather number of discrete objects (cells) per unit reference space (cells/mm^2) e.g. numerical density than a total number of cells per whole structure (Howard & Reed, 1998). We intended to test the reliability of “classical” 2D methods as compared to novel 3D stereological approach i.e. numerical density *vs* total number of cells. Thus we performed estimation of the neuronal density at single, randomly chosen horizontally and vertically cut section. The horozontal section was taken from the routine neuropathological series for the opposite olivary nucleus of the respective brain. The vertical sections were taken from the series of section used in stereological approach (*paper III &IV*).

a) horizontal section b) vertical section





In *paper III* an quantitative two-dimensional approach was used to estimate the density of GFAP positive astroytes (cells/mm^2). Randomly chosen sagittal cut slides were examined three times each, in 10 different systematic random points at 20x magnification with a grid of 0,2x0,2mm (adjusted to magnification), using the stereological principle for estimation of neuronal density. This method was used due to the lack of sufficient number of sections to fulfill the basic stereological requirements to estimate the total number of GFAP positive astrocytes.

3.4.3.Counting the percentage (%) of immunopositive cells.

We counted the percentage of positive cells (S100B in *paper III*) and CB, CR, PV positive neurons in (*paper IV*) through the total number of glial or neuronal cells, respectively. For this estimation we applied the principles of unbiased stereological approach, by counting immunopositive cells in 3 different sections at three different random start. The mean % of immunopositive cells was calculated (% = immunopositive cells/total number of counted cells x100).

3.5. ELISA

To detect the S100B concentration in normal and AD brains, we applied commercial sandwich Enzyme Linked ImmunoSorbent Assay (ELISA) measurements (BioVendor Laboratory Medicine, Inc. (Czech Republic). The protocol was followed exactly as described by the company and only original solutions from the kit were used. The absorbance was measured in 450nm wavelength. The standard curve ranged between 0,5pg/ml and 2500pg/ml. All frozen regions of PO were obtained from Huddinge Brain Bank and met the above mentioned diagnostic criterias for AD. Results obtained by ELISA were normalized against total protein content, as measured by BCA protein assay (Pierce, UK).

3.6. Statistical analysis

In all papers included this thesis, the statistical analyses of the results were performed by StatView 5.0.1 software, Macintosh, according to statistical principles by Altman & Bland (1996). In all studies the level of significance was chosen to be $p<0,05$. Precision of estimates was obtained by calculating coefficient of error (CE = SEM/mean), which provides the information necessary to determine whether more or less sampling should be carried out at the various levels of the sampling scheme, and should be less than 10% in all studies (Gundersen & Jensen, 1987, Gundersen, 1999).

<u>Paper I:</u> The unpaired Student's test was used to evaluate neuronal loss between: AD group *vs* total control group, AD *vs* age-matched control and young control *vs* elderly control groups. The age-related change in neuronal number was evaluated with Pearson's correlation coefficient (r). The sampling is considered optimal when the observed variance of the individual estimate, CE^2, is less than half the observed inter-individual variance, CV^2, where CV=SD/mean. In addition biological variability (BV) and proportion of biological variance contributing to the observed variance (BV^2/OCV^2) were estimated.

Paper II: To analyze the differences between CY, CO, matched AD and old AD we used non-parametric Kruskal-Wallis test to compare the number of oligodendroglia and astrocytes separately between the groups: CY *vs* CO; CY *vs* total AD; CO *vs* total AD the unpaired Mann-Whitney test was applied. The Spearman correlation coefficient (r) was used to estimate the relation between the following parameters: a) age *vs.* oligodendrocytes, b) age *vs.* astrocytes, c) neuron *vs.* oligodendrocytes, d) neuron *vs.* astrocytes.

Paper III: For immunohistochemical inter-group analysis Kruskal-Wallis non-parametric statistics were applied. To compare the groups (CY, CO, AD) one to one, the Mann-Whitney unpaired analysis for all S100B subgroups and GFAP. Spearman correlation coefficient was used to correlate GFAP-positive astrocytes and S100B-positive subgroups to neuronal number. Unpaired Student t-test was used to compare the S100B concentration between control and AD brains obtained by ELISA method.

Paper IV: Intergroup comparisons were performed using the Mann-Whitney non-parametric test. Spearman correlation coefficient was chosen to describe the correlation of CB positive neurons in relation to age in control group.

4. RESULTS AND DISCUSSION

4.1. Stereological assessment

4.1.1. 3D stereology: advantages and disadvantages

Many important observation of the histopathology of dementing illnesses have based on qualitative and semiquantitative observations. Quantitative morphometry is valuable because it provides objectivity and much greater degree of precision, and comparisons between different histological events and neurochemical data can be made (Anderson, 1997). The 3D based optical fractionator method makes it possible to estimate the total number of particles of interest, in our studies neuronal and glial cells, both by Nissl and immunostained. Thus the results obtained by 3D stereology enables to compare the results inbetween different studies in the same or any other laboratory. The only limitaiton on applying optical fractionator method is the need to have access to the entire anatomical structure of interest. The basis is to know the total number of sections per whole structure (*in toto*) which depends on the thickness of the section and

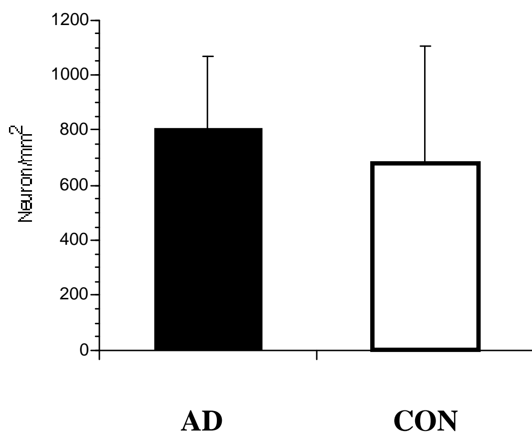
orientation of the structure in brain. The limitation is that whole structures are not always available for analysis. Thus there is a need to have a method which reflects changes similar to those achieved by estimation of total number. Thus we applied different quantitative approaches in human PO, in different orientation of cut (horizontal *vs* sagittal). We predicted that in addition to biological (few %) and technical (up to 50%) shrinkage, also orientation of the structure is important.

4.1.2. 2D stereology

It is known that 2D density estimation does not give the same results as 3D quantification. Since we already had our data using 3D (total number), we could apply 2D quantification in certain circumstances, such as investigating the amount of S100B positive astrocytes (*paperIII*) and neurons expressing Ca-binding in the same brains (*paper IV*) and calculate the ratio to total number of cells. Prior to this, we performed a pilot study to confirm that our density measurments would provide us results similar to those estimated by 3D method. Thus we performed density measurements both on horizontal and vertical sections, as described in 3.4.2. Since horizontal sections of PO are often used in routine pathology to determine pathological changes, we tested the reliability of this approach. Our results revealed no difference in neuronal number in AD compared to controls in horizontal sections (Fig1a). In contrast, the density measurement on sagittal sections, showed 32% reduction of neurons in AD brains (Fig 1b), which is very close to the 34% reduction found by 3D optical fractionator (Fig 3). Thus we can conclude that use of 1-3 sagittal but not horizontal sections give reliable results with the same precision as stereology, by plotting neuronal density (2D) *vs* total number of neurons (3D) (Fig.2). A positive correlation ($p<0,001$, $r^2=0,841$) among the neuronal density in the vertical oriented sections and total neuronal number (3D) indicates that single vertical section can be used in the quantitative analysis at least to investigate the pathology in principal inferior olivary nucleus in routine pathology.

Fig 1

a) horizontal section



b) vertical section

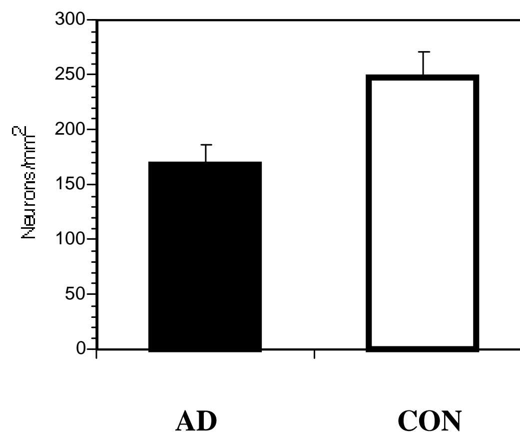


Fig2

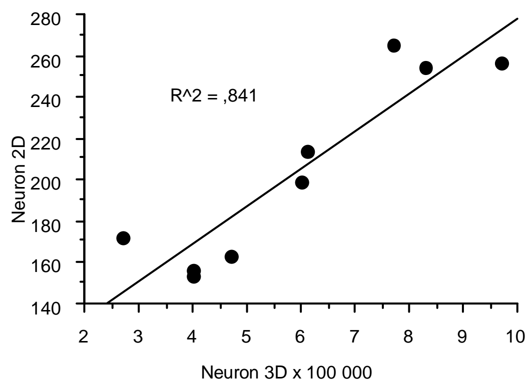
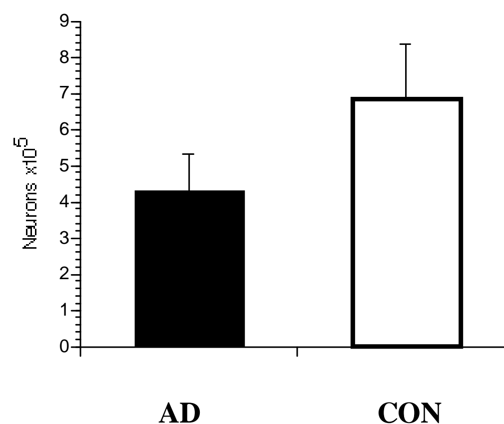


Fig3



In conclusion, we may speculate that tissue shrinkage due to the biological atrophy, but also during tissue procedure in AD in the PO, takes place mostly in the horizontal but not in vertical orientation. Due to the large horizontal shrinkage, neuronal loss in the PO in AD is overestimated. Note the difference in the neuronal number *per area* in horizontal *vs* sagittal sections. There is almost 3 times more neurons in the horizontal section per mm^2 than in the sagittal sections. This methodological observation is important for the further studies which intend to apply quantitative approaches on principal inferior olivary nucleus.

4.2. Morphological features of ION in AD and normal aging

4.2.1. Neuronal pathology in AD vs normal aging

In *paper I* we estimated the total number of neurons in human PO, and 34% reduction was obtained in AD brains compared to controls. This is one of the few regions analyzed in addition to cerebellum (Wegiel, 1999, Sjöbeck & Englund, 2001), and dentate gyrus (Simic et al., 1997), where neuronal loss occurs in the absence of classical neuropathological hallmarks, such as NPs and NFTs. Accumulation of lipofuscin granules is a common feature in aging brain, especially in inferior olive (Drach, 1998). There is increasing evidence that lipofuscin impairs neuronal function in multiple levels, although the content of lipofuscin in PO of AD patients is not increased compared to controls (Drach, 1998). However, according to our results the neuronal number and presence of CB and CR did not change during aging while PV was lost after midlife. This data suggests that neurons are not affected severely, but the loss of PV may be a prerequisite for increased susceptibility to $[Ca^{2+}]_i$ excess. It is unknown whether there is any relation between disappearance of PV and accumulation of lipofuscin. Our results in *paper I* were the first to report neuronal degeneration of PO in AD brains. In *paper IV* we performed neuronal staining with CBPs. The CBPs are known as neuronal vitality markers, and profound reduction of CB (ca 65%) and CR (ca 50%) were obtained in the PO of AD patients, with no decrease in normal aging or PD. In contrast, the PV positivity disappeared already in midlife in normal aging, which can contribute to increased cellular vulnerability in AD. In normal conditions the loss of PV can be compensated by other two CBPs (CR & CB) in aging, also suggested by others (Schwaller, 2002). There is sufficient evidence that the CBPs play a crutial role in cellular defense mechanisms by buffering $[Ca^{2+}]_i$ and cellular functional properties, such as excitability, synchronization and firing rate especially in pathological conditions. Thus our results suggest that in PO, that the neuronal vulnerability is increased and neuronal properties are impaired in AD. The fact that PO neurons function in ensemble, not each neuron alone, synchronization of entire neuronal population coupled to this network play crucial role in regulating the outcome of the nucleus, "pacemaking" the cerebellar function. Since there is no additional studies investigating neuronal pathology in PO of AD patients, the patomechanisms behind neuronal degeneration remain still unknown. To explore more in

detail possible pathological events, we performed immunostaining with iNOS and nitrotyrosine-3 (NT-3) antibodies, the markers related to neurotoxicity (Ghafourifar & Colton, 2003, Blanchard-Fillion et al., 2006). The presence of iNOS occurs only in pathological conditions, not in healthy brains (Brown & Bal-Price, 2003). The overproduction of S100B and other cytokines, mainly IL-1β are known to be strong triggers for iNOS production (Hu et al., 1996, 1997, Brown & Bal-Price, 2003,). In our AD brains the iNOS positivity was strong in most of neurons and in neuropil, while few astrocytes stained positively. (Fig 1.C below). In contrast control cases displayed no iNOS immunopositivity at all in gray matter, and only in one out of 8 brains small positivity existed in white matter (Fig.1A below). In PD only few iNOS positive spots in neuropil were present, while no intraneuronal or astrocyte staining occurred in any of investigated PD brains (Fig. B below). During chronic presence of iNOS high levels of nitric oxide ($NO^{\cdot}$) are produced, which exerts toxic effects on both neurons and glia, by inhibiting mitochondrial respiration chain and reducing ATP synthesis (Brookes et al., 1999). An irreversible damage to the mitochondrial respiratory chain may occur as a result of peroxynitrite generation, when $NO^{\cdot}$ reacts with superoxide ($O_2^{\cdot -}$) (Brookes et al., 1999, Brown & Bal-Price, 2003) what lead to cell death via apoptosis or necrosis, depending whether cellular ATP levels are maintained or depleted (Brown & Bal-Price, 2003). The production of peroxynitrite can be evidenced by evaluating the presence of nitrotyrosine (NT-3) (Salvemini et al., 2003). The presence of NT-3, one of the most common footprints of reactive nitrogene species and oxidative stress, is toxic for neurons in different diseases (Ghafourifar & Colton, 2003, Blanchard-Fillion et al., 2006). Our results revealed differential NT-3 positivity inbetween AD, PD and controls. Strong NT-3 immunopositivity appeared in both neurons and astrocytes in AD brains (Fig 2. C), while in PD (Fig 2. B) only few astrocytes were positive, in contast to control cases where glial cells, most likely microglia and oligodendroglia stained positively for NT-3. Interestingly in control brains a typical finding was positive microglia around non-stained astrocytes, and occasional NT-3 positive oligodendroglia in vicinity of non-stained neurons (Fig 2.A below). Taken together, the strong iNOS and NT-3 positivity indicates advanced neuronal and glial oxidative damage. On the other hand, in PD the minimal iNOS positivity in astrocytes only no neuronal peroxynitrite formation yet, thus can be marker of very early still reversible oxidative process, when the neurons are not affected. In control brains the staining pattern remains to be elucidated, due to the fact that iNOS is negative in all cell types of control brains, while NT-3 positive glial cells are common feature in all older

control cases, newborns did not show any NT-3 positivity (data not shown). Similar non-overlapping pattern of iNOS and NT-3 was also noted in cortical regions both in humans (Fernandez-Vizarra et al., 2004) and in rodent (Uttenthal et al., 1998). This finding needs further research, but may indicate that microglia degenerates in aging PO and astrocytes, neurons and oligodendroglia are affected in AD brains. Taken this together, we suggest that oxydative processes is a common finding in AD, PD and even in normal aging, although in different cell types and with different intensity. It could be possible that glial cells become affected in earlier stages of AD and already during normal aging and neuronal pathology occurs later on as a consequence.

Fig 1

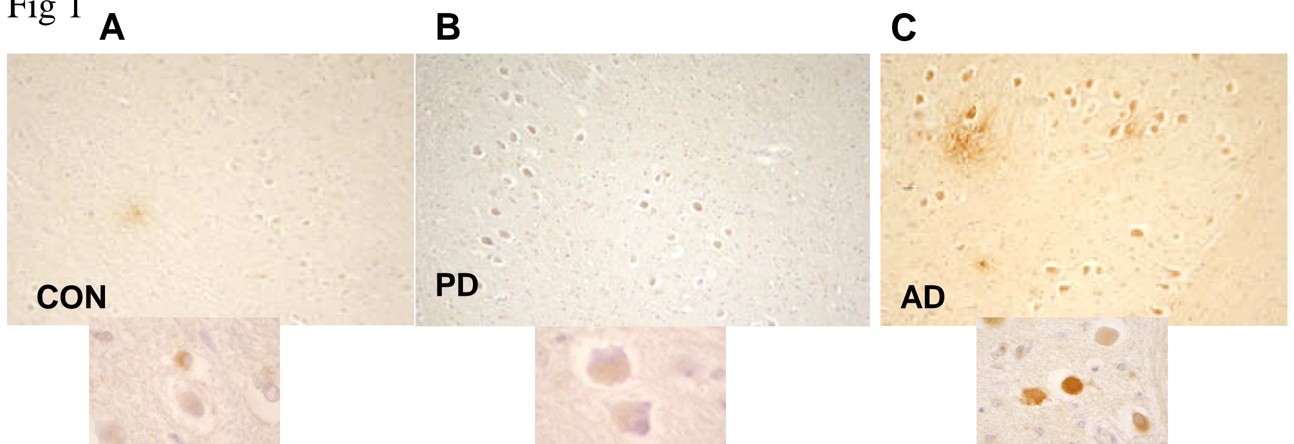
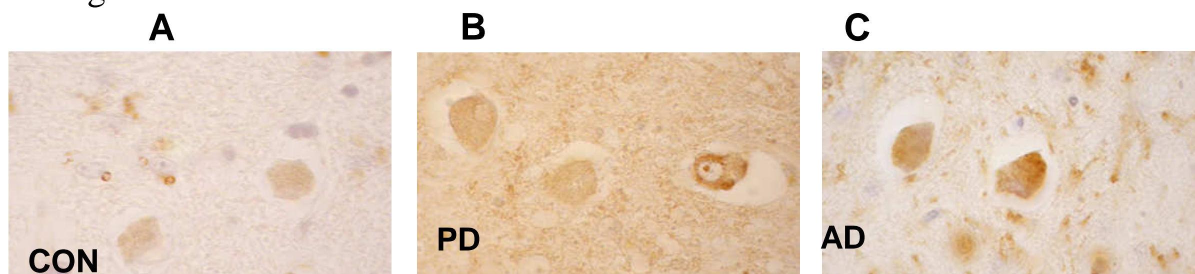


Fig 2

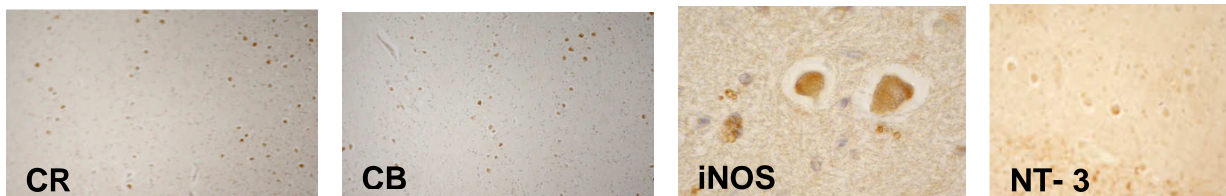


Ca^{2+} dyshomeostasis is likely involved in pathology of PO in AD.

We investigated changes in Ca^{2+}-related proteins in *paper III and IV*. The results indicate that both neuronal and glial Ca^{2+} dyshomeostasis is involved in PO in AD brains. In *paper III* the increase of S100B by 2 fold was measured by both stereology and ELISA. It is likely that in PO the $[Ca^{2+}]_i$ in neurons and glial cells become elevated for two reasons: 1) increased S100B production by astrocytes (*paper III*) in parallel to age-related $[Ca^{2+}]_i$ elevation and 2) diminished presence and function of CBPs in AD brains (*paper IV*). Taken together, it is likely that PO neurons which are defective in Ca-buffering systems, fail to handle increased $[Ca^{2+}]_i$ levels caused both by aging and elevated S100B, thus leading to Ca-related dysfunction and death. In aging, on the other hand, the age-related increase in $[Ca^{2+}]_i$ is accompanied by an increase of CB positivity what suggests intact neuronal adaptive mechanisms in normal aging brain, similarily found by Greene et al., 2001. Whether this upregulation is due to the loss of PV or not is unknown. Thus it is likely that neuroprotective increase of CB takes place in normal aging, but fails in AD. Moreover, the $[Ca^{2+}]_i$ dyshomeostasis is exacerbated by increased level of S100B which is known to increase $[Ca^{2+}]_i$ both in neurons and glial cells (Van Eldik & Griffin, 1994, Donato, 1999, 2003). It may be logical conclude that neurons in AD suffer from $[Ca^{2+}]_i$ overload due to the lack of CBPs and increase of S100B.

In summary, we can suggest that neurons in PO are affected both by decreased amount of CBPs and strong presence of neurotoxic iNOS and NT-3. It is reasonable to assume that in pathological conditions in which the overproduction of $NO^{\cdot}$ and related products occur, cellular energy metabolism and functions that rely on ATP may be compromised (Brooks et al., 1999). The primary trigger for this type of pathology needs to be elucidated, whether it is caused by loss of CBPs, which is often pre-requsite for neuronal dysfunction and death, or the presence of final end-product, such as NT-3 causing oxidative and metabolic dysbalance in PO, ending in downregulation and loss of CBPs. It has been shown that iNOS depletes neurons of PV, but not CB (Weldon et al., 1998). Moreover, normal high levels of IGF-I has been shown to maintain CB levels (Nieto-Bona et al., 1995, Hess, 2003), this mechanism may be affected in AD brains, due to the downregulation of IGF-I in AD (Selkoe, 1997). In conclusion there are multiple mechanisms involved in neuronal dysfunction and suffering in AD, but the main trigger remains to be elucidated in future studies.

Fig 3. Possible AD



4.2.2. Astroglial changes in AD *vs* normal aging

In *paper II* no significant astroglial changes occurred either in normal aging or AD probably due to the interindividual difference in AD group (range between 4,4-13,2 x10^6), especially in 5 of 11 cases where the number of astrocytes remained close to normal, around 8,8-13x10^6. However, six other cases displayed remarkably fewer astrocytes in PO (4,4-6,8x10^6) indicating rather decrease of total number of astrocytes than remaining stable, since in control group the number was in most cases close to 10x10^6. Thus the possibility of astroglial degeneration can be postulated. In other brain regions astrocytic apotosis has been reported (Koboyashi et al., 2002). Although the total number astrocytes has not been shown to be increased, it is known that neurodegeneration with various etiologies induces astrocytic reactivity (Ridet, 1997). In acute pathology the astrocyte activity is beneficial for neurons, but in chronic conditions it may become detrimental for both neurons and glia (Mrak, 2004). Thus in *paper III* we performed immunostainings for reactive astrocytes by applying S100B, GFAP and vimentin antibodies and the proportions of positive cells. Additional ELISA measurements were done to estimate the concentration of S100B in human PO. We found that S100B shows tendency to increase in normal aging, but did not reach significance (in young controls 22% *vs* 34% in older controls). In AD S100B is upregulated 2 fold compared to age-matched controls measured by ELISA. It is known from number of cellular and animal experiments that up to certain limit increase of S100B is beneficial for neurons, by being involved in neuronal plasticity and long-term potentiation, neurite outgrowth (Heizmann, 1999, Donato, 1999), while at some point when chronically overexpressed the beneficial effects are replaced by unfavorable effects, such as influx of intracellular Ca^{2+}, inducing iNOS and promoting self-ampification of inflammatory cascades (Hu et al., 1996, Mrak et al., 2004), (see scheme below). In AD brains 58% of astrocytes were positive for S100B, and as described in *paper III*, remarkable

qualitative shift of S100B staining occurred in AD brains, where astrocytes showed strong nuclear positivity in addition to strong cytoplasmic positivity. In cortex the increasd S100B levels have been shown to induce iNOS production and to increase $[Ca^{2+}]_i$ thus leading to neuronal death (Hu et al., 1996, Heizmann, 1999,). We tried to stain the neurons for apoptotic markers, such as caspse-3 (NovoCastra) and Cytodeath 30 (Peviva), with no positivity in PO of AD brains. This may be explained by a recent finding that only tangle-forming neurons stain with apoptotic markers, while NFT-negative neurons do not (Rohn et al., 2006, Hyman, 2006), thus we cannot exclude the occurrence of apoptosis in the PO of AD patients. In CSF the levels of S100B increase in early stages, and decrease in late stages (Van Eldik & Wainwright, 2003, Rothermundt et al., 2003). Furthermore in *paper III* we estimated density of GFAP-positive astrocytes (GFAP+), which showed 20% increase compared to all controls (111 cells/mm^2 *vs* 92cells /mm^2). It should be noted that in older control group the GFAP positivity showed tendency for decrease, although non-significantly compared to young controls (83 cells/mm^2 *vs* 99 cells/mm^2), what may suggest possible defects of protein turnover or increased GFAP degradation in aging (Sloane et al., 2000, Porschet, 2003). However, no vimentin re-expression was present in any of our brains, indicating the absence of classical reactivity process (Ridet, 1997). The increased GFAP and vimentin levels in AD are usually related to NPs and NFTs (Porchet, 2003), what may explain the low expression of both GFAP and vimentin in PO of AD patients.

To investigate further the possible pathology of astroglia and neurons, we performed immunostainings with iNOS and nitrotyrosine (NT-3) in controls, AD and PD brains as described above in part 4.2.1. It is known that S100B and activated microglia induce iNOS activity (Hu et al., 1996, 1997, Katsuse et al., 2003). The results are described in detail in part 4.2.1. iNOS is known to be produced mainly by astrocytes (Katsuse et al., 2003) and only in pathological conditions. Glial cells were iNOS positive only in AD brains, where numerous iNOS positive extracellular conglomerulates including astrocytes in neuropil and most neurons were iNOS positive in PO (Fig 1 C, above). Further NT-3 staining showed very strong positivity in astrocytes and neurons, and also in oligodendrocytes in both gray and surrounding white matter in AD brains. This indicates that both neurons and neuroglia suffer from oxidative damage that contributes to degeneration in AD. Microglial positivity was very rare in AD brains, and no typical pattern such as NT-3 positive microglia around negative astrocytes was noted in control brains was present in AD. In PD brains, the glia in white matter

showed moderate positivity, but remarably weaker than in AD, with no positivity in any cell type in gray matter ie. PO.

In conclusion, our results suggest that pathological nitric oxidation occurs in PO of AD patients, which quite likely leads to cellular damage. This mechanism is possibly accounts for both neuronal and glial pathology, although astrocytes are known to be more resistant to NT-3 than neurons (Bolaños et al., 1995).

4.2.3. Oligodendroglial loss AD *vs* normal aging

Oligodendrocytes are vulnerable even to slight dyshomeostasis of brain microenvironment, especially to oxygen radicals (including NO·), cytokines, tumor necrosis factor (TNF), causing oxidative processes and changes in $[Ca2+]_i$, leading either to apoptosis or necrosis (Oka et al., 1993, Ludwin, 1997). In *paper II* we estimated total number of oligodendroglial cells in aging and AD brains. Although the vulnerability of oligodendrocytes is believed to be increased in aging, we did not obtain any decrease in total number of oligodendrocytes. In most aged control brains occasional NT-3 positive oligodendroglia cells were obtained. This indicates the presence of oxidative stress in the normal aging brain, which may be a process limited to some but not affecting the majority of oligodendrocytes. In contrast, in AD group the total number of oligodendrocytes was decreased by 46% in PO, which correlated to neuronal reduction. Immunostaining with NT-3 revealed numerous stained oligodendroglial cells around neurons in AD brains, but not in control or PD cases. (Fig. 2). An overproduction of NO· has been shown to cause oligodendrocyte death via necrosis not apoptosis (Mitrovic et al., 1995). It is known by now that oligodendrocytes and neurons are more vulnerable to excitotoxicity mediated by free radical attack compared to astrocytes (Oka, 1993). The detailed pathomechanism of oligodendroglia death in PO remains unknown. In *paper II,* the oligodendroglial cell number paralleled the neuronal number, so these two cell types seem to be interdependent of each other, similarily noted in cortex by Pakkenberg and collegues (Pakkenberg et al., 2003, Pelvig et al., 2003). Our results indicate the close interdependence of neurons and oligodendrocytes, and both neurons and oligodendrocytes are severely affected in AD brains despite the absence of NPs and/or NFTs.

5. PO as a model for neurodegeneration

Most importantly, the PO is a region in AD, where the NPs and NFTs are absent even in late stage of sporadic AD cases. There was only one sporadic case which displayed NFTs and diffuse Aβ precipitations but no NPs in PO. However, NFTs formation in PO neurons may occur in AD brains with PS-1 mutation, with concomitant diffuse plaques but no NPs. Thus further research about the patomechanisms underlying AD may help to reveal the triggers and prerequisites for both neuronal and glial degeneration. Furthermore, the changes found up to now in PO, suggest that PO is affected in early AD (Fig 3). Our findings suggest that NP and NFT formation is not the primary event to cause neuronal degeneration, similarily suggested by others (Lee et al., 2004).

6. Possible therapeuric strategies

Since AD affects many brain regions, therapy should aim to have effects in all affected regions by improving neuronal energy storage and glucose utilization, as well as supporting mitochondrial function both reducing oxidative stress and excess of $[Ca^{2+}]_i$ and normalizing APP processing.

IGF-I

Glucose uptake and energy metabolism in the brain are regulated by insulin and insulin growth factor (IGF) (de la Monte et al., 2006). In AD brains the progressive deficit of insulin and IGF production is demonstrated (De la Monte et al., 2006) and decreased IGF-I actions reduce neuronal survival by diverse pathways (de la Monte et al., 2005). Insulin and IGF-I are strongly involved in neuronal growth, survival and metabolism (De la Monte et al., 2006) and impairments of glucose utilization and energy metabolism occur very early in AD. In olivo-cerebellar circuit the normal IGF-I levels are needed for normal development and maintenace of neurons in adult (Nieto-Bona et al., 1995, Fernandez et al., 1998). Peripherially, regardless of the route by which the IGF-I was injected, the neurons recovered in inferior olive and olivo-cerebellar function normalized. Thus IGF-I treatment is suggested to treat patients suffering from AD, PD and human cerebellar ataxia, even when substantial neuronal loss is present (Fernandez et al., 1998). Because IGF-I is linked to whole olivo-cerebellar circuit it is likely that the whole circuit will benefit from IGF-I treatment to

promote survival of interconnected populations of neurons within cerebellar circuit (Fernandez, 1998). IGF-I is present in red nucleus, inferior olivary nucleus, deep cerebellar nuclei, especially in long-axon projecting neurons (Bondy, 1991).

Mitochondrial protection and antioxidant therapy

Mitochondria play pivotal role in neuronal cell survival and death because they are regulators for both energy metabolism and apoptotic pathways. Accumulating evidence suggest that oxidative stess occurs (both in peripheral tissues and brain) prior the onset of symptoms and is early event that contributes to cascades leading to neuronal death in AD and other neurodegenerative disorders (Moreira et al., 2006, de la Monte et al., 2006). Thus the knowledge that mitochondrial dysfunction plays a predominant role in AD opened a window for new therapeutic strategies called "Mitochondrial medicine" (Moreira et al., 2006). Deficits in vitamin B_6, copper, iron, zink, biotin, pantothene acid have been shown to be decreased in aging and AD, thus contributing MK dysfunction (Ames et al., 2004). Mitochondria produce superoxide as part of their normal function and greater quantities when respiration is compromised, thus they are the major source of reactive oxygen species (ROS) in vulnerable neurons. (Zhu et al., 2006).

Levels of different antioxidants are inversely associated with cognitive performance in elderly (Ortega et al., 2002). Several studies show logarithmic age-dependent increase in oxidized proteins, lipids and DNA (Floyd & Hensley, 2002). In AD patients the levels of different antioxidants are decreased. Clinical trials with dietary intake of CoQ_{10}, vitamine C and E are shown to be preventive to develop AD (Smith et al., 1999, Engelhort et al., 2002, Morris et al., 2002, Beal, 2004). The early occurrence of oxidative damage in cognitive disorders may explain the disappointing effects of antioxidant therapies (Sies et al., 2003). A recent finding that the oxidative imbalance and especially lipid oxydation are more pronounced in early AD than in other neurodegenerative disorders (Practicó & Sung, 2004), indicates that antioxidative therapy should be effective in early AD when oxidative stress is still mild (Whitman & Cotman, 2004, Practicó and Sung, 2004).

In conclusion it has been proposed that combinations of antioxidants might be of greater potential benefits, especially if the agents worked in different cellular compartments or have complementary activity (Mecocci, 2004).

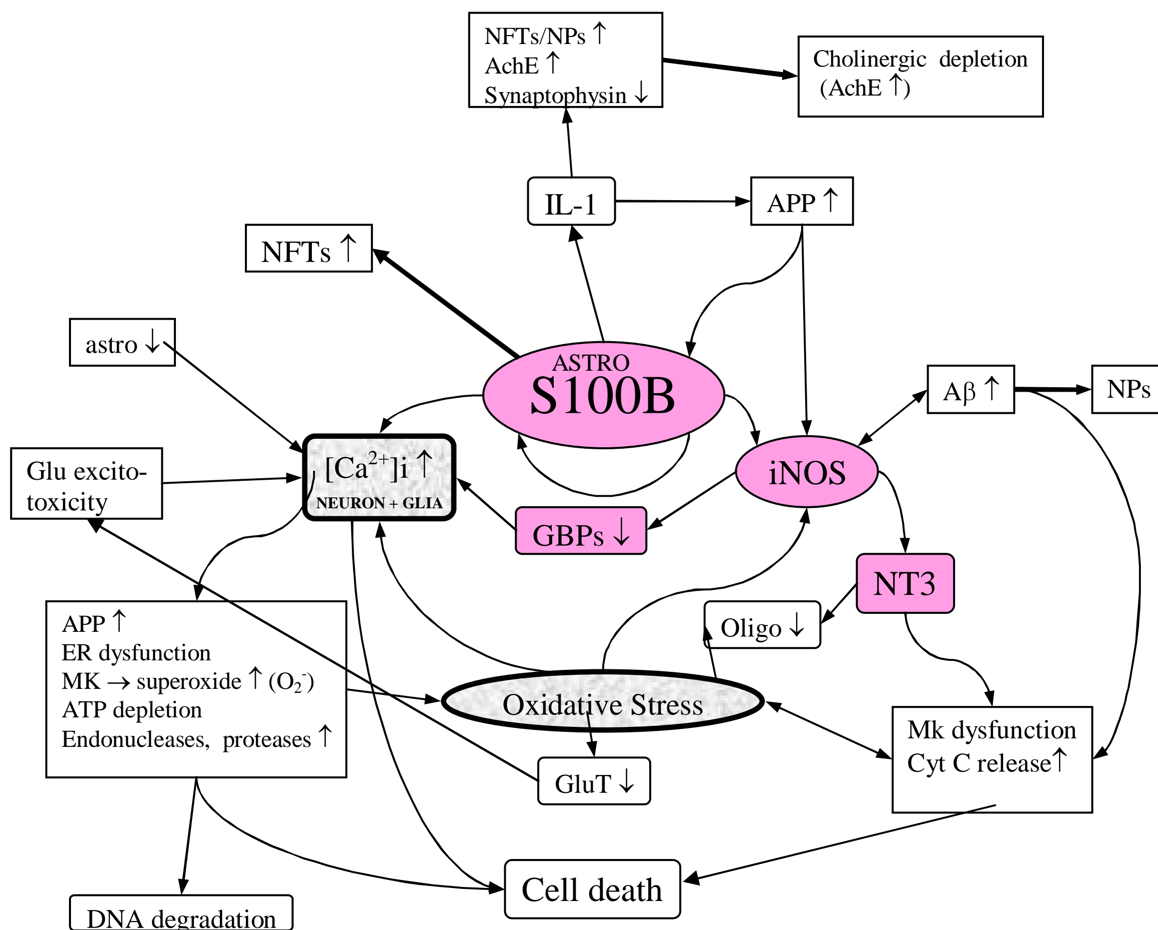
Reducing excess of [Ca2+]i

Normal Ca^{2+} levels play a pivotal role in neuronal signaling. But the excess of Ca^{2+} activates the destructive proteins which lead to disruption of cellular structure and altered APP processing. On the other hand, changed APP products can modify calcium homeostasis, indicating to reciprocal relationship between APP and calcium in AD (Brzyska and Elbaum, 2003). Thus the NPs formation can occur due to the Ca^{2+} dyshomeostasis. Degenerating neurons in AD brains show increased Ca^{2+} levels, usually colocalized with NFTs (Murray et al., 1992) and increased activation of Ca^{2+}-dependent enzymes (Nixon et al., 1994). Although neither β accumulation or NFTs are present in PO, the Ca^{2+} homeostasis is likely to be affected due to upregulation of S100B and downregulation of CBPs. ApoE increases intracellular Ca-levels dose-dependently (E4>E3>E2), (Muller et al., 1998). Ca^{2+-}dyshomesostasis is one of the factors that lead to dysfunction and stress of endoplasmic reticulum (ER). Since ER is not only Ca^{2+} storage and signaling compartment but also participates in protein processing and folding, it is likely to affect all metabolic events occurring within it (Brzyska and Elbaum 2003).

Targeting astrocytes: iNOS inhibitors & S100B supressors

Number of studies have demonstrated that iNOS inhibitors reduce significantly generation of NO·, thus suppressing oxidative damage of proteins and further formation of peroxynitrite and NT-3, thus reducing neurotoxicity (Ischiropoulos, 1998, Brown & Bal-Price, 2003). Presence of glutamate potentiates NT-3 formation (Ischiropoulos, 1998), indicating the contributing role for exotoxicity in oxidative stress. S100B, as a central key player to induce toxic cascades, is suggested to be a target in AD (Craft et al., 2005).

In summary, it is suggested that activation or stimulation of many different pathways in the mammalian CNS can finally lead to neuronal degeneration either necrotic or apoptotic. Multiple cascades are activated in AD, but at some point most of those pathways converge and are accompanied by prolonged increase in the $[Ca^{2+}]_i$ (D'Orlando, 2002). In these instances the increase of $[Ca^{2+}]_i$ is secondary, and speculatively more specific agents to protect mitochondria, improve antioxidative mechanisms and downregulate gliotoxic cascades (both S100B and iNOS) as mentioned above may be more effective to treat AD patients. Pathology involved in the PO of AD patients is described in following *circulus vitiosus*.



7. CONCLUSIONS AND FUTURE PERSPECTIVES

In this thesis we have investigated the state of PO in normal aging ad A D cases. Significant neuronal and oligodendroglial loss was found in PO of AD patients. Moreover, glial reaction and release of different agents occus in PO of AD cases. S100B upregulation by 2 times occurred compared to age-matched controls. It is known that NO overexpression via iNOS activation is a chronic process in AD, that via protein nitration alters membrane and cytosol proteins, affecting their physical and chemical nature and serving as an important link in the chain of events leading to oxidative damage observed in AD (Koppal et al., 1999). It is suggested that NO overproduction in AD is throughout the entire course of AD, including very early stages (Fig 3), with increasing nitration end-products in later stages (Fernández-Vizarra et al., 2004). It is suggested that accumulation of peroxynitrite in neurons leads to oxidative damage of lipids, proteins, DNA and different amino acids as well as inactivating mitochondrial key enzymes and triggering intracellular release of Ca^{2+} from mitochondria, thus causing depolarization of mitochondrial membrane, caspase activation and neuronal death (Fernández-Vizarra et al., 2004). According to our studies, the oxidative process was restricted in control and PD cases, but widespread in AD, affecting neurons, astrocytes and oligodendrocytes in PO. Whether overproduction of S100B is the trigger to induce iNOS cascade remains to be elucidated *in vivo* system. Up to now there is evidence for S100B to increase iNOS *in vitro* (Hu et al., 1996, 1997, Esposito et al., 2006), in micromolar concentrations, but whether increase of S100B found in our study is sufficient to trigger toxic cascades *in vivo* needs further investigations. In our studies we investigated several markers for possible toxic cascade in AD, PD and control brains. Furthermore we investigated the presence of CBPs in PO in AD, PD and normal aging. There was no differences in PD brains compared to controls. In contrast, in AD cases the expression of CB and CR were significantly decreased, indicating a deficit in calcium-buffering mechanism in neurons of PO. Possibly leading to excessive intracellular Ca^{2+} levels and eventually cellular death. Thus we may summarize, that both neuronal and glial pathology is involved in PO of AD patients.

Future perspectives regarding investigation of neurodegeneration in PO could be address towards:

1) Neuronal and glial state in other neurodegenerative diseases, such as Progressive Supranuclear Palsy, Olivo-ponto-cerebellar Atrophy, Multiple System Atrophy, PD, by applying stereological methods, to achieve total number of different cell types and synapses

2) Neuronal and glial number in MCI and very early AD patients, to prove or disprove whether PO degeneration is early or late event in AD.

3) Correlate neuronal loss in PO to neuronal number in other regions closely functionally involed: red nucleus, dentate nucleus and PCs.

4) Neuron-oligodendroglia ration by quantifying the distance of oligodendrocytes and astrocytes from neurons, applying Saucer method, as a marker of intercellular communication.

5) To quantify the presence of chemical markers in PO such as CBPs, IGF-I positive and CRF-positive neurons in MCI and AD patients.

6) Whether or not there is any morphological changes in aging related to IGF-I and CRF in PO.

7) To investigate state of microglia and levels of different cytokines in relation to possible inflammatory changes in PO.

8) (f)MRI analysis of PO in different clinical settings, and correlation with clinical picture

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References

Alonso D, Serrano J, Rodriguez I, Ruiz-Cabello J, Fernandez AP, Encinas JM, Castro-Blanco S, Bentura ML, Santacana M, Richart A, Fernandez-Vizarra P, Uttenthal LO, Rodrigo J.Effects of oxygen and glucose deprivation on the expression and distribution of neuronal and inducible nitric oxide synthases and on protein nitration in rat cerebral cortex. J Comp Neurol. 2002. 443(2):183-200.

Altman DG & Bland JM. Detecting skewness from summary information. BMJ. 1996. 313(7066):1200.

American psychiatric Association: Committee on Nomenclature and Statistics. Diagnostical and statistical manual of mental disorders 4th edn. Washington DC 1994. 133-145

Ames BN, Liu J. Delaying the mitochondrial decay of aging with acetylcarnitine. Ann N Y Acad Sci. 2004. 1033:108-16. Review.

Amstrong DM, Functional significance of connections of the inferior olive, Physiol Rev. 1974. 54: 358-417.

Andersen BB, Gundersen HJ, Pakkenberg B. Aging of the human cerebellum: a stereological study. J Comp Neurol. 2003. 466(3):356-65.

Anderson JR and Treip CS. Hypertrophic olivary degeneration and Purkinje cell degeneration in a case of long-standing head injury, J Neurol Neurosur Psych. 1973. 36:826-832.

Anderson JM. Morphometric methods and dementia. Ed By Esiri MM and Morris JH. 1997. The Neuropathology of dementia. Cambridge University Press.

Annunziato L, Amoroso S, Pannaccione A, Cataldi M, Pignataro G, D'Alessio A, Sirabella R, Secondo A, Sibaud L, Di Renzo GF. Apoptosis induced in neuronal cells by oxidative stress: role played by caspases and intracellular calcium ions. Toxicol Lett. 2003. 139(2-3):125-33.

Arendt T, Bigl V, Arendt A, Tennstedt A. Loss of neurons in the nucleus basalis of Meynert in Alzheimer's disease, paralysis agitans and Korsakoff's Disease. Acta Neuropathol (Berl). 1983. 61(2):101-8.

Azizi SA and Woodward DJ, Inferior olivary nuclear complex of the rat: Morphology and comments on the principles of organization within the olivocerebellar system, J.Comp.Neurol. 1987. 263:467-484.

Bacci A, Verderio C, Pravettoni E, Mateoli M: The role of glial cells in synaptic function. Phil Trans R Soc Lond. 1999. B354:403-409.

Back SA, Han BH, Luo NL, Chricton CA, Xanthoudakis S, Tam J, Arvin KL, Holtzman DM. Selective vulnerability of late oligodendrocyte progenitors to hypoxia-ischemia. J Neurosci. 2002. 22(2):455-63.

Ball MJ. Neurofibrillary tangles and the pathogenesis of dementia. A quantitative study. Neuropath Appl Neurobiol. 1976. 2:395-410.

Barger S.W. & Van Eldik L.J. S100β stimulates calcium fluxes in glial and neuronal cells. J. Biol. Chem. 1992. 267: 9689-9694.

Barnes CA. Normal aging: regionally specific changes in hippocampal synaptic transmission. Trends Neurosci. 1994.17(1):13-8.

Barres BA, Barde Y-A: Neuronal and glial cell biology. Curr Opin Neurobiol. 2000. 10:642-648.

Bartzokis G, Lu PH, Mintz J. Quantifying age-related myelin breakdown with MRI: novel therapeutic targets for preventing cognitive decline and Alzheimer's disease. JAD. 2004. 6:S53-9.

Batini C, Buisseret-Delmas C, Conrath-Verrier M.Olivo-cerebellar activity during harmaline-induced tremor. A 2-[14C]deoxyglucose study. Neurosci Lett. 1979. 12(2-3):241-6.

Bauman ML, Kemper TL. Neuroanatomic observations of the brain in autism: a review and future directions. Review. Int J Dev Neurosci. 2005. 23(2-3):183-7.

Beal MF. Mitochondrial dysfunction and oxidative damage in Alzheimer's and Parkinson's diseases and coenzyme Q10 as a potential treatment. J Bioenerg Biomembr. 2004. 36(4):381-6.

BehlC. Apoptosis and Alzheimer's disease. Review. J Neural Transm. 2000. 107(11):1325-44.

Benardo LS and Foster RE. Oscillatory behavior in inferior olive neurons: mechanism, modulation, cell aggregates. Brain Res Bull. 1986. 17(6):773-84

Benito-Leon J, Louis ED, Bermejo-Pareja F; Neurological Disorders in Central Spain Study Group.Elderly-onset essential tremor is associated with dementia. Neurology. 2006. 66(10):1500-5.

Bengtsson F. The cerebello-olivary feedback system. PhD thesis. 2005. Lund University.

Bengtsson, F and Hesslow, G. Cerebellar control of the inferior olive. 2006. Cerebellum. 5 (1)7-14

Blalock EM, Chen KC, Sharrow K, Herman JP, Porter NM, Foster TC, Landfield PW. Gene microarrays in hippocampal aging: statistical profiling identifies novel processes correlated with cognitive impairment. J Neurosci. 2003. 23(9):3807-19.

Berry M, Butt AM, Wilkin G, Perry VH: Structure and function of glia in the central nervous system. 2002. Greenfield's Neuropathology 7th Ed.

Bishop GA, McCrea GA and Kitai ST. A horseradish-peroxidase study of the cortico-olivary projection in the cat, Brain Res. 1976. 116:306-311.

Blalock EM, Chen KC, Sharrow K, Herman JP, Porter NM, Foster TC, Landfield PW. Gene microarrays in hippocampal aging: statistical profiling identifies novel processes correlated with cognitive impairment. J Neurosci. 2003. 23(9):3807-19.

Blanchard-Fillion B, Prou D, Polydoro M, Spielberg D, Tsika E, Wang Z, Hazen SL, Koval M, Przedborski S, Ischiropoulos H. Metabolism of 3-nitrotyrosine induces apoptotic death in dopaminergic cells. J Neurosci. 2006. 26(23):6124-30.

Bogdanovic N and Morris JH. Diagnostic criteria for Alzheimer's disease in multicentre brain banking. In Cruz-Sanches FF, Ravid R, and Cuzner ML (eds): Neuropathological Diagnostic Criteria for Brain Banking, Amsterdam: IOS Press, 1995. pp. 20-29.

Bolanos JP, Heales SJ, Land JM, Clark JB. Effect of peroxynitrite on the mitochondrial respiratory chain: differential susceptibility of neurones and astrocytes in primary culture. J Neurochem. 1995. 64(5):1965-72.

Bondy CA. Transient IGF-I gene expression during the maturation of functionally related central projection neurons. J Neurosci. 1991. 11: 3442-55.

Braak H, Braak E: Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol 1991. 82: 239-259.

Braak H and Braak E: Development of Alzheimer-related neurofibrillary changes in the neocortex inversely recapitulates cortical myelogenesis. Acta Neuropathol 1996. 92: 197-201.

Braak H, Rub U, Del Tredici K. Involvement of precerebellar nuclei in multiple system atrophy. Neuropathol Appl Neurobiol. 2003. 29: 60-76.

Braak H and Del Tredici K. Poor and protracted myelination as a contributory factor to neurodegenerative disorders. Neurobiol Aging 2004. 25: 19-23.

Braak H. and Braak E. Development of Alzheimer-related neurofibrillary changes in the neocortex inversely recapitulates cortical myelogenesis. Acta Neuropathol. 1996. 92: 197-201.

Brodal A. Experimentelle Untersuchungen über die olivo-cerebellare localisation, Z. Ges. Neurol. Psychiat. 1940. 169: 1-153.

Brodal P, Brodal A. The olivocerebellar projection in the monkey. Experimental studies with the method of retrograde tracing of horseradish peroxidase. J Comp Neurol. 1981. 201(3): 375-93.

Brown GC, Bal-Price A. Inflammatory neurodegeneration mediated by nitric oxide, glutamate, and mitochondria. Review. Mol Neurobiol. 2003. 27(3): 325-55.

Brookes PS, Bolanos JP, Heales SJ. The assumption that nitric oxide inhibits mitochondrial ATP synthesis is correct. FEBS Lett. 1999. 446: 261-3.

Brun A, Liu X, Erikson C. Synapse loss and gliosis in the molecular layer of the cerebral cortex in Alzheimer's disease and in frontal lobe degeneration. Neurodeg 1995. 4:171-7.

Brzyska M and Elbaum D. Dysregulation of calcium in Alzheimer's disease. Acta Neurobiol Exp. 2003. 63: 171-183.

Bu J, Sathyendra V, Nagykery N, Geula C. Age-related changes in calbindin-D28k, calretinin, and parvalbumin-immunoreactive neurons in the human cerebral cortex. Exp Neurol. 2003. 182(1): 220-31.

Bushara KO, Malik T, Exconde RE. The effect of levetiracetam on essential tremor. Neurology. 2005. 64(6):1078-80.

Burns JM, Galvin JE, Roe CM, Morris JC, McKeel DW. The pathology of the substantia nigra in Alzheimer disease with extrapyramidal signs. Neurology. 2005. 64(8):1397-403.

Casley CS, Canevari L, Land JM, Clark JB, Sharpe MA. Beta-amyloid inhibits integrated mitochondrial respiration and key enzyme activities. J Neurochem. 2002. 80: 91-100.

Cerminara NL, Rawson JA.Evidence that climbing fibers control an intrinsic spike generator in cerebellar Purkinje cells. J Neurosci. 2004. 24(19):4510-7.

Chu T, Hullinger H, Schilling K, Oberdick J. Spatial and temporal changes in natural and target deprivation-induced cell death in the mouse inferior olive. J Neurobiol. 2000. 43(1):18-30.

Clark CM, Ewbank D, Lener A, Doody R, Henderson VW, Panisset M, Morris JC, Fillenbaum GG, Heyman A. The relationship between extrapyramidal signs and cognitive performance in patients with Alzheimer's disease enrolled in the CERAD study. Neurology. 1997. 49: 70-75

Cole G, Neal JW, Singhrao SK, Jasani B, Newman GR. The distribution of amyloid plaques in the cerebellum and brain stem in Down's syndrome and Alzheimer's disease: a light microscopical analysis. Acta Neuropathol (Berl). 1993. 85(5):542-52

Colin F, Manil J, Desclin JC. The olivocerebellar system. I. Delayed and slow inhibitory effects: an overlooked salient feature of cerebellar climbing fibers. Brain Res. 1980. 187(1):3-27.

Cotman CW, Su JH. Mechanisms of neuronal death in Alzheimer's disease. Brain Pathology. 1996. 6: 493-506.

Craft J.M., Watterson D.M., Marks A. & Van Eldik L.J. Enhanced susceptibility of S100-B transgenic mice to neuroinflammation and neuronal dysfunction induced by intracerebroventricular infusion of human β-amyloid. Glia. 2005. 51: 209-216.

Deuschl G, Toro C, Hallett M. Symptomatic and essential palatal tremor. 2. Differences of palatal movements. Mov Disord. 1994. 9(6): 676-8.

Deuschl G, Wilms H. Palatal tremor: the clinical spectrum and physiology of a rhythmic movement disorder. Review. Adv Neurol. 2002. 89: 115-30.

Donato R. Functional roles of S100 proteins, calcium-binding proteins of the EF-hand type. BBA. 1999. 1450: 191-231.

Donato R. Intracellular and extracellular roles of S100 proteins. Microsc Res Tech. 2003. 60: 540-551.

Demer JL, Echelman DA, Robinson DA. Effects of electrical stimulation and reversible lesions of the olivocerebellar pathway on Purkinje cell activity in the flocculus of the cat. Brain Res. 1985. 346(1):22-31.

De Montigny C, Lamarre Y. Activity in the olivo-cerebello-bulbar system of the cat during ibogaline- and oxotremorine-induced tremor. Brain Res. 1974. 82(2):369-73.

De Montigny C, Lamarre Y.Rhythmic activity induced by harmaline in the olivo-cerebello-bulbar system of the cat. Brain Res. 1973. 53(1):81-95.

Deuchl G, Elble RJ. The pathophysiology of essential tremor. Review. Neurology. 2000. 54: S14-20.

De Zeeuw CI, Simpson JI, Hoogenraad CC, Galjart N,. Koekkoek SKE and Ruigrok TJH. Microcircuitary and function of the inferior olive. Trends Neurosci. 1998. 21: 391-400.

De Zeeuw CI, van Alphen AM, Hawkins RK, and Ruigrok TJH, Climbing fibre colleterals contact neurons in the cerebellar nuclei that provide a GABAergic feedback to the inferior olive. Neurosci. 1997. 80: 981-986.

D'Orlando C, Fellay B, Schwaller B, Salicio V, Bloc A, Gotzos V, Celio MR. Calretinin and calbindin D-28k delay the onset of cell death after excitotoxic stimulation in transfected P19 cells. Brain Res. 2001. 909: 145-58.

Drach LM, Bohl J, Wach S, Scglote W, and Goebel HH. Reduced intraneuronal lipofuscin content in dementia with Lewy bodies compared with Alzheimer's disease and controls, Dem Ger Cogn Dis. 1998. 9: 1-5.

Dringer R. Metabolism and functions of glutathione in brain. Prog Neurobiol 2000. 62: 649-671.

Du Y, Dreyfus CF: Oligodendrocytes as providers of growth factors. J Neurosci Res 2003. 68: 647-654.

Eccles JC, Llinás R, Sasaki K. The excitatory synaptic action of climbing fibers of the Purkinje cells of the cerebellum. J Physiol (Lond). 1966 Elble RJ. 1998 Animal models of action tremor. Review. Mov Disord. 1998. 3:35-9.

Elble RJ. Essential tremor frequency decreases with time. Neurology. 2000. 55(10): 1547-51.

Engelhart MJ, Geerlings MI, Ruitenberg A, van Swieten JC, Hofman A, Witteman JC, Breteler MM. Dietary intake of antioxidants and risk of Alzheimer disease. JAMA. 2002. 287(24): 3223-9.

Escobar A, Sampedro ED, and Dow RS, Quantitative data on inferior olivary nucleus in man, cat and vampire bat, J Comp Neurol. 1968. 13: 397-404

Esposito G, De Filippis D, Cirillo C, Sarnelli G, Cuomo R, Iuvone T. The astroglial-derived S100beta protein stimulates the expression of nitric oxide synthase in rodent macrophages through p38 MAP kinase activation. Life Sci. 2006. 78:2707-15.

Fernandez AM, de la Vega AG, Torres-Aleman I. Insulin-like growth factor I restores motor coordination in a rat model of cerebellar ataxia. Proc Natl Acad Sci U S A. 1998. 95(3):1253-8.

Fernandez-Vizarra P, Fernandez AP, Castro-Blanco S, Encinas JM, Serrano J, Bentura ML, Munoz P, Martinez-Murillo R, Rodrigo J. Expression of nitric oxide system in clinically evaluated cases of Alzheimer's disease. Neurobiol Dis. 2004. 15(2):287-305.

Finch AJ, Nicolson RI, Fawcett AJ.Evidence for a neuroanatomical difference within the olivo-cerebellar pathway of adults with dyslexia. Cortex. 2002. 38(4):529-39.

Finch C.E. Neurons, glia and plasticity in normal aging brain. Neurobiol. Aging. 2003. 24: S123-S127.

Floyd RA, Hensley K. Oxidative stress in brain aging. Implications for therapeutics of neurodegenerative diseases. Review. Neurobiol Aging. 2002. 23(5):795-807.

Fredette BJ, Adams JC, E. Mugnaini, GABAergic neurons in the mammalian inferior olive and ventral medulla detected by glutamate decarboxylase immunocytochemistry, J Comp Neurol. 1992. 321: 501-514.

Fukutani Y, Cairns NJ, Everall IP, Chadwick A, Isaki K, and Lantos PL, Cerebellar dentate nucleus in Alzheimer's disease with myoclonus, Dem. Ger. Cogn. Dis. 1999. 10: 81-88.

Gard AL, Solodushko VG, Waeg G, Majic T. 4-Hydroxynonenal, a lipid peroxidation byproduct of spinal cord injury, is cytotoxic for oligodendrocyte progenitors and inhibits their responsiveness to PDGF. Microsc Res Tech. 2001. 52(6):709-18.

Geinisman Y, Detoledo-Morrell L, Morrell F, Heller RE. Hippocampal markers of age-related memory dysfunction: behavioral, electrophysiological and morphological perspectives. Review. Prog Neurobiol. 1995. 45(3):223-52.

Geula C, Nagykery N, Wu CK, Bu J. Loss of calbindin-D28K from aging human cholinergic basal forebrain: relation to plaques and tangles. J Neuropathol Exp Neurol. 2003. 62(6): 605-16.

Ghafourifar P, Colton CA. Mitochondria and nitric oxide. Antioxid Redox Signal. 2003. 5(3):249-50.

Giasson BI, Lee VM, Trojanowski JQ. Interactions of amyloidogenic proteins. Review. Neuromolecular Med. 2003. 4(1-2):49-58.

Giannakopoulos P, Hof PR, Bouras C. Selective vulnerability of neocortical association areas in Alzheimer's disease. Microsc Res Tech 1998. 43:16-23

Giaquinta G, Casabona A, Smecca G, Bosco G, Perciavalle V. Cortical control of cerebellar dentato-rubral and dentato-olivary neurons. Neuroreport. 1999. 10(14):3009-13.

Gomez-Isla T, Hollister R, West H, Mui S, Growdon JH, Petersen RC., Parisi JE, and Hyman BT. Neuronal loss correlates with but exceeds neurofibrillary tangles in Alzheimer's disease. Ann Neurol. 1997. 41: 17-24.

Goyal M, Versnick E, Tuite P, Cyr JS, Kucharczyk W, Montanera W, Willinsky R, Mikulis D. Hypertrophic olivary degeneration: metaanalysis of the temporal evolution of MR findings. AJNR. 2000. 21(6):1073-7.

Graeber MB, Blakemore WF, Kreutzberg WG: Cellular pathology of the central nervous system. Greenfield Neuropathology, 7th. Eds Graham DI and Lantos P. 2002. 123-192.

Grandi D, and Arcari ML. Neuronal aspects and plasticity of inferior olivary complex and nucleus dentatus. Acta Biomed. de "L'Ateneo Parmense" 1992. 63: 17-25.

Greene JRT, Radenahmad N, Wilcock GK, Neal JW. Accumulation of calbindin in cortical pyramidal cells with ageing; a putative protective mechanism which fails in Alzheimer's disease. Neuropath and Appl Neurobiol. 2001. 27; 339-342.

Griffin WST, Sheng JG, Mrak R.E. Senescence-accelerated overexpression of S100β in brain of SAMP6 mice. Neurobiol. Aging. 1998. 19: 71-76.

Griffin WST, Sheng JG, Royston MC, Gentlemen SM, McKenzie JE, Graham DI., Roberts GW and Mrak RE. Glial-neuronal interaction in Alzheimer's disease: the potential role of a "cytokine cycle" in disease progression. Brain Pathol. 1998. 8: 65-72.

Gudovic R and Milutinovic B. Regressing changes in inferior olivary nucleus compared to changes of Purkinje cells during development in humans. J Brain Res. 1996. 37: 67-72.

Gundersen HJ. Stereology of arbitrary particles. A review of unbiased number and size estimators and a presentation of some new ones, in memory of William Thompson, J. Microsc. 1986. 143: 3-45.

Gundersen HJG, and Jensen EBV. The effiency of systematic sampling in stereology and its prediction, J. Microsc. 1987.147: 229-263.

Gundersen HJG, Jensen EBV, Kiêu K, and Nielsen J. The efficiency of systematic sampling in stereology - reconsidered, J. Microsc. 1999. 193: 199-211.

Haass C, Schlossmacher MG, Hung AY, Vigo-Pelfrey C, Mellon A, Ostaszewski BL, Lieberburg I, Koo EH, Schenk D, Teplow DB et al. Amyloid beta-peptide is produced by cultured cells during normal metabolism. Nature. 1992. 359(6393): 322-5.

Hansson E, Ronnback L. Glial neuronal signaling in the central nervous system. Review. FASEB J. 2003. 17(3):341-8.

Hardy R, Reynolds R.Neuron-oligodendroglial interactions during central nervous system development. Review. J Neurosci Res. 1993. 36(2):121-6.

Hardy J and Selkoe J. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Review. Science. 2002. 297(5580):353-6.

Headley PM, Lodge D, Duggan AW. Drug-induced rhythmical activity in the inferior olivary complex of the rat. Brain Res. 1976. 101(3): 461-78.

Heizmann CW. ”Ca2+-binding S100β proteins in the central nervous system. Neurochem. Res. 1999. 24: 1097-1100.

Hertz L, Dringen R, Schousboe A, Robinson SR.Astrocytes: glutamate producers for neurons. Review. J Neurosci Res. 1999. 57(4):417-28.

Hesslow G, Svensson P, Ivarsson M. Learned movements elicited by direct stimulation of cerebellar mossy fiber afferents Neuron. 1999. 24(1):179-85.

Hof PR, Bussière T, Morrison JH. Neuropahtology of aging brain. Neuropathology of Dementia, 2nd Ed. Editors: Esiri Margaret M. Esiri, Virginia M.-Y. Lee, John Q. Trojanowski. Cambridge University Press. 2004.

Howard CV and Reed MG. Unbiased stereology. Three-dimensional measurement in microscopy. BIOS Scientific Publishers. 1998.

Hu J, Castets F, Gueavra JL and Van Eldik L. S100β stimulates inducible nitric oxide synthase activity and mRNA levels in rat cortical astrocytes. J Biol Chem. 1996. 271: 2543-2547.

Hu J and Van Eldik LJ. S100β induces apoptotic cell death in cultured astrocytes via a nitric oxide-dependent pathway. BBA. 1996. 1313: 239-245.

Hu J, Ferriera A and Van Eldik LJ. S100β induces neuronal death through nitric oxide release from astrocytes. J Neurochem. 1997. 69: 2294-2301.

Hua SE, Lenz FA, Zirh TA, Reich SG and Dougherty PM. Thalamic neuronal activity correlated with essential tremor. J Neurol Neurosurg Psychiatry. 1998. 64(2):273-6.

Hyman BT, Spires T, Matsui T. Orne J, Pitstik R, Carlson GA, Ashe K. Neuritic alterations and dysfunctions in Alzheimer’s disease. Abstract, Symposia, The Journal of Alzheimer’s Association. Madrid. 2006. 2(3): S26

Ischiropoulos H. Biological tyrosine nitration: a pathophysiological function of nitric oxide and reactive oxygen species. Review. Arch Biochem Biophys. 1998. 356(1):1-11.

Ito M. Long-term depression. Annu Rev Neurosci. 1989. 12:85-102.

Jellinger KA, Stadelmann C. Problems of cell death in neurodegeneration and Alzheimer's Disease. JAD. 2001. 3(1):31-40.

Kandel ER, Schwartz JH and Jessell TM. Principles of neruoscience. 4th Ed. 2002.

Katsuse O, Dickson DW. Inferior olivary hypertrophy is uncommon in progressive supranuclear palsy. Acta Neuropathol (Berl). 2004. 108(2) :143-6

Katsuse O, Iseki E, Kosaka K. Immunohistochemical study of the expression of cytokines and nitric oxide synthases in brains of patients with dementia with Lewy bodies. Neuropathology. 2003. 23(1):9-15.

Keller JN, Guo Q, Holtsberg FW, Bruce-Keller AJ, Mattson MP. Increased sensitivity to mitochondrial toxin-induced apoptosis in neural cells expressing mutant presenilin-1 is linked to perturbed calcium homeostasis and enhanced oxyradical production. J Neurosci. 1998. 18(12): 4439-50.

Kelly KM, Nadon NL, Morrison JH, Thibault O, Barnes CA, Blalock EM. The neurobiology of aging. Review. Epilepsy Res. 2006. 1:S5-20.

King JS. The synaptic cluster (glomerulus) in the inferior olivary nucleus.J Comp Neurol. 1976. 165(3): 387-400.

Kinney HC, McHugh T, Miller K, Belliveau RA, Assmann SF. Subtle developmental abnormalities in the inferior olive: an indicator of prenatal brainstem injury in the sudden infant death syndrome. J Neuropathol Exp Neurol. 2002. 61(5):427-41.

Kim HS, Lee JH, Lee JP, Kim EM, Chang KA, Park CH, Jeong SJ, Wittendorp MC, Seo JH, Choi SH and Suh YH. Amyloid beta peptide induces cytochrome C release from isolated mitochondria. Neuroreport. 2002. 13: 1989-1993.

Kirchhoff F, Dringer R, Giaume C: Pathways of neuron-astrocyte interactions and their possible role in neuroprotection. Eur Arch Psych Clin Neurosci. 2001. 251: 159-169.

Kobayashi K, Hayashi M, Nakano H, Fukutani Y, Sasaki K, Shimazaki M, Koshino Y. Apoptosis of astrocytes with enhanced lysosomal activity and oligodendrocytes in white matter lesions in Alzheimer's disease. Neuropathol Appl Neurobiol. 2002. 28(3):238-51.

Kobayashi K, Hayashi M, Nakano H, Shimazaki M, Sugimori K, Koshino Y. Correlation between astrocyte apoptosis and Alzheimer's changes in gray matter lesions in Alzheimer's disease. JAD. 2004. 6: 623-632.

Koeppen AH. The pathogenesis of spinocerebellar ataxia. Review. Cerebellum. 2005. 4(1):62-73.

F.H. Kooy. The inferior oliva in vertebrates. Folia Neurobiol. 1917. 10: 205-369.

Koppal T, Drake J, Yatin S, Jordan B, Varadarajan S, Bettenhausen L, Butterfield DA. Peroxynitrite-induced alterations in synaptosomal membrane proteins: insight into oxidative stress in Alzheimer's disease. J Neurochem. 1999.72(1): 310-7.

Korbo L, Amrein I, Lipp HP, Wolfer D, Regeur L, Oster S, Pakkenberg B. No evidence for loss of hippocampal neurons in non-Alzheimer dementia patients. Acta Neurol Scand. 2004. 109(2): 132-9.

Kruman I, Guo Q, Mattson MP. Calcium and reactive oxygen species mediate staurosporine-induced mitochondrial dysfunction and apoptosis in PC12 cells. J Neurosci Res. 1998. 51(3): 293-308.

Lang EJ, Sugihara I and Llinas R. GABAergic modulation of complex spike activity by the cerebellar nucleo-olivary pathway in rat. J Neurophysiol. 1996. 1: 255-275.

Lang EJ. Organization of olivocerebellar activity the the absence of excitatory glutamatergic input. J Neurosci. 2001. 21(5): 1663-1675.

Lapresle J. Rythmic palatal myoclonus and the dentato-olivary pathway, J Neurol. 1979. 220: 223-130.

Lasn H, Winblad B, Bogdanovic N. The number of neurons in the inferior olivary nucleus in Alzheimer's disease and normal aging: A stereological study using the optical fractionator. JAD. 2001. 3: 159-168.

Lasn H, Winblad B, Bogdanovic N. Neuroglia in the inferior olivary nucleus during normal aging and Alzheimer's disease. JCMM. 2006.10: 145-56.

Lee HG, Casadesus G, Zhu X, Joseph JA, Perry G, Smith MA. Perspectives on the amyloid-beta cascade hypothesis. Review. JAD. 2004. 6(2): 137-45.

Lee JT, Xu J, Lee JM, Ku G, Han X, Yang DI, Chen S, Hsu CY. Amyloid-beta peptide induces oligodendrocyte death by activating the neutral sphingomyelinase-ceramide pathway. J Cell Biol. 2004. 164(1):123-31.

Liu L, Li Y, Van Eldik LJ, Griffin WST, Barger SW. S100B-induced microglial and neuronal IL-1 expression is mediated by cell type-specific transcription factors. J Neurochem. 2005. 92: 546-533.

Llinás R, Sasaki K. The Functional Organization of the Olivo-Cerebellar System as Examined by Multiple Purkinje Cell Recordings. Eur J Neurosci. 1989. 1(6): 587-602.

Llinás R, Volkind RA. The olivo-cerebellar system: functional properties as revealed by harmaline-induced tremor. Exp Brain Res. 1973. 18(1): 69-87.

Llinás R, Welsh JP. On the cerebellum and motor learning. Review. Curr Opin Neurobiol. 1993. 3(6):958-65.

Llinás R, Yarom Y. Electrophysiology of mammalian inferior olivary neurons in vitro. Different types of voltage-dependent ionic conductances. J Physiol. 1981. 315: 549-67.

Louis ED, Zheng W, Jurewicz EC, Watner D, Chen J, Factor-Litvak P, Parides M. Elevation of blood beta-carboline alkaloids in essential tremor. Neurology. 2002. 59(12):1940-4.

Ludwin SK. The pathology of the oligodendrocyte. J Neuropathol Exp Neurology. 1997. 56: 111-124.

Lyness SA, Zarow C, Chui HC. Neuron loss in key cholinergic and aminergic nuclei in Alzheimer disease: a meta-analysis. Neurobiol Aging. 2003. 24(1):1-23

Maccioni RB, Muños JP, Barberito L. The molecular basis of Alzheimer's disease and other neurodegenerative disorders. Arch Med Res. 2001. 32: 367-381.

Malchiodi-Albedi F, Domenici RM, Paradisi S, Bernardo A, Ajmone-Cat MA, Minghetti L. Astrocytes contribute to neuronal impairment in Aβ toxicity increasing apoptosis in rat hippocampal neurons. Glia. 2001. 34: 68-72.

Mann DMA, Yates PO, Stamp JE, The relationship between lipofucin pigment and aging in the human nervous system. J Neurol Sci. 1978. 37: 83-93.

Martin JA, Craft DK, Su JH, Kim RC, Cotman CW. Astrocytes degenerate in frontotemporal dementia: possible relation to hypoperfusion. Neurobiol Aging. 2001. 22(2): 195-207.

Martinez G, Carnazza ML, Di Giacomo C, Sorrenti V, Avitabile M, Vanella A. GFAP, S100β and vimentin proteins in rat after cerebral post-ischemic reperfusion. Int. J. Neurosci. 1998. 6: 519-526.

Mattson MP, Maudsley S, Martin B. BDNF and 5-HT: a dynamic duo in age-related neuronal plasticity and neurodegenerative disorders. TINS. 2004. 27(10):589-94.

Mattson MP. Pathways towards and away from Alzheimer's disease. Nature. 2004. 430: 631-739

Mattson MP and Chan SL. Good and bad amyloid antibodies. Science. 2003. 301: 1847-8.

Menet V, Prieto M, Privat A, Gimenez y Ribotta M. Axonal plasticity and functional recovery after spinal cord injury in mice deficient in both glial fibrillary acidic protein and vimentin genes. Proc Natl Acad Sci U S A. 2003. 100(15):8999-9004.

Menet V, Gimenez y Ribotta M, Chauvet N, Drian MJ, Lannoy J, Colucci-Guyon E, Privat A. Inactivation of the glial fibrillary acidic protein gene, but not that of vimentin, improves neuronal survival and neurite growth by modifying adhesion molecule expression. J Neurosci. 2001. 21(16): 6147-58.

Menet V, Gimenez Y Ribotta M, Sandillon F, Privat A. GFAP null astrocytes are a favorable substrate for neuronal survival and neurite growth. Glia. 2000. 31(3): 267-72.

McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work group under the auspices of the Department of Health and Human Services Task Force on Alzheimer's disease, Neurology. 1984. 34: 939-944.

Mirra SS, Hart MN, Terry RD. Making the diagnosis of Alzheimer's disease. Arch Pathol Lab Med. 1993. 117: 132-144.

Mitrovic B, Ignarro LJ, Vinters HV, Akers MA, Schmid I, Uittenbogaart C, Merrill JE. Nitric oxide induces necrotic but not apoptotic cell death in oligodendrocytes. Neuroscience. 1995. 65(2): 531-9.

Miwa H, Kubo T, Suzuki A, Kihira T, Kondo T.A species-specific difference in the effects of harmaline on the rodent olivocerebellar system. Brain Res. 2006. 1068(1):94-101.

Moatamed F. Cell frequencies in the human inferior olivary nuclear complex, JComp Neurol. 1966. 128: 109-116.

Monagle RD, H. Brody H. The effects of age upon the main nucleus of the inferior olive in the human, J Comp Neurol. 1974. 155: 61-66.

de la Monte SM, Wands JR. Molecular indices of oxidative stress and mitochondrial dysfunction occur early and often progress with severity of Alzheimer's disease. JAD. 2006. 9(2):167-81.

de la Monte SM, Wands JR. Review of insulin and insulin-like growth factor expression, signaling, and malfunction in the central nervous system: relevance to Alzheimer's disease. Review. JAD. 2005. 7(1):45-61.

de Montigny C, Lamarre Y. Rhythmic activity induced by harmaline in the olivo-cerebello-bulbar system of the cat. Brain Res. 1973. 53(1):81-95.

Monzon-Mayor M, Alvarez M, Arbelo-Galvan J, Romero-Aleman M, Yanes C, Plaza ML, Rodriguez JR, Rodriguez JJ, Toledano A. Long-term evolution of local, proximal and remote astrocyte responses after diverse nucleus basalis lesioning (an experimental Alzheimer model): GFAP immunocytochemical study. Brain Res. 2000. 865(2): 245-58.

Moreira PI, Cardoso SM, Santos MS, Oliveira CR. The key role of mitochondria in Alzheimer's disease. J Alzheimers Dis. 2006. 9(2):101-10.

Morris MC, Evans DA, Bienias JL, Tangney CC, Bennett DA, Aggarwal N, Wilson RS, Scherr PA. Dietary intake of antioxidant nutrients and the risk of incident Alzheimer disease in a biracial community study. JAMA. 2002. 287(24): 3230-7.

Mouser PE, Head E, Ha KH, Rohn TT. Caspase-mediated cleavage of glial fibrillary acidic protein within degenerating astrocytes of the Alzheimer's disease brain. Am J Pathol. 2006. 168(3):936-46.

Mrak RE, Sheng JG, Griffin WST. Correlation of astrocytic S100β expression with dystrophic neurites in amyloid plaques of Alzheimer’s disease. J Neuropathol Exp Neurol. 1996. 55: 273-279.

Mrak RE, Griffin WST. The role of activated astrocytes and of the neurotrophic cytokine S100β in the pathogenesis of Alzheimer’s Disease. Neurobiol Aging. 2001. 22: 915-922.

Mrak RE, Griffin WST. Trisomy 21 and the brain. J Neuropath Exp Neurol. 2004. 63: 679-685.

Muller W, Meske V, Berlin K, Scharnagl H, Marz W, Ohm TG. Apolipoprotein E isoforms increase intracellular Ca^{2+}-differentially through a omega-agatoxin Iva-sevsitive Ca^{2+}-channel. Brian Pathol 1998. 8: 641-653.

Murray FE, Landsberg JP, Williams RJ, Esiri MM, Watt F. Elementar analysis of neurofibrillary tangles in Alzheimer’s disease using proton-induced X-ray analysis. Ciba Found Symp. 1992. 169:201-210.

Nag TC, Wadhwa S. Ontogeny of two calcium-binding proteins (calbindin D-28K and parvalbumin) in the human inferior olivary complex and their distribution in the adults. J Chem Neuroanat. 2004. 27(3):183-92.

Navarro A, Del Valle E, Tolivia J. Differential expression of Apolipoprotein D in human astroglial and oligodendroglial cells. J Histochem Cytochem. 2004. 52: 1031-1036.

Nieto-Bona MP, Busiguina S, Torres-Aleman I. Insulin-like growth factor I is an afferent trophic signal that modulates calbindin-28kD in adult Purkinje cells. J Neurosci Res. 1995. 42(3):371-6.

Nissl, F. Über die sogenannten Granula der Nervenzellen. Neurologisches Centralblatt, Leipzig, 1894. 13: 676-685.

Nixon RA, Saito KI, Grynspan F, Griffin WR, Katayama S, Honda T, Mohan PS, Shea TB, Beermann M. Calcium-activated neutral proteinase (calpain) system in aging and Alzheimer's disease. Ann NY Acad Sci. 1994. 747: 77-91.

Noble M. The possible role of myelin destruction as a precipitating event in Alzheimer's disease. Review. Neurobiol Aging. 2004. 25(1):25-31.

O'Hearn E, Molliver ME. Degeneration of Purkinje cells in parasagittal zones of the cerebellar vermis after treatment with ibogaine or harmaline. Neuroscience. 1993. 55(2):303-10.

Oka A, Belliveau MJ, Rosenberg PA, Volpe JJ. Vulnerability of oligodendroglia to glutamate: pharmacology, mechanisms, and prevention. J Neurosci. 1993. 13(4):1441-53.

Ondo WG, Jankovic J, Connor GS, Pahwa R, Elble R, Stacy MA, Koller WC, Schwarzman L, Wu SC, Hulihan JF; Topiramate Essential Tremor Study Investigators. Topiramate in essential tremor: a double-blind, placebo-controlled trial. Neurology. 2006. 66(5):672-7.

Ortega RM, Requejo AM, Lopez-Sobaler AM, Andres P, Navia B, Perea JM, Robles F. Cognitive function in elderly people is influenced by vitamin E status. J Nutr. 2002. 132(7): 2065-8.

Overmyer M, Helisalmi S, Soininen H, Laakso M, Riekkinen P, Alafuzoff I: Astrogliosis and the ApoE Genotype. Dem Ger Cogn Dis 1999. 10: 252-257.

Ozawa T, Paviour D, Quinn NP, Josephs KA, Sangha H, Kilford L, Healy DG, Wood NW, Lees AJ, Holton JL, Revesz T. The spectrum of pathological involvement of the striatonigral and olivopontocerebellar systems in multiple system atrophy: clinicopathological correlations. Brain. 2004. 127: 2657-71.

Pakkenberg B, Pelvig D, Marner L, Bundgaard MJ, Gundersen HJG, Nyengaard JR. Regeur L: Aging and the human neocortex. Exp Gerontol 2003. 38: 95-99

Pelvig DP, Pakkenberg H, Regeur L, Oster S, Pakkenberg B. Neocortical glial cell numbers in Alzheimer's disease. Dem Ger Cogn Dis 2003. 16: 212-219.

Perea G, Araque A. Synaptic regulation of the astrocyte calcium signal. Review. J Neural Transm. 2005. 112(1):127-35.

Perea G, Araque A. Glial calcium signaling and neuron-glia communication. Review. Cell Calcium. 2005. 38(3-4):375-82.

Perea G, Araque A. Synaptic information processing by astrocytes. Review. J Physiol Paris. 2006. 99(2-3):92-7.

Peskind ER, Griffin WS, Akama KT, Raskind MA, Van Eldik LJ. Cerebrospinal fluid S100B is elevated in the earlier stages of Alzheimer's disease. Neurochem Int. 2001. 39(5-6):409-13.

Pettersson A. Motor function and cognition. Aspects on gait and balance. PhD thesis. Karolinska Institute. 2005.

Pettersson A, Olsson E, Wahlund L.-O. Motor function in subjects with mild cognitive impairment and early Alzheimer's disease. Dement Geriatr Cong Disord. 2005. 19: 299-304.

Pierot L, Cervera-Pierot P, Delattre JY, Duyckaerts C, Chiras J, Brunet P.Palatal myoclonus and inferior olivary lesions: MRI-pathologic correlation. J Comput Assist Tomogr. 1992. 16(1):160-3.

Pinto AD, Lang AE, Chen R. The cerebellothalamocortical pathway in essential tremor. Neurology. 2003. 60(12):1985-7.

Porchet R, Probst A, Bouras C, Draberova E, Draber P, Riederer BM. Analysis of glial acidic fibrillary protein in the human entorhinal cortex during aging and in Alzheimer's disease. Proteomics. 2003. 3(10):2067.

Powers RE, DeSouza EB, Walker LC, Price DL, Vale WW, Young WS 3rd. Corticotropin-releasing factor as a transmitter in the human olivocerebellar pathway. Brain Res. 1987. 415(2):347-52.

Powers RE, O'Connor DT, Price DL. Noradrenergic innervation of human inferior olivary complex. Brain Res. 1990. 523(1):151-5.

Pranzatelli MR. Serotonin and human myoclonus. Rationale for the use of serotonin receptor agonists and antagonists. Review. Arch Neurol. 1994. 51(6):605-17.

Pratico D, Sung S. Lipid peroxidation and oxidative imbalance: early functional events in Alzheimer's disease. JAD. 2004. 6(2):171-5.

Pratt BM, McPherson JM. TGF-beta in the central nervous system: potential roles in ischemic injury and neurodegenerative diseases. Cytokine Growth Factor Rev. 1997. 8(4):267-92.

Raman IM, Bean BP. Properties of sodium currents and action potential firing in isolated cerebellar Purkinje neurons. Ann N Y Acad Sci. 1999. 868:93-6.

Ramon y Cajal S. Histologie du systeme nerveux de I'homme et des vertebres. 1909. Vol.1. Maloine Paris.

Ramos AJ, Tagliaferro P, Lopez-Costa JJ, Lopez EM, Pecci Saavedra J, Brusco A. Neuronal and inducible nitric oxide synthase immunoreactivity following serotonin depletion. Brain Res. 2002. 958(1): 112-21.

Reisberg B, London, E, Ferris MJ, Borenstein J, Scheier L, de Leon MJ, The Brief Cognitive rating Scale: language, motoric and mood concomitants in primary degenerative dementia. Psychopharmacol Bull. 1983. 19: 702-708.

Ridet J.L., Malhotra A., Privat A. & Gage F.H. Reactive astrocytes: cellular and molecular cues to biological function. TINS. 1997. 20, 570-7.

Riga D, Riga S, Halalau F, Schneider F. Brain lipopigment accumulation in normal and pathological aging. Review. Ann N Y Acad Sci. 2006. 1067:158-63.

Rissman RA, Poon WW, Blurton-Jones M, Oddo S, Torp R, Vitek MP, LaFerla FM, Rohn TT, Cotman CW. Caspase-cleavage of tau is an early event in Alzheimer disease tangle pathology. J Clin Invest. 2004. 114(1):121-30.

Rivera EJ, Goldin A, Fulmer N, Tavares R, Wands JR, de la Monte SM.Insulin and insulin-like growth factor expression and function deteriorate with progression of Alzheimer's disease: link to brain reductions in acetylcholine. JAD. 2005. 8(3):247-68.

Robertson LT, Stoler WA. The structure and connections of the developing inferior olivary nucleus of the rhesus monkey (Macaca mulatta). J Comp Neurol. 1974. 158(2): 167-90.

Rogers JD, Brogan D, Mirra SS. The nucleus basalis of Meynert in neurological disease: a quantitative morphological study. Ann Neurol. 1985. 17(2): 163-70.

Rohn TT, Rissman RA, Davis MC, Kim YE, Cotman CW, Head E. Caspase-9 activation and caspase cleavage of tau in the Alzheimer's disease brain. Neurobiol Dis. 2002. 11(2):341-54.

Rohn TT, Head E, Su JH, Anderson AJ, Bahr BA, Cotman CW, Cribbs DH. Correlation between caspase activation and neurofibrillary tangle formation in Alzheimer's disease. Am J Pathol. 2001. 158(1): 189-98.

Rothermundt M, Peters M, Prehn JHM, Arlot V. S100B in brain damage and neurodegeneration. Review. Micr Res Tech. 2003. 60: 614-632.

Royston MC, McKenzie JE, Gentleman SM, Sheng JG, Mann DMA, Griffin WST, Mrak RE. Overexpression of S100β in Down’s syndrome: correlation with patients age and with β -amyloid deposition. Neuropathol Appl Neurobiol. 1999. 25:387-393.

Rub U, Del Tredici K, Schultz C, Thal DR, Braak E, Braak H: The autonomic higher order processing nuclei of the lower brain stem are among the early targets of the Alzheimer's disease-related cytoskeletal pathology. Acta Neuropathol (Berl). 2001. 101: 555-64.

Rub U, Gierga K, Brunt ER, de Vos RA, Bauer M, Schols L, Burk K, Auburger G, Bohl J, Schultz C, Vuksic M, Burbach GJ, Braak H, Deller T. Spinocerebellar ataxias types 2 and 3: degeneration of the pre-cerebellar nuclei isolates the three phylogenetically defined regions of the cerebellum. J Neural Transm. 2005. 112(11):1523-45.

Ruigrok TJH. Cerebellar nuclei: the olivary connection. Progress in Brain Res. 1997. 114: 167-192.

Sadowinski Pine S, Landing BH, Shankle WR. Reduced inferior olivary neuron number in early down syndrome. Pediatric Pathol Lab Med. 1997. 17: 537-545.

Salter MG, Fern R. NMDA receptors are expressed in developing oligodendrocyte processes and mediate injury. Nature. 2005. 438(7071): 1167-71.

Salvemini D, Ischiropoulos H, Cuzzocrea S. Roles of nitric oxide and superoxide in inflammation. Methods Mol Biol. 2003. 225: 291-303.

Samuel M, Torun N, Tuite PJ, Sharpe JA, Lang AE. Progressive ataxia and palatal tremor (PAPT): clinical and MRI assessment with review of palatal tremors. Brain. 2004. 127:1252- 68.

Sastry BR, Morishita W, Yip S, Shew T. GABA-ergic transmission in deep cerebellar nuclei. Review. Prog Neurobiol. 1997. 53(2):259-71.

Scarmeas N, Albert M, Brandt J, Blacker D, Hadjigeorgiou G, Papadimitriou A, Dubois B, Sarazin M, Wegesin D, Marder K, Bell K, Honig L, Stern Y. Motor signs predict poor outcomes in Alzheimer disease. Neurology. 2005. 64(10): 1696-703.

Scarmeas N, Hadjigeorgiou GM, Papadimitriou A, Dubois B, Sarazin M, Brandt J, Albert M, Marder K, Bell K, Honig LS, Wegesin D, Stern Y. Motor signs during the course of Alzheimer disease. Neurology. 2004. 63(6):975-82.

Scheibel ME, Scheibel AB. Observations on the intracortical relations of the climbing fibers of the cerebellum; a Golgi study. J Comp Neurol. 1954. 101(3): 733-63.

Schwaller B, Meyer M, Schiffmann S. 2002. 'New' functions for 'old' proteins: the role of the calcium-binding proteins calbindin D-28k, calretinin and parvalbumin, in cerebellar physiology. Studies with knockout mice. Review. Cerebellum. 1(4): 241-58.

Schwarz C, Schmitz Y. Projection from the cerebellar lateral nucleus to precerebellar nuclei in the mossy fiber pathway is glutamatergic: a study combining anterograde tracing with immunogold labeling in the rat.
J Comp Neurol. 1997. 381(3): 320-34.

Scott SA. Dendritic atrophy and remodeling of amygdaloid neurons in Alzheimer's disease.Dementia. 1993. 4(5):264-72.

Selkoe DJ. The molecular pathology of Alzheimer's disease. Review.
Neuron. 1991. 6(4):487-98.

Selkoe DJ. Amyloid beta-protein precursor: new clues to the genesis of Alzheimer's disease. Review. Curr Opin Neurobiol. 1994. 4(5):708-16.

Selkoe DJ. Alzheimer's disease: genotypes, phenotypes, and treatments. Review. Science. 1997. 275(5300):630-1.

Serrano-Duenas M. Fluoxetine-induced tremor: clinical features in 21 patients. Parkinsonism Relat Disord. 2002. 8(5):325-7.

Seubert P, Vigo-Pelfrey C, Esch F, Lee M, Dovey H, Davis D, Sinha S, Schlossmacher M, Whaley J, Swindlehurst C, et al. Isolation and quantification of soluble Alzheimer's beta-peptide from biological fluids. Nature. 1992. 359(6393): 325-7.

Sheng JG, Mrak RE, Rovnaghi CR, Kozlowska E, Van Eldik LJ, Griffin WST. Human brain S100β and S100β mRNA expression increase with age: pathogenic implications for Alzheimer’s disease. Neurobiol Aging. 1996.17: 359-363.

Sies H, Stahl W, Polidori MC: Oxidative stress: Antioxidants in degenerative neurological and ophthalmological disorders; in Bachmann C, Koletzko E (eds): Genetic Expression and Nutrition. Nestlé Nutrition Workshop Series. Philadelphia, Lippincott Williams & Wilkins, 2003, vol 50, pp 107-118

Simic G, Kostovic I, Winblad B, Bogdanovic N. Volume and number of neurons of the human hippocampal formation in normal aging and Alzheimer's disease, J Comp Neur. 1997. 379: 482-494.

Sjöbeck M, Dahlén S, Englund E. Neuronal loss in the brainstem and cerebellum - part of the normal aging process? A morphometric study of the vermis cerebelli and inferior olivary nucleus. J. Gerontol. 1999. 54: B363-B368.

Sjöbeck, Englund E. Alzheimer's disease and the cerebellum: a morphologic study on neuronal and glial changes. Dement Geriatr Cogn Disord. 2001. 12(3):211-8.

Sloane JA Hollander W, Rosene DL, Moss MB, Kemper T, Abraham CR. Astrocytic hypertrophy and altered GFAP degradation with age in subcortical white matter of the shesus monkey. Brain Res. 2000. 862: 1-10.

Smale G, Nichols NR, Brady DR, Finch CE, Horton WE Jr. Evidence for apoptotic cell death in Alzheimer's disease. Exp Neurol. 1995. 133(2):225-30.

Smith RA, Porteous CM, Coulter CV, Murphy MP. Selective targeting of an antioxidant to mitochondria. Eur J Biochem. 1999. 263(3): 709-16.

Su JH, Anderson AJ, Cummings BJ, Cotman CW. Immunohistochemical evidence for apoptosis in Alzheimer's disease. Neuroreport. 1994. 5(18): 2529-33.

Sugihara I. Organization and remodeling of the olivocerebellar climbing fiber projection. Cerebellum. 2006. 5: 15-22.

Szteyn S. Types of neurons in nucleus olivaris inferior of the Europan bison. J Hirnforsch. 1988. 29: 353-356.

Tacconi MT. Neuronal death: is there a role for astrocytes? Review. Neurochem Res. 1998. 23(5):759-65.

Tang Y, Nyengaard JR, Pakkenberg B, Gundersen HJ.Age-induced white matter changes in the human brain: a stereological investigation. Neurobiol Aging. 1997. 18(6):609-15.

ten Donkelaar HJ. Evolution of the red nucleus and rubrospinal tract. Review. Behav Brain Res. 1988. 28(1-2): 9-20.

Toescu EC, Myronova N, Verkhratsky A. Age-related structural and functional changes of brain mitochondria. Review. Cell Calcium. 2000. 28(5-6):329-38.

Tomlinson BE, Irving D, Blessed G. Cell loss in the locus coeruleus in senile dementia of Alzheimer type. J Neurol Sci. 1981. 49(3):419-28.

Trelles JO. La oliva bulbar: su estructura function y pathologia. Rev Neuro-Psycat. 1943. 6:433-521.

Trojanowski JQ. Mattson MP. Overview of protein akkggregation in single, double and triple neurodegenerative brain amyloidosis. 2003. NeuroMolecular Medicine. 4: 1-5.

Tsolaki M, Kokarida K, Iakovidou V, Stilopoulos E, Meimaris J, Kazis A. Extrapyramidal symptoms and signs in Alzheimer's disease: prevalence and correlation with the first symptom. Am J Alz Dis Other Dem. 2001. 16(5):268-78.

Unger JW. Glial reaction in aging and Alzheimer’s Disease. Microsc Res Tech. 1998. 43: 24-28.

Ulfig N, Chan WY. Differential expression of calcium-binding proteins in the red nucleus in the developing and adult human brain. Anat Embryol. 2001. 203: 95-108.

Uttenthal LO, Alonso D, Fernandez AP, Campbell RO, Moro MA, Leza JC, Lizasoain I, Esteban FJ, Barroso JB, Valderrama R, Pedrosa JA, Peinado MA, Serrano J, Richart A, Bentura ML, Santacana M, Martinez-Murillo R, Rodrigo J. Neuronal and inducible nitric oxide synthase and nitrotyrosine immunoreactivities in the cerebral cortex of the aging rat. Microsc Res Tech. 1998. 43(1):75-88.

Xu J, Chen S, Ahmed H, Chen H, Ku G, Goldberg MP, Hsu C. Amyloid-β peptides are toxic to oligodendrocytes. J Neurosci. 2001. 21: 1-5.

Van Eldik LJ, Griffin WST. S100β expression in Alzheimer's Disease: relation to neuropathology in brain regions. BBA. 1994. 1223: 398-403.

Van Eldik LJ, Wainwright MS. The Janus face of glial-derived S100B: beneficial and detrimental functions in the brain. Review. Restor Neurol Neurosci. 2003. 21(3-4):97-108.

Vereecken TH, Vogels OJ, Nieuwenhuys R. Neuron loss and shrinkage in the amygdala in Alzheimer's disease. Neurobiol Aging. 1994. 15(1):45-54.

Verkhratsky A, Toescu EC. Calcium and neuronal aging. TINS. 1998. 21(1): 2-7.

Verkhratsky A, Kettenmann H. Calcium signalling in glial cells. Review. Trends Neurosci. 1996. 19(8):346-52.

Walz W. Controversy surrounding the existence of discrete functional classes of astrocytes in adult gray matter. Review. Glia. 2000. 31(2):95-103.

Weiss M, Pellet J. Raphe - cerebellum interactions. II. Effects of midbrain raphe stimulation and harmaline administration on single unit activity of cerebellar cortical cells in the rat. Exp Brain Res. 1982. 48(2):171-6.

Wegiel J, Wisniewski HM, Dziewiatkowski J, Badmajew E, Tarnawski M, Reisberg B, Mlodzik B, De Leon MJ, Miller CD. Cerebellar atrophy in Alzheimer's disease - Clinicopathological correlations. Brain Res. 1999. 818: 41-50.

Weldon DT, Rogers SD, Ghilardi JR, Finke MP, Cleary JP, O'Hare E, Esler WP, Maggio JE, Mantyh PW. Fibrillar beta-amyloid induces microglial phagocytosis, expression of inducible nitric oxide synthase, and loss of a select population of neurons in the rat CNS in vivo. J Neurosci. 1998. 18(6): 2161-73.

Welsh JP, Chang B, Menaker ME, Aicher SA. Removal of the inferior olive abolishes myoclonic seizures associated with a loss of olivary serotonin. Neuroscience. 1998. 82(3):879-97.

Welsh JP, Llinas R. Some organizing principles for the control of movement based on olivocerebellar physiology. Review. Prog Brain Res. 1997. 114: 449-61.

Welsh JP, Lang EJ, Suglhara I, Llinas R. Dynamic organization of motor control within the olivocerebellar system. Nature. 1995. 374(6521):453-7.

Welsh JP, Placantonakis DG, Warsetsky SI, Marquez RG, Bernstein L, Aicher SA. The serotonin hypothesis of myoclonus from the perspective of neuronal rhythmicity. Review. Adv Neurol. 2002. 89:307-29.

Welsh JP, Ahn ES, Placantonakis DG. Is autism due to brain desynchronization? Review. Int J Dev Neurosci. 2005. 23(2-3):253-63.

Vernadakis A. Glia-neuron intercommunications and synaptic plasticity. Prog Neurobiol. 1996. 49: 185-214.

Whitehouse PJ, Vale WW, Zweig RM, Singer HS, Mayeux R, Kuhar MJ, Price DL, De Souza EB. Reductions in corticotropin releasing factor-like immunoreactivity in cerebral cortex in Alzheimer's disease, Parkinson's disease, and progressive supranuclear palsy. Neurology. 1987. 37(6):905-9.

Whitman GT, Cotman CW. Oligodendrocyte degeneration in AD. Neurobiol Aging. 2004. 25(1): 33-6.

Wilhelmsson U, Li L, Pekna M, Berthold CH, Blom S, Eliasson C, Renner O, Bushong E, Ellisman M, Morgan TE, Pekny M. Absence of glial fibrillary acidic protein and vimentin prevents hypertrophy of astrocytic processes and improves post-traumatic regeneration. J Neurosci. 2004. 24(21): 5016-21.

West MJ, Slomianka L, Gundersen HJ. Unbiased stereological estimation of the total number of neurons in the subdivisions of the rat hippocampus using the optical fractionator. The Anatomical Record 1991. 231: 482-497.

West MJ, Kawas CH, Stewart WF, Rudow GL, Troncoso JC. Hippocampal neurons in pre-clinical Alzheimer's disease. Neurobiol Aging. 2004 Oct;25(9):1205-12.

Zadikoff C, Lang AE, Klein C. The 'essentials' of essential palatal tremor: a reappraisal of the nosology. Review. Brain. 2006. 129: 832-40.

Zhu X, Perry G, Moreira PI, Aliev G, Cash AD, Hirai K, Smith VA. Mitochondrial abnormalities and oxidative imbalance in Alzheimer disease. JAD. 2006. 9(2):147-53.