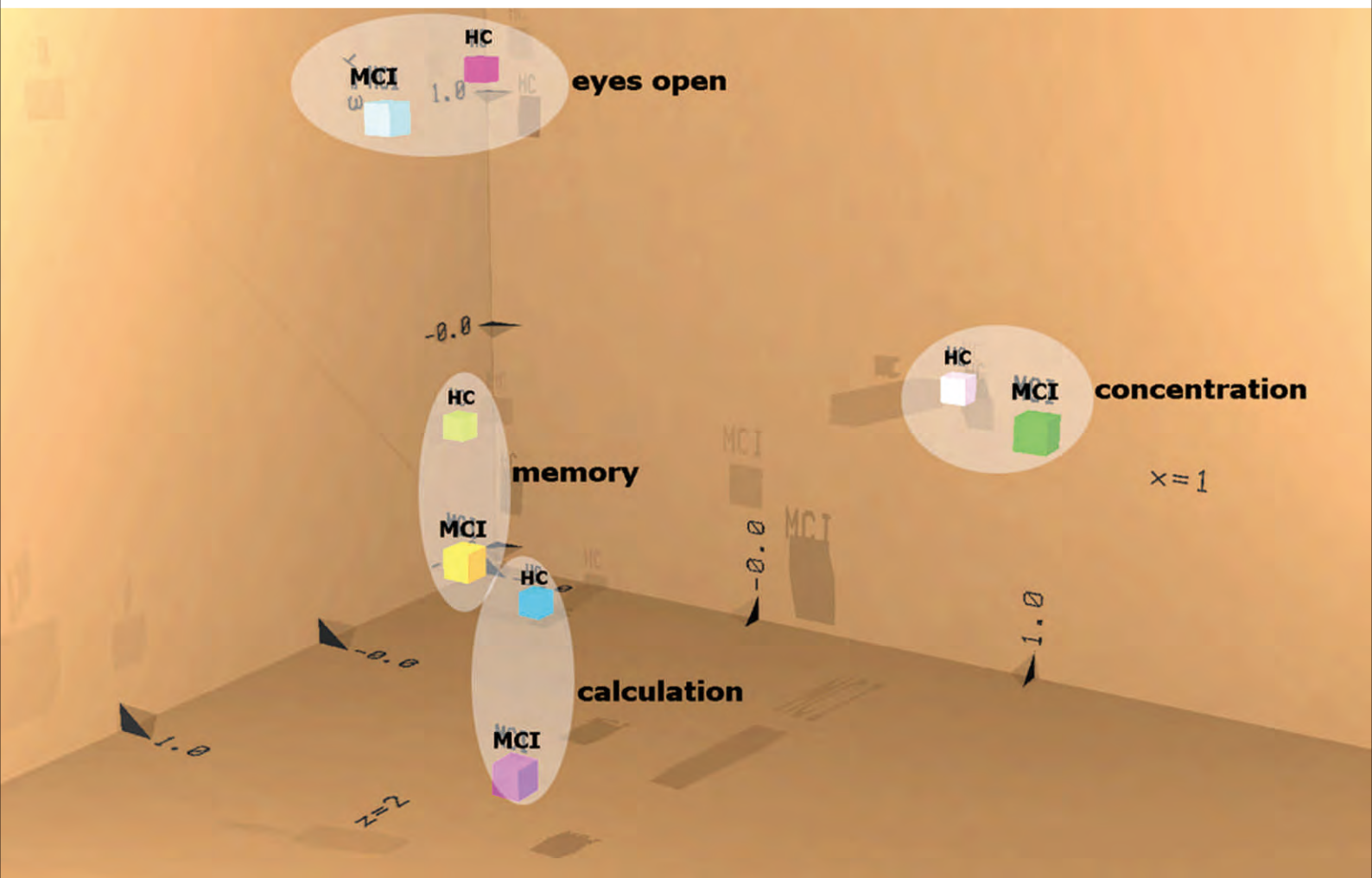


Advances in Alzheimer's Disease



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The Drug Development Based on Pathogenetic Research in Alzheimer's Disease

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Abstract

Neuropathologically, Alzheimer's disease is characterized by the presence of extracellular deposits of amyloid- β peptides, intracellular neurofibrillary tangles and atrophy of the basal forebrain cholinergic neurons. The research of pathogenesis of Alzheimer's disease inspires potential clinical drugs for treatment. To block the progression of the disease, drugs under development have to interfere with the pathogenic steps responsible for the clinical symptoms, including cholinergic deficit, calcium dysregulation, inflammation and oxidative damage, and the deposition of amyloid- β plaques and of neurofibrillary tangles. In this review, the pertinent literature about drugs targeted on relieving symptoms above is reviewed. We aim to discuss possible research priorities in the future.

Keywords

Alzheimer's Disease, A β , Protein Tau, ULMWH, MT-Stabilizing Agents

1. Introduction

Alzheimer's disease (AD) is described as one of the most common neurodegenerative disorders, with a prevalence of 5 percent after sixty-five years of age, increasing to almost 30 percent in people over age eighty-five [1]. The clinical symptoms of AD include loss of memory, progressive cognitive impairment, various behavioral disturbances and neurological disorders. Typically, AD starts with mild memory deficits, then gradually progresses to severe dementia and stupor. Generally speaking, about nine years after clinical diagnosis, the AD patients may face death caused by respiratory complications [2]. Neuropathologically, AD is characterized by senile plaques (SP) composed by amyloid- β peptides (A β), neurofibrillary tangles (NFTs) generated by hyper-phos-

phorylated forms of protein tau [3] and degeneration or atrophy of the basal forebrain cholinergic neurons.

APP is overexpressed in AD [4]. After the cleavage of APP by two proteases, denoted as β -secretase (BACE1) and γ -secretase, the sequential action will aggregate into toxic $A\beta$. The two major subtypes are $A\beta_{1-40}$ and $A\beta_{1-42}$. The shorter one comes from the cutting of typical APP in endoplasmic reticulum, while the longer one is formed across the Golgi network. Compared with each other, $A\beta_{1-40}$ is more common, but $A\beta_{1-42}$ is even more relevant to the disease. Tau is a component of microtubules; it stabilizes growing axons and is necessary for neuritis [4]. In AD, tau is abnormally hyperphosphorylated and forms insoluble fibrils, which contribute to the earliest cytoskeletal changes in NFTs formation. This abnormal spiral structure interfere the normal function of neurons, ultimately leading to neuronal cell death. Cholinesterase is the key enzyme in biological nerve conduction. In the cholinergic synaptic cleft, it degrades acetylcholine, induces the termination of excitability role of the neurotransmitter on postsynaptic membrane, which ensures normal convey of nerve signals *in vivo*. Acetylcholinesterase catalyzes the cleavage reaction of acetylcholine, resulting in the lack of acetylcholine, thus interfere nerve signal transmission.

Alzheimer's disease pathology changes major involve cholinergic nerve pathways from frontal base to the cerebral cortex and hippocampus. As we all known, these pathways are associated with attention, learning ability, memory and other cognitive processes. The loss of basal forebrain cholinergic cells in AD patients leads to reduction of synaptic availability of acetylcholine. So, AD patients always suffer the cognitive impairment.

Scientists have been working on the pathophysiological processes of Alzheimer's disease for more than a century. A large number of theories have been discovered to explain what is happening in the brain of AD patients. For example, $A\beta$ causes calcium dysregulation and oxidative stress in central nervous system cells, inflammatory changes can be observed in the brain, even diabetes and insulin-resistance may have connection with AD through GSK3 β . Nevertheless, there is a long way to go before we discover the exactly and comprehensive functions of $A\beta$ and tau in the process of AD. In other hand, the significantly effective drugs for AD clinical treatment are less than enough. In this article, we focus on the molecular and cellular alterations involved in neuronal dysfunctions caused by $A\beta$ and tau in AD. More importantly, the drug development based on pathogenetic research in AD, and possible research priorities in the future will be mentioned.

2. Cholinergic Drugs

Neurochemical research shows that the brain in AD patients have obvious shortage of central cholinergic neurotransmitter, causing loss of memory, directional force, behavior and personality change as a result. Enhancing cholinergic effects is an important way for the treatment of AD. Current research focuses on acetylcholinesterase inhibitors (AChEI), which can increase ACh concentration in the synaptic cleft by reducing its degradation, thus improve the central activity of choline. It is by far the most commonly used and is considered to be one of the most promising drug treatments of AD. The second generation of AChEI is widely applied in clinical treatment, that is Donepezil hydrochloride (aricept) [5], Rivastigmine Tartrate (exelon) [6], Galantamine and Huperzine A.

Donepezil hydrochloride is a highly selective AChEI. It is approved for use in mild-to-moderate AD patients [7] [8]. It has shown some benefit in slowing hippocampal atrophy and protecting nerve cells. Rivastigmine Tartrate has easy BBB permeability and is approved for mild-to-moderate AD [9]. Rivastigmine Tartrate can selectively enhance the effect of acetylcholine in cerebral cortex and hippocampus. Furthermore, cholinesterase inhibitors can slow down the the formation of the amyloid precursor protein (APP) fragment. After combining with its target enzyme into covalent compounds, Rivastigmine Tartrate causes a temporary loss of activity of the enzyme. Galantamine has allosteric nicotinic receptor modulation properties. Galantamine may increase ACh release by regulating brain external nicotinic acid receptor. Research showed that galantamine may be safe for the treatment of elderly patients with severe AD, which improves cognitive function in patients. However, everyday life parameters change is not obvious. Another ChEI, Huperzine A, is considered to be one of the treatment for memory disorders [10]. However, for its lacking of proprietary patent for the treatment of AD, it is considered as a nutraceutical supplement in the US. Clinical and preclinical toxicities are to be established in the future.

AD is a multifactorial disease, so the innovative model is to achieve the goal of "one molecule, multiple targets". Ideally, hybrids can provide parent compounds more potency such as BBB permeability, additional receptors or epitopes [11]. Human studies are planned.

3. Calcium Regulation as a Therapeutic Approach for AD

There is considerable evidence that $A\beta$ induces calcium dysregulation in neuron. Sequential cleavages of APP by secretases generate $sAPP\alpha$, $A\beta$ and AICD. Intracellular APP domain (AICD) can modify nuclear gene transcription and further more perturb Ca^{2+} homeostasis. On the other hand, $sAPP\alpha$, generated from APP by α -secretase, is normally produced to active K^+ channels, thereby hyperpolarizing the membrane and reducing Ca^{2+} influx [12]. The production of $sAPP\alpha$ plays a protecting role in neurons; however, amyloidogenic processing may prevent this program. $A\beta$ oligomers enhance calcium ion influx by Ca^{2+} -permeable channels, which can be facilitated by binding to phosphatidylserine (PtdS). Cell-surface exposure of PtdS is usually indicative of apoptotic cells. Mitochondrial, the energy supply station of cells, once effected by $A\beta$, will induce superoxide anion radical ($O_2^{\bullet-}$) production, Ca^{2+} overload, and decreased ATP production. In this condition, PtdS will flip from the inner portion to the cell surface of the plasma membrane. In turn, neurons with lower ATP level are particularly susceptible to $A\beta$ toxicity [13]. $A\beta$ can also generate hydroxyl radical (OH) in the presence of Fe^{2+} and Cu^+ [14] [15]. As a result, the function of ATPases dependent ion channels (Na^+ and Ca^{2+} pumps) can be impaired by toxic aldehydes generated by membrane lipid peroxidation (LP). Therefore, the membrane becomes depolarized and toxic amounts of Ca^{2+} flux into the cytoplasm through the open channels, glutamate receptor channels (N-methyl-D-aspartate receptor, NMDAR) and voltage-dependent Ca^{2+} channels (VDCC) open. Inside the neurons, Presenilins (PS) functions as an endoplasmic reticulum (ER) Ca^{2+} leak channel. In familial Alzheimer's disease, PS mutations cause excessive accumulation of Ca^{2+} and then enhance Ca^{2+} release by inositol 1, 4, 5-trisphosphate receptors (IP_3R) channels and ryanodine receptor (RyR). $A\beta$ can also block the response of nicotinic acetylcholine receptors (nAChRs) and induces sustained Ca^{2+} levels increases in presynaptic through IP_3 [16] (Figure 1).

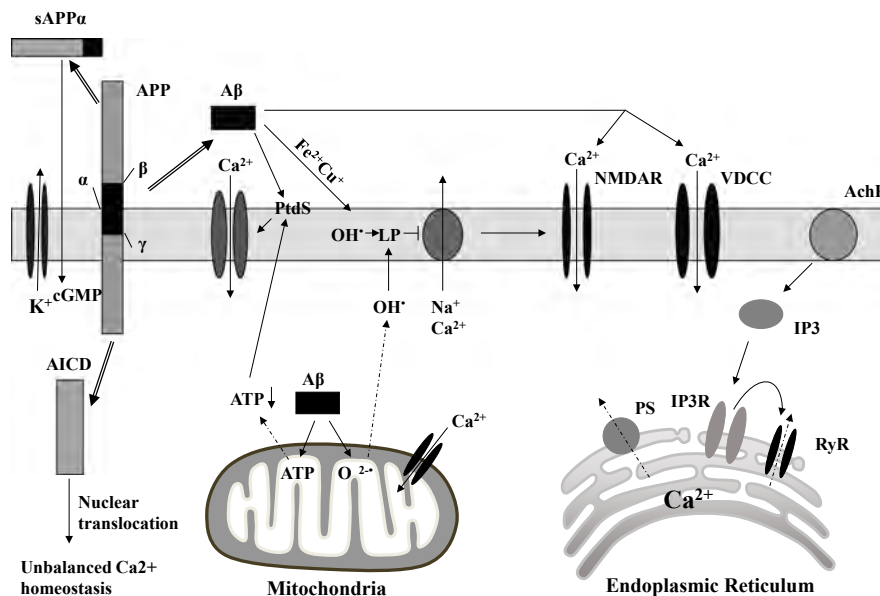


Figure 1. Molecular and cellular alterations involved in neuronal dysfunction in AD. The amyloid- β precursor protein (APP) can be cleaved by β -secretase and γ -secretase, resulting in the liberation of $sAPP\alpha$, the amyloid- β peptide ($A\beta$) and intracellular APP domain (AICD). AICD can translocate to the nucleus and perturb Ca^{2+} homeostasis. $sAPP\alpha$ may activate K^+ channels. $A\beta$ oligomers enhance calcium ion influx into the cell by the formation of Ca^{2+} -permeable channels. $A\beta$ will also induce superoxide anion radical ($O_2^{\bullet-}$) production, Ca^{2+} overload, and decreased ATP production in mitochondria. $A\beta$ can also interact with Fe^{2+} and Cu^+ to generate hydrogen peroxide and hydroxyl radical (OH). As a result, the function of Na^+ and Ca^{2+} pumps can be impaired by toxic aldehydes generated by membrane lipid peroxidation (LP), resulting the Ca^{2+} flux through N-methyl-D-aspartate receptor (NMDAR) and voltage-dependent Ca^{2+} channels (VDCC). In familial Alzheimer's disease, Presenilins (PS) mutations cause excessive accumulation of Ca^{2+} in the endoplasmic reticulum (ER) and then enhance Ca^{2+} release through inositol 1,4,5-trisphosphate receptors (IP_3R) channels and ryanodine receptor (RyR). $A\beta$ can also induce sustained nAChR-mediated increases in presynaptic Ca^{2+} levels through IP_3 .

Inspired by the above theory, methods to stabilize neural intracellular calcium homeostasis may be one of the treatments for AD. So far, a variety of drugs have therapeutic potential *in vivo* or *in vitro* experiments.

Since excessive activation of glutamate system causes excitotoxic neuronal death, NMDA open channel blocker, can antagonize glutamate excitotoxicity. Memantine, which belongs to NMDA receptor antagonist, is different from the cholinesterase inhibitor. It is the first and only drug that is approved for treatment of moderate to severe AD. Clinical studies have demonstrated that it benefits in cognitive and behavioral outcomes in patients, either as monotherapy or in combination with donepezil [17]. It also shows good effect to main types of dementia: Alzheimer's dementia, vascular dementia and AIDS dementia, which indicate that it has an advantage over other kinds of AD drugs on the market. Animal experiments showed that Nimodipine, an L-type VGCC inhibitor, plays a role as calcium antagonist. It decreases the intracellular calcium ion concentration, promotes the regeneration of injured neurons, enhance the plasticity of the aging central nervous system. In the clinical treatment, Nimodipine has obvious curative effect on memory impairment due to AD [18]. Recently, nimodipine was reported to selectively stimulate secretion of $A\beta_{1-42}$ slightly [19]. But the exact mechanism has not yet been elucidated.

In vitro experiments, ultra-low-molecular-weight heparin (ULMWH) partly reduced the $[Ca^{2+}]_i$ increase induced by glutamate, this suggests that ULMWH may inhibit external Ca^{2+} influx mediated by NMDA receptor. In addition, the IP_3R induced $[Ca^{2+}]_i$ was significantly suppressed ULMWH, suggests that ULMWH can regulate calcium balance by inhibiting calcium ion release. So ULMWH may be speculated as a specific IP_3R antagonist just like heparin. In this way, less IP_3 binds to IP_3Rs and the increase of $[Ca^{2+}]_i$ was blocked. Therefore, factors such as ULMWH are expected to have good effect on AD.

4. Anti-Amyloid Therapies

As $A\beta$ is the heart of the amyloid hypothesis of AD, the formation of $A\beta$ oligomers is a directly AD killer. The mainly function of β -secretase and γ -secretase is to produce toxic $A\beta_{1-42}$. Currently the focus of scientists is the inhibition of activity of β -secretase and γ -secretase. β -secretase (BACE1) initiates the amyloidogenic pathway. Activation of nuclear peroxisome proliferator-activated receptor γ (PPAR γ) can suppress expression of β -secretase. Rosiglitazone and pioglitazone, which belong to oral drugs for type 2 diabetes, can act as β -secretase inhibitors by stimulating PPAR γ [20]. The therapeutic effects of PPAR γ agonists in AD may be caused by their effect of increasing insulin sensitivity and reducing concentrations of insulin.

Present study is generally believed that insulin can not only pass through the blood brain barrier, but also be synthesized within the brain tissue. The insulin receptor (IR) and receptor signal transduction molecules exist in brain tissue [21]. Insulin can not only regulate sugar metabolism and energy metabolism, but also have various biological functions. The function of brain insulin is complex, including supporting the surviving of mature neurons. Cascade control apoptosis is one of the important roles of insulin. The common pathological mechanism of diabetes encephalopathy and AD is an obstacle of insulin signal transduction pathways, namely the insulin resistance, which can cause metabolic disorders and cognitive dysfunction. In a word, insulin signaling induces the phosphorylation and inhibition of glycogen synthase kinase 3 (GSK3). It would therefore promote tau phosphorylation, leading to aggregation and tangle formation, as well as contributing to $A\beta$ peptide production and plaque formation [22] (Figure 2). The related content remains further research.

Using $A\beta$ antibody in treatment of AD is a current research hot spot. Active and passive immunization clinical research is ongoing in several pharmaceutical companies. According to animal tests, vaccination of synthetic $A\beta_{1-42}$ immunization can produce $A\beta_{1-42}$ antibody, thus causes activation of monocyte or microglia. As a result, immune therapy has become a new method for treatment of AD, which has entered clinical trials in the United States. Preclinical and early clinical trial results show $A\beta$ immunotherapy has great potential to overcome the AD. Active immunizations include injection of synthetic $A\beta$ peptide, $A\beta$ fragment joined with carrier protein or adjuvant. It will stimulate the host to produce antibodies against $A\beta$. Passive immunization is to directly inject $A\beta$ specific antibody into the host, thus activate the host immune system. The common basic principle of active and passive immunotherapy is removing $A\beta$ from the brain. In clinical trials of AN-1792 vaccine, the number of $A\beta$ has decreased in the brains of patients. However, some patients appeared subacute aseptic meningitis within II period clinical trials [23]. Better than AN-1792 vaccine, CAD-106 vaccine, did not show such side effect in the early human trials [24]. A new strategy to increase the security of active immunization is to optimize the drug delivery way. It has been proved that, intranasal administration of $A\beta$ peptide, without adjuvant,

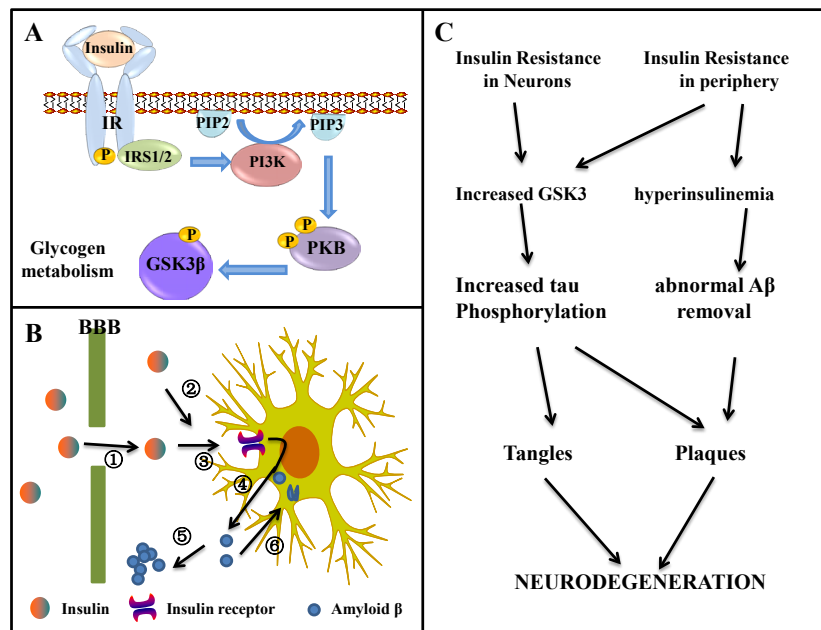


Figure 2. The role of insulin receptor (IR) in neurodegeneration, which is expressed in both neurons and glia. (A) Schematic representation of insulin signaling; (B) The potential role of insulin in the pathogenesis of AD. Insulin is transported actively across the blood-brain barrier (①), it can also be produced locally in the brain (②). Insulin act through cerebral insulin receptors (③). Thus stimulates the secretion of $A\beta$ into the extracellular space (④) where it can aggregate into senile plaques (⑤). Alternatively, excessive $A\beta$ can be cleared through endocytosis (⑥); (C) The connection between insulin resistance and neurodegeneration.

leads to appropriate immunoreaction. Gene immunotherapy, a kind of active immunization in essence, has much potential. Initially, $A\beta$ -DNA vaccine was coded by adenovirus or adeno-associated virus vector. Researchers are focusing on the development of non-viral plasmid vector, because of the large-scale low-cost production and no risk of infection or transfection [25].

As for passive immunization, many monoclonal antibody is in development and testing, such as Bapineuzumab (AAB-001) [26] and Solanezumab (LY2062430) [27] [28]. But the production of monoclonal antibodies cost great and need repeated injection. What's worse, it is hard to choose the appropriate target and pass through the BBB. It also has a risk of bleeding.

5. Drugs to Target Tau Protein

Tau protein is a kind of microtubule associated protein whose primary function is to stabilize the conformation of microtubules (MTs). The phosphorylation level of tau protein in AD patients' brain is three times higher than normal. Excessive phosphorylated tau protein may dissociate from microtubules and aggregates into nerve toxic NFTs [29]. As a consequence, microtubule is disintegrated and cytoskeleton is damaged, too.

There are two main therapeutic approaches aiming at tau protein: inhibitors of phosphorylase kinase of tau protein and compounds that inhibit tau aggregation or promote its disassembly. Inhibition of the excessive phosphorylation of tau protein is the major research direction for the treatment of AD. GSK3, and specifically its beta isoform ($GSK3\beta$), have been shown to closely connected with phosphorylate tau [30]. Animal studies have confirmed that in the early pathological changes of tau protein, lithium salt, one kind of non-specific GSK3 inhibitor [31], can prevent excessive phosphorylation of tau protein and block the further progress of the disease. But lithium salt has little effect during the late stage, so researchers have turned to develop specific inhibitors of phosphorylase kinase of tau protein. According to the research results, some kind of small molecular compounds can prevent the interaction of tau protein, thus inhibit its aggregation. For example, methylene blue, a widely used histology dye, can not only prevent polymerization of tau protein into oligomers, but also reduce $A\beta$ level

in the brain [32]. Methylene blue also has antioxidant properties and was effective in improving learning deficits either used alone or in combination with rivastigmine in animals [33]. So it is considered to be a promising new AD treatment. It has a high bioavailability when tested in a phase 2 study in patients with moderate AD [34] and its safety and clinical efficacy need to be further confirmed.

In addition to the two kinds of medicine mentioned above, new drugs designed on counteracting the functional loss of tau protein are promising. As we all know, the primary function of tau protein is to stabilize the conformation of MTs. Over the past decades, several classes of MT-stabilizing products have been used as anti-neoplastic drug. However, people are worried about dose-limiting toxicities if this class of therapeutics is used in long-term treatment of tauopathy patients. Paclitaxel, which belongs to taxanes natural products, binds to the lumen of the MT at β -tubulin subunit [35]. Importantly, paclitaxel is found to having a function of promoting MT stabilization instead of tau protein. [36]. The actin mode of epothilones is similar to paclitaxel. *In vitro*, both paclitaxel [37]-[41] and epothilones [42]-[44] have been found to protect neurons against neurotoxicity mediated by tau protein or $A\beta$. However, paclitaxel could not cross the BBB while epothilone D shows to be a brain-penetrant MT-stabilizing agent [45]. Considering that tau pathology is primarily in the brain, only epothilone D can be suitable as a therapeutic candidate for human tauopathies. As to the consideration of dose-limiting toxicities, one important observation made *in vivo* studies in epothilone D [46] indicated that low doses of epothilone D may produce optimal therapeutic effects. Overstabilization of MTs by high dose of agents on the other hand become counterproductive and may be accompanied by side effects. As a growing number of MT-stabilizing products are being discovered, a particular attention should be paid to these agents to find more useful treatment for AD and other tauopathies.

6. Summary and Prospective

The study of the pathophysiological processes of AD has been attracting people's attention for more than a century. $A\beta$ and NFTs have been observed to have extensively damage to normal function of central nervous system, such as cholinergic deficit in the CNS, intracellular Ca^{2+} disequilibrium, inordinate oxidative stress and inflammatory processes. These pathophysiological processes of AD provide multifarious targets for therapeutic or preventative agents. Besides those five drugs which are currently ratified for use in the treatment of AD (donepezil (Aricept[®]), galantamine (Reminyl[®]), rivastigmine (Exelon[®]), tacrine (Cognex[®]) and memantine (Namenda[®])), many other kinds of drugs aiming at processes mentioned above have potentials in AD treatment, for example, GAG mimetic, NSAIDs, and hypoglycemic agents (Table 1).

According to the past failures we have met in AD drug clinical trials that were conducted over the last decades, we can come to the conclusion that it is questionable to conducting large clinical development programs in AD blindly. AD is the result of the interaction of pathogenic factors. It is necessary to be better appreciating the complicacy of the disease. In our way to find significant treatment for AD, it is important to better understand the relationship between tau, $A\beta$ and other factors. In a word, the competing risk factors, physiological factors such as age and genetics, and environmental factors all play non-ignorable roles in the progress of AD. As a result, drugs with wide range of targets always appear little effect in the treatment.

The failures in AD drug clinical trials also forced people to turn to another approach to the treatment of AD. There are three kinds of behaviors that are considered to reduce the risk of AD [56]. Those are cognitive stimu-

Table 1. Different classes of products and their stages of development as potential candidates for AD.

Main mechanisms of action	Candidate drugs	Stage of development in trails
Acetylcholinesterase inhibitors	Huperzine A	RCTs completed [10]
Specific IP ₃ R antagonist	ULMWH	Vitro experiments [47]
β -secretase inhibitors	Rosiglitazone	RCTs completed [48]
	Pioglitazone	Phase 2 RCT in MCI [48]
Active immunotherapy that increase $A\beta$ clearance	AN1792 and CAD-106	RCTs completed [49] [50]
Passive immunotherapy that increase $A\beta$ clearance	AAB-001 and LY2062430	RCTs completed [51] [52]
Non-specific GSK3 inhibitor	Lithium salt	RCTs completed [53]
Anti-tau that decrease tau fibrillization	Methylene blue	RCTs completed [54]
MT-stabilizing products	Paclitaxel	Vitro animal model [55]
	Epothilone D	Phase 1b clinical trail [46]

RCT: Randomized Controlled Trial. MCI: Mild Cognitive Impairment.

lation, mental and physical exercise, and dietary energy restriction. They are thought to show beneficial effect by activating neurotransmitters, growth factors, and hormone receptors. In this way, the synaptic activity in nerve cell networks is increased and neurons will be protected against oxidative and metabolic stress.

What's more, most compounds showed some benefits in mild AD, either than moderate AD. These discoveries inspire us that the early we carry out therapeutic trials, the more possible it will be to block the course of the disease. As a consequence, the identification of more accurate tools for early diagnosis is needed. If we have new markers that can be detected in the blood or other body fluids at the mild phase of AD, it will be a useful aid for the diagnosis and management of patients with AD.

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Neurophysiological Biomarker of Mild Cognitive Impairment*

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Abstract

Mild cognitive impairment is sometimes regarded as related to aging. However, statistically every second case turns into full dementia, which still is resistant to any treatment. It is therefore desirable to recognize deviations from normality as early as possible. This might be feasible by using quantitative EEG analysis in the presence of mental work. The present retrospective data analysis revealed a new quantitative biomarker indicating the degree of impairment. Current source density was calculated from 16 channel EEG using CATEEM® software. Four different conditions were analyzed: relaxed state, performing a d2-concentration test, a calculation performance test and a memory test for 5 min each. Subjects older than 40 years were divided into two groups according to their DemTect score: 13 - 18 (HC; n = 44) or 8 - 12 (MCI; n = 45). Spectral power was chopped into six frequency ranges (delta, theta, alpha 1, alpha 2, beta 1 and beta 2). Average spectral power was enhanced in the MCI group in comparison to healthy subjects with respect to delta ($p = 0.05$) during relaxed state when all electrode positions were regarded. With respect to EEG recording during performance of three different psychometric tests it was recognized that mainly spectral changes during performance of the d2-concentration test were related to mild cognitive impairment. With regard to all electrode positions statistically significantly lower spectral power values were reached during the d2-test for delta ($p = 0.001$), theta ($p = 0.0001$) and alpha 1 waves ($p = 0.08$) in impaired subjects in comparison to healthy subjects. Regarding regions of interest increases of delta and theta power were seen in the fronto-temporal brain during performance of the d2-concentration test. These increases disappeared when looking at MCI data. In the centro-parietal region decreases of alpha and beta 1 power emerged, which were even larger in MCI subjects. No MCI-dependent changes were observed in the other two tests. A correlation was found between psychometric performance of the d2-test and the DemTect score ($r = 0.51$). MCI subjects had statistically significant worse performance in all three mental challenges in comparison to healthy volunteers. It is concluded that MCI can be characterized at an early stage by EEG recording in the relaxed state. High spectral delta and theta power in general and specifically at fronto-

*Data was presented as poster at the 6th World Congress on Controversies in Neurology, Vienna, Austria, March 8-11, 2012 and awarded the best one in the category dementia.

temporal electrode positions (especially at T₃) was recognized as a biomarker for MCI. A DemTect score of 8-12 was validated as indicative for MCI.

Keywords

DemTect, Cognition, Psychometry, EEG, Source Density, Mild Cognitive Impairment (MCI), Alzheimer's Disease, CATEEM

1. Introduction

Mild cognitive impairment is regarded as a possible transitional stage during the development of Alzheimer's disease [1]. According to literature about every second subject suffering from this impairment develops manifest dementia [2]-[4]. Since progression from mild cognitive impairment to dementia occurs fluently, the problem arises to find a quantitatively defined diagnosis of this stage in order to recognize possible parameters for the risk of development of dementia later on. This early brain dysfunction can be described by considering three different approaches. The first approach consists in using an interactive questionnaire like mini-mental state (MMS [5]) or DemTect [6] [7]. The second approach is represented by use of psychometric tasks (cognitive testing) like the d2-test for concentration, a memory test or performance of arithmetic calculations [8]. The third approach very often is covered by a neurophysiological methodology, usually recording of quantitative electroencephalography [9] [10] or biochemical parameters from cerebrospinal fluid [11]. Thus, a combination of measurements on these three levels should be able to provide a quantitative definition of mild cognitive impairment and fulfil the criteria for an early diagnosis and recognition of the risk potential. Results should also provide the quantitative base for testing of drugs or food supplements aiming at the improvement of these early deficits.

The present investigation deals with the comparison of data from 45 healthy control subjects (HC group; DemTect score 13 - 18) with 44 subjects rated as cognitively impaired on the base of a DemTect score between 8 and 12 (MCI group). Concomitant use of psychometric performance and current source density imaging [12] [13] is used to validate this score range for the diagnosis of mild cognitive impairment as proposed by the DemTect guideline in comparison to being mentally healthy. With respect to EEG, basic conditions (recording during the relaxed eyes open condition) as well as event related EEG during performance of mental tasks [14] are used in order to learn more on possible deficits with respect to special demands. The analysis is based on data from 89 subjects using recordings of the first visit in the laboratory during several clinical studies (EudraCT-Nr.: 2007-004753-29 and EudraCT-Nr. 2009-015827-97).

2. Methods

2.1. Subjects

Eighty-nine subjects were taken from five consecutive clinical studies. They were asked to perform an interactive questionnaire developed for recognition of mild cognitive impairment (DemTect) before they entered the study consisting of a combined technology of EEG recording in the presence of mental performance of three different psychometric tasks. Volunteers were grouped according to the proposal of the developers of the test and according to the validation results published. Subjects having a score from 8 - 12 were assembled into the cognitive impaired group (MCI), those scoring higher from 13 - 18 were taken as healthy control group (HC). Both groups were compared with respect to electric power under the different recording conditions and with respect to psychometric performance.

2.2. Experimental Procedure

Subjects (HC group 17 male/27 female, average age 50.5 and 47 years, respectively; MCI group 25 male/20 female, average age 58.1 and 56.6 years, respectively) were sitting alone in a quiet separate room in a comfortable easy chair. The light was dimmed. Baseline recording of 6 min under the condition of eyes open was followed by

the performance of the d2-test, a mathematical calculation task and a memory test. All experiments took place at the same time of the day (starting at 8 am).

2.3. EEG Recording

The EEG was recorded bipolarly from 17 surface electrodes according to the international 10/20 system [15] against a common average reference calculated from Cz against all other electrodes as proposed by Lehmann [16] (Computer aided topographical electroencephalometry: CATEEM[®] from MEWICON CATEEM-Tec GmbH, A-4164 Schwarzenberg, Austria) using an electrocap. EEG recording was performed as reported earlier [17]. Setting was kept constant for each individual throughout the experiment.

2.4. Current Source Density Analysis

In this study the EEG was processed not in the potential mode based on voltage [18], but in a surface charge mode obtained by Laplacian estimates also known as current source density analysis (CSD), [12] [13]. Charge is the 2nd derivation of the potential and gives the curvature of the potential curve according to space. Under the condition of using a homogenous, steadily conducting medium surface charge mode provides the source density of the electric flow on the cortex surface. Whereas the EEG in the potential mode tends to produce a more extensive and diffuse picture of changes, Laplacian estimate acts as a spatial filter emphasizing local sources over distant sources (for review see [19]). There is a sharply contrasted display of cortex areas with highly activated generators in the depth of the brain and brain areas with less intensely working generators. Harmony *et al.* [12] were able to demonstrate, that spectral parameters obtained from the CSD showed higher correlations with computer tomography measures than those calculated from the potential mode of the EEG. We therefore used this methodology in order to describe the focal changes of brain activity.

Brain Imaging was achieved by conversion of numerical values of spectral EEG power into spectral colours and additive colour mixture according to RGB as used in TV settings [20]. Data acquisition and analysis were carried out simultaneously and provide topographical maps displayed on-line on the computer screen. The maps show the relative, time averaged changes of electrical brain activity of each recording condition during mental work in % of the reference period during relaxation with open eyes.

2.5. Psychometric Testing

The d2 attention test is a well-known standardized validated test. Number of correct answers and number of lines were evaluated as performance index including quality and quantity of answers. Arithmetic test (Concentration Performance Test (CPT) was carried out as described by Düker and Lienert, [8]. Number of solved tasks and correctness gave a performance index. The memory test was applied according to the following schema: a combination of 8 numbers and/or letters (for example: Dv8L3oPX) was presented on the screen for 4 s. After this no information was given for 10 s. Finally, a fourfold multiple choice including the correct answer was presented for decision. Number of tasks and correctness were evaluated to give a performance index. Each test was presented for 5 minutes. The row of order was kept constant for the sake of direct comparisons of the results under identical conditions.

2.6. Statistics

Since EEG data are not normally distributed, the non-parametrical Wilcoxon test was chosen for comparison between the two groups. Data were averaged for each of the recording periods of 5 minutes during eyes open and the different challenges (*i.e.* in separate for each psychometric test). Statistics gave p values, which are presented at the appropriate site. The absolute power values under the recording condition “eyes open” were taken as reference values and set to 100% when psychometric tests were performed. Thus, possible physiological changes during test performance are given in % of these reference values.

In order to differentiate results from healthy subjects and those suffering from mild cognitive impairment data were fed into linear discriminant analysis according to Fischer. Results from the first three discriminant functions were depicted in space (x, y and z coordinates). Results from the 4th to 6th functions were transformed into colour according to the RGB mode (like in TV).

3. Results

3.1. Electric Power during the Recording Condition “Eyes Open”

Comparison of the absolute electric power values with respect to all 17 electrode-positions of the source density EEG of subjects with mild cognitive impairment (MCI; $n = 45$) with the healthy control group (HC; $n = 44$) revealed massive differences. Higher values of electric power at single electrodes were generally seen in the group of subjects with MCI in comparison to healthy subjects. Global median values regarding all electrode positions were higher in MCI subjects than in HC subjects but did not reach such high statistical significance as with respect to delta (Table 1).

Table 1. Absolute power values for each electrode position under the recording condition of “eyes open” in relaxed position for every frequency range from delta, through theta, alpha 1, alpha 2, beta 1 and beta 2. Electrode positions: C = central, P = parietal; F = frontal; T = temporal; O = occipital. Even numbers represent the right hemisphere, uneven numbers the left hemisphere. Statistically significant differences with respect to median values regarding all electrodes from healthy subjects (HC; $n = 44$) in comparison to individuals with mild cognitive impairment (MCI; $n = 45$) are marked by stars before the number. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Electrode	Eyes Open [μV^2]											
	Delta		Theta		Alpha 1		Alpha 2		Beta 1		Beta 2	
	HC	MCI	HC	MCI	HC	MCI	HC	MCI	HC	MCI	HC	MCI
Cz	2.71	3.23	0.58	0.58	0.55	0.66	0.36	0.56	0.62	0.67	0.89	1.15
Fz	3.03	3.29	0.72	0.74	0.74	0.86	0.49	0.67	0.67	0.78	0.96	1.33
F3	2.93	*3.89	0.64	0.81	0.76	0.97	0.51	0.66	1.04	1.17	2.08	2.49
C3	2.10	*3.65	0.52	0.56	0.58	0.77	0.63	1.04	1.24	1.40	1.76	2.04
P3	1.71	*2.21	0.42	0.40	0.52	0.63	0.48	0.65	0.88	0.72	0.77	0.63
Pz	2.49	2.48	0.54	0.53	0.58	0.75	0.52	0.69	0.67	0.70	0.66	0.62
P4	1.73	2.17	0.35	0.42	0.44	0.55	0.44	0.73	0.76	0.71	0.73	0.74
C4	2.36	2.89	0.50	0.55	0.57	0.78	0.70	1.04	1.39	1.45	1.96	2.31
F4	3.52	4.12	0.72	0.78	0.86	0.91	0.62	0.82	1.11	1.33	2.60	3.75
F7	7.07	10.59	1.39	1.41	1.38	1.79	1.15	1.42	1.98	1.87	4.75	3.16
T3	4.16	4.62	0.88	0.89	1.22	1.31	1.23	1.32	2.44	1.78	3.36	3.10
T5	3.19	3.79	1.10	0.96	1.74	1.51	1.37	1.25	2.07	1.70	1.84	1.34
O1	3.10	***5.88	0.73	*0.88	0.68	1.17	0.70	*0.99	1.30	1.60	1.85	2.65
O2	4.00	**5.46	0.79	0.86	0.90	0.91	0.87	1.10	1.49	1.83	2.20	2.40
T6	3.07	2.88	0.77	0.69	1.14	1.51	1.04	1.36	1.86	1.50	1.72	1.70
T4	3.79	4.12	0.93	0.89	1.20	1.38	1.18	1.11	2.37	2.13	3.31	3.35
F8	7.84	*11.88	1.57	1.60	1.61	1.92	1.42	1.40	2.67	2.01	6.18	3.66
global median	3.14	*3.62	0.71	0.74	0.83	0.91	0.75	0.86	1.33	1.43	1.81	1.92

3.2. Spectral Power during Performance of the d2-Concentration Test

Regarding the median of all electrode positions during performance of the d2-concentration test statistically different values for the MCI subjects in comparison to healthy control were obtained when recordings in the relaxed state were taken as a reference (100%). Highest statistical significance for the difference between the healthy and the cognitively impaired group is reached in the delta ($p < 0.001$) and theta frequency range ($p < 0.001$). But also lower values for alpha 2 power ($p < 0.08$) were seen during this challenge. Lower spectral power in the alpha 1 frequency range did not reach statistical significance. No statistically significant differences between MCI subjects and healthy controls were observed with the calculation performance test (CPT) or the memory test (Table 2).

During performance of the d2-test healthy volunteers (HC-group) were able to **increase** fronto-temporal delta and theta power taking the recording condition “eyes open” as reference (100%). Highest statistical significance was reached by increases of theta power in frontal and temporal areas of the brain as documented in Figure 1 by statistical analysis for each location in separate. At the same time **attenuation** of electric power were observed with respect to alpha waves mainly in central areas of the brain.

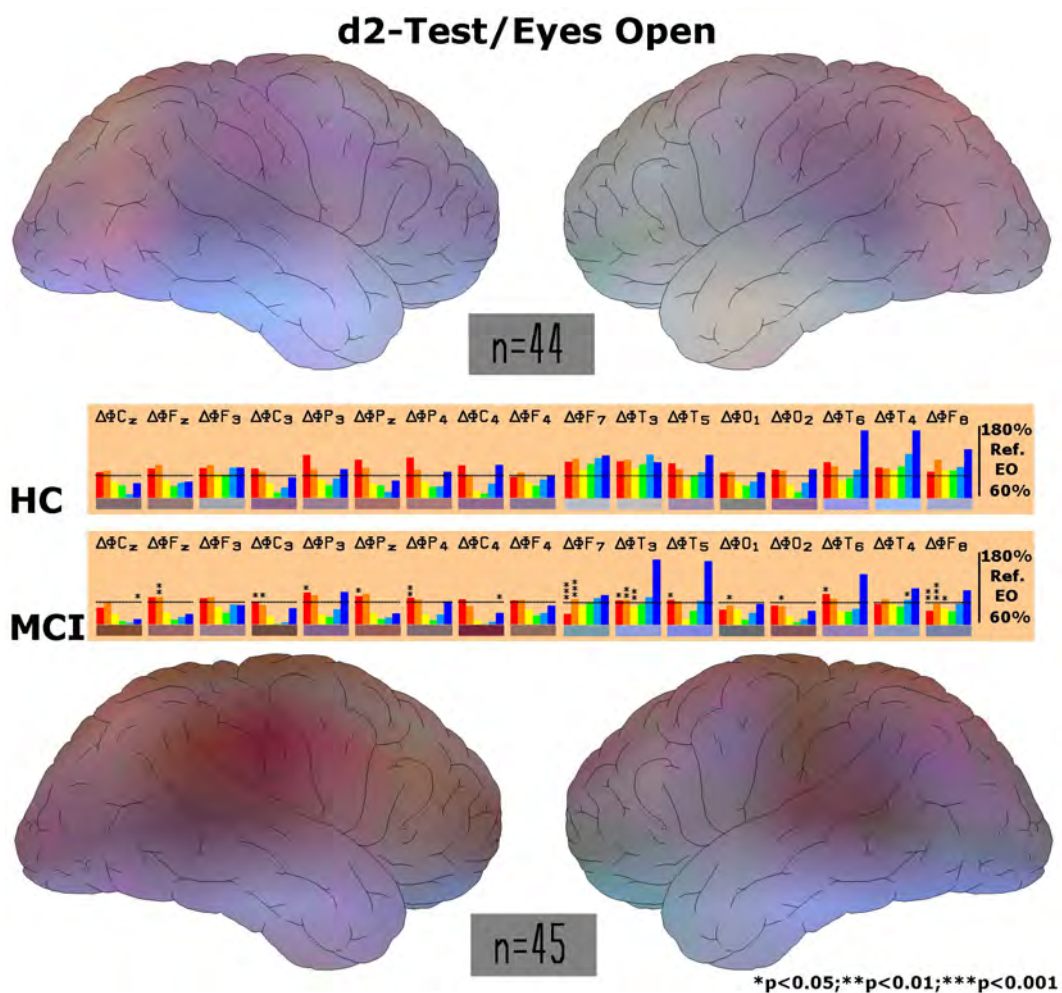


Figure 1. Documentation of statistically significant differences in healthy subjects in comparison to subjects suffering from MCI with respect to every single electrode position under the recording condition of the d2-test. Statistically significant differences between HC and MCI are documented by stars. Brain maps were constructed using the recording condition eyes open as reference. Differences are shown between healthy subjects (HC) and subjects with mild cognitive impairment (MCI) during performance of the d2-test. Please note fronto-temporal brightening in the left hemisphere (right side of upper map) in healthy people compared to lack of such feature in mild cognitive impairment (lower map).

Opposite to this, subjects diagnosed to have MCI according to “DemTect” score produced significant less electric power regarding delta and theta frequencies during performance of this test. Differences in changes of power with respect to single electrode locations are depicted in **Figure 2** for the HC control group (upper panel) and the MCI group (lower panel), respectively. Statistical significance is given in the lower part for each location of recording.

During the course of brain research it has become obvious that electric activity depends on the region where one looks at. Different mental challenges have been recognized to induce quite different patterns of electric activity. This is also seen during performance of a concentration test (**Figure 1**). Under this recording condition eminent higher delta spectral power values are observed at parietal areas (electrode positions P_{3,4}) as well as delta and theta spectral power in fronto-temporal areas represented by the electrode positions F_{7,8,2} and T_{3,4}. In MCI subjects these increases disappear nearly completely in a highly statistically significant manner. In addition, alpha waves are depressed in the parietal region during the d2test. This decrease became even more pronounced in mentally impaired subjects. This difference also becomes visible when looking at the electric maps

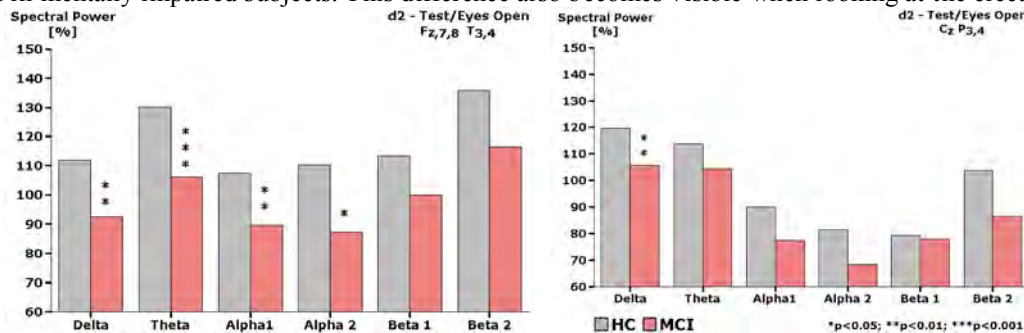


Figure 2. Differences in spectral EEG power between healthy subjects (HC) and subjects suffering from mild cognitive impairment (MCI) with respect to two regions of interest (ROI) when performing the d2-concentration test. Data from the relaxed state are taken as reference. Fronto-temporal region is represented by the electrode positions F_{7,8} and centro-parietal region is represented by the positions C_z and P_{3,4}.

Table 2. Differences of frequency changes (test condition in % relative to eyes open condition) between healthy subjects (HC) and the MCI group are given for each psychometric test as median values taking all electrode positions in consideration. Statistical significance is given as p-values for the comparison between healthy people (HC) and subjects suffering from mild cognitive impairment (MCI).

	d2-Test/Eyes Open [%]											
	Delta		Theta		Alpha 1		Alpha 2		Beta 1		Beta 2	
	HC	MCI	HC	MCI	HC	MCI	HC	MCI	HC	MCI	HC	MCI
global median	116.26	98.41	110.67	99.66	93.77	88.74	86.82	76.67	88.07	81.75	118.80	100.54
p <	0.001		0.001								0.076	
	CPT/Eyes Open [%]											
	Delta		Theta		Alpha 1		Alpha 2		Beta 1		Beta 2	
	HC	MCI	HC	MCI	HC	MCI	HC	MCI	HC	MCI	HC	MCI
global median	117.49	113.91	102.32	106.54	90.32	81.79	80.66	72.70	85.53	86.86	98.19	103.84
p <												
	Memory/Eyes Open [%]											
	Delta		Theta		Alpha 1		Alpha 2		Beta 1		Beta 2	
	HC	MCI	HC	MCI	HC	MCI	HC	MCI	HC	MCI	HC	MCI
global median	116.60	98.50	97.72	88.91	88.08	81.03	90.39	75.68	92.32	90.30	101.95	94.97
p <												

calculated for both groups (**Figure 1**). Due to the absence of theta power a large difference is seen in fronto-

temporal areas. Thus, under the recording condition “d2-concentration test” massive differences with respect to spectral power in fronto-temporal and parietal brain regions are visible. The numerical values for these two regions of interest are given in separate as median of the respective fronto-temporal and parietal electrode positions (Figure 2).

3.3. Electric Power during the Recording Condition “CPT”

During performance of the calculation-performance-test (CPT) **healthy** volunteers were able to increase frontal delta and theta power in comparison to the recording condition “eyes open” (set to 100%). As depicted in the middle bar chart of Figure 3, electrode locations F₇, F₈, T₅ and T₆ show increases of delta and theta power. With respect to this hardly a difference was observed in cognitively impaired volunteers. However, when looking at the brain map depicted in Figure 3, left frontal increases of slow power were attenuated in mildly impaired subjects. Despite the impression of lower production of delta and theta waves in the impaired group (as also documented in Figure 4) this difference was not statistically significant. The same is true for beta power. Global

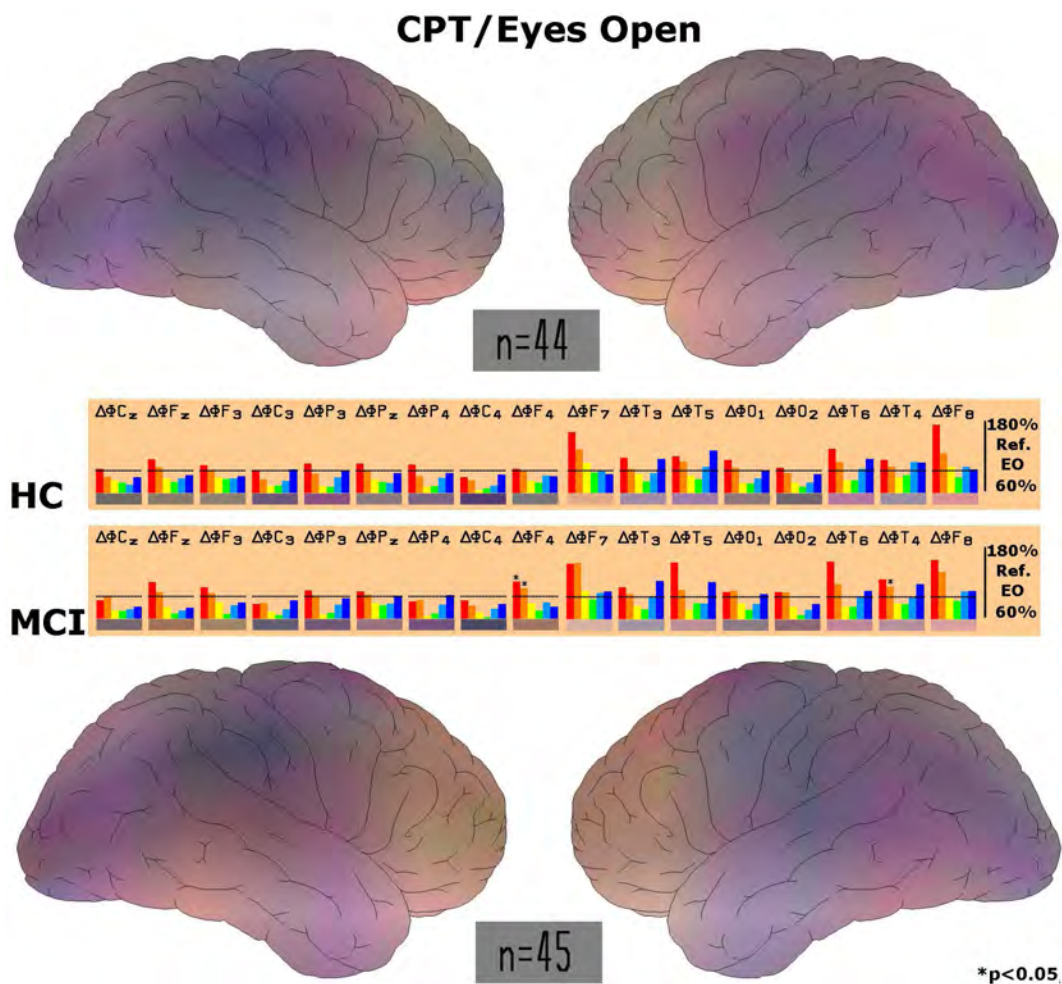


Figure 3. Documentation of statistically significant differences in healthy subjects in comparison to subjects suffering from MCI with respect to every single electrode position under the recording condition of the calculation performance test (CPT). Statistically, significant differences between HC and MCI are documented by a star. Brain maps constructed using the recording condition eyes open as reference. Differences are shown between healthy subjects (HC) and subjects with mild cognitive impairment (MCI) during performance of the d2-test. Please note that only marginal differences between healthy people subjects suffering from mild cognitive impairment are seen.

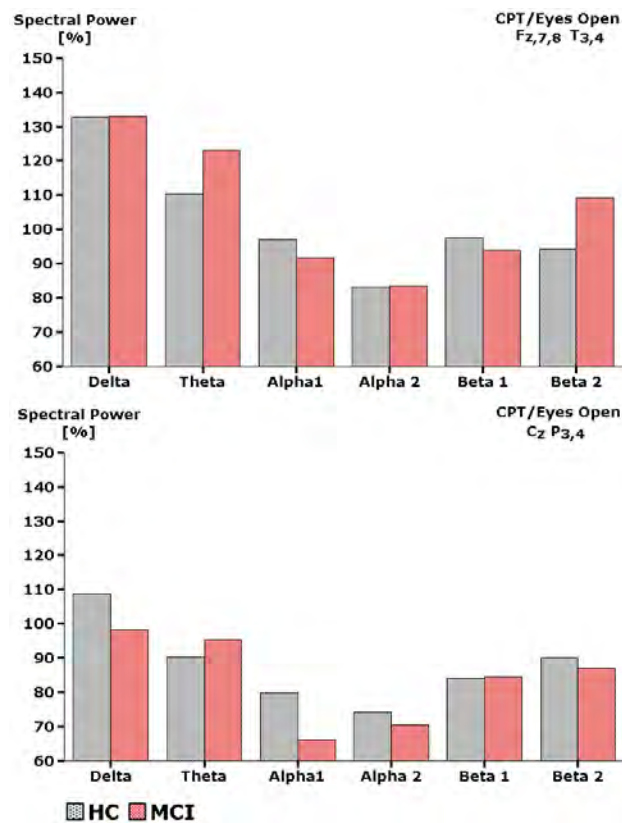


Figure 4. Differences in spectral EEG power between healthy subjects (HC) and subjects suffering from mild cognitive impairment (MCI) with respect to two regions of interest (ROI) when performing the calculation performance test. Data from the relaxed state are taken as reference. Fronto-temporal region is represented by the electrode positions $F_{z,7,8}$ and centro-parietal region is represented by the positions C_z and $P_{3,4}$.

median values of electric power with regard to all electrode positions during the performance of the calculation test are given in **Table 2** (middle part). Despite some further reduction of alpha 1 and alpha 2 power in mildly impaired subjects this difference in comparison to healthy subjects did not become statistically significant.

This result is underlined by the spectral changes as observed in the two regions of interest. Despite some lower value with respect to alpha 1 power in mildly impaired subjects, the difference did not become statistically significant as depicted in **Figure 4**.

3.4. Electric Power during the Recording Condition “Memory Test”

During performance of the memory test, **healthy** volunteers were able to increase frontal delta power ($F_{7,8}$) and to some extent also theta power in comparison to the recording condition “eyes open” (set to 100% in **Figure 5**). A similar feature was observed in cognitively impaired volunteers. But with respect to delta and theta spectral power cognitively impaired subjects produced somewhat less spectral power (bar chart in the middle of **Figure 5**). Electric maps in mildly impaired subjects reveal less red colour in the frontal brain due to some but statistically not significant delta decreases. Differences between healthy controls and impaired subjects are also obvious from the regions of interest in **Figure 6**. Obviously, the lower spectral delta power is statistically not significantly different between the two groups. However, alpha 2 spectral power was significantly less in impaired subjects. When regarding global median values (calculated from all electrode positions) also a tendency of attenuation of alpha 2 spectral power emerged, but which did not become statistically significant at this global measurement (**Table 2**).

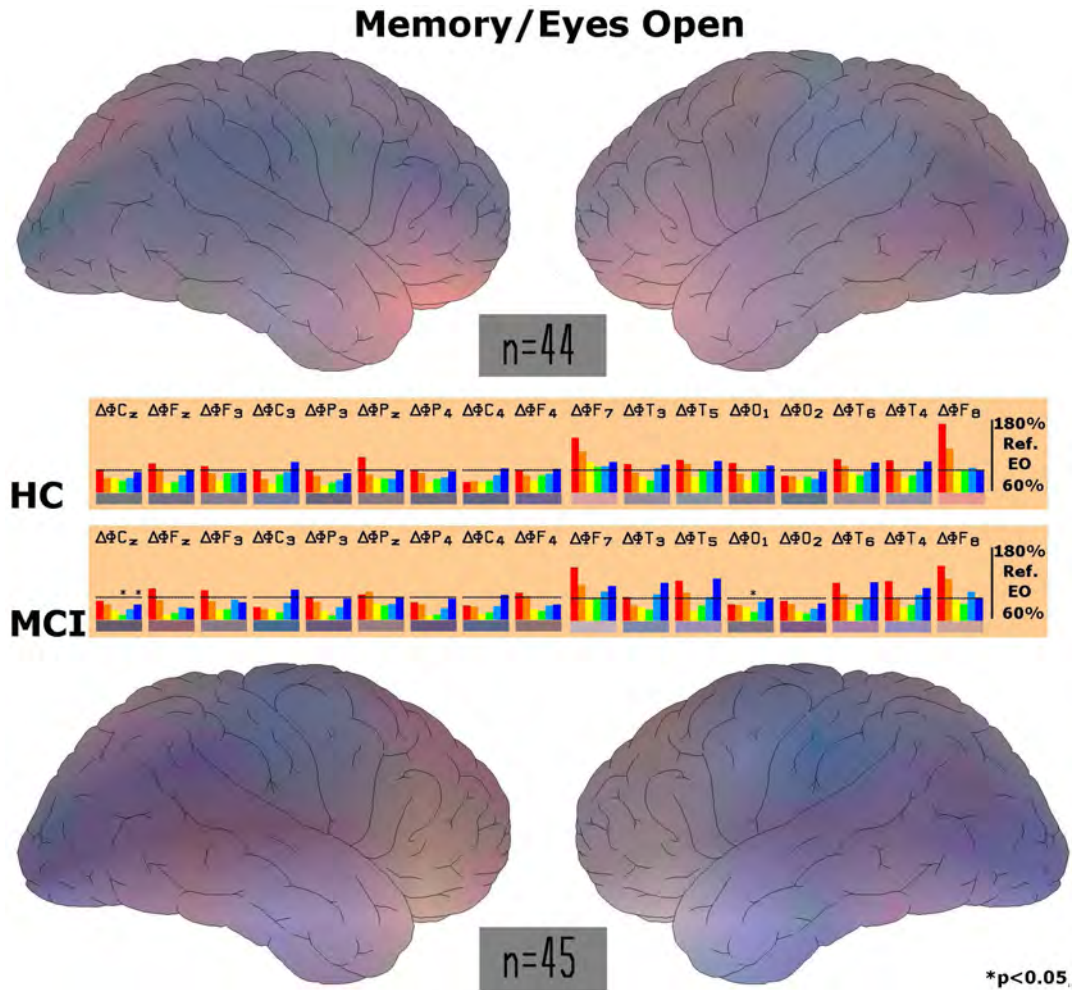


Figure 5. Documentation of statistically significant differences between healthy subjects and subjects suffering from MCI with respect to every single electrode position. Statistically significant differences between HC and MCI subjects are documented by stars. Brain maps are constructed using the recording condition eyes open as reference (100%). Differences are shown between healthy subjects (HC) and subjects with mild cognitive impairment (MCI) during performance of the memory test. Please note only marginal differences between healthy people and subjects suffering from mild cognitive impairment.

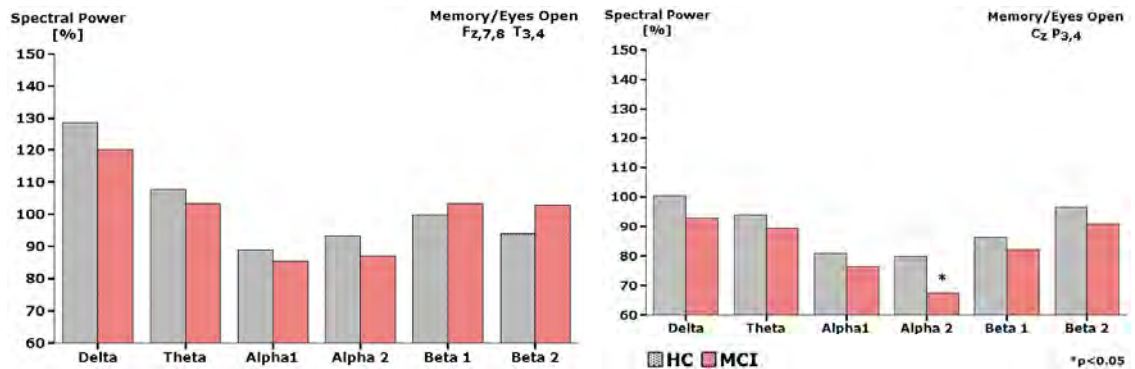


Figure 6. Differences in spectral EEG power between healthy subjects (HC) and subjects suffering from mild cognitive impairment (MCI) with respect to two regions of interest (ROI) when performing the memory test. Data from the relaxed state are taken as reference (100%). The frontotemporal region is represented by the electrode positions F_{z,7,8} and the centroparietal region is represented by the positions C_z and P_{3,4}.

3.5. Comparison by Means of Discriminant Analysis

Discriminant analysis is a mathematical tool, which allows statistical evaluation of a large set of parameters. Since this quantitative EEG analysis consists of 102 parameters (17 electrode positions \times 6 frequency bands) absolute spectral power data from all participants of the studies were fed into this type of analysis. As is documented in **Figure 7** healthy controls and impaired subjects can be discriminated from each other with respect to all 4 recording conditions. It is also obvious that the different recording conditions lead to different types of electric brain states. For example the state of “eyes open” can easily discriminated from the state during performance of these psychometric tests.

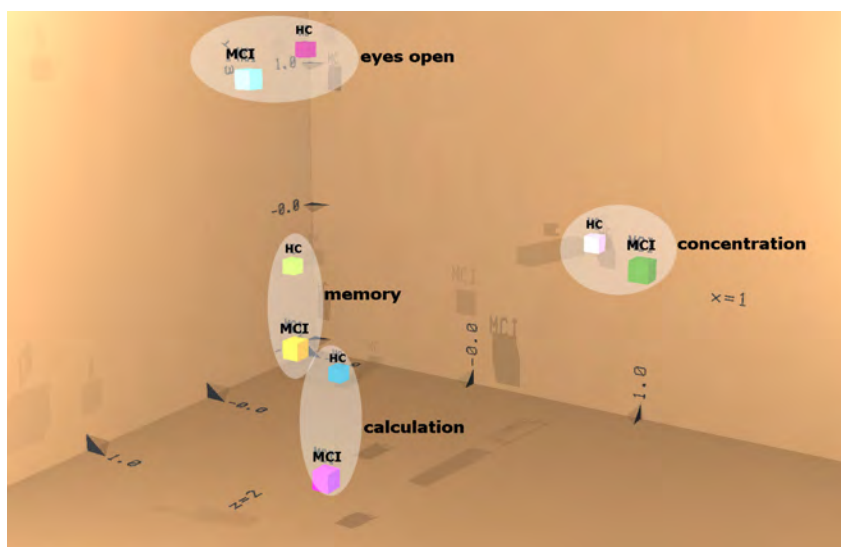


Figure 7. Documentation of results of discriminant analysis for both groups of subjects (HC = healthy controls; MCI = mild cognitive impairment) with respect to performance of psychometric tests and the recording condition relaxed state with “eyes open”. Results from the first three discriminant functions are depicted in space (x, y and z coordinates). Results from the 4th to 6th discriminant functions are depicted using the RGB mode (additive colour mixture of red, green and blue for the result of each function). Single test conditions are marked by arbitrarily brightening.

3.6. Evaluation of Psychometric Results

Psychometric performance was documented for each of the three mental tests according to the definition given under “Material and Methods”. As described in **Table 3** performance of cognitively impaired subjects was always significantly lower in all three tests with respect to an index calculated on the base of quality and quantity of the answers.

In addition, a statistically significant correlation was observed between the psychometric performance during these tests and the score of the “DemTect”. These data confirm the cognitive impairment as indicated by the “DemTect” score, since a correlation was observed between the DemTect score and these psychometric results ($r = 0.53$; $p = 0.000001$) as depicted in **Figure 8**.

Table 3. Result for psychometric testing (details under material and methods). Mean values and deviation are given besides the statistical significance (p-values) on the left side.

	Mean HC	SEM HC	Mean MCI	SEM MCI	Statistical Significance
d2-test	11.20	0.48	8.21	0.43	$p < 0.0001$
CPT	4.46	0.59	2.93	0.49	$p < 0.03$
Memory	10.38	0.49	8.36	0.64	$p < 0.02$

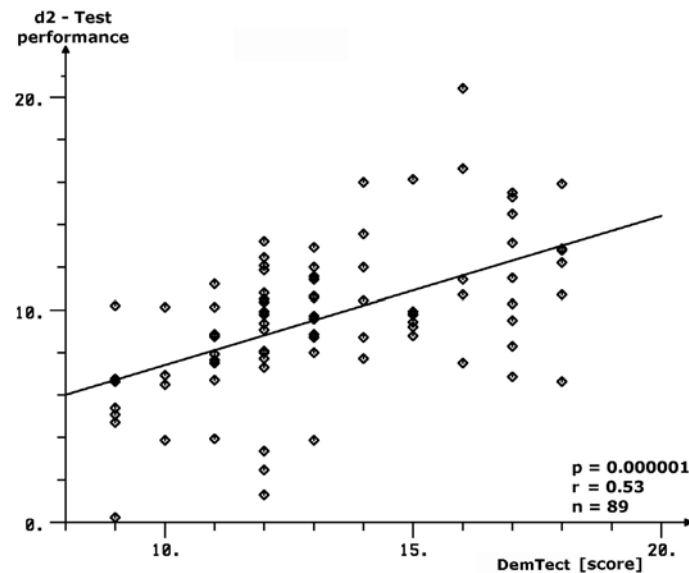


Figure 8. Statistically significant correlation between DemTect score values and psychometric performance in the d2-test. The rank correlation value according to Spearman is given with statistical significance.

4. Discussion

Two groups of subjects as defined according to the result of the interactive DemTect questionnaire already differed with respect to absolute voltage in the delta frequency range providing higher values for the MCI group. Largest differences were seen in frontal delta and theta power (electrode positions F_7 and F_8). This feature of higher delta and theta power has been reported using the same methodology for demented patients in comparison to normal aged matched controls [21]. Other authors also have recently documented higher delta values for subjects suffering from mild cognitive impairment in comparison to healthy volunteers and reported on further alterations typical for those seen in Alzheimer's disease [22]. Higher power in the delta frequency range indicates lower activity of the cholinergic transmitter system [23]. In animal lesion studies, increased delta activity has been reported after destruction of the Nucleus Basalis [24], the main production site of acetylcholine [25]. A comparison of cortical source EEG with MR-based measurements of lobar brain volume (white and grey matter) revealed a negative correlation between the frontal white matter and the amplitude of the delta sources across MCI and Alzheimer subjects (AD), which support a transition hypothesis of brain structural and functional continuity between MCI and AD [26].

Changes of delta and theta waves were also related to pathological changes in cholinergic brain regions. A significant increase of these frequencies was found in patients with the highest total cholinergic burden as well as in patients with highest capsular pathway damage [27]. Abnormal high frontal delta waves **under basic relaxed recording conditions** can therefore be regarded as indicative for a biochemical and pathological brain dysfunction involving the cholinergic transmitter system.

This also applies to theta waves, which also have been reported to be significantly higher in demented patients than in controls and related to decreased performance in all cognitive domains [28]. In this analysis increase of theta power was observed in most of the brain regions and the difference between the MCI group and the healthy control group (HC) was however not statistically significant with respect to global median values. Very strong evidence for theta power increase as indicator for cognitive decline comes also from longitudinal studies in normal elderly with subjective complaints [1]. With respect to EEG frontal theta power a negative linear correlation was reported to hippocampal volumes for patients suffering from MCI or AD compared to control also indicating fluent transitions for these diseases [29]. These data clearly contradict impressions from nuclear magnetic resonance pictures suggesting only one frontal brain area to be involved in cognitive function. In addition, electric features of brain activity recorded under different performance conditions provide evidence for the involvement of different transmitter activities within different brain regions. There is evidence that theta waves are under the control of the norepinephrine alpha 2 receptor [30]. Also changes of beta frequency ranges have

been used to successfully predict the clinical status of subjects with MCI over a two years period [31]. However, changes of beta activity did not become statistically significant in my analysis.

Thus, EEG source density measurements in the presence of a relaxed state confirmed deviations of electric power within several frequency ranges reported in the literature so far. But like an engine can only be tested under load, brain function should be tested also under “mental” load in order to characterize possible dysfunctions in a more relevant manner. This is possible by recording the EEG in the presence of cognitive performance. Using a battery of different psychometric tests it was recognized long ago that frontal delta and theta power increase under those conditions in healthy volunteers and were related to the difficulty of the task [14]. Interestingly, it was then observed, that demented patients were only able to produce power increases, which were significantly lower than those observed in age matched healthy controls [32]. At the same time a significant correlation was recognized to the severity of the disease as indicated by the interactive questionnaires MMS and ADAS. The present analysis reveals a similar picture. All three psychometric tests induced fronto-temporal increases of delta and theta power in healthy controls. Increases of fronto-central electric theta power have also been observed during other memory demands [33]. Reflection of cognitive and memory performance in the EEG has also been described in detail by others. For example, retrieval of lexical semantic information was linked to theta increases [34]. Furthermore, it was suggested that the encoding of new information is reflected by theta oscillations in hippocampal-cortical feedback loops, whereas search and retrieval processes in (semantic) long-term memory are reflected in upper alpha (alpha 2) oscillations in thalamo-cortical feedback loops [35]. According to animal data alpha 2 frequencies are under the control of dopamine.

In the group of mild cognitive impairment, however, performance of the d2-test led to considerable smaller increases of delta and theta power. Similar results were described for individuals with mild cognitive impairment in the literature [36]. As already recognized in demented patients and confirmed by a correlation analysis between basic theta and event related induced theta [32] this lacking production of fronto-temporal theta waves presumably derives from too high baseline values during the relaxed eyes-open condition. There is obviously a ceiling effect, which prevents further increase of theta power after reaching a physiologically limited maximum. Interestingly, low performance in attention testing was reported to be associated with reduced grey matter density of the left inferior frontal gyrus [37]. In demented patients under the condition of mental load, theta changes were related to the MMS questionnaire. In subjects suffering from mild cognitive impairment a close correlation between theta changes and the score of the DemTect is now observed. This parallel feature speaks in favor of fluent transient states from being healthy via mild cognitive impairment to dementia. But, according to the literature only about every second individual suffering from a decline in cognition develops dementia. In summary, there is compelling evidence, that this change in theta power reactivity can be taken as an indicator for decline of cognition. A longitudinal analysis of future recordings from our subjects will tackle this question.

It can be concluded from our data, that deficits in concentration seem to be the first and most important sign of mild cognitive impairment represented by aberrations in theta activity, followed by already some deficits in memory recognized by deviations in alpha 2 reactivity, whereas arithmetic deficits are not so obvious at this early stage of cognitive impairment. In summary, cognition is a rather complex process, which involves several parts of the brain with increases of electric power in frontal delta and theta waves but also decreases of power in central alpha 2 waves governed by different neurotransmitters. Which of the differences between healthy and mildly impaired subjects are indicative for final development into dementia will hopefully be discovered in future longitudinal studies. But we have now clear neurophysiological parameters to follow in future measurements.

5. Conclusion

The present analysis of current source density of the EEG resulted in the detection of quantitative parameters, which are suitable to diagnose mild cognitive impairment at a very early stage. Lower production of theta waves during performance of the d2-test as paper pencil version seems to be the most sensitive neurophysiologic indicator of a cognitive decline. This parameter can now also be used as a non-invasive biomarker for early diagnosis and for testing new drugs aiming at the prevention of development of MCI into dementia.

Competing Interest

There is no conflict of interest.

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Lipolysaccharide-Induced Neuroinflammation Is Associated with Alzheimer-Like Amyloidogenic Axonal Pathology and Dendritic Degeneration in Rats

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Abstract

Chronic neuroinflammation is thought to play an etiological role in Alzheimer's disease (AD) which is characterized pathologically by amyloid and tau formation, as well as neuritic dystrophy and synaptic degeneration. The causal relationship between these pathological events is a topic of ongoing research and discussion. Recent data from transgenic AD models point to a tight spatiotemporal link between neuritic and amyloid pathology, with the obligatory enzyme for β -amyloid ($A\beta$) production, namely β -secretase-1 (BACE1), being overexpressed in axon terminals undergoing dystrophic change. However, the axonal pathology inherent with BACE1 elevation seen in transgenic AD mice may be secondary to increased soluble $A\beta$ in these genetically modified animals. Further, it is unclear whether the inflammation seen in AD is the result of, or the cause of

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neuritic dystrophy. Here we explored the occurrence of AD-like axonal and dendritic pathology in adult rat brains affected by LPS-induced chronic neuroinflammation. Unilateral intracerebral LPS injection induced prominent inflammatory response in glial cells in the ipsilateral cortex and hippocampal formation. BACE1 protein levels were elevated in the ipsilateral hippocampal lysates in the LPS-treated animals relative to controls. BACE1 immunoreactive dystrophic axons appeared in the LPS-treated ipsilateral cortex and hippocampal formation, colocalizing with increased β -amyloid precursor protein and A β antibody (4G8) immunolabeling. Quantitative Golgi studies revealed reduction of dendritic branching points and spine density on cortical layer III and hippocampal CA3 pyramidal neurons in the LPS-treated ipsilateral cerebrum. These findings suggest that Alzheimer-like amyloidogenic axonal pathology and dendritic degeneration occur in wildtype mammalian brain in partnership with neuroinflammation following LPS injection.

Keywords

Amyloid Pathogenesis, Neuritic Dystrophy, Neurodegeneration, Neuroplasticity, Synaptic Pathology

1. Introduction

Neuroinflammation has been linked to many neuropsychiatric disorders, including neurodegenerative diseases such as Alzheimer's diseases (AD), Parkinson's disease (PD), multiple sclerosis and traumatic brain injury [1]-[8]. Aging is a major risk factor for many age-related diseases, and is associated with a certain degree of chronic inflammation [9] [10]. In general, chronic inflammation is considered to mount lasting stress on neurons and synapses, and may lead to brain dysfunction, including cognitive deficits [5] [11]-[14]. The causal relationship between chronic inflammation and some of the hallmark pathological lesions in neurological diseases is under intensive investigation. For example, oxidative or inflammatory stress is suggested to promote cerebral amyloid pathology via increased production and/or impaired clearance of A β , involving both neurons and glial cells [15]-[19]. A β products including soluble and aggregated variants may also act as proinflammatory factors [20] [21].

Loss of synapses and their connectivity best correlate with cognitive deficits in AD [22]-[29]. The density of dendritic spines appears to be reduced readily at prodromal stages of the disease [30]. Besides synaptic degeneration, axonal elements including presynaptic terminals undergo aberrant sprouting and dystrophic expansion [30]-[32]. Recent data from transgenic AD models, nonhuman primates and human subjects show that upregulation of the amyloidogenic proteins, especially the rate-limiting enzyme β -secretase-1 (BACE1), appears to be an molecular cascade tightly associated with axonal sprouting and dystrophy, suggestive of a driving role for amyloidogenic axonal pathology in plaque formation [33]-[35].

The bacterial endotoxin lipopolysaccharide (LPS) can induce chronic neuroinflammation in rodents [36]-[38]. LPS administration also causes learning and memory deficits in the animals, providing an excellent model system for studying cognitive dysfunction associated with chronic neuroinflammation [39]-[41]. Neuroinflammation is considered to play an early or inductive role in the development of AD pathologies, although the anatomic evidence remains to be better formulated. Therefore, the present study was set to address whether intracerebral LPS injection in adult rats may induce axonal and dendritic pathologies similar to that seen in AD [32]. Specifically, we aimed to identify whether dystrophic axonal pathology inherent with amyloidogenic modulation and degenerative dendritic/spine changes occur on cerebral principal neurons in LPS-treated adult rats.

2. Materials and Methods

2.1. Animals and Intracerebral Injection

In-house bred male adult Sprague-Dawley rats ($n = 24$) weighing ~ 200 g (Animal Center of Central South University) were used. Rats were maintained in temperature ($20^{\circ}\text{C} - 26^{\circ}\text{C}$), humidity (30% - 60%) and lighting (12:12 hours light/dark cycle) controlled rooms, with food and water freely available. For intracerebral injections, rats were placed in a stereotaxic apparatus under sodium pentobarbital anesthesia (50 mg/kg, i.p.). LPS

from *Escherichia coli* serotype 055:B5 (L2637, Sigma-Aldrich, St. Louis, MO, USA) was dissolved (2.5 µg/µl) in sterile phosphate-buffered saline (PBS, 0.01 M, pH 7.2) (vehicle) [41]. LPS (10 µg in 4 µl) was injected through the neocortex into the right hippocampus with a microsyringe in each animal (n = 12), using the following coordinates: 3.5 mm lateral to the sagittal cranial suture; 5 mm caudal to the bregma and 3 mm below the dura mater. Controls (n = 12) were injected with the same amount of PBS. Brain examination was carried out 30 days post intracortical injection. The experimental protocol was approved by the Animal Care and Use Committee of the Central South University and were in compliance with NIH guidelines.

2.2. Immunoblot

Hippocampi ipsilateral to the injection side were dissected out following decapitation (n = 4/group). Tissue was weighed and homogenized in a commercial protein extraction buffer at 1 to 10 w/v ratio (CW0883, Kangwei Century Company, Beijing), followed by centrifuge at 15,000 g. Protein concentrations in the supernatants were determined by DC protein assay (Bio-Rad Laboratories, Hercules, CA, USA). A total of 25 µg protein from each sample was separated electrophoretically in 10% SDS-polyacrylamide gel and transferred to polyvinylidene fluoride membrane. Membranes were blocked with 1% non-fat milk and 5% bovine serum albumin (BSA) in 0.1 M Tris-buffered saline containing 0.1% Tween-20 (TBS-T) for 2 hours at room temperature. Membranes were then incubated overnight at 4°C in the same buffer containing rabbit anti-BACE1 (1:2000) [33] [34] [42] [43], rabbit anti-gial fibrillary acidic protein (GFAP) (G9269, Sigma-Aldrich, St Louis, MO, USA, 1:4000) or rabbit anti-β-tubulin (Sigma-Aldrich, T2200, 1:10000). Membranes were washed thoroughly with TBS-T, then were incubated for 2 hours at room temperature with horseradish peroxidase-conjugated goat anti-rabbit IgGs (1:20,000; Bio-Rad Laboratories). Bound antibodies were detected by enhanced chemiluminescence (ECL kit, GE Healthcare Life Sciences, Piscataway, NJ, USA). The membranes were exposed to X-ray films developed subsequently in a darkroom. The films were scanned, with optical density (OD) of immunoblot bands measured using Image-J, followed by standardization to the internal references.

2.3. Immunohistochemistry

Rats were perfused transcardially with PBS followed by 4% phosphate-buffered (0.1M) paraformaldehyde (pH 7.4). Brains were removed and postfixed for 12 hours and transferred into 30% sucrose for cryoprotection. Thirty micrometer-thick coronal sections were cut in a cryostat, with 12 sets of sections collected for cresyl violet stain and for immunohistochemical studies. For immunohistochemistry with the avidin-biotin complex (ABC) method, free-floating sections were soaked in 1% H₂O₂ in PBS for 30 minutes to diminish endogenous peroxidase activity, and preincubated in PBS buffer containing 0.2% Triton X-100 and normal horse serum for 1 hour. The sections were then reacted with one of the following primary antibodies: 1) Mouse monoclonal antibody against major histocompatibility complex class II molecules (MHC-II) at 1:1000 (ab55152, Abcam, Cambridge, MA, USA); 2) Rabbit anti-human BACE1 at 1:2000; 3) mouse anti-β-amyloid precursor protein (APP) monoclonal antibody 22C11 (MAB348, EMD Millipore, Billerica, MA, USA, 1:4000); 4) mouse anti-Aβ17-24 monoclonal antibody 4G8 (#39240, Signet, Dedham, USA, 1:4000); 5) rabbit anti-GFAP (G9269, Sigma-Aldrich, 1:2000). The sections were further reacted with a biotinylated pan-specific secondary antibody (BA-1300, Vector Laboratories, Burlingame, CA, USA, 1:400) for 2 hours, and subsequently with the ABC reagents (PK-6100, Vector Laboratories, 1:400) for one hour. Immunoreactivity was visualized in 0.05% 3,3'-diaminobenzidine (DAB) and 0.003% H₂O₂.

Selected sections were processed for double immunofluorescence beginning with blocking nonspecific reactivity by incubation in PBS buffer containing 5% normal donkey serum. Sections were further reacted overnight at 4°C with a pair of primary antibodies raised in different species in PBS containing 0.2% Triton X-100 and the blocking serum. The antibody pairs included: 1) mouse anti-MCH-II (1:1000) and rabbit anti-CD11b (MABT149, EMD Millipore, 1:1000); 2) mouse anti-MCH-II and rabbit anti-GFAP (G9269, 1:2000); 3) rabbit anti-BACE1 and mouse anti-APP 22C11; 4) rabbit anti-BACE1 and mouse anti-Aβ 4G8; 5) rabbit anti-BACE1 and mouse anti-synaptophysin (MAB329, EMD Millipore, 1:4000); 6) rabbit anti-BACE1 and mouse anti-microtubule associated protein-2 (MAP2) (M9942, Sigma-Aldrich, 1:2000). On the second day, the sections were rinsed with PBS and incubated at room temperature for 2 hours with Alexa Fluor® 488 and Alexa Fluor® 594 conjugated donkey anti-mouse and anti-rabbit IgGs (1:200, Invitrogen, Carlsbad, CA, USA). Sections were then counter-stained with bisbenzimidazole (Hoechst 33342, 1:50000, Catalog #B2261, Sigma-Aldrich), washed thoroughly, and mounted with anti-fading medium before microscopic examination.

2.4. Rapid Golgi-Cox Stain

Brains were removed following a vascular rinse with PBS. Blocks containing the middle 1/3 cerebrum of both hemispheres were rinsed briefly in double distilled water, and processed with the FD Rapid Golgi stain TM Kit (FD Neuro Technologies, Ellicott City, MD) following the manufacturer's instruction. The brain blocks were immersed in freshly made mixture of Solutions A and B (1:1) in the dark at room temperature for 2 weeks, and then in Solution C at 4°C in the dark for 3 days. After silver impregnation, the blocks were cut slowly into frontal sections at 100 µm thickness in a vibratome. Sections were collected alternatively in 10 sets in Solution C, mounted on gelatin-coated microslides, dehydrated through ascending concentrations of ethanol, cleared in xylene, and sealed with Permount™ mounting medium.

2.5. Imaging and Data Analysis

An Olympus (BX53) microscope equipped with imaging system (CellSens Standard, Olympus) was used for examining sections stained with the ABC and fluorescent methods. Double immunofluorescence was also imaged on a confocal microscope (Nikon, DIGITAL ECLIPSE C1 plus, 5 µm thickness scan). Immunolabeling in sections around the level of injection in the rostrocaudal dimension was comparatively examined between the ipsilateral and contralateral cerebral hemispheres, using the needle track as a reference. Golgi-impregnated sections were examined on a Zeiss Axioplan microscope equipped with the Neurolucida and a high-resolution motorized stage for 3D neuronal reconstruction (MicroBrightField China). Two sections nearest to the injection coordinates (in the rostrocaudal dimension) were selected from each brain for neuronal morphometric analysis. Golgi-stained pyramidal neurons that met the following criteria were selected for reconstruction: 1) they were located in layer III of the parietal cortex overlying the mid-hippocampus and in the middle portion of CA3 (*i.e.*, around the dorsal to ventral turning area); 2) they were among the labeled cells with the widest dendritic field by overall visual judgment; 3) they were well separated from other impregnated cells such that their dendritic tree was not or minimally overlapped with the processes from other cells; 4) the somata and dendritic processes were well-impregnated throughout the section thickness, with no apparent truncation of the dendritic arbor. Ten pyramidal neurons per region/brain were reconstructed with the aid of the Neurolucida software. Subsequently, somal area, total length of the dendritic tree, branching nodes and spine density (per 10 µm length) of the apical and basal dendrites, were obtained from a given selected neuron.

2.6. Statistical Analysis

Imaging and numerical data for comparing groups were processed, with the mean ± SD calculated. Means were statistically analyzed by Student-*t* test or one-way ANOVA with posthoc Duncan's multi-group comparisons when applicable. $P < 0.05$ was considered statistically significant. Figures were assembled with Photoshop 7.1, with brightness and contrast adjusted as needed.

3. Results

3.1. LPS Injection Induced Immunoinflammatory Cellular/Molecular Changes

To confirm the occurrence of chronic neuroinflammation, cerebral sections from the LPS and PBS groups were processed under identical conditions for the detection of immunoinflammatory proteins. Compared to PBS controls, increased immunoreactivity for MCH-II (**Figures 1(A)-(E)**), CD11b (not shown) and GFAP (not shown) occurred in the ipsilateral cortex and hippocampal formation in the LPS-injected brain sections. Specifically, MCH-II immunoreactive cells appeared to be largely glial cells (**Figure 1(D)** and **Figure 1(E)**). Double immunofluorescence showed that the majority of MCH-II labeled cells co-expressed the microglial marker CD11b (**Figures 1(F)-(H)**), although a few also colocalized with GFAP immunoreactivity, suggestive of a colocalization in astrocytes (**Figures 1(I)-(K)**).

3.2. LPS Injection Elevated BACE1 Protein Levels

BACE1 protein levels were immunoblotted with a well-characterized rabbit antibody, which detects mature BACE1 protein migrating at ~70 kd [33] [34] [42] [43]. In the lysates of the ipsilateral hippocampi, immunob-

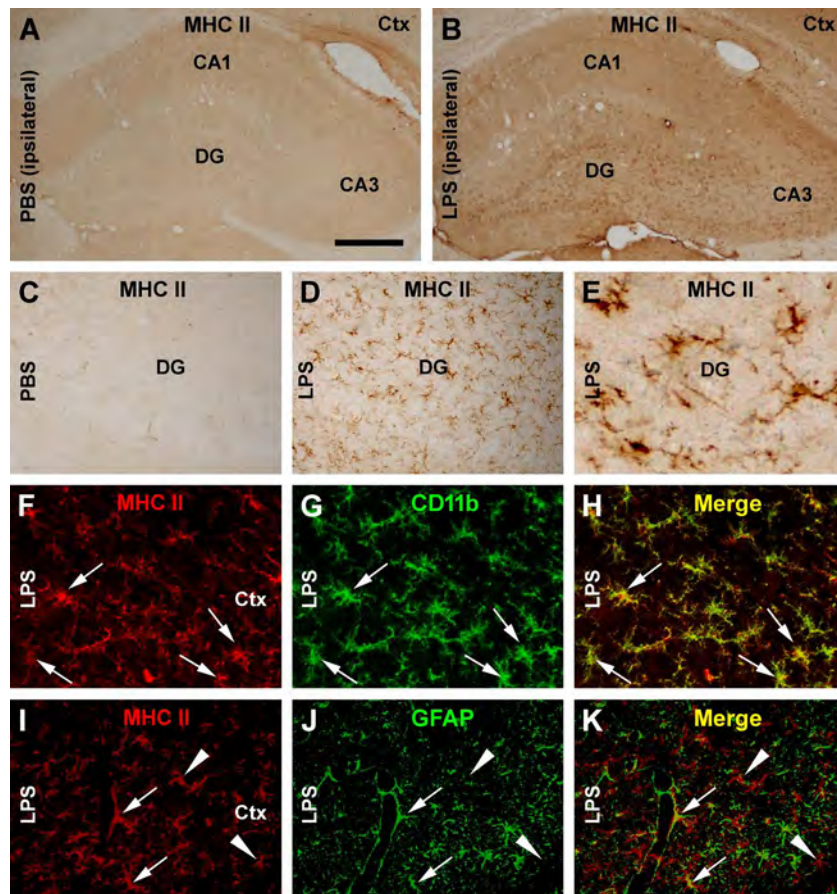


Figure 1. Representative images illustrating immunoinflammatory cellular/molecular changes in rat cortex and hippocampal formation following intracerebral lipopolysaccharide (LPS) injection. Panel (A) shows minimal immunoreactivity of major histocompatibility complex class II molecules (MHC II) in the hippocampal CA1 to CA3 areas, the dentate gyrus (DG) and the overlying cortex (Ctx) in a PBS-injected control rat, with the area of the dentate gyrus enlarged as (C). MHC II immunolabeling is increased in both the cortex and hippocampal formation in the LPS-injected animal (B), with the labeled cellular profiles appeared as glial cells at high magnifications ((D), (E)). Confocal double immunofluorescence shows a great extent of colocalization of MHC II reactivity among CD11b labeled microglial cells in the LPS-treated cerebrum ((F)-(H)). A small amount of MHC II labeled cells exhibit glial fibrillary acidic protein (GFAP) immunoreactivity, suggestive of a colocalization in activated astrocytes ((I)-(K)). Scale bar = 500 μ m in A applying to B; equivalent to 250 μ m for ((C), (D)); and to 75 μ m for ((E)-(K)).

lotted BACE1 signal was significantly increased relative to PBS-treated counterparts (**Figure 2(A)**). The mean optic density of BACE1 in the LPS group increased one fold ($199.0\% \pm 52.8\%$) relative to control ($100.0\% \pm 12.2\%$) ($P = 0.043$, $t = 3.40$, $df = 3$; two-tailed paired t-test) (**Figure 2(A)** and **Figure 2(B)**). Serving as an experimental as well as assay control [36]-[38], GFAP levels in the same cerebral samples were checked. In the ipsilateral hippocampal lysates, levels of GFAP were significantly ($P = 0.027$, $t = 4.03$, $df = 3$; two-tailed paired t-test) elevated in the LPS-treated ($141.7\% \pm 11.4\%$) relative to PBS-treated ($100\% \pm 13.4\%$) samples (**Figure 2(A)** and **Figure 2(C)**).

3.3. LPS Injection Induced Axonal Pathology with Enhanced Amyloidogenic Protein Expression

In normal mammalian brains BACE1 immunoreactivity is largely expressed in the neuropil in a diffuse pattern

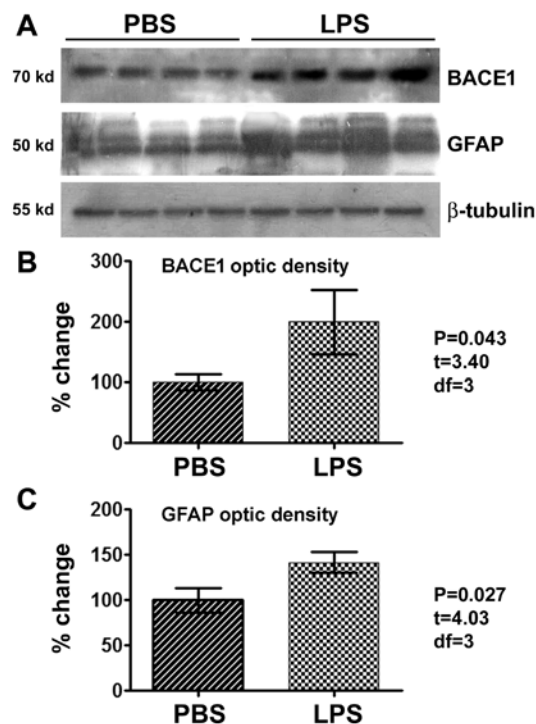


Figure 2. Western blot analysis showing elevation of the levels of β -secretase-1 (BACE1) and GFAP in the LPS-injected relative to PBS-treated (control) brains ($n = 4/\text{group}$), as assayed using hippocampal lysates ipsilateral to the injection. BACE1 levels in the LPS-treated group are about 2 times of that in the control ((A), (B)), while GFAP levels in the LPS group approach to 1.5 times of the control ((A), (C)), with the changes being significant for both proteins (paired two-tail t-tests).

except for a distinct heavy labeling at selected neuronal terminal fields including the mossy fiber terminals and olfactory glomeruli. In human and transgenic animal brains with amyloid plaque pathology, BACE1 immunoreactivity is increased preferentially in swollen and sprouting axonal terminals or axonal dystrophic neurites [33]-[35] [42]. In the present study, BACE1 labeling in both cerebral hemispheres in the PBS injected brains and the contralateral cerebrum in the LPS-injected animals exhibited the aforementioned normal distribution pattern (Figure 3(A) and Figure 3(B)). On the contrary, BACE1 labeled neuritic structures emerged in the ipsilateral hemisphere of the LPS-treated brains in a site-specific manner. Thus, increased BACE labeling appeared in both the cortex and hippocampal formation especially evident around the needle track. At high magnification, these labeled profiles appeared as swollen sphericles and neuritic processes (Figures 3(B)-(F)). In the white matter, these neuritic processes appeared to spread from the needle track for a considerable distance (Figure 3(B) and Figure 3(D)). In the ipsilateral hippocampus, the BACE1 labeled swollen neurites occurred in the stratum pyramidale (s.p.) to stratum-lacunosum-moleculare (s.l.m.) of CA3 and the adjacent CA1 area (Figure 3(C)). A large amount of swollen sphericles extended along the stratum oriens (s.o.) of CA3 in the LPS-injected animals (Figure 3(E) and Figure 3(F)). At high magnifications, these BACE1 labeled neurites were mostly round or oval in shape, and varied in size. Some smaller sphericles were sometimes arranged in chains resembling enlarged axonal varicosities (Figure 3(C), Figure 3(D) and Figure 3(F)).

In double immunofluorescence, the BACE1 immunoreactive swollen neurites exhibited a great extent of colocalization with APP (Figures 4(A)-(H)). In addition, the $A\beta$ antibody 4G8 (which detects mouse APP, $A\beta$ and likely APP β -C-terminal fragments, ref. 34) showed increased immunoreactivity in the same area with BACE1 labeled neurites (Figures 4(I)-(K)). A colocalization of BACE1 and 4G8 labeling was detected among some individual neuritic profiles. Notably, there existed a considerable amount of diffuse extracellular $A\beta$ labeling (not colocalized with BACE1 reactivity), some of which appeared punctate (Figure 4(L)). The BACE1 labeled

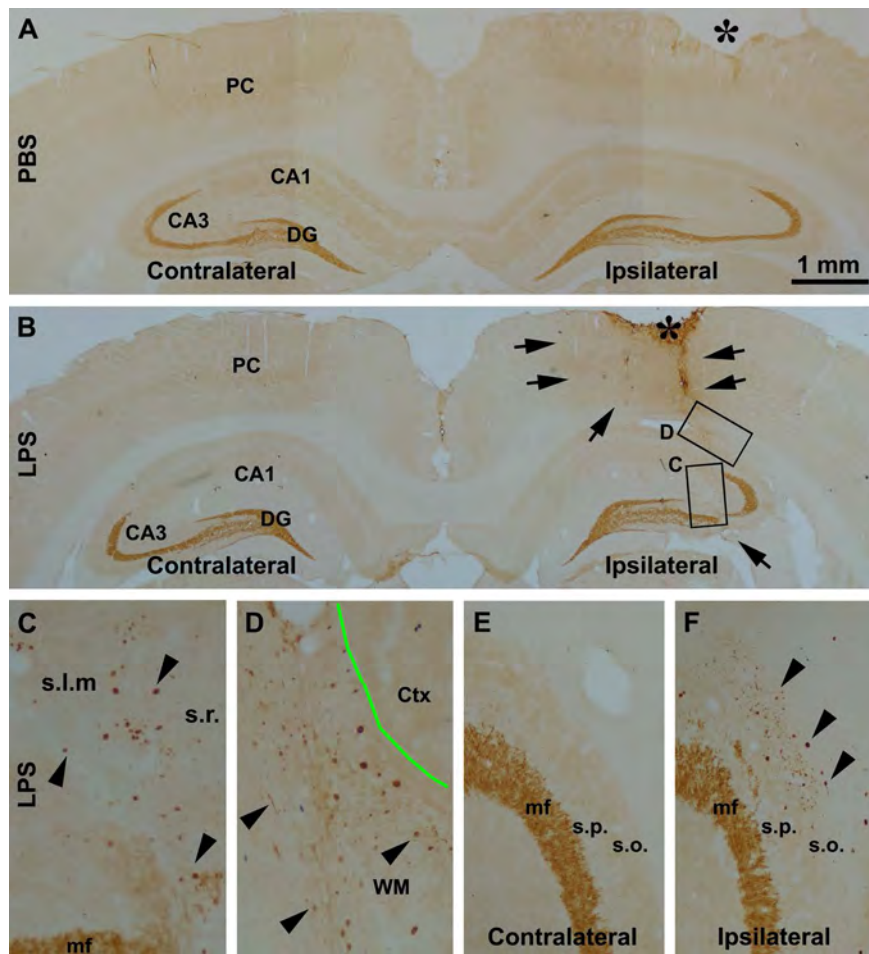


Figure 3. Representative images illustrating the occurrence of BACE1 immunoreactive neuritic pathology after focal LPS injection in rat cerebrum. In the vehicle control (injection of phosphate-buffered saline, PBS), the pattern of BACE1 immunoreactivity is comparable between the ipsilateral and contralateral cortex and hippocampal formation, which is generally associated with neuropil except for a heavy labeling at the hippocampal mossy fiber (mf) terminals (A). In the LPS injected animal (B), BACE1 immunoreactivity is increased in the neuropil in the ipsilateral relative to contralateral cortex and hippocampal formation, especially apparent around the needle track (outline by arrows). At high magnification, swollen neurites are found in the cortex and hippocampal formation in the ipsilateral side, especially evident in the white matter, stratum radiatum (s.r.), stratum-lacunosum-moleculare (s.l.m.) and the striatum oriens (s.o.) that show little labeling in the normal conditions ((C), (D), (F)). Panels ((E), (F)) are taken from the section that is $\sim 480 \mu\text{m}$ ($12 \times 30 \mu\text{m}$) apart from (B), showing the distinct difference regarding the neuritic pathology between the two sides. The needle entry at the cortical surface is marked with an asterisk (*). Ctx: cortex (grey matter); DG: dentate gyrus; PC: parietal cortex; s.l.m.: stratum-lacunosum-moleculare; s.p.: stratum pyramidale. WM: white matter; Scale bar = 1 mm in (A) applying to (B); equivalent for $400 \mu\text{m}$ for ((C), (D)) and $200 \mu\text{m}$ for (E).

sphericles and swollen processes were partially colocalized with the presynaptic marker synaptophysin (**Figures 4(M)-(P)**). In contrast, the BACE1 labeled swollen neurites did not colocalize with MAP2, which is clearly expressed in dendrites and somata of nearby pyramidal neurons (**Figures 4(R)-(U)**).

3.4. LPS Injection Induced Somatodendritic Changes in Cerebral Principal Neurons

In our Golgi preparations, pyramidal neurons around layer III were consistently impregnated in all brain samples (**Figure 5(A)**). In the hippocampal proper, the curving area of CA3, whereby the s.p. continues dorsally from the

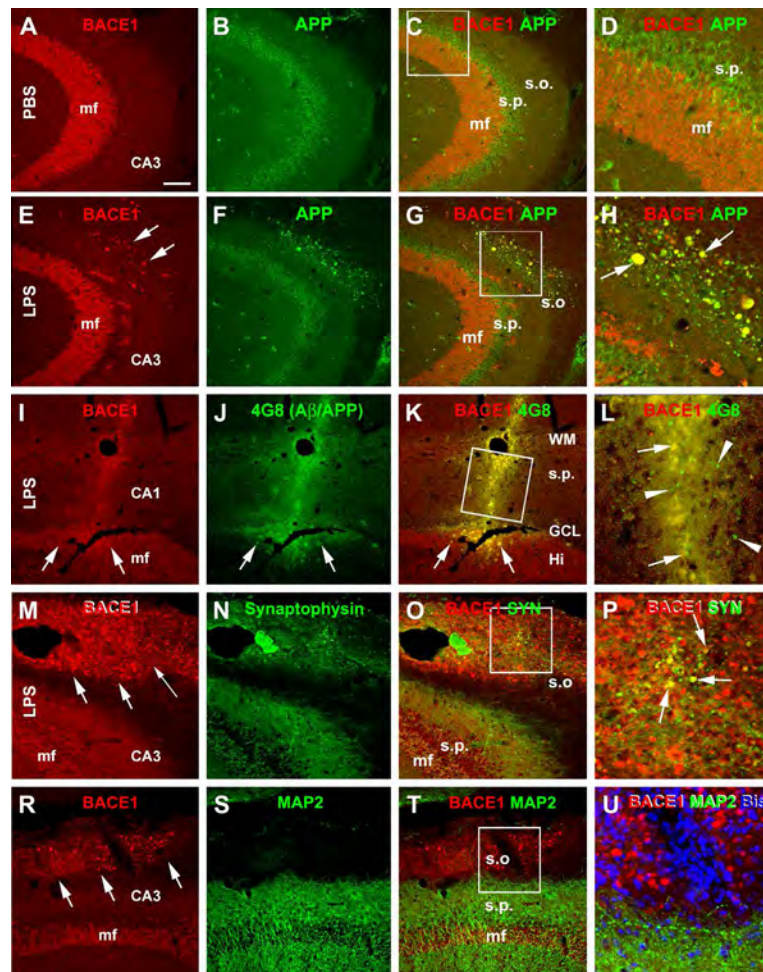


Figure 4. Confocal double immunofluorescent images showing LPS induced axonal pathology associated with increased labeling of amyloidogenic proteins. All images are taken from the CA3 area of the hippocampus ipsilateral to the intracerebral injection of PBS ((A)-(C)) or LPS ((E)-(U)). Antibody markers and color channels are as indicated. Panels ((A)-(C)) show double labeling of BACE1 and β -amyloid precursor protein (APP) in ipsilateral CA3 of the control animal, with the former expressed predominantly in the mossy fiber (mf) terminals, and the latter largely in somata of CA3 pyramidal neurons. Note that no abnormal neurites are present in the stratum oriens (s.o.). Panels ((E)-(H)) illustrate the occurrence of BACE1/APP double labeled swollen neurites (examples are indicated by arrows) in the s.o. of the ipsilateral hippocampus of the LPS injected rat. Panels (I-L) show that these BACE1 positive neurites are locally associated with increased 4G8 labeling within (pointed by arrows) as well as outside (arrowheads) the swollen terminals in the cortex and CA1. Panels ((M)-(P)) show that a partial coexpression of synaptophysin (SYN) among the BACE1 labeled swollen neurites in the s.o. of the LPS injected ipsilateral hippocampus, which appear in yellow in the merged image (arrows, P). Panels (R-U) show that there is no colocalization of the microtubule associated protein-2 (MAP2) in the BACE1 labeled swollen neurites. MAP2 labeling is distinctly associated with the somata and dendrites of pyramidal neurons in the stratum pyramidale (s.p.). DAPI counterstain is included in panels (L) and (U), showing that the BACE1 labeled elements are not somatic. Scale bar = 200 μ m in (A) applying to ((B), (C), (E)-(G) and (R)-(T)), equivalent to 100 μ m for (I)-(K), (M)-(O)), 50 μ m for ((D), (H), (U)) and 25 μ m for ((L), (P)).

CA1 direction and turns ventrally toward the dentate gyrus, could be defined systematically across brains. Therefore, we decided to use Golgi-stained pyramidal neurons in cortical layer III and around the dorsoventral turning area of CA3 for automated NeuroLucida reconstruction and morphometry. Measurements from reconstructed layer III and CA1 pyramidal neurons in the ipsilateral hemisphere of the LPS and PBS treated brains

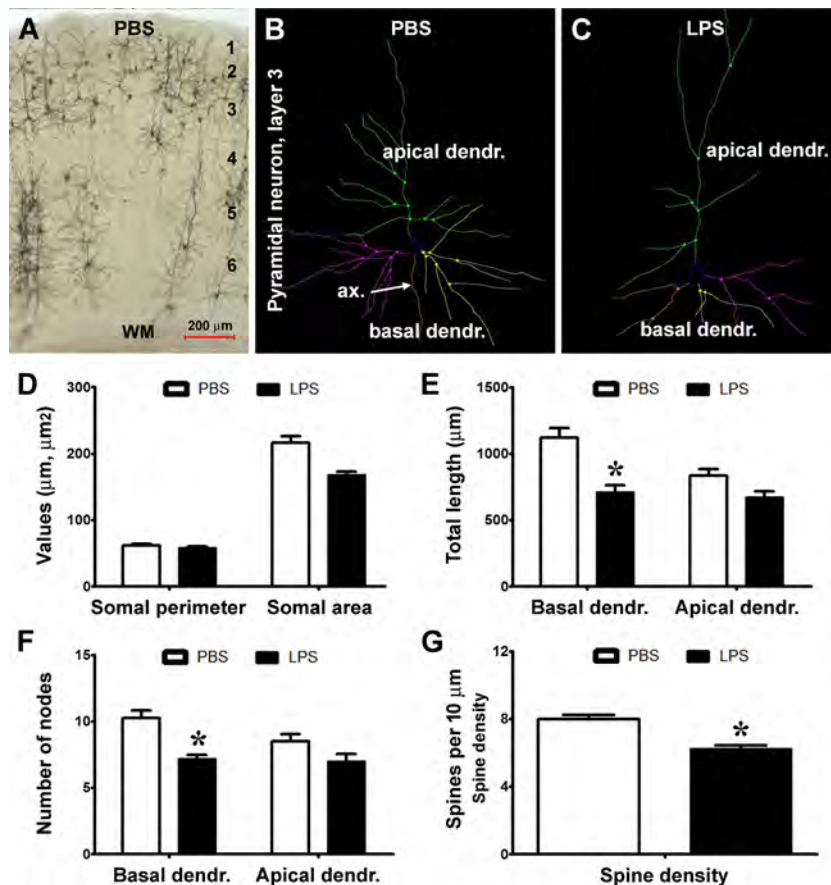


Figure 5. Quantitative Golgi study of somal and dendritic changes in cortical layer III pyramidal neurons in LPS-injected relative to PBS-injected rats. Panel (A) shows an example of Golgi stain in the parietal neocortex (ipsilateral to PBS injection), note that layer III pyramidal neurons are relatively well-labeled. Panels (B), (C) show representative Neurolucida reconstructions of impregnated layer III pyramidal neurons from a LPS (B) and a PBS (C) injected brains, with their apical and basal dendrites and dendritic branching points illustrated in color. Bar graphs ((D)-(G)) summarize the parameters obtained from 10 constructed neurons in each brain ($n = 4/\text{group}$). The means of somal perimeter and somal area show a trend of reduction in the LPS relative to PBS (control) groups, although the difference is not statistically significant (E). The means of total dendritic length tends to reduce in the LPS group relative to control, with significant difference for that of the basal dendritic tree (F). The branching nodes (points) are reduced in the LPS group on the basal dendritic tree (G). Overall spine density is significantly reduced on the dendritic tree of Golgi-impregnated cortical pyramidal neurons in the LPS group relative to control (H). Scale bar = 200 μm in (A).

(10 cells per region per brain) were compared quantitatively (Figures 5(A)-(C) and Figures 6(A)-(D)).

The somal perimeters of the layer III pyramidal neurons were comparable between the LPS ($58.6 \pm 7.4 \mu\text{m}$, mean \pm S.D., same format below) and PBS ($61.7 \pm 9.9 \mu\text{m}$) groups ($P > 0.05$, two-tail paired t-test; same statistical test below) (Figure 5(D)). The mean somal area of layer III pyramidal neurons was reduced in the LPS ($168.4 \pm 21.6 \mu\text{m}^2$) relative to the PBS ($216.5 \pm 45.3 \mu\text{m}^2$) groups ($P < 0.001$). The total length of the dendritic processes of the layer III pyramidal neurons tended to be reduced in the LPS relative to PBS groups (Figure 5(E)). Thus, the total length of the apical dendrites was $647.4 \pm 182.9 \mu\text{m}$ in the LPS group as compared to $834.9 \pm 228.8 \mu\text{m}$ in the PBS group, with the means not statistically different ($P > 0.05$). The means of the total length of the basal dendrites were $711.4 \pm 226.9 \mu\text{m}$ and $1122.0 \pm 320.7 \mu\text{m}$ in the LPS and PBS groups, respectively, showing a significant difference ($P < 0.001$). The means of the branching points (nodes) on the apical dendrites were 7.0 ± 2.5 and 8.5 ± 2.4 in the LPS and the PBS groups, respectively, while they were 7.2 ± 1.3

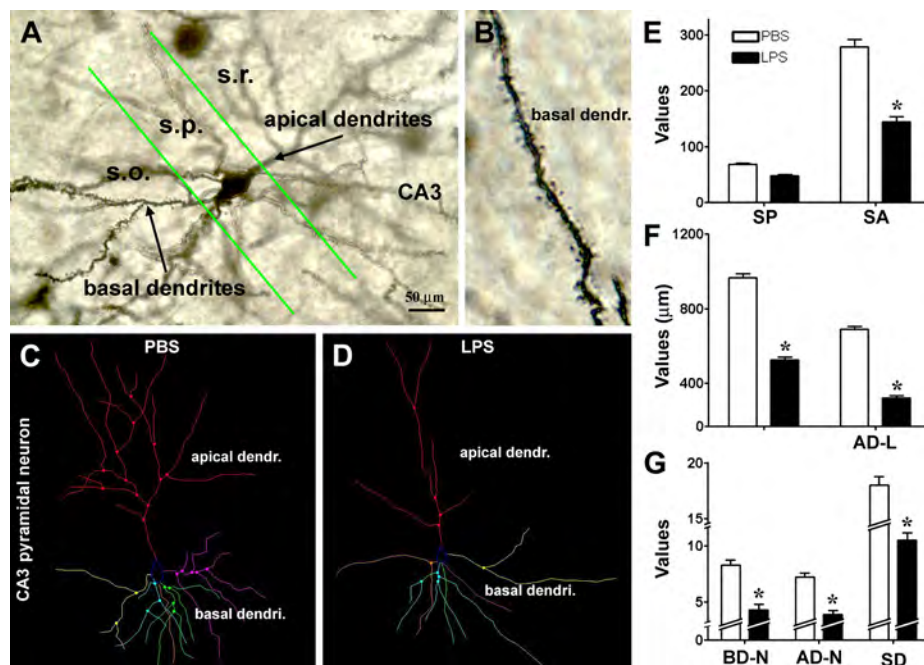


Figure 6. Quantitative Golgi study of somal and dendritic changes in CA3 pyramidal neurons in LPS-injected relative to PBS-injected rat brains. Panel (A) shows an example of Golgi-stained CA3 pyramidal neurons located around dorsal to ventral turning area of the stratum pyramidale (s.r.). Basal dendrites in the stratum oriens (s.o.) and apical dendrites in the stratum radiatum (s.r.) are visible, with dendritic spines clearly labeled at high resolution (B). Panels (C), (D) show examples of Neurolucida reconstructions of CA3 pyramidal neurons from LPS (C) and a PBS (D) injected brains. The reduction in mean somal area (SA) in the LPS relative to PBS groups is statistically significant, whereas the reduction in the somal perimeter (SP) is not (E). The reduction in the total dendritic length in the LPS relative to PBS groups is significant for both the basal and apical trees (F). Scale bar = 50 μm in (A) and 5 μm for (B).

and 10.6 ± 2.7 for the two groups on the basal dendrites. Statistical tests reported a significant difference between the two groups for the means of the basal dendritic but not the apical measurements (Figure 5(F)). The number of spines per unit length of dendrite was reduced significantly on the layer III pyramidal neurons in the LPS relative to PBS groups (Figure 5(G)). Data from the apical and basal dendrites combined, showed spine density to be 6.3 ± 0.9 vs. 8.0 ± 1.1 for the LPS relative to PBS groups ($P < 0.0001$).

For the reconstructed CA3 pyramidal neurons (Figures 6(A)–(D)), both the somal perimeter and somal area of the pyramidal neurons tended to reduce in the LPS relative to PBS groups (Figure 6(E)). Thus, the mean somal perimeters were 47.8 ± 11.0 μm (mean \pm S.D., same format below) and 67.8 ± 12.3 μm in the LPS and PBS treated animals, respectively ($P > 0.05$, two-tail paired t-test, same statistical test below). The reduction in the somal area of CA3 pyramidal neurons was significant ($P < 0.001$) in the LPS group (144.8 ± 40.4 μm^2) compared to the PBS group (278.3 ± 60.2 μm^2). There were significant decreases in the total lengths of the apical dendrites (320.0 ± 52.6 vs. 690.0 ± 63.6 μm ; $P < 0.005$) and the basal dendrites (534.6 ± 70.6 vs. 967.0 ± 90.6 μm ; $P < 0.001$) in the LPS compared to PBS groups (Figure 6(F)). The number of branching points on the apical dendrites (4.3 ± 2.1 vs. 8.3 ± 2.1 ; $P < 0.001$) and basal dendrites (3.9 ± 1.5 vs. 7.2 ± 1.7 ; $P < 0.001$) of CA1 pyramidal neurons was significantly reduced in the LPS relative to PBS groups (Figure 6(G)). Finally, the density of spines on the apical and basal dendrites of the CA3 pyramidal neurons was significantly reduced ($P < 0.001$) in the LPS animals (10.5 ± 3.2) relative to PBS controls (18.0 ± 3.3) (Figure 6(H)).

4. Discussion

4.1. The LPS Model for Studying Chronic Neurodegeneration and Alzheimer's Disease

The LPS model is increasingly used in mechanistic and translational studies into chronic neurodegenerative dis

eases such as AD and PD [15] [16] [38] [44]-[50]. This relatively simple experimental approach offers yet a number of advantages in elucidating the neurobiology of aging and age-related diseases. This model recapitulates many cellular/molecular events of neuroinflammation, which is considered an early, perhaps fundamental, causal factor in chronic neuronal and synaptic degeneration [8]-[14] [36]-[41]. The model also reproduces some defining clinical manifestations of human neurodegenerative diseases in rodents [44]-[46]. For instance, peripheral and central LPS administration induces learning/memory deficits in experimental animals analogous to AD-like cognitive decline [39]-[41]. Importantly, LPS treatment elicits neuropathological and behavioral changes in *wild-type* animals [47]-[50]. As neurodegenerative diseases occur mostly in a sporadic nature, the LPS model serves an ideal system to address pathogenic mechanisms underlying common human neurological disorders. Specifically, this study examined whether inflammation is a consequence or cause of dystrophic amyloidogenic neuritis.

We have shown in a recent study that unilateral intracerebral injection of ~10 µg LPS is sufficient to induce spatial learning and memory impairments in adult rats. Upregulation of immunoinflammatory molecules including PirB and GFAP is evident in the LPS-treated brains, occurring largely in the ipsilateral cortex and hippocampal formation [41]. In the present study we observe that this same dose of LPS injection dramatically induces MHC II expression in the ipsilateral neocortex and hippocampal formation. This modulation appears to be largely associated with microglial activation, a key finding in the initial studies that establish this model of neurological diseases [36] [39]. Consistent with other reports [51], LPS-induced MHC II upregulation also appears to occur to a lesser extent in activated astrocytes that express high levels of GFAP. Thus, our data are consistent with the notion that intracranial LPS injection induces profound glial immunoinflammatory responses in the brain.

4.2. Axonal and Dendritic Pathology in LPS-Induced Neuroinflammation

Besides glial responses, neuronal and synaptic alterations have been reported in rodent LPS models anatomically and electrophysiologically. Local LPS infusion causes increased cell/neuronal death in the entorhinal cortex [52] and substantia nigra [53] in rats as well as the hippocampus in ICR mice [54]. In BALB/c mice, the length and branching points of apical dendrites of CA1 pyramidal neurons are reduced in old but not young adults 72 hours following intraperitoneal LPS injection, while no change in spine density is found on these neurons [55]. In C57BL/6 mice, an increase in the density of thin spines on the dendrites of adult-born granule cells is found in the inner but not outer molecular layer 28 days following intrahippocampal LPS injection [56]. In adult rats, intrahippocampal LPS injection does not alter the intrinsic membrane properties, dendritic arborization or spine formation of the newly generated granule cells, but affect the overall network activity in the hippocampal neural circuitries [57]. LPS treatment can alter hippocampal synaptoplasticity in rats as measured by long-term potential and long-term depression [52] [58]-[60].

Here we demonstrate pre- and post-synaptic alterations among cortical and hippocampal neurons in LPS relative to PBS injected rats. Microscopically evident axonal pathology is featured by aberrant sprouting and swelling, and increased expression of APP, BACE1 and synaptophysin, but not MAP2. These abnormal axonal profiles seen following LPS treatment resemble the dystrophic neurites seen in models of injury that exhibit an increase in APP or A-beta [61] [62], although they do not arrange in typical rosette-like clusters as seen around established compact plaques [32]. Our Golgi data indicate that focal LPS injection causes a significant decrease in dendritic length and nodes on the basal tree as well as a reduction of spine density on the entire dendritic tree of layer III cortical principal neurons. The impact of focally injected LPS appears to be greater on CA3 pyramidal neurons, including significant reductions of somal size, lengths of basal and apical dendrites, branching points of the basal and apical dendrites, and overall spine density. The vulnerability of CA3 pyramidal neurons to LPS toxicity might relate to an intrinsic property of these cells in regard to synaptoplasticity. The dendrites and spines of CA3 pyramidal neurons belong to one of most plastic network systems in the brain—they receive major presynaptic inputs from the mossy fiber terminals that are constantly renewed in the process of adult neurogenesis of the dentate granule cells [63]. Alternatively, the observed variable somatodendritic changes between the cortical and hippocampal pyramidal neurons may reflect a potential spatial or dose-related effect by the injected toxin (*i.e.*, the proximity to the injection site), which can be expected since the inflammation and BACE1 upregulation are not evident in the contralateral hemisphere.

4.3. Amyloid Pathogenesis in LPS-Induced Neuroinflammation

LPS administration has been shown to influence amyloid pathogenesis via mixed cellular modulations in wild-type and transgenic rodents. Chronic intraventricular LPS infusion in rats induces intraneuronal A β immunoreactivity in the hippocampus [37]. Repeated intraperitoneal LPS injections in ICR mice result in increased intraneuronal and extracellular A β 42 immunoreactivity in the cortex and hippocampus, with biochemical data indicating reduced α -secretase activity, increased γ -secretase activity, and elevated BACE1 protein and activity in tissue lysate [64]. In transgenic models of AD, LPS administration is shown to increase APP and A β immunoreactivity in neuronal somata [65], elevate γ -secretase activity [66] and accelerate plaque deposition [67]. However, other reports show reduced amyloid deposition via enhanced A β clearance by activated microglia after LPS administration [68] [69].

Being the obligatory enzyme for A β genesis, BACE1 is at the crossing point potentially linking cellular and signaling substrates to amyloid pathogenesis. BACE1 elevation is associated with axonal pathology during plaque development in the brains of transgenic AD models as well as aged and AD human subjects [32] [70]. As elaborated in the preceding section, we have identified axonal sprouting and swelling associated with enhanced APP, BACE1 and A β antibody reactivity in the ipsilateral cortex and hippocampal formation in LPS treated rat brains, especially evident around the needle track. This finding clearly indicates that chronic neuroinflammation can act as an inductive factor for amyloidogenic axonal pathology in wild-type adult mammalian brain.

More basic questions can be asked as to why axonal sprouting/dystrophy, dendritic/spine degeneration and glial activation develop in partnership under neuroinflammatory conditions. One possibility is that the toxin interrupts axonal transport causing accumulation of the amyloidogenic proteins and other neuronal molecules [31]. This is consistent with the observation that the presynaptic marker synaptophysin is present only among a subset of the BACE1 immunoreactive swollen neurites. One may also speculate that this pathogenic correlation potentially involves the triadic organization of the synapse—presynaptic and postsynaptic terminals engulfed by glial processes. Neuroinflammation could initially induce some disengagement between the synaptic terminals and glial scaffold. For instance, inflammation-associated structural and functional changes in the glial cells may disrupt the fitness, integrity and plasticity of the pre- and post-synaptic terminals. This may eventually lead to major degenerative changes in the postsynaptic components, resulting in dendritic shrinkage and spine loss. The presynaptic components may also largely regress in the course of synaptic degeneration. However, some axon and presynaptic terminals might otherwise undergo an aggressive regenerative attempt because of the loss of appropriate postsynaptic targets. In this sense, the aberrant sprouting and dystrophy of the axonal terminals may be considered as a form of maladaptive neuronal regeneration, with the activated amyloidogenic machinery being a part of the its complex molecular dyshomeostasis.

In summary, the present study demonstrates that chronic neuroinflammation induced by intracerebral LPS injection promotes the amyloidogenic cascade in dystrophic neurites by upregulation of BACE1 and APP in the brain. This modulation is evident in axon terminals that exhibit dystrophic-like morphology. LPS injection also induces degenerative changes in postsynaptic components manifested as dendritic shrinkage and spine loss among cortical and hippocampal pyramidal neurons. Together with the immunoinflammatory responses of the glial cells, the LPS model recapitulates multiple cellular and molecular deficits seen in aging and AD brains. The synaptic triad could be the initial site as well as center of neural degeneration and aberrant regeneration, with its progression ultimately leading to overt neuritic amyloid pathology and cognitive deficits.

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Prevalence of Dementia and Its Associated Factors in Cotonou Teaching Hospital, Benin

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Abstract

Introduction: Dementia constitutes a public health hazard in developing countries. There is little data in the sub-Saharan region of African especially in Benin. **Objective:** Determining dementia hospitalization prevalence and identifying its associated factors in CNHU-HKM, Cotonou. **Method:** It was a cross-sectional, prospective, descriptive and analytical research conducted from October 2012 to July 2013 in the neurology department; it involved 251 patients aged 50 and above. Dementia screening was conducted using a modified and adapted Mini Mental Scale Examination (MMSE). Dementia clinical and etiological diagnoses were respectively conducted based on DMS-IV and HACHINSKI criteria. **Results:** Patients were averagely aged 60.9 ± 8.1 . Sex ratio (Male/Female) was 1.07. Dementia prevalence was 8.8%. This rate increased proportionally with age, from 5.3% with patients aged below 60 to 12.7% with patients aged above 60. Degenerative dementia was the most predominant type (50%). Following multi-varied analysis, smoking (RC = 6.05 [IC 95% = 1.26 - 29.38] p = 0.0001) and stroke past records (RC = 6.05 [IC 95% = 1.26 - 29.38] p = 0.001) revealed to be the factors associated with dementia. **Conclusion:** This research showed that dementia affects a significant part of the aging population in CNHU-HKM. It is imperative to combat its associated factors so as to defuse its prevalence.

Keywords

Prevalence, Dementia, Elders, MMSE, DSMIV, Cotonou, Benin

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1. Introduction

In sub-Saharan Africa, the gradual improvement of living conditions is correlated with life span increase. The end result is the increasing emergence of certain pathologies associated with age, among others, dementia [1]. Dementia syndrome is defined as a global cognitive decline in a person with a normal state of consciousness. Its occurrence and development are progressive. The Pathophysiology is dependent on the cause. Dementia occurs through accelerating apoptosis. AD is the commonest among neurodegenerative dementias; it is predominantly cortical. There are two types of brain damage resulting in AD: senile plaques (amyloidogenesis) and neurofibrillary tangles. Preferentially, the lesions affect the limbic system. In parallel, during fibrillary degeneration, pathologic filaments comprised of TAU proteins build up around the cell bodies of neurons. Several factors such as ageing, environment and genetic interrelate then causing dementia [2]. Dementia diagnosis is referred to a progressive and acquired memory deterioration associated with a disorder of at least one of the other cognitive functions. It results in social and occupational impairment, as well as difficulties in carrying out daily activities, not forgetting loss of autonomy [3]. It has become a public health hazard. In 2005 the number of dementia patients was estimated at 24.3 million worldwide, and 4.6 million new cases recorded yearly. This figure will reach 81.1 million by 2040 [2]. Worldwide, dementia is one of the major causes of disability and dependency in elderly population. It has physical, psychological, social and economic consequences on care givers, families and society. There is hardly any epidemio-clinical data on dementia in Africa [3] [4] particularly in Benin [5] [6]. So, this research was initiated to determine dementia hospitalization prevalence and identify its associated factors.

2. Method

It consists in a cross-sectional, prospective, descriptive and analytical research conducted from October 1, 2012 to July 30, 2013 in Hubert Koutoukou Maga teaching hospital of Cotonou Benin. Benin is a French-speaking country located in West African (**Figure 1**), sharing border with Nigeria on the east. It boasts of 9 million inhabitants with an area of 112,622 km². The population subjected to the research consisted of patients who went through consultation in the neurology department during research period. The sample size was calculated using Daniel Schwartz formulae $n = Z\alpha^2 pq/i^2 = 182$ with $p = 13.79\%$ (dementia prevalence in neurology department of CNHU-HKM in 1991 [5], $\alpha = 5\%$ and $I = 5\%$). The total number of subjects enrolled in the research was 251. But during the study period, we performed a systematic enrollment of all patients who met the inclusion criteria and consented to participate in the research, up to the number expected.

Inclusion criteria

- Be aged 50 and above.
- Having been consulted in the neurology department during research period.

Exclusion criteria

- Having experienced language disorder.
- Having been diagnosed as experiencing continuous confusional episode, chronic psychosis and depressive syndrome.



Figure 1. Position of Benin in Africa.

Diagnosis Criteria

Patients were subjected to their cognitive functions evaluation through Mini Mental State Examination (MMSE) in its modified version which is adapted to the research cultural era. Patients who recorded $MMSE \leq 24/30$ are likely to have dementia and subjected to DSM IV criteria [7] for dementia diagnosis. Its severity is appreciated in conformity with MMSE score. In fact,

- a score $20/30 \leq MMSE \leq 24/30$ with a patient matching DMS IV criteria, is considered as “moderate dementia”.
- a score $MMSE \leq 19/30$ with a patient matching DMS IV criteria, is considered as “severe dementia”.

HACHINSKI score was used to determine the nature of the effect:

- >7 : vascular dementia.
- Between 4 and 7: mixed dementia.
- <7 degenerative dementia.

The Variables Studied Were

Dependent variable: dementia.

Independent variables: they were:

- Socio-demographic: age, gender, ethnicity, profession, residence, marital status, number of children, education level.
- Clinical: Hypertension, diabetes, obesity, stroke, cardiomyopathy, sinusitis, epilepsy hyperlipidemia.
- Types of dementia: curable dementia, vascular dementia, degenerative dementia, mixed dementia.

Data Processing and Analysis

Data processing was conducted through EPI-DATA. Data analysis was done using statistics software STATA/IC 11.0. A descriptive analysis was completed with regard to the variables which were studied. So, for qualitative variables, frequencies and proportions were determined. Either chi 2 or FISHER test was used if only expected values are lower than 5. For quantitative values, averages together with their typical gaps, medians, minima and maxima have all been described. STUDENT test was utilized for comparisons purpose. The study of associated factors was conducted using logistical regression model in unvaried and multi-varied analyses. The multi-varied analysis was conducted by inserting into the model all variables, of which p value in unvaried analysis is less or equal to 20%, due to the exploratory nature of the study. The break-even point in terms of significance was 5% and confidence gap rated at 95%.

Ethical Considerations

Each patient or his/her next of kin submitted a written notice of consent upon explanation of the research objectives and modalities. Moreover, patients’ right as well as confidentiality was highly respected.

3. Results

All in all, 251 patients were enrolled. They were aged between 50 to 86 years with an average of 61 years ± 8.1 . There were 130 (51.8%) males against 121 females (48.2%) meaning a sex-ratio of 1.07. The other socio-demographic characteristics are shown in **Table 1**.

Clinically, 59 (23.5%) complain of mental disorders. 173 (68.9%) and 73 (29.1%) respectively had hypertension and stroke past records. **Table 2** shows the population clinical characteristics. 28 (11.2%) out of the 251 patients enrolled had MMSE score below or equal to 24. Indeed, 22 out of the 28 patients matched DSM IV dementia criteria, representing 8.8% (**Figure 2**). The prevalence was 5.3% with patients aged below 60 and 12.7% with those aged above 60 (**Figure 3**). All the 22 patients suffering from dementia were aged between 55 and 84, meaning an average age of 66 ± 9.1 . There were 11 males (50%) and 11 females (50%). 18 (81.2%) were married; 21 (95.5%) lived in a family and 9 (41.0%) already retired from work. **Table 3** shows the classification of other socio-demographic characteristics of dementia patients. 18.2% revealed to have behavioral and mental disorders. **Table 4** shows clinical characteristics of dementia patients. Total MMSE score varied from 7 to 23 with a median of 22. **Table 5** shows the classification of MMSE various items of dementia patients. 19 (86.3%) showed memory disorders against 13% showing temporal and spatial disorders. **Table 6** shows the prevalence rate of cognitive disorders of dementia patients. Etiologically, 6 (27.3%) and 11 (50%) suffered respectively from curable and degenerative dementia. **Figure 4** shows classification of dementia etiologies. Within curable etiologies, there were three chronic hydrocephaly cases, two chronic subdural hematomas and a single cerebral tumor (**Figure 5**).

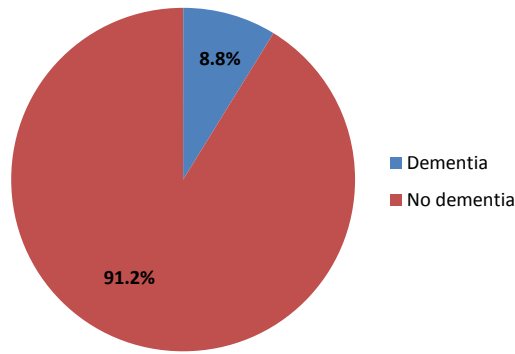


Figure 2. Dementia prevalence, Cotonou 2013.

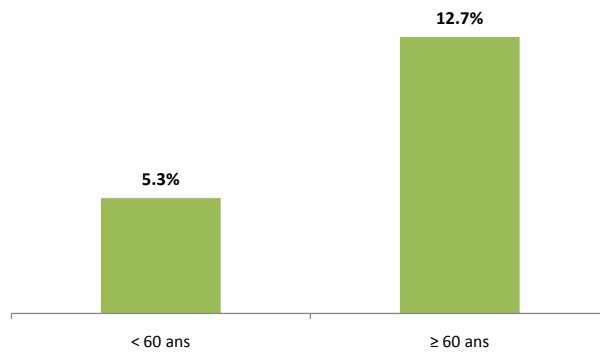


Figure 3. Dementia prevalence as per age, Cotonou 2013.

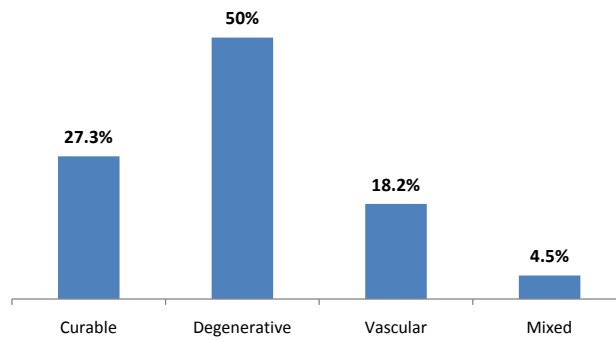


Figure 4. Classification of dementia etiologies, Cotonou 2013.

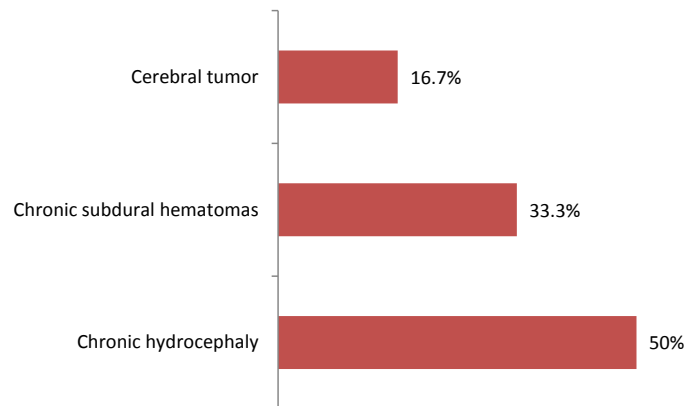


Figure 5. Classification of curable dementia etiologies, Cotonou 2013.

Table 1. Socio-demographic characteristics of the population, Cotonou 2013.

	Percentage
Marital status	
Married	75.7%
Widower	15.9%
Single	4.3%
Divorced	1.1%
Life pattern	
Lives in family	88.8%
Lives alone/single life	11.2%
Residence	
Urban locality	85.3%
Rural locality	14.7%
Level of education	
Uneducated	10.4%
Primary	25.9%
Secondary	38.6%
University	25.1%
Profession	
Civil servant	24.7%
Private worker	11.2%
Craftsman	9.6%
Housewife	17.5%
Retired	30.7%
Trader	63%

Table 2. Clinical characteristics of the population, Cotonou 2013.

Clinical characteristics	Total	Percentage
Motive of consultation		
Mental disorders	59	23.5%
Behavioral disorders	21	8.4%
Past records		
Hypertension	173	68.9%
Diabetes	42	16.7%
Obesity	58	23.1%
Hyperlipidemia	59	23.5%
Cardiomyopathy	21	8.4%
Epilepsy	10	3.9%
Stroke	73	29.1%
Smoking	17	6.8%
Alcoholism	71	28.3%

Table 3. Socio-demographic characteristics of dementia patients, Cotonou 2013.

	Total	Percentage (%)
Matrimonial status		
Single	2	9.1%
Married/concubine	18	81.8%
Widower	2	9.1%
Life pattern		
Lives alone/Single	1	4.5%
Lives in family	21	95.5%
Profession		
Civil servant	1	4.5%
Private worker	3	13.6%
Craftsman	2	9.1%
Housewife	6	27.3%
Retired	9	41.0%
Trader	1	4.5%
Residence		
Urban locality	18	81.8%
Rural locality	4	18.2%
Other	7	31.8%
Level of education		
Uneducated	5	22.7%
Primary	6	27.3%
Secondary	6	27.3%
Higher	5	22.7%

Table 4. Clinical characteristics of dementia patients, Cotonou 2013.

Clinic characteristics	Total	Percentage
Motive of consultation		
Mental disorders	4	18.2%
Behavioral disorders	4	18.2%
Past records		
Hypertension	16	72.7%
Diabetes	3	13.6%
Obesity	6	27.3%
Hyperlipidemia	5	22.7%
Cardiomyopathy	3	14.3%
Epilepsy	1	4.5%
Stroke	14	63.6%
Smoking	4	18.2%
Alcoholism	5	22.7%

Table 5. MMSE characteristics of dementia patients, Cotonou 2013.

MMSE items	Median score [Mini - Max]
Time orientation	4 [0 - 5]
Space orientation	4 [0 - 5]
Learning	3 [2 - 3]
Attention and calculation	0 [0 - 2]
Memory retention	0 [0 - 1]
Language	7 [2 - 8]
Constructive practices	1 [0 - 1]

Table 6. Classification of cognitive disorders in dementia population, Cotonou 2013.

Types of disorder	Total	Percentage
Time orientation	13	59.1%
Space orientation	13	59.1%
Learning	1	4.6%
Attention and calculation	21	95.4%
Memory retention	19	86.3%
Language	14	63.6%
Constructive practices	6	27.3%

3.1. Factors Associated with Dementia

The only one socio-demographic factor associated with dementia was age exceeding 60 years (IC 95% = 2.61 [1.03 - 6.67], $p = 0.036$). **Table 7** shows such data.

Clinically, stroke past records (IC 95% = 5.04 [2.01 - 12.62], $p = 0.0001$) and smoking (IC 95% = 3.69 [1.09 - 12.50], $p = 0.049$) were closely associated with dementia as shown in **Table 8**.

3.2. Predictive Factors of Dementia

After multi-varied analysis of associated variables in unvaried analysis, those which are individually associated with mortality were stroke past records (IC 95% = 7.66 [2.46 - 23.85], $p = 0.0001$), and smoking (IC 95% = 6.05 [1.26 - 29.38], $p = 0.001$). **Table 9** shows these data.

4. Discussion

It was a cross-sectional, descriptive and analytical research. The method we implemented is really adapted to this kind of research in the sense that, there was no plan to follow-up the patients; instead the strategy was to conduct a questionnaire after consultation. During the research period, we exhaustively screened all patients fulfilling our inclusion criteria. This enabled us to incorporate 251 patients. However, only 22 patients showed dementia based on DMS IV diagnosis criteria. Such a small number of patients did not allow us to highlight some factors associated with dementia, which were however observed in the paper.

4.1. Prevalence

In our research dementia prevalence was 8.8%. This result is close to 8.1% obtained by Mbelesso *et al.* [8] in a cross-sectional research conducted in the 3rd district of Bangui (Central African Republic) in 2012, on dementia epidemiology in elderly population. Similarly, Molero *et al.* [9] recorded in 2007 a prevalence of 10.3% in a re-

Table 7. Socio-demographic factors associated with dementia, Cotonou 2013.

	Dementia n (%)	No dementia n (%)	RC [IC 95%]	p value
Age				0.036*
<60 years	7 (5.3%)	126 (94.7%)	1	
≥60 years	15 (12.7%)	103 (87.3%)	2.61 [1.03 - 6.67]	
Sex				0.860
Male	11 (8.5%)	119 (91.5%)	1	
Female	11 (9.1%)	110 (90.1%)	1.08 [0.45 - 2.59]	
Marital status				0.390
Single	2 (18.2%)	9 (81.8%)	1	
Married	18 (9.5%)	172 (90.5%)	2.12 [0.42 - 10.59]	
Widower	2 (5%)	38 (95%)	0.50 [0.11 - 2.25]	
Divorced	0 (0%)	10 (100%)	-	
Life pattern				0.250
Lives alone	1 (3.6%)	27 (96.4%)	1	
Lives in family	21 (9.4%)	202 (90.6%)	2.80 [0.36 - 21.71]	
Profession				0.160
Civil servant	1 (1.6%)	61 (98.4%)	1	
Private worker	3 (10.7%)	25 (89.4%)	7.32 [0.73 - 73.78]	
Craftsman	2 (8.3%)	22 (91.7%)	5.54 [0.47 - 64.22]	
Housewife	6 (13.6%)	38 (86.7%)	9.63 [1.11 - 83.13]	
Retired	9 (11.7%)	68 (88.3%)	4.35 [0.25 - 73.98]	
Trader	1 (6.2%)	15 (93.7%)	8.07 [0.99 - 65.58]	
Education level				0.295
Uneducated	5 (19.2%)	21 (80.8%)	1	
Primary	6 (9.2%)	59 (90.1%)	4.31 [0.35 - 63.98]	
Secondary	6 (6.2%)	91 (93.8%)	2.80 [0.36 - 21.71]	
Higher	5 (7.9%)	58 (92.1%)	0.50 [0.11 - 2.25]	

*Significant result.

search conducted on elderly population living on the Caribbean coast of Venezuela. In 2012, Coume M. *et al.* [7] recorded 10.8% as prevalence rate in a cross-sectional research on the estimation of cognitive prevalence within an elderly Senegalese population. Our record of 8.8% dementia prevalence is lower than 24.3% registered by Uwakwe R. *et al.* [10] in a cross-sectional research conducted in 2009 on dependency epidemiology in elderly Nigerian population. It is also lower than 43.2% recorded by Ndiaye *et al.* [11] in a research conducted from January 2004 to June 2005 on the operation of a Senegalese memory clinic in Fann teaching hospital psychiatry department (Dakar, Senegal). In fact, many reasons may justify this difference in terms of the prevalence rate. First of all, in Uwakwe R. *et al.* [10] research, patients were aged 65 and above and the sample was made up of 1238 patients who went through 10/66 protocol; knowing that the specificity and sensitivity of such a protocol are higher than those of MMSE we implemented. The same goes with Ndiaye *et al.* [11] who utilized in their research Senegal Test, which sensitivity and specificity are higher than those of MMSE. Moreover, in their re-

Table 8. Clinical factors associated with dementia, Cotonou 2013.

	Dementia n (%)	No dementia n (%)	RC [IC 95%]	p value
Hypertension				0.68
Yes	16 (9.3%)	157 (90.7%)	1.22 [0.46 - 3.23]	
No	6 (7.7%)	72 (92.3%)	1	
Diabetes				0.67
Yes	3 (7.1%)	39 (92.9%)	0.76 [0.22 - 2.73]	
No	19 (9.1%)	190 (90.9%)	1	
Obesity				0.63
Yes	6 (10.3%)	52 (89.7%)	1.28 [0.45 - 3.42]	
No	16 (8.3%)	177 (91.7%)	1	
Epilepsy				0.89
Yes	1 (10%)	9 (90%)	1.2 [0.14 - 9.64]	
No	21 (8.7%)	220 (91.3%)	1	
Stroke past records				0.0001*
Yes	14 (19.2%)	59 (80.8%)	5.04 [2.01 - 12.62]	
No	8 (4.5%)	170 (95.5%)	1	
Smoking				0.049*
Yes	4 (23.5%)	13 (76.5%)	3.69 [1.09 - 12.50]	
No	18 (7.7%)	216 (92.3%)	1	
Alcoholism				0.53
Yes	5 (7.0%)	66 (93.0%)	0.72 [0.26 - 2.05]	
No	17 (9.4%)	163 (90.6%)	1	

*Significant result.

Table 9. Dementia predictive factors. Results of multi-varied analysis, Cotonou 2013.

Variables	Total	Dementia (n%)	RC _{Gross} [IC _{95%}]	RC _{adjusted} [IC _{95%}]	p value
Stroke					0.0001*
Yes	73	14 (19.2)	5.04 [2.01 - 12.62]	7.66 [2.46 - 23.85]	
No	178	8 (4.5)	1	1	
Smoking					0.001*
Yes	17	4 (23.5)	3.69 [1.09 - 12.50]	6.05 [1.26 - 29.38]	
No	234	18 (7.7)	1	1	

search, enrolled patients (132 in total) were selected among those who showed memory disorders. All this could explain the higher rate recorded in these two researches. Notwithstanding our 8.8% prevalence is higher than the figure recorded by other authors. Thus, Paraiso *et al.* [6] recorded 2.6% in rural area (Djidja) and 3.7% in urban area (Cotonou) in a door-to-door research conducted in Benin in 2010 on dementia epidemiology with patients aged 65 and above, whilst Guerchet *et al.* [12] recorded 6.7% in a cross-sectional research conducted in the year 2012 on dementia epidemiology within elderly population living in Brazzaville (Congo). Longdon *et al.* [13]

registered 6.4% prevalence in a cross-sectional community research on dementia prevalence in rural Tanzania. El Tallawy *et al.* [14] also registered 2.3% in a research conducted in 2011 on dementia prevalence in Kharga (Egypte) in 2012, whilst Yusuf *et al.* [15] estimated dementia prevalence at 2.8% in a community research on dementia prevalence and subtypes dementia within elderly population living in northern Nigeria. These prevalence rates which are very lower than what we recorded in our research could be explained by the fact that most of these researches were conducted within a vast population with samples above 1000 patients (1198, 8173, and 1139 patients were respectively enrolled by Longdon, El Tallawy and Paraíso). In addition, the test utilized in these researches is CSID, which has a weaker specificity than MMSE.

4.2. Socio-Demographic Characteristics

In our research dementia patients average age was 66 years \pm 9.1. This result is close to 67 years \pm 17 observed by Ndiaye *et al.* [11] as well as 67 years \pm 7.5% recorded by Coume *et al.* [7]. The same result is lower to 76 years \pm 7.1 recorded by Guerchet *et al.* [12]. This result is explained by the fact that the patients enrolled in the research were 65 years and above against 50 years and above in our research. It comes out that age is the sole risk factor which was corroborated. In our research, 62% of dementia patients were aged beyond 60 years. Moreover, dementia prevalence increased with age. The figure varied from 5.3% below 60 years to 12.7% beyond 60 years. El Tallawy [14] recorded that dementia prevalence of 2.3% increased drastically to 18.5% with subjects aged beyond 80 years. Libre *et al.* [16] confirmed that beyond 65 years, dementia prevalence doubles anytime age increases by 5 years. Other authors such as Longdon, Coume, Yusuf et Paraíso [6] [7] [13] [15] recorded similar results. Out of 22 dementia patients 11 were males; meaning a sex ratio of 1. This result is dissimilar to the ratio recorded by most researches which revealed a female predominance. Thus, Mbeleso *et al.* [8] recorded 82.35% female predominance whilst Stewart *et al.* [17] recorded 60.8%. Letenneur *et al.* [18] as well as Nitri *et al.* [19] corroborated this female predominance which according to Stewart *et al.* [17] might be explained by the biological and hormonal differences between the two sexes, particularly estrogens. Our result which is quite different from others' could be explained by the fact that the population was male biased and not large enough. Indeed 18 (81.8%) of dementia patients were married. This result is similar to that of Ndiaye *et al.* (76.5) and Coume *et al.* (79%). However, it is different from most authors' findings. Thus, Fratiglioni [20] maintains that being a widower or a divorcee implies an under-performance regarding MMSE; such under-performance is not recorded with elderly married persons. We could then bring up the hypothesis of marriage playing a role of social balance. Van Gelder *et al.* [21] in a research conducted in 2006 in Finland noted that men who lost their partner, those who weren't married and those who live single life or lived single life for at least 5 years were much more exposed to cognitive decline, unlike married men or men who lived with a partner. This predominance of married persons has its explanation in our socio-cultural realities. In fact, it is rare to see persons aged 50 and above who are not married. Out of the 22 dementia patients, 21 (95.5%) lived in a family. Fratiglioni *et al.* [20] proved that single patients were twice exposed to dementia comparing to those living in a family. They also found out that a closed social network increases by 60% the risk. Dementia risk is therefore correlated with the significance of social network. Baiyewu *et al.* [3] exposed that in Africa elderly persons live in extended family made up of several generations. 6.7% live alone; which contrast the prevalence rate in Europe (35% - 39%). Our results reflect the importance of social network in Africa particularly in Benin. Such a rich social network may explain the low dementia prevalence in our societies. Unfortunately, this social network tends to ebb with a progressive urbanized lifestyle leading to individualism. However, we should not forget that these maladies are under-diagnosed. 5 (22.7%) of the dementia patients were uneducated. Among the 17 (77.3%) who were educated, 6 (27.3%) had primary education only. In Egypt, El Tallawy *et al.* [14] conducted a research which revealed that dementia prevalence rate was significantly higher among uneducated persons (6.35%) than literate persons (0.6%). They asserted that the low education level played a predominant role in dementia occurrence in elderly population especially when they did not reach elementary school. This assertion is supported by Guerchet *et al.* who demonstrated that, not acquiring elementary education was significantly associated with dementia. On the contrary, a research conducted in Kenya revealed that there was no proof of little education being associated with dementia occurrence [22].

4.3. Dementia Etiologies

Results from our research revealed that degenerative dementia cases (50%) were mostly prevalent, followed by

vascular dementia (31.8%) then curable dementia (27.3%) and finally mixed dementia (18.2%). This result is similar to that of Shelley *et al.* [23] who recorded 57.1% and 26.9% respectively for degenerative and vascular dementia. Alzheimer disease (AD) is the commonest neurodegenerative dementia; it is cortically predominant and represents dementia major cause in elderly population. AD is fundamentally caused by two types of cerebral lesions: senile plaques and fibrillary neurodegeneration. Senile plaques are extracellular deposits of amyloid substance, mostly made up of peptide polymer $A\beta$ (or β -amyloid). This substance settles down progressively in the brain predominantly in the gray substance. These insoluble deposits are not metabolized by the body and destroy progressively adjacent nervous fibers. Parallel to this, in the fibrillary degeneration process, pathologic filaments made of protein build up around the cell body of the neurons. These neurons depolymerize then aggregate in the cytoplasm. Generally, this degeneration begins from the hippocampus and spreads across temporal regions. Other researches also revealed a high prevalence of AD. Thus, Yusuf *et al.* [15] recorded 66.7% AD while Alladi *et al.* [24] and Shelley *et al.* [23] respectively recorded 38.39% and 52.6%. Vascular dementia (VD) was the second etiology of dementia syndromes after degenerative dementia of Alzheimer type. Vascular dementia encompasses the whole dementia states secondary to cerebrovascular lesions. El Tallawy *et al.* [14] recorded 28.7% of vascular dementia cases. They are caused by the consequences of cerebrovascular affection (blockage of vessels or cerebral hemorrhage). In general they worsen discretely (during a new stroke occurrence). Neuro-imaging plays a predominant role in diagnosing by showing lesions of vascular origin. Curable dementia cases are those of which the etiology was identified. The most frequent causes are neurosurgical: cerebral tumors, adult hydrocephaly, and subdural hematoma. These causes are easily revealed by the neuro-imaging. The systematic results of research about infectious dementia (mainly syphilis, HIV-Aids or hardly ever Whipple disease), endocrine or deficient dementia are rarely productive because these types of dementia can totally be reversible. The discovery of curable cause for dementia does not always guarantee a complete recovery from the disease. However, systematic results from the research allow finding and treating reversible concomitant affections and disorders which can contribute to worsening dementia.

4.4. Factors Associated with Dementia

In our research two factors were revealed to be associated with dementia. There were stroke past records and smoking. 63.6% had stroke. This result is similar to the record of the University of Oxford in the United Kingdom [25], following a research conducted by the department of research on diseases prevention with stroke inpatients. The prevalence of dementia with these patients was comparable to the rate of prevalence recorded with stroke free patients who were 10 years older. Only one stroke occurrence provokes no diffuse decline of cognitive functions leading to dementia. It is the repetitive occurrence of such incidents with the same patient which can provoke cognitive decline. We then refer to multiple infarction dementia or multi-infarction dementia. In a research conducted by Altieri *et al.* [26], about 30% of stroke survivors suffered from dementia thereafter. The risk is high after stroke occurrence, but it is higher even after the embolic thrombosis occurrence. Post stroke dementia risk factors are not well known. However, three theoretical reasons could explain post stroke dementia occurrence. First of all, dementia could be the direct consequence of cerebral lesions of vascular origin. Then it could be due to the combination of degenerative lesions of Alzheimer type in a pre-clinical stage; some post stroke dementia states are progressive. Finally, white substance anomalies could also contribute to the decline in the sense that, they are associated with a high risk of vascular relapses and could stimulate neuropsychological disorders. Vascular lesions, white substance and lesions of Alzheimer type anomalies all put together, could stimulate post stroke dementia occurrence [27]. 18% were addicted to smoking. This result is similar to Juan *et al.* [28] findings, as they maintained that smoking revealed to be a risk factor. Likewise, Rusanen *et al.* [29] in 2011 established that smoking increases Alzheimer disease risk occurrence by 2. These Finnish scientists conducted a broad research between 1978 and 2008. In this research, 21,123 patients aged 50 to 60 years were thoroughly followed during 23 years. They diagnosed 5367 senile dementia cases of which 1136 Alzheimer diseases. Having reviewed different risk factors associated with dementia, researchers discovered that smoking played an important role. In fact, dementia risk increased by hundred percent (100%) concerning heavy smokers consuming over two packets of cigarette a day. Globally, smokers presented a risk of vascular dementia multiplied by 2.72. The same researchers showed that smoking affected the brain directly and increased both oxidative stress and inflammation which were two risk factors related to Alzheimer disease [29]. Nonetheless, the effect of smoking in dementia occurrence is not well explained. We only know that smoking has cerebrovascular effects

and amplifies the cholinergic metabolism by dysfunctioning brain nicotine receptacles. A Chinese research [30] proved that second-hand smoking constitutes a risk factor which could increase by 29% the occurrence of severe dementia.

5. Conclusion

Dementia with elderly population aged 50 and above, remains a pathology to which very little attention is paid in Benin health system. This research through its theme originality highlights dementia in the intention of developing adapted and effective national strategies to combat it. There is a need to conduct other researches about this theme in developing countries, where, just like in the case of Benin no consideration is given to this pathology.

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Abbreviation List

Abbreviations	Full meaning
CNHU-HKM	Centre National Hospitalier et Universitaire Hubert Koutoukou Maga
MMSE	Mini Mental State Examination (MMSE)
DSM-IV	Diagnostic and Statistical Manual of mental disorders, 4th edition
AD	Alzheimer Disease
VD	Vascular Dementia
HIV	Human Immunodéficiciency Virus



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