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Neurophysiological Correlates of Motor Behaviour in Alzheimer's Disease

A Thesis submitted to the University of Liège in fulfillment
of the requirements for the degree of
Doctor in Medical Sciences

By

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December 2015

ACKNOWLEDGEMENTS

I would like to thank Professor Jean Louis Pepin and Professor Alain Maertens de Noordhout for the supervision of my thesis. Their sustained guidance and their scientific inspiration have been very important and extremely helpful to me throughout this scientific study. In addition, I greatly appreciate the support of all the members of my thesis committee, particularly Professor Eric Salmon for his enlightening contribution. I am also grateful to Victor de Pasqua for his technical aid during my experiments and to my sister Eleana Balla for her efficacious contribution to the editing of my dissertation script. Finally, I would like to thank all the patients and their families for their participation in the study as without them, nothing could have been accomplished. Last but not least, I want to express my gratitude to the members of my own family for their continuing love and support not only during the writing process, but also throughout the three years of work when I had to make regular journeys between Greece and Belgium.

"The roots of education are bitter, but the fruit is sweet"

Aristotle

TABLE OF CONTENTS

ABSTRACT 3

CHAPT. 1. INTRODUCTION

1.1. EPIDEMIOLOGICAL DATA OF ALZHEIMER'S DISEASE 4

1.2. PREDISPOSING FACTORS 4

1.3. PATHOPHYSIOLOGY 5

1.4. CLINICAL CHARACTERISTICS 7

CHAPT. 2. BACKGROUND OF THE STUDY

2.1. MOTOR BEHAVIOUR IN ALZHEIMER'S DISEASE..... 8

2.2. THE MOTOR BEHAVIOUR OF AD PATIENTS THROUGH THE MODEL OF BROWN AND PLUCK 11

2.3. TRANSCRANIAL MAGNETIC STIMULATION IN THE STUDY OF MOTOR CONTROL IN AD 13

2.3.1. Previous TMS studies on Alzheimer's disease 14

CHAPT.3. THE STUDY 16

3.1. THE AIM OF THE STUDY 17

3.2. THE METHODOLOGY 17

3.3. THE EXPERIMENTS 19

3.3.1. MAIN TMS EXPERIMENT 19

A. Patients 19

B. Methodology 20

C. Results 24

3.3.2. SUBEXPERIMENT 1 32

A. Patients and Methodology 33

B. Results 33

3.3.3. SUBEXPERIMENT 2 36

A. Patients 37

B. Methodology 37

C. Results 38

CHAPT. 4. DISCUSSION

4.1. THE ALTERED EXCITABILITY OF MOTOR CORTEX IN EARLY AD **41**

4.2. THE ROLE OF THE CHOLINERGIC SYSTEM IN THE DECREASED CORTICAL EXCITABILITY **45**

4.3. EVALUATION OF MOTOR BEHAVIOUR IN EARLY AD BEYOND EXCITABILITY: ASSESSMENT OF CORTICAL INHIBITION **50**

4.4. CHOLINERGIC SYSTEM AND IMPAIRED CORTICAL INHIBITION IN EARLY AD **54**

4.5. THE CONNECTION BETWEEN DECREASED CORTICAL EXCITABILITY AND IMPAIRED CORTICAL INHIBITION OF THE MOTOR CORTEX..... **59**

4.6. A PATHOPHYSIOLOGICAL MODEL ABOUT THE ALTERED MOTOR FUNCTION IN EARLY AD..... **61**

CHAPT. 5. FINAL CONCLUSIONS **64**

REFERENCES **69**

APPENDIX A

PUBLISHED ARTICLE: MOTOR CORTEX EXCITABILITY CHANGES IN MILD ALZHEIMER'S DISEASE ARE REVERSED BY DONEPEZIL

APPENDIX B

PUBLISHED ARTICLE: AN INVESTIGATION OF THE LATE EXCITATORY POTENTIALS IN THE HAND FOLLOWING TRANSCRANIAL MAGNETIC STIMULATION IN EARLY ALZHEIMER'S DISEASE

ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative disorder that dramatically affects the cognition of the patients. Its effect on the motor cortex is not clearly established despite clinical observations implying some dysfunction at disease onset. From the mild stage, AD patients display a motor behavior different from normal, that is, restricted movement with slowness, delayed reaction to external stimuli and diminished facial expression. This pattern gradually changes as the disease progresses; in fact, at the more advanced stages the patients show an increased mobility with nervous movements, pacing, akathisia and falls. These observations have been the basis of our investigation of the function of the motor cortex through transcranial magnetic stimulation (TMS) in a group of AD patients with mild disease. Patients were compared to a group of normal individuals in order to find a neurophysiological correlate of their altered motor behavior. The experiments were performed in two phases, before and after the oral administration of an acetylcholinesterase inhibitor (donepezil) taking into consideration the significant role of the cholinergic hypothesis in the pathogenesis of AD. The active motor threshold (aMT) which reflects cortical excitability and the silent period (SP) which reflects cortical inhibition were measured during the TMS experiments. These measurements give an overview of the motor control in early AD, from the activation of the pyramidal cells in the primary motor area to the temporary inhibition of the contraction of the peripheral muscle. An increased aMT was observed in the early AD patients representing decreased excitability of the primary motor cortex. Also, an increased duration in SP due to its being scattered by multiple electromyographic breakthroughs called late excitatory potentials (LEP) was measured, representing impaired cortical inhibition. The administration of donepezil restored both neurophysiological parameters to normal indicating a key role of the cholinergic system in the regulation of the mechanisms which determine motor control in early AD. Additional neurophysiological and pharmacological sub experiments that we performed completed these observations. Our results combined with recent data from the literature argue in favor of a functional disturbance in the cholinergic system instead of cholinergic neuronal loss in early AD. The recently demonstrated existence of a direct connection between the basal forebrain and the primary motor area enable us to present an original physiological model explaining our findings. This model gives a complete explanation of the changes in the function of the primary motor cortex at the early stages of AD under the regulation of the cholinergic system.

CHAPTER 1.

INTRODUCTION

1.1. EPIDEMIOLOGICAL DATA OF ALZHEIMER'S DISEASE

Alzheimer's disease (AD) is the most common degenerative disorder of the brain and it has an immense societal impact worldwide. The prevalence of AD increases with age, being most frequent in individuals older than 60 years [1, 2]. Epidemiological data estimates the prevalence of Alzheimer's disease at 1-4 % in people younger than 65. This rapidly increases to 5% - 10% between the ages of 65-74 years, to 44 % between75-84 and as high as 46-50 % at the age of 85 and older. The average duration of the symptoms from diagnosis to death is around 10 years within a range of 4 to 16 years. The proportion of women suffering from Alzheimer's disease in the general population is larger than that of men, explained partially by the fact that the average life expectancy of women exceeds that of men. No significant difference has actually been found between genders in the new cases of Alzheimer's disease emerging every year [3].

1.2. PREDISPOSING FACTORS

Predisposing factors of the disease, apart from old age, are familial history, traumatic brain injury, common vascular risk factors (hypertension, diabetes, elevated homocysteine, and hypercholesterolemia) and depression [1, 2, 6, 7]. Educational and socioeconomic status has been thoroughly examined as another possible predisposing factor. Most of the studies concluded that low educational level increases the incidence of Alzheimer's disease [8, 9]. The results of the impact of the socioeconomic level are not consistent in the various studies [10, 11]. As regards the hereditary predisposition of AD, mutations have been described in three

genes: the amyloid precursor protein (APP) gene on chromosome 21, the presenilin 1 (PS1) gene on chromosome 14 and the presenilin 2 (PS2) gene on chromosome 1. These mutations result in an autosomal dominant form of the disease beginning at a young age, often in the third decade of life. For late onset Alzheimer's disease, the main known genetic risk factor is the presence of the ApoE gene located on chromosome 19 which exists in three forms: ApoE ε2, ApoE ε3, and ApoE ε4. The ε4 polymorphism has been associated with the more typical sporadic and familial forms of Alzheimer's disease, usually beginning after age 65. Nevertheless, more than 90% of cases of AD are sporadic without any demonstrated genetic factor.

1.3. PATHOPHYSIOLOGY

Regarding pathophysiology, the hallmarks of the disease are senile neuritic plaques and neurofibrillary tangles [1, 2, 3]. Senile neuritic plaques are spherical lesions of amorphous material surrounded by enlarged axonal endings (neurites). The main protein found in the core of these lesions is a β-peptide, amyloid (Aβ), which is derived from a transmembrane protein, the amyloid precursor protein (APP) by proteolysis through α,β and γ-secretase. Amyloid is also found scattered throughout the cerebral cortex in a "diffuse" form and additionally is detected in the walls of small blood vessels near the plaques (argyrophilic angiopathy). Neurofibrillary tangles are fibrillary intracytoplasmic structures within the neurons. These structures are made of a hyperphosphorylated form of the microtubular protein "tau" and appear as pairs of helicoidal filaments (Figure 1.)

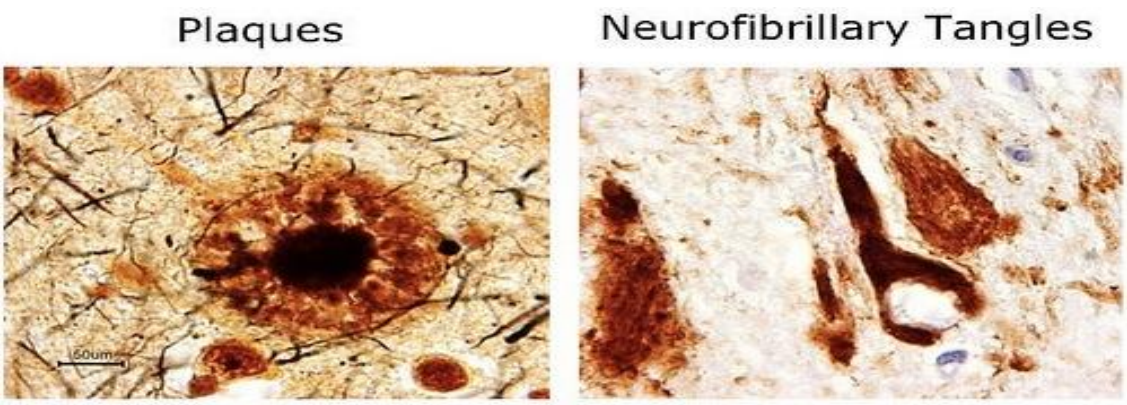

***Figure 1**: pathological hallmarks of AD: plaques and tangles*

Biochemically, the abnormality which is most prevalent in a brain affected by AD pathology is the significant reduction of the activity of the enzyme choline acetyltransferase (ChAT). This enzyme is responsible for the biosynthesis of the neurotransmitter acetylcholine and is found in cholinergic neurons. The vast majority of the cholinergic innervation of the human brain originate in the basal forebrain (medial septal nucleus, diagonal band of Broca, nucleus basalis of Meynert).There is no general cholinergic deficit in AD but a selective loss loss of the cholinergic projection pathway from the deep nuclei in the septum and diagonal band of Broca to the hippocampus and from the nucleus basalis of Meynert to the cerebral cortex. The cholinergic innervation of the striatum and of thalamus remains relatively intact.

The severity of cognitive loss is roughly proportional to the loss of choline acetyltransferase [12, 13, 14]. This observation had been the basis for the formulation of the cholinergic hypothesis, a very widely accepted hypothesis for the pathogenesis of Alzheimer's disease. The cholinergic hypothesis links the cognitive deficit of the affected individuals to a cholinergic deficit. The therapeutic effect of acetylolinesterase inhibitors upon the cognitive functions of the patients affected by AD supports the cholinergic hypothesis [15, 16, 17]. However, the pathogenesis of the disease cannot be based solely on the cholinergic deficit. It is far more complex, including the crucial, though not fully understood, role of amyloid and Tau protein in the whole physiopathological process.

The areas of the brain predominantly affected by the degenerative process during the course of Alzheimer's disease are the associative cortical areas of temporal, parietal and frontal lobes and the limbic system. Neuritic plaques and neurofibrillary tangles are found from the very early stages in olfactory areas, particularly the olfactory bulb which is considered as the area of the brain where the degeneration begins in AD. Along with neuronal loss, plaques and tangles progressively occupy the hippocampal formation including layer II of the entorhinal cortex and also amygdala, cingulate gyrus, nucleus basalis of Meynert and the associative temporo-parietal and frontal areas of the neocortex.

Primary visual areas and motor cortex appear to be spared the neurodegenerative process [19] (Figure 2.).

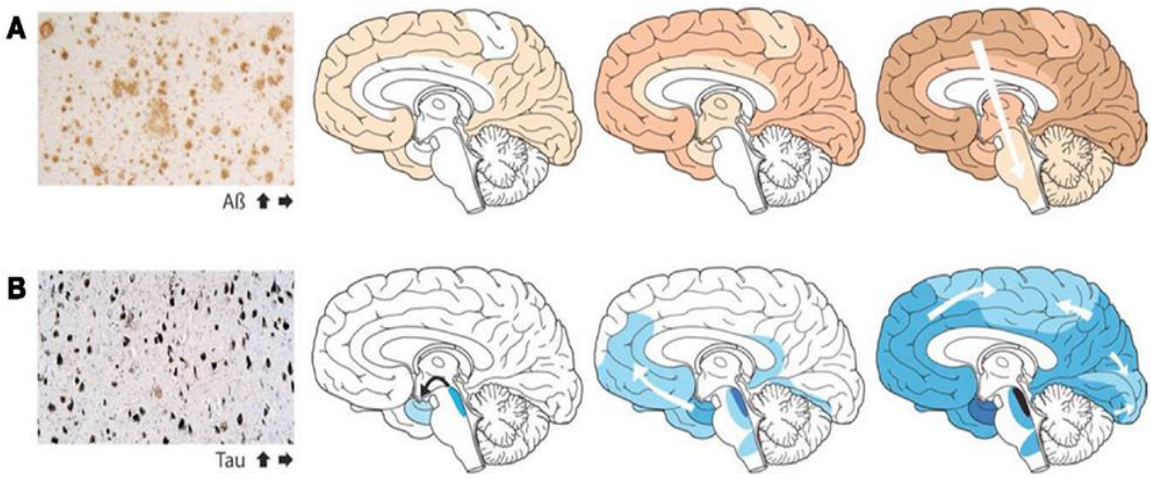

***Figure 2**: progression of pathological hallmarks of AD during the course of the disease*

1.4. CLINICAL CHARACTERISTICS

The clinical manifestations of AD evolve from the earliest signs of impaired episodic memory to severe cognitive impairment [4, 20]. The course of the disease is progressive leading to complete incapacity and death. In the early stages of the disease, the most commonly referred symptom is impaired episodic memory, meaning memory deficit for newly acquired information, whereas memory for remote events is relatively well preserved. As the disease progresses, other cognitive functions besides memory are seriously affected, such as language, both oral and written, abstract reasoning, ability for sound judgment and executive function. Along with the degeneration of certain areas of the brain, the classical triad of apraxia–aphasia-agnosia is finally established. In the more progressed stages other symptoms commonly exhibited are sleep disturbances, delusions, visual and auditory hallucinations, agitation and sometimes psychotic events. Depression and anorexia occur in 5% to 8% of patients regardless of the severity of the disease.

CHAPTER 2.

BACKGROUND OF THE STUDY

2.1. MOTOR BEHAVIOUR IN ALZHEIMER'S DISEASE

Given that the primary motor cortex is supposed to be spared the neurodegenerative process, one would assume that no motor abnormalities would be expected in the clinical picture of AD patients. Besides, disturbances in motor function of these patients rarely if ever are included in the standard symptomatology of the disease which focuses mainly on the cognitive and behavioural dysfunction. However, in everyday clinical practice it is evident that the motor behaviour of the patients who suffer from Alzheimer's disease differs in comparison with normal individuals of the same age. Balance and gait are affected with a positive correlation to the stage of the disease [21, 22]. Falls are much more common in AD patients [23, 24] and focal motor signs not attributed to a specific lesion can also be detected in these patients [25]. Some studies based on animal models [26, 27,28, 29] provide experimental evidence, contrary to what was traditionally believed, which supports the clinical observations concerning the involvement of the motor system in the course of Alzheimer's disease.

It may be observed that often patients in advanced stages of Alzheimer's disease exhibit some kind of hyperkinesia. Their muscle tone may be increased, they pace and wander without specific purpose, they exhibit a lot of stereotypical behaviours, they engage in searching behaviour and they are rarely able to relax, often giving a picture of akathisia. This kind of motor behaviour, when evaluated as an isolated element distinct from the cognitive deficit, could be safely viewed as disinhibited normal motor behaviour. A possible explanation for this hyperkinetic state could be some lack of inhibition of the motor cortex. In Alzheimer's disease

besides the neurodegenerative process (plaques and tangles) detected in the associative cortex and the limbic system, there is also a serious disturbance or a complete loss of the neuronal connections from the frontal, temporal and parietal areas to the primary motor cortex .These areas of the brain schedule, prepare and continually regulate the motor functions whereas the motor cortex is responsible for finally implementing the movement. Nevertheless, it is not clear from currently available studies [30, 31] whether the alteration of motor function observed in progressed AD represents a primary or a secondary defect of the primary motor area. It is questionable whether the changes in the motor behaviour of progressed AD patients are due to a secondary infliction of the neurodegeneration of the affected areas of the brain and their associated neural connections on the motor cortex, or whether they are caused by a primary dysfunction of the motor cortex itself.

Regarding changes in motor behaviour during the course of AD, specific mention has to be made about the extrapyramidal features which are displayed, to a certain degree, by some AD patients. These features show great variability. Scarmeas et al [32] evaluated the motor signs of extrapyramidal origin exhibited by the patients during the course of AD. In this study, the clinical motor features examined in a large group of patients followed for 13 years were; speech, facial expression, posture and bradykinesia/ hypokinesia. It was found that as the disease progressed, the prevalence and severity of the abnormal motor signs increased accordingly, reaching 71% in the last year of the study. In that study patients were selected with great care so as to exclude, as far as possible, Lewy body pathology comorbitidy which could have caused misinterpretation of the results or scientific bias. The investigators attributed their results to extranigral lesions which involved mesocortical dopaminergic pathways, loss of striatal dopaminergic transporter sites and reduced dopaminergic D2 receptors in the putamen. An alternative explanation was that the dopaminergic system may have been involved but through AD and not Lewy body pathology given that senile plaques were reported in the putamen, caudate nucleus and substantia nigra [33] and neurofibrillary tangles were noted in substantia nigra [34].

An increased awareness of Alzheimer's disease over the last 20 years, has led more patients to seek expert advice while still in the initial stages of the disease. Apart from altered cognition it has been clinically observed that early AD patients more often exhibit a type of motor behaviour different from the usual hyperkinesia of advanced AD. The patients in the early stages of the disease appear less reactive to external stimuli. The initiation of a motor reaction and also the velocity of the execution of a certain movement take longer when compared to normal individuals [35]. These patients often exhibit diminished facial expression but not in the form of the usual extrapyramidal hypomimia. They display a narrow range of movement of the facial muscles responsible for expressing emotion and alertness of the perceived external environment often presented as apathy. Their whole motor function from their ability to react fast when in their baseline calm state [36] to performing a simple or complex movement [37] and walking [38] is slower, exhibiting a form of hypokinesia.

A recent study of Vidoni et al. [39] supports the clinical observations of impaired motor function in early AD patients from a neuroradiological point of view. The authors examined a group of early-stage AD patients using functional MRI while they were performing a visually-directed, simple motor task and compared them to a group of normal individuals in order to investigate AD-related differences in regional brain activation during motor performance. The results of this study have shown that the early AD patients displayed increased co-activation of bilateral motor and visual regions of the cerebral cortex. These findings could either represent inefficiency in the motor network as a consequence of the disease or could be interpreted as compensatory activation. Nevertheless, they provide further evidence that in the early stages of AD, motor function is altered even during simple motor tasks, suggesting an impaired connectivity between the primary motor area (M1) and other areas of the brain. The results of this recent study are in accordance with an older study by Agosta et al [40] who used fMRI to examine possible changes in the sensory and motor cortex of AD patients. They actually identified functional changes in areas of the brain traditionally considered to be spared in early AD, in a form of an initial phase of hyperactivation of the sensorimotor cortex in patients

suffering from amnestic mild cognitive impairment, followed by a phase of hypoactivation of this area in AD patients.

2.2. THE MOTOR BEHAVIOUR OF AD PATIENTS THROUGH THE MODEL OF BROWN AND PLUCK

A publication of Brown and Pluck [41] about the pathology of motivation and goal-directed behaviour in neuropsychiatric disorders which display 'negative' symptoms, including Alzheimer's disease, provides some original ideas which could be used to explain the pathophysiology of the motor behaviour of AD patients. The authors propose that goal-directed behaviour and the details of its execution can be theorized as a reflection of cognitive and motor function in close interaction. It is proposed that preparation, initiation and termination of a goal–directed movement requires active co-operation between the anatomical areas of the limbic system (amygdala, hippocampus) and those of the striato-thalamo-cortical circuit in the form of a functional network in order to first inspire, then schedule, prepare and finally execute the movement. This complete procedure initially involves the affective areas of the brain (limbic system), then the cognitive areas (prefrontal cortex, cingulated gyrus) and finally the motor areas (striatum – premotor, motor cortex). The interaction between those brain circuits is constantly dynamic in order to achieve a certain goal which could be a simple or complex movement or a series of expressions and motor reactions. Given that the motor cortex is the final area of the brain responsible for the execution of movement we can assume that any divergence from the normal motor behaviour observed in AD patients could lead to an altered functioning of their motor cortex. The degree to which the possible changes in the function of motor cortex alters the motor behaviour of AD patients is determined by the existing direct or indirect connections between the various areas of the brain involved in the goal-directed movement and their anatomical and functional connection to the primary motor cortex.

A schematic presentation of the model proposed by Brown and Pluck demonstrating the interactions among various areas of the brain is displayed in the Figure. 3.

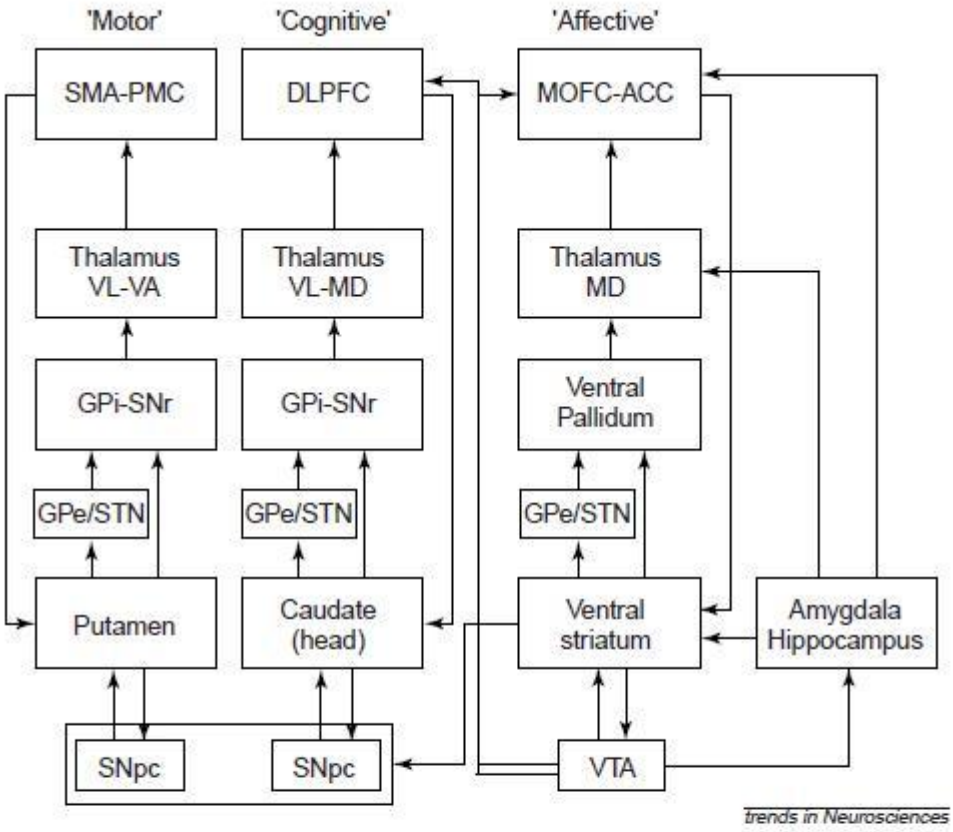

Fig 3. ***Striato-thalamo-cortical circuits and their interactions with limbic structures.*** *The amygdala and other limbic structures involved with motivational and emotional processes, provide input via the ventral striatum, thalamus and cortex. These offer many direct and indirect opportunities for the emotional and motivational processses to influence the activity of other circuits including those concerned with cognition and motor function. Abbreviations: ACC, anterior cingulate cortex; DLPFC, dorsolateral prefrontal cortex; GPe, globus pallidus external section; GPi, globus pallidus internal segment; MD, mediodorsal; MOFC, medial orbitofrontal cortex; PMC, premotor cortex; SMA, supplementary motor area; SNpc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; STN, subthalamic nucleus; VA, ventroanterior; VL, ventrolateral; VM, ventromedial; VTA, ventral tegmental area.*

The significant alterations in normal motor behaviour which are already present from the disease's onset in patients suffering from AD, if viewed through the aforementioned model of Brown and Pluck, outline an important early effect of Alzheimer's disease upon the function of the primary motor cortex. The pathophysiology of this effect is probably very distinct and not connected to or dependent on the usual neuropathological findings traced in certain areas of the

brain as in early AD there is no evident neuronal loss in the primary motor cortex. At this stage the neuropathological abnormalities are limited to the limbic areas and the hippocampus [19]. A new pathophysiological explanation must be given for the changes in motor behaviour of early AD patients given that the motor cortex is not affected by amyloid plaques, neurofibrillary tangles and neuronal loss early in the course of the disease.

2.3. TRANSCRANIAL MAGNETIC STIMULATION ON THE STUDY OF MOTOR CONTROL IN AD

An efficacious way to explore changes in motor control and motor behaviour in Alzheimer disease is through neurophysiological studies. Transcranial magnetic stimulation (TMS) is a neurophysiological tool which is easily accessible, painless, safe, not costly, simple in its application and gives fast results when used on individuals. The stimulator produces an electromagnetic induction that generates electric currents using a rapidly changing magnetic field. An electromagnetic coil is held close to the skull of the individual tested and short electromagnetic pulses are delivered through the coil. The magnetic pulse passes unattenuated through the scalp and skull, and induces small electrical currents stimulating the neurons of the targeted area of the brain [42]. In this way, TMS can activate cortical motor areas and the corticospinal tract giving valuable information about the motor excitability in the central nervous system. A very important factor is that the whole procedure causes only minimal discomfort to the subject.

TMS activates the pyramidal cells of the primary motor area (M1) and following the corticospinal tract the electric current activates the alpha motor neurons in the spinal level causing contractions in contralateral body muscles. This results in a motor evoked potential (MEP) -compound muscle potential (CMAP) in the activated muscle, which is recorded by a conventional electromyography device. The most commonly used muscles from which CMAPs are recorded during TMS are the small muscles of the hand: first dorsal interosseous (FDI), abductor pollicis brevis (APB) and abductor digiti minimi (ADM) [43].

Different types of coils and stimulators can be used to perform TMS. A detailed description exceeds the purpose of this work; though it can be found in certain textbooks specializing in TMS [44, 45]. The evaluation of motor function in the central nervous system by TMS is achieved by the measurement of some standard neurophysiological parameters such as the central motor conduction time, the motor threshold and the silent period. Another parameter often measured is Short-latency afferent inhibition (SAI). Central Motor Conduction Time (CMCT) is defined as the time from the motor cortex to the spinal motor neurons [44]. Motor threshold (MT) is defined as the minimal stimulus intensity that produces a motor evoked potential (MEP) greater than 100 μV in 5 out of 10 trials in muscle [45]. It is called resting (rMT) or active (aMT) motor threshold dependent on whether the tested muscle is at rest or at an isometric contraction during TMS. Silent period (SP) is defined as the duration of interruption of electromyographic activity of a moderately tonically active muscle (40- 50% of maximum contraction) when the contralateral motor cortex is stimulated by TMS intensities of 110%–160% of motor threshold [46]. Short-Latency Afferent Inhibition (SAI) refers to the suppression of the amplitude of a MEP produced by a conditioning afferent electrical stimulus applied at the median nerve at the wrist approximately 20 ms prior to TMS of the hand area of the contralateral motor cortex [47].

2.3.1. Previous TMS studies on Alzheimer's disease.

Various studies have assessed the excitability of the motor cortex in Alzheimer's disease by calculating the motor threshold and silent period, in other words, the TMS parameters that more directly reflect cortical excitability and cortical inhibition respectively. Systematic research in this field was started in the 90's by Perretti et al. [48]. Their results pointed towards an increased resting motor threshold in the patient group, demonstrative of decreased cortical excitability in AD. De Carvalho et al. [49] in 1996 presented different results to those of Perreti et al. showing a decrease instead of increase in motor threshold of the patient group.

Pepin et al. [50] in their study in 1999 found that both TMS parameters were significantly reduced in AD patients when compared to normal individuals leading to the conclusion that there is an increased excitability of motor cortex in Alzheimer's disease. Similar results to the study of Pepin et al. in terms of motor threshold can be seen in the study by Pennisi et al. [51] in 2002 and the study of Ferreri et al. [52] in 2003.

However, in the study performed by Nardone et al. [53] in 2008 both rest and active motor threshold were found to be increased in the group of AD patients compared to controls, even though this increase did not reach statistical significance. Apart from motor threshold, short latency afferent inhibition (SAI) was also examined as an indicator of the function of the cholinergic pathways in the motor cortex. The amount of SAI was significantly smaller in early AD patients than in controls leading to the conclusion that a central cholinergic dysfunction occurs in the earlier stages of AD. The results of Nardone et al. were in accordance with those of older studies conducted by Di Lazzaro et al. [54, 55, 56,57]. The latter explored the excitatory and inhibitory neuronal pathways that control the motor cortex function in AD patients and the role of the cholinergic system by performing their experiments under pharmacological manipulation with an acetylcholinesterase inhibitor.

More recently, Khedr et al. [58] in 2011 conducted a TMS study in AD patients categorizing them by the stage of the disease and evaluating the motor threshold and silent period. A significant positive correlation was noted between the rest and active motor threshold and disease progression as rMT and aMT were both increased in mild dementia and significantly decreased in advanced AD compared to normal subjects. The correlation with the evolution of the disease was negative for the silent period. Given these particular results, the investigators assumed that advanced AD is associated with hyperexcitability of the motor cortex and they attributed an important role to certain neurotransmitters as γ-aminoboutyric acid (GABA) and Glutamate.

CHAPTER 3.

THE STUDY

It is evident from the previous chapter that the neurophysiological TMS studies which have investigated the function of the primary motor cortex in Alzheimer disease, give conflicting evidence regarding the excitability of the cortical motor areas and the related neuronal pathways that lie beneath them. Some studies point towards a general increase in excitability of the motor cortex in AD [49, 50, 51, 52] while others [48] exhibit the opposite result suggesting a decrease in cortical excitability and there are yet others [58], which correlate the excitability of the motor cortex to the stage of Alzheimer's disease. Possible reasons for the variability of the results in these studies could be the different methodologies used by different investigators, the technical restrictions of each study and also the heterogeneity of the participating patients in terms of the progression of the disease.

Given that Alzheimer's disease is an evolutionary process, the modifications in neuropathology and biochemistry during its course could have a very significant effect upon the excitability of the primary motor cortex in each stage of the disease.

In a previous study where TMS was applied on severely affected patients [48] the observed modulation of cortical excitability was attributed to cortical atrophy. It has been suggested that cortical atrophy increases the distance between the site of the stimulation on the scalp and the TMS activated pyramidal cells of the primary motor area leading to an increased motor threshold. However, this is not the case when such TMS studies are performed in patients suffering from mild and moderate Alzheimer's disease. In the early stages no measurable cortical atrophy is evident particularly in the motor areas. It is known from the literature [19] that the primary motor cortex is free from senile plaques and neurofibrillary tangles in mild AD.

Interestingly enough some experimental paradigms [53, 54, 55, 56, 57,] have attributed a regulating role to certain brain neurotransmitters such as acetylcholine, GABA and glutamate, upon the motor control of AD patients. However, a clear hypothesis about the specific neuronal circuits involved has not been formed.

3.1. THE AIM OF THE STUDY

Our study was motivated by the clinical observations of altered motor behaviour in AD patients at the mild stage of the disease. As cortical atrophy is not implicated, the changes in cortical excitability rely probably on dysfunctional neuronal pathways. The aim of the study was:

1. To identify and confirm changes in the function of the motor cortex in early AD patients.
2. To provide an explanation for these changes revealing the responsible pathophysiological mechanisms

The question that our research was seeking to answer is: “Could some specific neuronal pathways which utilize certain brain neurotransmitters be at the root of the altered motor behaviour that the AD patients exhibit at the early stage of the disease?”

3.2. THE METHODOLOGY

In order to carry out our study we chose the method of Transcranial Magnetic Stimulation (TMS) because of the many advantages it offers, in terms of accessibility, safety, validity and convenience for the patient. The prospect of comparing our results with previous TMS studies which provided conflicting evidence about motor control in AD was indeed challenging.

Our study consists of three experiments, a main TMS experiment which answers our basic research question and two subexperiments to verify and enhance the validity of our main experimental body.

Considering the importance of the cholinergic hypothesis in the pathogenesis of Alzheimer's disease, we decided to perform our main TMS experiments on a group of AD patients at the early stage of the disease. First, we examined them before the administration of any treatment and then after treating them with an acetylcholinesterase inhibitor (donepezil). In this way we would be able to observe the possible effect that the cholinergic intervention exerts upon the function of motor cortex in early AD. More importantly, we would be able to form a hypothesis about the underlying neuronal pathways which determine the function of motor cortex and affect the motor behaviour of the patients.

In the subexperiment 1, we examined the effect of another pharmaceutical substance (memantine) upon motor cortex applying the same TMS experimental procedure in another smaller sample of early AD patients. As memantine acts through a different neuronal pathway than the cholinergic one, this subexperiment would provide further evidence for the specific role of certain neuronal pathways in the regulation of the motor control in Alzheimer's disease.

In the subexperiment 2 we examined whether the changes in the function of motor cortex displayed at the early stages of AD have a clinical implication in motor behaviour. In a group of early AD patients, distinct from the one we had used in our main TMS experiment, we examined the motor reaction time after a visual stimulus was given to the patients. The experiment was performed before and after treating the patients with donepezil and the results were compared with a group of normal individuals separate than the one we used for our TMS experiment.

All our experiments were carried out in CHR Citadelle in Liege.

3.3. THE EXPERIMENTS

An analytical description of the 3 experiments carried out in the study can be found below, each followed by a small discussion of the results.

3.3.1. MAIN TMS EXPERIMENT

A. Patients

The sample for our study was selected from patients from the outpatient memory clinic of the neurological department. It included patients who were diagnosed with probable Alzheimer's disease based on the criteria of the National Institute of Neurological and Communicative Disorders and Stroke – Alzheimer's disease and Related Disorders Association. According to the Clinical Dementia Rating Scale, they were in the early stage of the disease. The Mini-Mental State Examination (MMSE) was performed to all patients. Any patient who exhibited signs of other neurological diseases was excluded from the sample. The same exclusion was applied to anyone who was under pharmaceutical therapy with medications which could have a possible impact on cognitive function or could in any way affect the excitability of the nervous system.

We finally recruited thirteen (13) patients, six (6) men and seven (7) women. Their median age was 75 years (with limit ages 54-83 y) and they were all right-handed. For all the recruited patients the neurological examination of the motor system was normal. Their median MMSE score was 24 (limits: 18-26) (table 1), thus being classified as suffering from mild AD. The patients were generally independent when dealing with the basic activities of daily life even though some more complex activities were impaired. All patients were examined with a brain MRI which excluded the presence of cerebral vascular lesions or any cause of reversible dementia.

The control group that we recruited for our study consisted of thirteen (13) age-matched normal subjects, six (6) men and seven (7) women, (median age: 72

years with limit ages 55-82 y). They were all right–handed. They had no history of neurological disease and their neurological examination was completely normal.

B. Methodology

We performed our experiment in two phases. In the first phase, all AD patients were tested before any treatment was started. Immediately after the first phase, all patients received treatment with the cholinesterase inhibitor donepezil administered orally at 10 mg/day after the required titration. In the second phase of the experiment the whole procedure was repeated after 2 months of treatment in all patients except one (patient 11) who was lost to follow-up.

The protocol was approved by the local ethic's committee and, after being provided with detailed information about the aspects and the goal of the study all patients and normal subjects were willing and able to participate in the electrophysiological procedure.

Prior to the electrophysiological examination, the patients and normal subjects were seated comfortably on a chair in a quiet examination room. A Nicolet Viking IV IES 405-1 EMG machine was used to record the compound muscle action potential (Cmap) of the right abductor digiti minimi in order to measure various parameters of motor evoked potential induced by TMS. TMS was applied by a Magstim 200 stimulator (Magstim Ltd., Withland Dyfed, UK) in accordance with the consensus guidelines [60].

For our TMS experiment we used a circular coil with a diameter of 9 cm which was centered at the vertex of the scalp. The circular coil was chosen as opposed to a more focal eight-shaped so as to reduce the duration of the whole procedure and the related possible discomfort felt by the individuals tested. The centre of the coil was measured on the line between the nasion and the inion point in each patient to ensure the reproducibility of the procedure from the first session to the second one performed 2 months later. The current in the coil was circulating counterclockwise.

The subjects were asked to perform a slight isometric voluntary contraction of the right abductor digiti minimi at around 20% of the maximum voluntary muscle contraction during the application of the TMS. Both auditory and visual feedbacks of EMG activity were used to ensure the accuracy of the procedure.

We measured two TMS parameters, the active motor threshold (aMT) and the silent period (SP).

Active motor threshold (aMT)

Active motor threshold is defined as the minimal intensity of cortical stimulation, which produces MEPs greater than 100μV and approximately to 200 μV in 50% of the consecutive trials during isometric contraction of the tested muscle when the muscle is at about 20% of the maximum voluntary contraction. The aMT was determined by gradually lowering the stimulator output from 60% of the maximal output.

As mentioned before, motor threshold is a neurophysiological TMS parameter which reflects the excitability of motor cortex. Its measurement in patients with Alzheimer's disease can give very important information about the way this neurodegenerative disease affects the function of the cortical motor areas and consequently alters the motor behaviour of the patients.

We chose to perform our experiment measuring the active motor threshold instead of the resting motor threshold (rMT) in order to avoid any technical errors arising from possible difficulties that patients with Alzheimer's disease could exhibit in maintaining absolute rest in their muscles during the experimental procedure.

Silent period (SP)

Once the active motor threshold was determined we proceeded with the calculation of the silent period (SP) applying single-pulse TMS in the same groups of AD patients and normal subjects.

Silent period is defined as the duration of interruption of electromyographic activity which follows the motor evoked potential (MEP) elicited in a muscle sustaining isometric voluntary contraction after the application of TMS in the

contralateral primary motor area (M1). Despite the inter-individual and intraindividual variation, the usual duration of the silent period in healthy individuals is around 200-220 msec.

It is assumed that the early part of the silent period is mediated by spinal mechanisms while the latter part is mediated by mechanisms interacting at the cortical level. The duration of the first part of the SP called the 'spinal' SP is almost always stable at around 50 -70 msec. It is ascribed primarily to multiple segmental mechanisms, including Renshaw cell recurrent inhibition, and activation of inhibitory Ia interneurons of the spinal level. The second part is determined by the interaction between interneurones at the cortical level which activate cortical inhibitory mechanisms. It is more variable in duration and is considered as the 'cortical' silent period [62].

Silent period is considered as a direct indicator of cortical inhibition. It also provides useful evidence about the pathophysiological mechanisms that regulate cortical inhibition when measured in subjects under pharmacological agents which affect basic neurotransmitters such as acetylcholine or glutamatergic acid [53, 59].

However, SP has not been extensively studied in AD patients until now. The main reasons for this were the considerable inter-individual variation of SP duration, the high degree of intraindividual asymmetry and the variable results when different examiners apply the TMS in the same subject [61]. It should also be noted that some patients with Alzheimer's disease have difficulty collaborating efficiently. In our study we have tried to lessen the impact of these restricting parameters by recruiting AD patients in the early stage of the disease. Furthermore, in order to secure good collaboration and to avoid even subtle differences in the application of TMS, the same examiner was always used to carry out the procedure in both groups of patients and normal subjects.

In our experiment we adapted the most widely used technique to determine SP. Immediately after the measurement of the aMT we raised the intensity of the output of the magnetic stimulator to 150 % of the motor threshold. Some seconds before the electromagnetic current was applied by the stimulator, the patients were

asked to perform a voluntary contraction of the targeted muscle (abductor digiti minimi) of about 50 % of the maximal voluntary contraction assessed by amplitude of the EMG response. The base time of the recordings was 500 milliseconds.

The duration of the silent period is one of the main parameters used to assess cortical inhibition. SP duration is independent from the level of baseline EMG contraction, it increases with the strength of stimulation and is defined as the difference between SP onset and SP offset. However, the methods used to determine the onset and offset of SP vary widely in different studies. SP onset has been defined by: a) the onset of TMS, b) the MEP onset, c) the MEP offset, or d) when electromyography (EMG) drops below the volitional pre-TMS EMG level. Similarly, the SP offset has been defined by: a) the first return of any volitional EMG, b) the absolute return of EMG to the pre-TMS level, or c) when EMG no longer significantly differs from pre-TMS EMG level [63, 64].

The evaluation of the whole SP duration is also difficult to determine as sometimes it can be temporarily interrupted by an electromyographic breakthrough of short duration and low amplitude. This EMG breakthrough is called late excitatory potential (LEP) [65] and its appearance could lead to a miscalculation of the true duration of silent period. Sometimes, several LEPs can scatter the SP in a lot of valleys before the final return of full electromyographic activity.

In our study we chose to measure the SP 'valley' defining the onset of SP by MEP offset and the offset of SP by the return of the electromyographic activity in amplitude which no longer differed from pre–TMS level, and not by the first return of any volitional EMG. In this way we avoided errors in our calculation of SP caused by possibly measuring false shorter valley duration due to the appearance of late excitatory potentials which would be incorrectly taken as the return of EMG activity. The duration of the silent period was analyzed as well as the presence or absence of LEPs. In the cases where LEPs appeared, their latency, amplitude and duration were also calculated.

C. Results

All the results of the active motor threshold and silent period in the group of normal subjects and the group of AD patients were compared and statistically analyzed using the unpaired Student T test. In addition, we investigated a possible correlation between the MMSE score and the motor threshold in untreated AD patients using the Pearson correlation test.

Results of the Active motor threshold (aMT)

The results of each AD patient's MMSE scores and active motor threshold before and after donepezil are summarized in Table 1.

Table 1: *Description of age, MMSE score and Active Motor Threshold (aMT) in the studied patients with mild AD.*

Patient	Age, years	MMSE score before donepezil	MMSE score after donepezil	aMT before donepezil	aMT after donepezil
1	73	26	26	34	34
2	83	21	25	33	30
3	58	26	28	33	35
4	66	24	23	42	27
5	75	18	18	40	40
6	81	23	22	37	33
7	70	23	24	39	36
8	74	25	27	38	35
9	75	21	22	33	30
10	81	24	25	37	37
11	81	25	NA	47	NA
12	54	26	26	37	32
13	72	21	22	34	30

In the group of normal subjects the mean active motor threshold (aMT) was 32.8 % (SD +/- 7.7).

For the AD patients before treatment with donepezil the mean aMT was 37.6 % (SD +/- 3, 9) .

The observed difference in the aMT between normal subjects and AD patients before treatment was statistically significant ($p < 0.05$).

For the 12 AD patients who underwent the experiment after being treated for 2 months with donepezil the mean aMT was 33.5 % (SD +/- 3.5).

The difference in the aMT between normal subjects and treated AD patients was not statistically significant. The observed difference in the aMT in AD patients before and after treatment with donepezil was statistically significant ($p < 0.05$), regardless of the inclusion or exclusion of patient 11 (Figure.4).

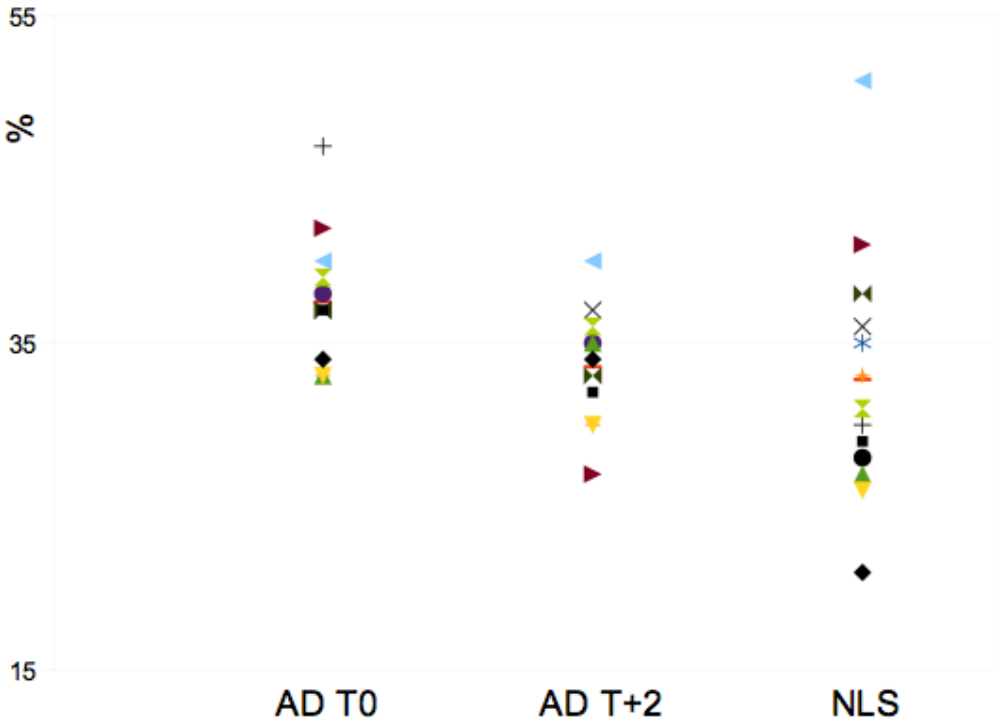

Figure 4. *Distribution of individual values of aMT (expressed in %) in AD patients before treatment with donepezil (AD T0), in AD patients after 2 months of donepezil treatment (AD T+2) and in normal subjects (NLS).*

Similarly, the mean MMSE score of the treated AD patients was slightly increased (24.00 ± 2.76) compared with the MMSE score of these patients before treatment (23.6 ± 2.40). However, this difference was not so great as to reach statistical significance.

When a Pearson correlation test was performed between the MMSE value and the value of the aMT in untreated patients, the obtained R showed a weak value of 0.04. Nevertheless, this was a positive value indicating a trend of the aMT to increase with the MMSE score (Figure.5).

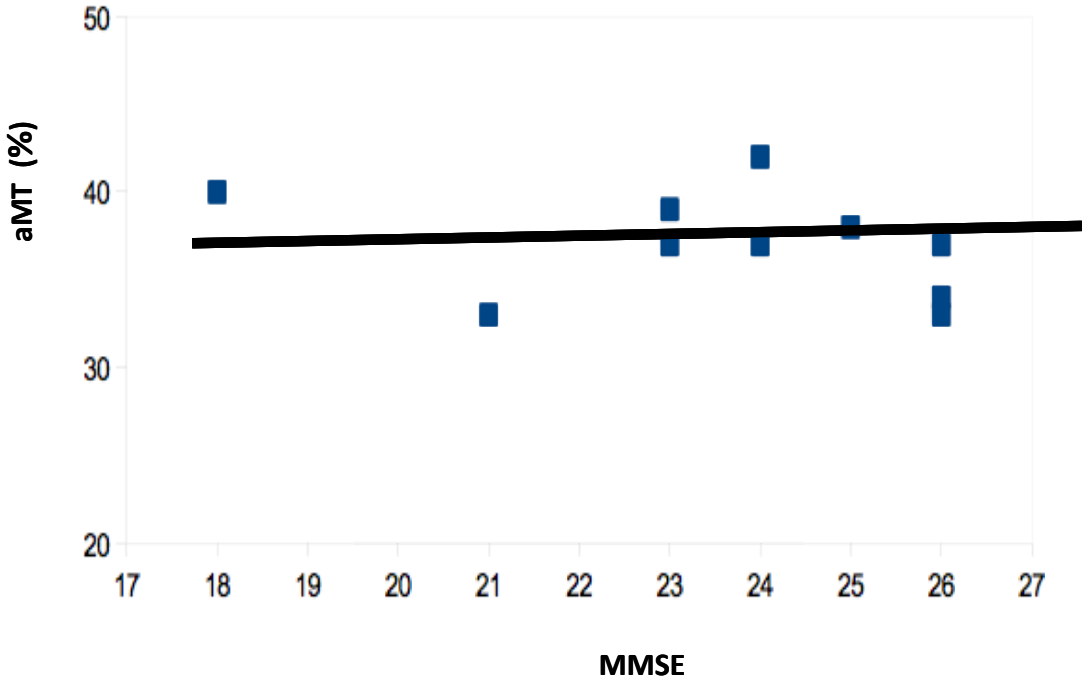

Figure 5: *Relationship between values of MMSE in untreated AD patients and the aMT. A weak positive correlation (R = 0.04) was found.*

Results of the Silent period (SP)

The results concerning the Silent period (SP) for each group of subjects are presented below, starting with the results for the control group, continuing with the results for the AD patients before treatment and ending with the results for the AD patients after treatment.

Control group - normal subjects:

For the control group (Table 2.) the EMG following TMS generally showed the pattern of a MEP at a latency of around 20 ms followed by a silent period of variable

duration. LEP was observed in 1/13 subjects (7%). For the one subject (L. 70 y.o.) who presented LEP the duration of silent period was 229 ms.

The mean latency of the LEP was 67 ms, the amplitude was 487 μV and the duration was 111 ms These results, concerning the latency of the LEPs, were within the range described by the related literature [65, 66]. For the normal subjects the mean (+/- SD) duration of SP was 107.5 +/- 42.2 ms. When subject L. was included, the duration of SP was 116.8 +/- 52.6 ms.

Table 2. *SP duration and LEPs for normal subjects (control group).*

Normals	**LEP**	**SP duration (ms)**	**LEP duration (ms)**	**LEP amplitude (μV)**	**LEP latency (ms)**	**SP duration if LEP present (ms)**	**SP duration if LEP absent (ms)**
1	NO	125					125
2	NO	132					132
3	NO	58					58
4	NO	122					122
5	NO	187					187
6	NO	89					89
7	YES	229	111	487	67	229	
8	NO	47					47
9	NO	71					71
10	NO	145					145
11	NO	136					136
12	NO	116					116
13	NO	62					62
Mean		116.8	111	487	67	229	107.5
SD		52.6					42.2

Patients group - AD patients before treatment

For the AD patients before treatment with donepezil (Table 3.), the mean duration of SP was 186.4 +/- 88.2 ms.

Table 3. *SP duration and LEPs for AD patients before treatment with donepezil.*

AD before treatment	LEP	SP duration (ms)	LEP duration (ms)	LEP amplitude (μV)	LEP latency (ms)	SP duration if LEP present (ms)	SP duration if LEP absent (ms)
1	YES	262	18	1100	46	262	
2	NO	45					45
3	YES	182	27	798	112	182	
4	NO	86					86
5	YES	162	32	812	98	162	
6	YES	176	42	625	61	176	
7	YES	364	55	563	181	364	
8	YES	173	86	650	62	173	
9	NO	95					95
10	YES	238	74	1800	94	238	
11	YES	223	68	625	102	223	
12	YES	285	49	368	134	285	
13	NO	132					132
Mean		186.4	50.1	815.7	98.9	229.4	89.5
SD		88.2	22,8	420.5	41.5	66.3	35.7

The obvious increase observed in SP duration by comparison to normal subjects was proved significant statistically ($p < 0.001$).

In 9 out of 13 Alzheimer's disease patients (69%) LEPs were evident. The mean latency of the LEP was 98.9 +/- 41.5 ms, the mean amplitude was 815.7 +/- 420.5 μV and the mean duration was 50.1 +/-22.8 ms.

In the 9 patients who presented LEP the SP valley appeared fragmented in multiple segments before the true return of full electromyographic activity. In these patients the mean duration of the SP was 229.4 +/- 66.3 ms. A statistically significant difference was established by comparison to the duration of SP in normal subjects ($p<0.001$).

For the 4 patients who did not exhibit any LEP, the mean duration of the SP was 89.5 +/- 35.7 ms, a result which did not reach significant difference statistically compared to normal subjects.

Patients group - AD patients after treatment

For the patients after treatment with donepezil (Table 4.) the mean duration of the silent period was 150.5 +/ 85.0 ms. There was no statistically significant difference with the duration of SP of normal subjects or with that of the AD patients before treatment.

LEPs occurred in 5 out of 12 patients (42%) with a mean latency of 96.8 +/- 29.2 ms, mean amplitude of 860.4 +/- 225.9 μV and a mean duration of 46.6 +/- 21.9 ms. There was no statistically significant difference in latency, amplitude or duration of the LEPs between the patients before and after treatment with donepezil.

In the 5 patients who presented LEP, the mean duration of the SP was 221.2 +/- 87.1 ms which was not significantly different from the duration of SP in AD patients with LEP before treatment.

In the 7 patients without LEP, the mean duration of the SP was 90,6 +/- 28.6 ms which was also not significantly different compared to the 4 patients without LEP before treatment and to the normal subjects.

Table 4. *SP duration and LEPs for AD patients after treatment with donepezil.*

AD after treatment	LEP	SP duration (ms)	LEP duration (ms)	LEP amplitude (μV)	LEP latency (ms)	SP duration if LEP present (ms)	SP duration if LEP absent (ms)
1	YES	274	52	662	137	274	
2	NO	112					112
3	YES	139	23	854	73	139	
4	NO	140					140
5	YES	261	36	614	102	261	
6	NO	54					54
7	YES	214	41	1120	64	214	
8	NO	68					68
9	YES	284	81	1052	108	284	
10	NO	94					94
12	NO	86					86
13	NO	80					80
Mean		150.5	46.6	860.4	96.8	221.2	90.6
SD		85.0	21,9	225.9	29.2	87.1	28,6

In the figures below there is an illustration of examples of SP in a normal individual (Figure 6.a.) and in two untreated AD patients where LEPs are present (Figure 6.b.).

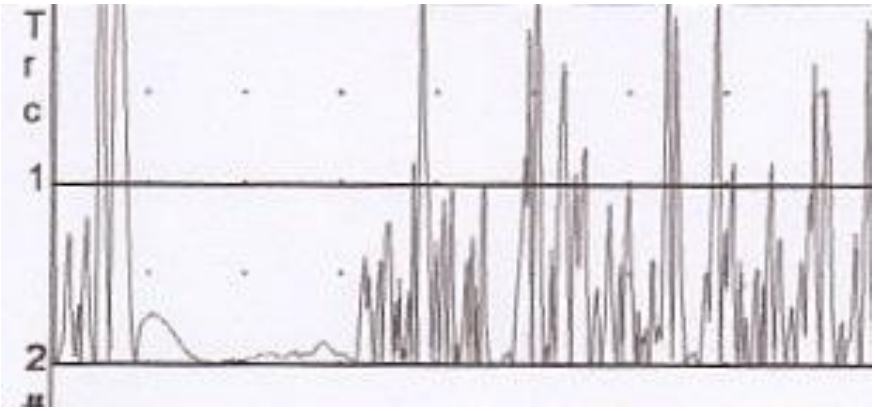

Fig.6a. *SP in a normal individual (base time: 50 ms/division; amplitude: 200 µV/division).*

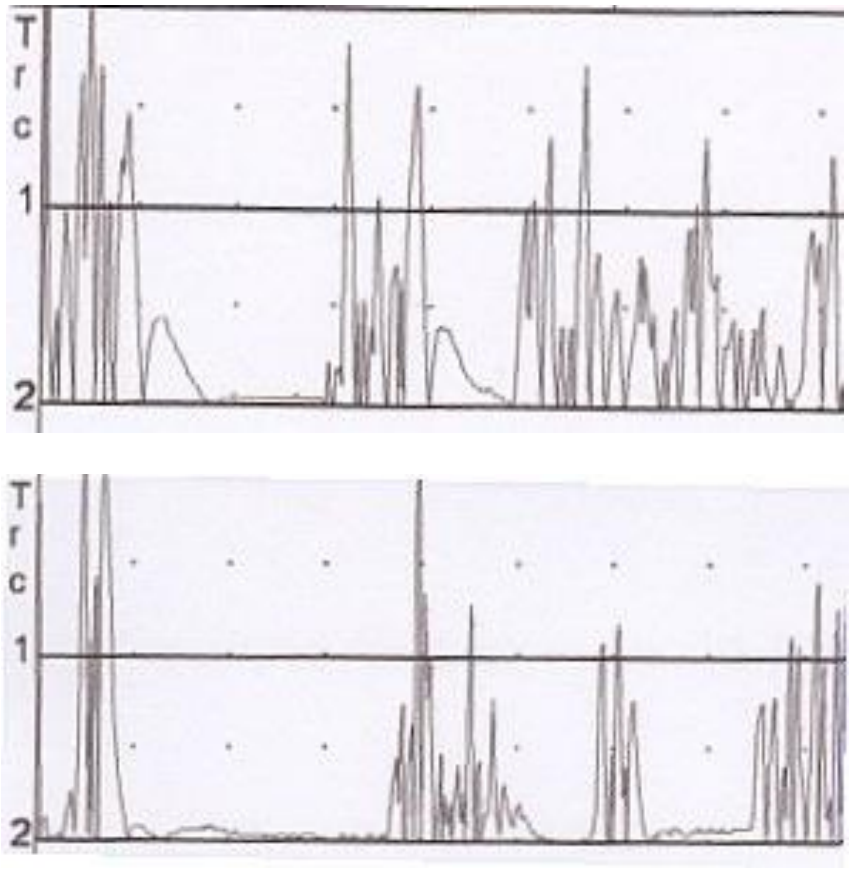

Fig.6b. *SP with presence of LEPs in two untreated AD patients (base time: 50 ms/division; amplitude: 200 µV/division).*

After performing our TMS experiment in the early AD patients under pharmacological manipulation with donepezil, the results showed a statistically significant difference for active motor threshold between AD patients and normal subjects and also between untreated AD patients and those treated with donepezil. Notably, an increase in the active motor threshold for the early AD patients who had never received any treatment was observed in comparison with normal subjects of the same age.

After 2 months of treatment with 10 mgr of donepezil the motor threshold decreased significantly in the group of Alzheimer's disease patients resembling the active motor threshold of the normal patients. Also a difference was noted in the duration of the silent period between AD patients before treatment with donepezil and normal individuals of the same age. In early AD patients the SP valley was found to be significantly longer in duration and also scattered with multiple LEPs when compared to the control group. After treatment with donepezil both the duration and shape of the SP resembled that of the control group more.

Taking into consideration that the motor threshold reflects the excitability of the motor cortex, the increased aMT indicates a decreased cortical excitability in the early stage of Alzheimer's disease. The restoration of aMT after treatment with donepezil to levels close to those of the control group raised the issue of an important effect of the cholinergic mechanism of action of donepezil upon cortical excitability.

3.3.2. SUBEXPERIMENT 1.

The statistically significant decrease in the active motor threshold of early AD patients after receiving the achetylcholinesterase inhibitor donepezil enabled us to assume the existence of a cholinergic mechanism in the regulation of cortical excitability in early AD. Thus, we performed a supplementary experiment with a smaller group of patients in the early stage of Alzheimer's disease who, instead of being treated with donepezil, would be treated with memantine for 2 months. In this way we could observe the subsequent results of cortical excitability after the

prescription of a medication with a different mechanism of action from that of an achetylcholinesterase inhibitor. Unlike donepezil, which exerts its action by directly affecting the cholinergic system of the brain, memantine exerts its action in demented patients by mainly affecting the neurotransmitter glutamate.

It is hypothesized that in the etiology of Alzheimer's disease a dysfunction of glutamatergic neurotransmission is involved, manifested as a neuronal excitotoxicity due to the excess of glutamate in the brain. Consequently, the targeting of the glutamatergic system and specifically the NMDA receptors represents a new therapeutic option for AD. Memantine is an NMDA receptor antagonist which binds to NMDA receptors on brain cells and blocks the activity of glutamate, therefore protecting neurons from excitotoxicity. In addition to its main mode of action, memantine also exerts an effect on the cholinergic system of the brain being an antagonist at alpha-7 nAChR. This could explain the initial worsening of cognitive function during early memantine treatment. However, alpha-7 nAChR up-regulates quickly in response to antagonism, which could explain the cognitive-enhancing effects of chronic memantine treatment.

Taking into consideration the mode of action of memantine, the performance of our TMS experiment after treating early AD patients with this medication would lead to conclusions about the effect that certain neurotransmitters have on the function of the motor cortex. Also the comparison of the results of this sub-experiment with those obtained by the experiment conducted under donepezil treatment might enable us to form a better understanding of the neuronal circuits involved in the regulation of the primary motor cortex.

A. Patients and Methodology

For our experiment with memantine we recruited five (5) more AD patients, four (4) women and one (1) man. The median age was 74 years (limit ages: 68-75).The patients were in the early stage of the disease with a median MMSE score of 24 (limits: 21-26).

We applied single-pulse TMS using the same methodology as in our experiment with donepezil performing the procedure in two phases, before and after treatment with memantine. We also calculated the same TMS neurophysiological parameters of active motor threshold and the silent period.

As control group for this sub-experiment we used the same group of normal patients that was used in our experiment with donepezil.

The subsequent results of this experiment between the group of normal subjects and the group of AD patients were also compared and statistically analysed by using the unpaired Student T test.

B. Results

Results of the Active motor threshold

The mean active motor threshold (aMT) of the 5 AD patients before the initiation of treatment with memantine was 38,6 (SD +/- 5,94).

After treatment with memantine for two months the mean aMT was 39, 2 (SD +/- 3, 96).

The observed difference in the aMT between normal subjects and AD patients before treatment with memantine was statistically significant ($p < 0.05$).

No statistically significant difference was observed in the results regarding active motor threshold for the AD patients before and after treatment with memantine.

Results of the Silent Period

AD patients before treatment

The duration of the silent period for the 5 AD patients before treatment with memantine was 245, 8 +/- 83,7 ms. (Table 5).

In comparison with the duration of SP in the group of normal subjects, this observed increase reached statistical significance ($p<0, 05$). However, we have to take into consideration the limitations of the small number of participants in the AD group in our interpretation of the results.

3 out of the 5 patients presented LEP ,with a mean duration of 51 +/- 5, 57 ms, a mean amplitude of 1250,67 +/- 279,39 µV and a mean latency of 117, 33 +/- 48,69 ms. In these 3 patients the SP appeared fragmented and its duration was 289 ,66 +/- 75,95 ms.

For the 2 patients who did not display LEP, the duration of SP was 180 +/- 45,25 ms.

Table 5: *results of aMT, SP duration and presence of LEPs in AD patients before treatment with memantine.*

Patients	Age	MMSE	Threshold	SP Duration	LEP	SP Duration, LEP present	SP Duration LEP absent	LEP Duration	LEP Amplitude	LEP Latency
1	75	24	31	253	1	253		56	1542	132
2	68	25	44	212	0		212			
3	75	23	38	377	1	377		45	985	157
4	71	26	45	148	0		148			
5	74	21	35	239	1	239		52	1225	63
Mean	72.6	23.8	38.6	245.8		289.7	180	51	1250.7	117.3
SD	3.05	1.9	5.9	83.7		75.9	45.2	5.6	279.4	48.7

AD patients after treatment

The duration of the silent period for the 5 AD patients after treatment with memantine was 238, 2 +/- 43, 7 ms. (Table 6.).

There was no significant difference in the duration of SP between untreated patients and those treated with memantine.

4 out of 5 AD patients presented LEP after treatment with memantine with a mean duration of 51,25 +/- 8, 47 ms, a mean amplitude of 1151,25 +/- 278,26 µV and a mean latency of 113, 33 +/- 24,067 ms. No significant difference is observed concerning the mean duration, latency and amplitude of LEP between untreated AD

patients and those treated with memantine. The duration of SP for the 4 patients who presented LEP was 244, 25 +/- 48 ms. For the one patient who did not display LEP the duration of SP was 214 ms.

Table 6: *results of aMT , SP duration and presence of LEPs in AD patients after treatment with memantine.*

Patients	Age	MMSE	Threshold	SP Duration	LEP	SP Duration LEP present	SP Duration LEP absent	LEP Duration	LEP Amplitude	LEP Latency
1	75	23	33	236	1	236		62	1024	120
2	68	25	42	214	0		214			
3	75	25	40	301	1	301		53	850	142
4	71	24	43	255	1	255		42	1236	105
5	74	23	38	185	1	185		48	1495	85
Mean	72.6	24	39.2	238.2		244.25		51.2	1151.2	113
SD	3.05	1	3.96	43.7		48		8.4	278.3	24.7

The results of the Subexperiment 1 show that memantine intake does not have any effect upon active motor threshold or the silent period of early AD patients. These results combined with the results by our experiment with donepezil, show that in early AD patients the excitability of the primary motor cortex is regulated through cholinergic neuronal pathways.

3.3.3. SUBEXPERIMENT 2.

As the motor threshold reflects cortical excitability, our experimental finding of decreased motor cortical excitability in early AD was in accordance with what we had clinically observed in these patients in terms of diminished movement. In order to accumulate further evidence in our TMS study about the alertness of the

motor cortex to produce a movement in early Alzheimer's disease we decided to perform another experiment not linked to TMS. This would provide more direct answers about the ability of the motor cortex of early AD patients to provide a motor reaction. We also performed this experiment under the pharmacological manipulation of donepezil, keeping in line with our standard procedure so far. In this way we could further examine our early observations that the 'hypomovement' which is clinically present in early AD is linked to a decreased excitability of the primary motor cortex and is probably influenced by the cholinergic system.

Thus, we developed an experiment where we studied the reaction time after giving a simple visual stimulus to a group of early AD patients and we compared them to a group of normal individuals. We specifically measured the single motor reaction time (sRT) and the single muscular movement time (sMT).

A. Patients

We recruited eight (8) more AD patients, two (2) men and (6) women. Their mean age was 69 years (limit ages: 62-81). They were all in the initial stage of their disease with a median MMSE of 26 (limits 24-29).

The control group consisted of fifteen (15) normal individuals, six (6) men and eight (8) women with a median age of 71 years (limit ages 63-77).

B. Methodology

We applied the same inclusion criteria in the patients group which had been applied to the patients of the previous TMS experiments so, we ensured that the patients did not suffer from any other neurological disease, their neurological examination was completely normal, they had a normal brain MRI and they were not under treatment with any medication that could possibly have an effect on the central nervous system or affect the cortical excitability in any way.

All patients and all normal subjects agreed to participate in the experiment and their consent was approved by the local ethics committee. We performed the

experiment before any treatment was started and also after 2 months of treatment with 10 mgr of donepezil and we compared the results.

We assessed Simple reaction time (sRT) by using a random visual stimulus. Patients and normal subjects were comfortably seated in a chair and they placed their right index finger on a button. A surface electrode was stuck on the skin above the body of the extensor indicis proprius muscle in order to record the electromyographic activity produced by the movement of this muscle which moves the index finger. In this way the simple muscular movement time (sMT) could be determined. This experiment was performed with the same Nicolet Viking IV IES 405-1 EMG machine previously used for our TMS experiments.

The patients and normal subjects were instructed to push the button as quickly as possible after a randomly computer generated visual red flash. The latency for the movement response (sRT) and the latency for the electromyographic response (sMT) were both recorded by a CED1401 system.

C. Results

The mean simple reaction time (sRT) was 207, 3 +/- 25, 5 msec in normal subjects. (Table 7.)

The mean sRT was 253, 0 +/- 32, 5 ms in AD patients before treatment with donepezil was initiated (Table 8.)

The difference in sRT between these two groups reached statistical significance ($p < 0,001$).

The mean sRT was 229, 9 +/- 30, 6 ms in AD patients after treatment with donepezil. A statistically significant difference between the treated and untreated patients ($p < 0,002$) was established.

The difference in sRT between not treated with donepezil patients and normal subjects was even more significant statistically ($p < 0,001$).

The mean single muscular movement time (sMT) in normal subjects was 159,3 +/- 29,3 msec.

The mean sMT for the untreated patients was 203, 8 +/- 29, 0 msec. The difference by comparison to normal subjects was very significant statistically ($p < 0,001$).

The mean sMT was 180, 8 +/- 32, 2 msec in AD patients after 2 months treatment with donepezil. a statistically significant difference in sMT between the treated and untreated AD patients ($p<0,001$) and between treated AD patients and normal subjects ($p< 0,001$) was established.

Tables 7 and 8 summarize the results of subexperiment 2.

Table 7: *Simple reaction time and simple movement time in the group of normal subjects*

	Sex	**Age**	**sRT**	**sMT**
1	F	76	245	198
2	M	71	220	175
3	M	68	204	154
4	F	77	251	199
5	F	70	200	154
6	M	77	205	161
7	M	68	168	147
8	F	66	174	130
9	M	72	189	140
10	F	76	202	142
11	M	73	248	201
12	F	72	221	172
13	F	71	200	141
14	F	67	184	132
15	F	63	199	144
Mean		71.33	207.33	159.33
SD		4.24	25.48	24.31

Table 8: *Simple reaction time and simple movement time in the group of AD patients, before and after treatment with donepezil.*

	Sex	**Age**	**MMSE before donepezil**	**MMSE After donepezil**	**sRT before donepezil (SD)**	**sRT after donepezil (SD)**	**sMT before donepezil (SD)**	**sMT after donepezil (SD)**
1	F	66	24	24	278.28 (32.420	217.97 (11.97)	233.74 (25.55)	169.72 (22.33)
2	M	75	25	24	255.46 (31.35)	226.78 (36.45)	215.68 (28.45)	191.23 (30.58)
3	F	81	29	28	240.45 (49.38)	239.18 (66.03)	197.93 (36.22)	183.08 (56.56)
4	M	68	26	27	239.67 (19.56)	224.76 (22.62)	192.45 (25.39)	172.76 (23.74)
5	F	70	26	27	244.04 (29.8)	236.75 (17.8)	211.15 (31.2)	198.6 (26.99)
6	F	62	27	26	221.69 (23.79)	224.76 (22.62)	168.31 (23.95)	170.69 (22.37)
7	F	74	25	26	285.15 (39.52)	256.73 (39.01)	196.14 (35.56)	174 (37.4)
8	F	64	29	27	259.69 (34.46)	212.37 (28.29)	215.03 (25.66)	286.31 (37.47)
Mean		70	26.37	26.12	253.05 (32.53)	229.91 (30.59)	203.8 (28.99)	180.79 (32.18)

The results of Subexperiment 2, which show significantly increased sRT and sMT in early AD patients compared to normal subjects of the same age, confirm the clinical observation of decreased mobility in early AD patients. The improvement of sRT and sMT after donepezil intake supports a cholinergic modulation of the alertness of the primary motor cortex in the early stage of Alzheimer's disease.

CHAPTER 4.

DISCUSSION

4.1. THE ALTERED EXCITABILITY OF THE MOTOR CORTEX IN EARLY AD

The results of our TMS experiments show a difference in the active motor threshold of patients in the initial stage of Alzheimer's disease in comparison to age-matched normal subjects. Given that the motor threshold is a direct reflection of cortical excitability our experimental study indicates a modulation in the excitability of the primary motor cortex in early AD. Our results are in accordance with a fair number of TMS studies performed prior to ours. Regardless of the differences in the various studies in terms of the methodology, the stage of the disease of the participating patients and whether the modulation of cortical excitability increases or decreases, the common factor is a change in the excitability of the motor cortex in Alzheimer's disease. The TMS experimental paradigms, starting from the first study conducted by Perretti et al. [48], continuing with the studies of De Carvalho et al. [49], Pepin et al. [50], Pennisi et al. [51], Ferreri et al. [52] and more recently those of Nardone et al. [53] and Khedr et al. [58], have all shown an alteration in motor threshold in patients suffering from Alzheimer's disease.

In our TMS study we found a statistically significant increase in the active motor threshold in the group of AD patients compared to the control group which is suggestive of a decreased excitability of the motor cortex in early AD. Most of the older studies [49, 50, 51, 52] with the exception of the study of Perreti [48] have shown a decrease in motor threshold leading the investigators to assume an increased excitability of the motor cortex in Alzheimer's disease. This discrepancy between our study and the others can be explained if we look more carefully into some details in the organization of each study. In our experimental paradigm we recruited patients in a very early stage of Alzheimer's disease (mean MMSE score of 24).The majority of the previous studies which identified decreased motor threshold

in Alzheimer's disease had recruited more mixed groups of patients. Pepin et al. [50] and Pennisi et al [51] in their experiments applied single-pulse TMS in a heterogenous group of patients with moderate and advanced Alzheimer's disease. Ferreri et al. [52] included patients with both mild and moderate disease severity.

However, the study conducted by Nardonne et al. in 2008 [53] has shown similar results to ours regarding active motor threshold. Even though the results of Nardonne et al. did not reach the statistical significance of our study, an increase both in resting and active motor threshold was evident in their group of AD patients when compared to normal subjects. This increase was more profound for the active motor threshold (aMT), which is similar to our results.

The study of Khedr et al. [58] supports our results concerning increased active motor threshold in early AD patients. These investigators organized their study by separating their patients into 3 different groups according to which stage of the disease they were classified: mild, moderate or advanced disease. It was revealed that the active motor threshold was increased in the early (mild) stage of AD compared to normal individuals even though the increase did not reach statistical significance. However, their findings clearly showed decreased motor cortical excitability in the initial stages of AD which is in agreement with our experimental results. More interestingly it was shown by Khedr et al. that the initial increase of the active motor threshold noted in the mild stage of AD, was followed by a gradual decrease of this parameter from mild to moderate and finally to more advanced stages. After the early stages, the more the disease progressed the more the motor threshold diminished exhibiting an increase in the excitability of the motor cortex as the disease evolved.

While the results of our study, which are supported by those of Nardonne and Khedr, demonstrate a decrease in the excitability of the motor cortex in the early stages of Alzheimer's disease, other studies performed with more advanced patients [50, 51,52] revealed different results. These, especially the study of Perreti which included severely affected patients, displayed results which showed a significant increase in the excitability of the motor cortex in advanced disease. Thus, it can be concluded that an awareness of the exact stage of the disease of the

patients who participate in any TMS experiment which evaluates motor threshold, and consequently cortical excitability, is crucial. The stage of the disease seems to be determining factor for the modulation of the excitability of the primary motor cortex in Alzheimer's disease. The careful review of the methodology and results of all the previous studies in comparison with ours indicates that AD in the initial stages induces a decrease in the excitability of the primary motor cortex followed by a gradual increase as neurodegeneration evolves. This outcome allows us to conclude that, as regards cortical excitability, Alzheimer's disease is an evolving process. The pattern that it follows resembles the one described for amyotrophic lateral sclerosis (ALS), a neurodegenerative disease which has also been examined for the effect it exerts upon the excitability of the motor cortex. It was found that in ALS the threshold of motor cortex varies throughout the evolution of the disease displaying a pattern of decreased threshold in the initial stages (increased cortical excitability) followed by a significant increase as the disease evolves (decreased cortical excitability) [67]. Thus, even if Alzheimer's disease is not primarily a motor system disease like amyotrophic lateral sclerosis, it seems to affect the excitability of motor cortex in a similar manner to ALS but in terms of qualitative instead of quantitative characteristics. This is an interesting observation which could indicate that the phenomenon of variability in cortical excitability in relation to the state of the disease might possibly be a common factor in other neurodegenerative diseases of the central nervous system as well.

The established conclusion that the alteration of the excitability of the motor cortex in AD is directly connected to the stage of the disease not only presents a new view of Alzheimer's disease as a dynamic evolving process regarding its effect upon the motor areas of the brain, but also expands our understanding of certain clinical aspects of the disease. Our finding of decreased excitability of the primary motor cortex in the early stages of AD can be associated with the clinical observations of the motor behaviour that the patients exhibit at disease onset.

As already stated, the patients in the initial stages of Alzheimer's disease display a pattern of restricted motor behaviour. They exhibit less facial micro expressions when they express emotion or when they participate in a situation that

demands alertness. They are slower both in the initiation of their motor reaction and in the execution of a certain movement when compared to unaffected individuals of the same age. If we combine the results of our study of motor threshold with the model suggested by Brown and Pluck [41] which views goal-directed behaviour as the outcome of a close interaction between cognitive and motor function, we could link the decreased excitability of the motor cortex in early AD with the observed changes in the motor behaviour of these patients. The primary motor area (M1) is responsible for the final implementation of any goal–directed movement which has been formerly inspired and programmed under the dynamic co-operation between the limbic system and the striato-thalamo-cortical circuit. Therefore, any change in the excitability of M1 causes an alteration in the motor behavior of the individual. According to this line of thought, the increased active motor threshold in early AD reflects a hypo-excitable motor cortex. A hypo-excitable motor cortex is less reactive to any external stimulus rendering the patient less likely to respond with the proper latency, amplitude and velocity of any necessary movement. This finally leads to a pattern of hypo-movement in the affected individual. Hence, our neurophysiological finding of decreased excitability of the motor cortex in the early AD patients pathophysiologically explains the differences from normal motor behaviour in the form of diminished movement.

The correlation we have hypothesized exists between the excitability of the motor cortex and the type of motor behaviour exhibited by AD patients is further enhanced by the TMS studies which were conducted in individuals in more advanced stages revealing an increase in the excitability of the motor cortex at these stages [50, 51, 52]. We have already said that the patients with advanced disease display increased mobility in a form of disinhibited movement when compared to normal individuals. They have increased muscle tone, they engage in stereotypical and searching behaviour and they pace and wander incessantly often resulting in falls. These patients displayed increased excitability of the motor cortex when tested with TMS. Using the same analogy that we formerly used for the early stages of the disease we can assume that the excessive movement of the more severely affected AD patients is a reflection of a hyperexcitable -disihibited motor cortex. A more

excitable motor cortex is more prone to react with excessive movement in any given situation, illustrating the hyperactive, 'hyperkinetic' AD patient that most of the clinicians are very familiar with.

4.2. THE ROLE OF THE CHOLINERGIC SYSTEM IN THE DECREASED CORTICAL EXCITABILITY

The probable correlation between the decreased excitability of the primary motor cortex and the motor behaviour of the early AD patients, gave rise to the question of how exactly Alzheimer's disease induces the decrease in the excitability of the motor cortex in the early stages given that in these stages the primary motor cortex is spared from the neuropathological hallmarks of the disease [19]. Various previous studies [52, 54, 56, 57] have used pharmacological agents to examine the possible biochemical pathways which may affect cortical excitability in AD. The cholinergic hypothesis that connects the disturbed acetylcholine output with the impaired cognitive function of the affected individuals from disease onset is well known [68, 69]. Also well known is the beneficial effect of the acetycholinesterase inhibitors in the cognitive function of the AD patients [70, 71, 72]. Hypothesizing a possible additional role of the cholinergic system upon the motor control of AD patients as well, we conducted our TMS experiment in our patient group before and after the daily treatment with 10 mg of the acetylcholinesterase inhibitor donepezil. Our experimental results confirmed our hypothesis as they clearly showed that after two months of treatment with 10 mg of donepezil daily, the active motor threshold of the AD patients significantly decreased to values close to the aMT of the normal individuals.

The alteration in the form of 'normalization' of the active motor threshold after the oral administration of donepezil demonstrates the existence of a functional pathophysiologic mechanism in the regulation of the excitability of the motor cortex in early AD. This mechanism seems to be under the control of the cholinergic system. Thus, by attributing this critical role to the cholinergic system, a plausible explanation is given for the altered excitability of the primary motor cortex in early

AD. Besides, no subclinical cortical atrophy or any other neuropathology can be traced in the motor areas of the patients in the initial stages of the disease which could explain the changes in cortical excitability as it is the case in the more advanced stages. So, based on our experimental results, we assumed that the function of the cholinergic system is at the root of the decreased excitability of the motor cortex in early AD.

In order to further test the validity of our hypothesis concerning the critical role of acetylcholine upon cortical excitability and motor function in AD, we conducted our first supplementary TMS sub-experiment on a small group of AD patients by administering a pharmacological agent (memantine) which does not implicate the cholinergic system. Memantine, as a NMDA receptor antagonist, mainly affects the neurotransmitter glutamate. Our results showed no difference for active motor threshold in the group of the AD patients before and after 2 months of treatment with memantine. This result validated our hypothesis regarding the significant role of the cholinergic system in the regulation of the excitability of the motor cortex in AD. It is also in accordance with the existing literature relating to the capacity of memantine to affect the motor threshold [71]. Additionally, our sub-experiment with memantine provided more experimental data about the difference in the active motor threshold between early AD patients and normal subjects. The 5 early AD patients who participated in the experiment with memantine displayed increased active motor threshold when compared to the normal subjects, like the 13 AD patients of our main experiment with donepezil. This augmented the sample of our patient group giving further statistical validation to our results.

The next question which should be answered was about the exact manner in which the cholinergic system exerts such an impact on the excitability of the motor cortex of the affected individuals. According to the cholinergic hypothesis, the cognitive dysfunction of the AD patients is attributed to the degeneration of the cholinergic neurons in the basal forebrain (nucleus basalis of Meynert, medial septal nucleus and diagonal band of Broca) and the loss of cholinergic transmission in the neocortex. This hypothesis is supported by studies pointing out that pharmacological agents which act by potentiating the central cholinergic function (donepezil,

rivastigmine and galantamine) have a positive symptomatic effect in the treatment of AD patients, especially in the early stages[72, 73].

It is known from the literature [74] that the degeneration of the cholinergic pathways which is traditionally believed to occur in Alzheimer's disease is also observed in normal ageing but to a far lesser extent. The cholinergic neurons of basal forebrain have been assumed to undergo moderate degenerative changes during normal ageing resulting in hypofunction of the cholinergic system which is related to the deterioration of the memory. However, a recent study conducted by Schliebs and Arendt in 2011 [75], presented results from experiments in humans and in rats which seriously challenge the commonly accepted view about cholinergic neuronal loss during normal ageing. This study suggests that whereas in pathological ageing, such as Alzheimer's disease, an actual loss of cholinergic neurons of the basal forebrain is evident, normal ageing and mild cognitive impairment are not characterized by cholinergic neuronal loss but by a functional impairment of the cholinergic synapse. These investigators stated that although in moderate and advanced stages of Alzheimer disease a severe impairment of the cholinergic innervation of the basal forebrain is extensively displayed, this is not the case in mild AD; in the very early stages of AD no true cholinergic neuronal loss is evident in the basal forebrain. Instead, a process similar but more intense to that of normal ageing and mild cognitive impairment seems to take place. According to these investigators, a modulation of the synaptic cleft associated with a dysfunction of the cholinergic neurons and a loss of signalling by the nerve growth factor is what happens in these neurons. This results in a dysfunction of the cholinergic neurons without true neuronal loss in mild AD. More interestingly, Arendt and Shliebs have stated that the cholinergic dysfunction is triggered by the presence of amyloid therefore connecting the biochemical dysfunction with the protein which holds the key role in the etiology of AD. Indeed, they provided abundant evidence that amyloid may trigger cholinergic dysfunction through action on a7-nAChR, by affecting NGF signalling, mediating tau phosphorylation, interacting with achetylcholinesterase, and specifically affecting the proteome in cholinergic neurons.

It is common knowledge that the basal forebrain BF), the area of the brain which is richer in cholinergic neurons, is connected to the primary motor cortex (M1) indirectly via basal ganglia and prefrontal cortex. Though, evidence recently obtained from animal experiments [76, 77] have shown that besides this indirect pathway, there are also direct connections from the basal forebrain and particularly from the nucleus basalis of Meynert to the motor cortex in rats. Through those direct connections, a possible alteration in the cholinergic function of basal forebrain can directly affect the function of the primary motor cortex causing changes in cortical excitability. In the light of this new experimental evidence, the increased active motor threshold in our group of early AD patients could be seen as a reflection of a defective function of these connections between BF and M1 due to cholinergic deficit which according to Arendt and Schliebs is caused not by cholinergic neuronal loss but is due to a functional disturbance of the cholinergic neurons. The immediate restoration of the aMT, after treatment with donepezil, to values close to those of normal subjects is in keeping with our hypothesis. The existence of a direct pathway between the basal forebrain and the primary motor area provides a very persuasive explanation for the effect that a cholinergic dysfunction exerts upon the excitability of the motor cortex in early AD patients. A reduced cholinergic output results in decreased excitability of the motor area which is clinically translated into altered motor behaviour of the patients in the form of restricted movement.

After correlating the results of our TMS experiment concerning increased motor threshold in early AD patients with the hypo-movement that these patients display and subsequently assuming the role of the cholinergic system as the underlying regulating factor, we judged it necessary to explore our conclusions further by implementing an experimental procedure other than TMS. Thus, we performed our second sub-experiment by examining the simple reaction time in a group of patients in the early stages of Alzheimer's disease before and after donepezil intake. The two parameters we assessed, the latency of the movement response (sRT) and the latency of the muscular response (sMT) were found to be significantly longer in the patient group than the control group. These findings are

supported by relevant literature which shows a prolongation of the reaction time in people affected by neurodegenerative cognitive disorders, Alzheimer's disease included [78, 79]. One could argue that this differentiation in reaction time may reflect a distraction of attention or defective visual processing rather than a dysfunction of the motor system. Although this could happen in some neurodegenerative disorders such as Lewy Body Dementia due to the specific impairment of the anatomical structures involved in attention focusing and visual processing, it is not the case with Alzheimer's disease. While some studies show some differences in visually evoked potentials between normal individuals and patients with Alzheimer's disease [81] there is no clear and consistent evidence from the literature suggesting specific visual or attention deficit in AD in the early stages of the disease.

The prolongation of both sRT and sMT reflects a delayed motor response in the AD patients after a simple visual stimulus by comparison to normal subjects. This delayed motor response is indicative of a lower capacity of the primary motor cortex of the affected individuals to react as fast as the normal subjects when responding to a certain visual stimulus. This finding is in agreement with the results of our TMS experiment showing increased active motor threshold in early AD patients. It is obvious that an increased motor threshold reflects a hypo-excitable motor cortex which is less able to react quickly after a certain stimulus, leading to a delayed motor reaction of the affected individual. In this way our reaction time experiment serves as further confirmation of the main argument of our TMS experiment about decreased excitability of the primary motor cortex in patients at the initial stages of Alzheimer's disease. It also supports the correlation we propose between the impaired function of the motor cortex in early AD and the impaired motor behaviour exhibited by the affected individuals in the form of hypo-movement.

We also observed that after treating the patients in early AD for 2 months with donepezil daily, both parameters (sMT) and (sRT) significantly decreased in duration, reaching a level that very much resembled that of the control group. The normalization of reaction time after donepezil intake outlines the important role of

the cholinergic system in the regulation of the mechanisms which control the ability of the primary cortex to provide a certain motor reaction in a certain time frame. This is in agreement with the results of our TMS experiment concerning normalization of the active motor threshold after donepezil intake. It is evident from the reaction time experiment that the restitution of acetylcholine renders the motor cortex of early AD patients more excitable compared to the state before treatment. A more excitable motor cortex can react faster to a stimulus, is more ready to initiate a certain movement in a reduced latency and finally, more able to perform the motor task in a shorter time frame. Thus, this experiment strongly supports our argument that the cholinergic system is the regulating factor in the function of primary motor cortex in early AD, being responsible for the differentiation in motor behaviour that these patients exhibit by comparison to normal individuals.

4.3. EVALUATION OF THE MOTOR BEHAVIOR IN EARLY AD BEYOND EXCITABILITY: ASSESSMENT OF CORTICAL INHIBITION

In order to explore the exact manner in which the motor behaviour of the early AD patients is closely connected to the particular function of their motor cortex more thoroughly, we wanted to examine the neuronal circuit which is involved in a generated movement holistically This circuit starts from the pyramidal cells of the primary motor area (M1), passes through the alpha-motoneurons of the spinal cord and terminates in the contralateral contracted muscle. The motor threshold is a measure of the ability of the pyramidal cells to produce descendant volleys when they are stimulated by TMS. The descendant volleys produced activate the alpha-motoneurons at the spinal level. This leads to the genesis of a motor evoked potential (MEP) in the targeted muscle as a final response to the stimulation of the contralateral motor area of the brain. However, we did not want to form our conclusions about motor function in early AD based only upon the initial part of the neuronal circuit. This would have been the case if we had restricted the evaluation of the motor threshold as a reflection of the ability of the pyramidal cells to fire volleys to the spinal alpha-motoneurons. Instead, we decided to proceed further by

examining whether voluntary muscle contraction could be impaired in AD in other areas of its functional route apart from altered cortical excitability and, if so, to identify the possible causes of such impairment. Given that TMS activates different excitatory and inhibitory corticospinal pathways which influence the voluntary contraction, we also decided to calculate the silent period in addition to the active motor threshold.

Silent period is a neurophysiological TMS parameter which is considered as an indicator of cortical inhibition. The duration of silent period defined as the difference between SP onset and SP offset is the parameter which is more widely assessed. It provides useful information about the specific ways by which the cortical inhibitory mechanisms affect voluntary muscle contraction. In addition to its role as an indicator of cortical inhibition, silent period is a more 'active' test for the assessment of voluntary muscle contraction. By definition, silent period is the duration of interruption of electromyographic activity which follows the production of a motor evoked potential (MEP) in a muscle sustaining isometric contraction after the application of TMS in the contralateral primary motor area (M1). Thus, by evaluating the silent period we were able to observe the voluntary muscle contraction in a more dynamic and functional way at a postsynaptic level, surpassing the 'static' approach that offers the sole evaluation of the excitability of MI through the calculation of the motor threshold only. Subsequently, a connection between the decreased excitability of the motor cortex in early AD and the state of function of the inhibitory mechanisms involved in the regulation of the movement could be established.

Our TMS study has shown that the duration of silent period of the patients at the initial stages of Alzheimer's disease was significantly increased in comparison with the SP of normal individuals of the same age. A more thorough and detailed study of our results revealed that not only the duration but also the morphology of the silent period was significantly different in AD patients. In particular, we have made the interesting observation that the 'valley' of the silent period which is defined as the interval between MEP offset and return of full electromyographic activity, was more heterogeneous in the group of patients than it was in normal

individuals . The SP valley in AD patients, instead of having the shape of an isoelectric almost flat line due to complete cessation of any electromyographic activity, actually displayed a fragmentation in various segments due to the frequent appearance of late excitatory potentials (LEPS).

A late excitatory potential (LEP), as already stated in a previous chapter, is an electromyographic breakthrough of short duration and low amplitude which, when it appears, scatters the valley of the silent period spoiling its linear form. LEPs are produced only when the tested muscle actively performs a voluntary contraction. LEPs are absent when the tested muscle is at rest [65]. As SP emerges after the stimulation of M1 at the 150% of the motor threshold (which in our study was the active motor threshold) this can justify the appearance of LEP in our control group even though it was rare (only one person in the control group presented LEP). The TMS studies which have assessed silent period in normal individuals [81, 82,] and those which have assessed SP in AD patients [55, 58, 59], have calculated the resting motor threshold instead of active motor threshold as we did. This can explain the scarcity of evidence from the literature regarding the appearance of LEP in AD patients and their appearance in our own study. Regarding the etiology of LEPs, the related literature [64, 65, 66], provides some possible explanations for their appearance attributing them to the activation of slow motor pathways or to the activation of reflex pathways. The most prominent hypothesis though, addresses them to cortical disinhibition [65]. The malfunction of the inhibitory mechanisms of the brain which regulate the muscle contraction permits the release of late excitatory potentials during a period which should be characterized by total silence in electromyographic activity.

In our experimental TMS study the appearance of LEPs was rare in the group of normal subjects as already stated. Only 1 out of the 13 subjects (7%) which had been tested displayed them. This result is not very distinct from that referred in the available literature (2-3%) [64]. On the contrary, in the group of early AD patients LEPs were very frequent as they appeared in 9 out of the 13 patients tested (69%). A very interesting observation was that the presence of LEPs was in line with the prolongation in the duration of silent period for both groups. The absence of LEPs

was compatible with a silent period significantly shorter in duration. This observation allowed us to conclude that the increased duration of the silent period in our early AD patients is closely related to the frequent appearance of late excitatory potentials which scatter the SP valley in various segments before the final return of full electromyographic activity. Attributing the appearance of LEP to cortical disinhibition, as the data from the literature suggests, we can assume a disturbance in the function of the cortical inhibitory mechanisms of the brain in the early stages of Alzheimer disease which results in a more frequent appearance of late excitatory potentials. LEPs scatter the SP valley in multiple segments finally leading to a prolongation of silent period in comparison with normal individuals.

However, there has not been extensive research about the evaluation of silent period in Alzheimer's disease using TMS experiments so far. The relevant literature is rather scarce [48, 58, 83]. Most probably this can be attributed to technical restrictions arising during the procedure of the calculation of SP [73] and also to the difficulties that the AD patients have in terms of good collaboration which is necessary for the assessment of this parameter. The first TMS study in Alzheimer's disease performed by Peretti et al [48] indicated a silent period with decreased duration in the patient group in comparison with the normal subjects. However, we have to keep in mind that this study included a large number of patients in advanced AD. Liepert et al [83] have concluded no difference in the duration of silent period between AD patients and normal subjects. The study of Khedr in 2010 [58] investigated the silent period in AD patients in each stage of their disease (mild, moderate, advanced). This study displayed a clear increase in the duration of the silent period in the patient group by comparison to normal individuals. More importantly, this increase was already prevalent in the mild stage, thus supporting the validity of our own study about increased duration of silent period in patients at the initial stages of Alzheimer's disease. Nevertheless, although the study of Khedr confirmed our results concerning the prolonged duration of SP in early AD, no information was given about the shape of the SP valley. Until now, with the exception of our experimental paradigm there has not been any evidence available from previous TMS studies suggesting that the appearance of LEP can cause a

fragmentation and finally a prolongation of the silent period. Maybe the frequent appearance of LEPs in early AD should be considered as a potential biomarker in the future but more studies with larger number of patients are required to investigate this further.

4.4. THE CHOLINERGIC SYSTEM AND IMPAIRED CORTICAL INHIBITION IN EARLY AD

The prolongation of the silent period in the group of early AD patients had to be attributed to a certain pathophysiologic mechanism. The second phase of our experiment, when we reexamined all our patients after treating them with the acetylcholinesterase inhibitor donepezil for two months, provided a very plausible explanation. In particular we observed that after treatment with donepezil the duration of the silent period of the AD patients was significantly decreased in comparison with its duration prior to treatment. The duration of SP actually became much closer to that of the normal subjects to a point where there was no statistically significant difference between the SP of normal individuals and the SP of the AD patients treated with donepezil. The shortening of SP duration was due to the decrease in the occurrence of LEPS after donepezil intake. Our experiment has shown that in the group of untreated patients, 9 out of 13 displayed LEPs while in the group of patients treated with donepezil this occurrence dropped to 5 out of 13 patients (a reduction from 69% to 42%).The restoration of the silent period both in morphology and duration after treatment with donepezil, was clearly indicative of a functional change in the inhibitory mechanisms of the brain given that silent period is a reflection of cortical inhibition. The fact that donepezil is an acetylcholinesterase inhibitor enabled us to assume a key role of the cholinergic system in the regulation of the inhibitory neuronal pathways of the brain.

The only studies which have investigated the direct impact of cholinergic pharmacological agents upon the silent period of patients suffering from Alzheimer's disease are ours and that of Liepert et al [83]. In the study of Liepert, the investigators calculated the silent period in the patient group before and after the

administration of donepezil for one week. No difference in the duration of SP was found between AD patients and normal subjects either before or after the administration of donepezil. Due to the scarcity of relevant literature, and given the contradictory results between our study and that of Liepert, our sub-experiment with memantine was very significant in order to test our conclusion concerning the important effect of the cholinergic system upon the duration and morphology of silent period in early AD. This experiment helped to create a clearer picture of certain biochemical circuits which regulate the inhibitory mechanisms of the brain during voluntary muscle contraction given that memantine acts through glutamate. After treating the AD patients with 10 mgr of memantine daily for two months, no significant change was observed either in the duration of silent period or in the shape of silent period regarding the appearance of LEP. Late excitatory potentials not only did not show any inclination to diminish after memantine treatment but on the contrary their appearance increased. These results support the conclusion derived from our main TMS experiment that it is indeed the cholinergic system which regulates cortical inhibition in the brain during voluntary muscle contraction.

In addition, our sub-experiment with memantine provided more evidence to our experiment with donepezil regarding the significant difference in the duration and morphology of silent period between early AD patients and normal individuals of the same age. The 5 untreated patients participating in the sub-experiment with memantine displayed the same pattern in their SP as the untreated patients in the experiment with donepezil. This added 5 more patients to our initial patient group of 13 patients thus increasing the statistical value of our results.

Although based almost solely on our experimental results, we did not hesitate to conclude that, at least in the early stages of Alzheimer's disease, the cholinergic system exerts important control upon the inhibitory neuronal circuits of the motor cortex. The cholinergic dysfunction which characterizes the early stages of Alzheimer's disease causes a malfunction of the inhibitory neuronal pathways of the brain. This provokes a scattering of the silent period by electromyographic breakthroughs (LEPs) as a complete cessation of electromyographic activity is difficult to sustain. It becomes evident that cortical disinhibition which is caused by a

cholinergic deficit results in a silent period fragmented and longer in duration in early AD patients when compared to normal individuals of the same age.

Given the scarcity of previous research on the topic, we carefully reviewed the literature for the existence of any scientific evidence addressing, even indirectly, the impact of Alzheimer's disease on the various inhibitory neuronal pathways of the brain and the possible role of the related neurotransmitters in the regulation of the function of these pathways. Di Lazarro was the first investigator who experimentally proved a close relationship between the cholinergic system and the inhibitory mechanisms of the cerebral cortex [84]. He observed, after the intravenous administration of scopolamine in normal individuals, a very significant reduction in the amount of short-latency afferent inhibition (SAI) evoked by somatosensory input from the hand. Short-latency afferent inhibition is based on coupling electrical peripheral nerve stimulation with motor cortex stimulation by TMS. SAI refers to the suppression of the amplitude of a MEP caused by a conditioning afferent electrical stimulus upon the median nerve of the wrist of the contralateral hand area. The conditioning electrical stimulus is exerted at the median nerve approximately 20 ms prior to the application of TMS. Scopolamine is a medication which blocks the muscarinic receptors of acetylcholine. Given that short latency afferent inhibition (SAI) reflects inhibition in the level of interaction between the sensory and motor system of cerebral cortex, this experimental study proved the central role of cholinergic system in cortical inhibition.

After establishing the strong connection between SAI and cholinergic function in the normal brain, Di Lazzaro evaluated short-latency afferent inhibition in Alzheimer's disease patients. His experimental study of 2002 [54] revealed that Alzheimer's disease patients had a significantly reduced amount of SAI by comparison to normal subjects. After the oral administration of the acetylcholinesterase inhibitor rivastigmine, an elevation in SAI was observed. Another experiment performed by this group has shown that short-latency afferent inhibition is influenced by GABAergic drugs as well. Particularly, when benzodiazepine lorazepam was administered in healthy subjects, a significant reduction of SAI was induced [85]. Di Lazzaro et al. explained these changes in SAI

attributing a central role to acetylcholine in its regulation. [54]. They also proposed an involvement of other neurotransmitters, such as glutamate and GABA, in the whole process [57]. Despite various assumptions, a certain model of the exact way the cholinergic system interacts with neuronal pathways mediated by other neurotransmitters in the regulation of cortical inhibition was not proposed by Di Lazarro. Nevertheless, his work provided important evidence of a serious dysfunction of the inhibitory mechanisms in the level of interaction between sensory and motor areas of the brain in patients suffering from Alzheimer's disease. His work also underlined the serious impact of the lack of acetylcholine in the etiology of this dysfunction serving as indirect support for our own results concerning impaired inhibition in early AD.

Nardonne, in his experimental study in 2008 [53], confirmed the conclusions of Di Lazarro et al. regarding the decreased short-latency afferent inhibition in Alzheimer's disease. More interestingly, he conducted his experiment focused on early AD as the patients he recruited were all in the initial stages of the disease. The mean amount of SAI was significantly smaller in the group of early AD patients in Nardone's study compared to normal subjects suggesting an early impairment of cholinergic function in AD which seriously affects the SAI. More importantly, the study of Nardone serves as a very significant argument to our own results of decreased cortical inhibition in patients in early AD because of the similar methodology. The experimental studies of Di Lazzaro and Nardone assessing SAI allow us to conclude that despite the different level of inhibition that silent period and short latency afferent inhibition refer to, the former post-synaptically, the latter pre-synaptically, we can assume a global dysfunction of the inhibitory mechanisms of the cerebral cortex in early AD closely related to cholinergic dysfunction.

Di Lazarro and Nardone in their TMS studies, apart from assessing the short-latency afferent inhibition as an indicator of cortical inhibition in the sensorimotor level and a marker of cholinergic function, also assessed short-interval intra-cortical inhibition (SICI) in AD patients [54,55,86]. Short-interval cortical inhibition is a paired–pulse TMS paradigm [87]. It involves a subthreshold conditioning stimulus that precedes a suprathreshold test stimulus adjusted to produce an average MEP of

0.5–1.5 mV peak-to-peak amplitude in the contralateral muscle. In order to measure short-interval cortical inhibition, conditioning stimuli are applied to the motor cortex before the test stimulus at inter-stimulus intervals (ISIs) between 1 ms and 4 ms. These investigators presented results showing that SICI was reduced in AD patients by comparison to normal individuals. Interestingly, the study of Pepin et al. [50] which included more advanced AD patients showed no difference of SICI between patients and normal subjects. Taking into account that Narbone performed his TMS study with early AD patients as we did in our study, our conclusions about a dysfunction of the inhibitory cortical mechanisms in early AD are further supported.

The results of Nardone and Di Lazzaro about decreased short interval cortical inhibition (SICI) in Alzheimer's disease are in accordance with the much older study of Liepert at al. [83]. According to the literature SICI is likely to be mediated by GABAergic inhibition at the intra-cortical level [88, 89]. Furthermore, it has been proved through experiments with pharmacological agents [90] that short-interval cortical inhibition is related to GABA-A receptor–mediated inhibitory neurotransmission. Reduced SICI suggests a dysregulation of the intra-cortical GABAergic inhibitory circuits.

Collating all the aforementioned studies with the results of our own study, we concluded that indeed there is impairment in various levels of cortical inhibition in Alzheimer's disease from the early stages. The reduction of short-latency afferent inhibition (SAI) represents impaired inhibition in the brain areas between the motor and sensory system in a presynaptic level and is related to central cholinergic activity. Reduced short interval intracortical inhibition (SICI) suggests a dysregulation of the intra-cortical GABAergic inhibitory circuitries with GABA-A mediation predominantly involved. The prolongation of silent period due to its fragmentation by the presence of multiple LEP reflects an impaired, long lasting cortical inhibition at the postsynaptic level and is mediated predominantly by GABA-B receptors [91, 92].

4.5. THE CONNECTION BETWEEN DECREASED EXCITABILITY AND IMPAIRED INHIBITION OF THE MOTOR CORTEX

As silent period represents in a dynamic manner cortical inhibition during voluntary muscle contraction caused by TMS stimulation of the contralateral motor area, our experimental results were indicative of a dysregulation of the mechanisms responsible for the muscle contraction in early AD. This dysregulation was attributed to a dysfunction of the motor cortex in early AD patients closely related to a cholinergic dysfunction. Moreover, by evaluating the active motor threshold of these patients we have also shown that a decrease in the excitability of the primary motor cortex exists in early AD patients. The decreased cortical excitability was also closely related to a dysfunction of the cholinergic neurons in early AD. So, a very interesting challenge arose: to investigate the existence of a connection between the impaired cortical inhibition at the postsynaptic level that the scattered and prolonged silent period demonstrated with the reduced excitability of the primary motor area that the increased aMT suggested in early AD. The discovery of such a connection would create a model which could convincingly describe the changes that Alzheimer's disease, from the early stages, causes in the function of primary motor during a motor action from its initiation to its implementation. Furthermore, it would give a clearer view of the neuronal pathways that are involved in the regulation of a motor action and the specific neurotransmitters these pathways utilize in their function.

In our effort to establish this connection we should briefly review the physiology of the TMS procedure. The process starts with the transynaptical activation of the pyramidal cells in primary motor area (M1) by TMS, continues with the genesis of the indirect (I) waves which travel through the corticospinal tract activating the alpha-motoneurons at the spinal level and is terminated with the genesis of a MEP in the tested muscle of the contralateral area. The genesis of the MEP represents the muscle contraction as a response to the TMS stimulation. When we stimulate the M1 with stimulus intensity of 150 % of the motor threshold while the individual performs a voluntary muscle contraction, silent period emerges due to

the activation of certain inhibitory mechanisms so as to improve the plasticity of the movement. It is widely accepted that SP and MEP are correlated to some extent. The question arises though as to the anatomical basis of the relationship between SP and MEP. Given that MEP is closely related to threshold reflecting cortical excitability while SP represents cortical inhibition in the postsynaptic level, the answer to this question would provide us with a fundamental formula to describe the connection between the decreased cortical excitability and the impaired cortical inhibition observed in early AD.

It has been well recognized in the past that both SP duration and MEP amplitude are linearly related to the intensity of the TMS stimulation [46, 62, 94, 95, 96]. Orth and Rothwell in their study in 2004 [97] observed that this correlation was stably present for all the individuals tested and was independent of the pulse waveform of the TMS stimulus. By calculating ratios of silent period duration and the corresponding MEP area these investigators managed to reduce the variability between subjects and the magnetic stimulator for the current flow direction when TMS was applied. This led them to suggest that the factors which were causing variation in the MEP were the same as those which caused variation in the duration of the silent period. The most probable explanation they provided was that the corticospinal outflow that produces the MEP is also responsible for the generation of the SP.

It is already known that TMS primarily activates the fast–conducting pyramidal neurons leading to the genesis of MEP. However, from experiments in cats [98] it is also known that recurrent collaterals of these fast conducting neurons exert an inhibitory effect on slower-conducting pyramidal neurons most probably by exciting intercalated inhibitory neurons. As slowly-conducting pyramidal neurons are responsible for the maintenance of tonic voluntary muscle contraction, the inhibition of those neurons is presumed to be responsible for the occurrence of the silent period. The proposed model of Orth and Rothwell, suggesting that the occurrence of silent period is in close relation to the genesis of a MEP in the targeted muscle during TMS stimulation was later confirmed by other TMS studies [63, 64]. This model provided us with the necessary, adequate neurophysiological basis to

connect cortical excitability with cortical inhibition. However, in order to form a complete picture about the mechanisms of the changes which take place in the primary motor cortex of early AD patients, we should expand and support this neurophysiological model with knowledge of neuroanatomy about the neuronal pathways which lie underneath.

4.6. A PATHOPHYSIOLOGICAL MODEL CONCERNING ALTERED MOTOR FUNCTION IN EARLY AD.

We know from neuroanatomy, further confirmed by animal experiments in rats that there is a very rich presence of cholinergic fibers in layer I of the primary motor area which lessens significantly in layers II-III [99]. The apical dendrites of pyramidal cells (PC) which are rich in cholinergic afferents are located in layers II-III. A strong release of ACH from cholinergic axons located in layer I can therefore stimulate the apical dendrites of the PC found in layers II-III by acting on their muscarinic receptors. However, layers II-III of M1, apart from containing the apical dendrites of pyramidal cells, are also very rich in GABAergic neurons [100,101]. From the various types of GABAergic neurons located in these layers a specific cell type, called basket GABAergic neurons, connects its axons to the apical dendrites of the PC of this area forming a common neuronal circuit. Combining this information from the literature we proceeded to form our hypothesis.

We suggest that when acetylcholine is released from the cholinergic axons of the layer I of M1, it activates not only the apical dendrites of the pyramidal cells of the inferior layers II-III but also certain GABAergic neurons of these layers as parts of the common neuronal circuit that these GABAergic neurons form with the dendrites of the pyramidal cells. It is well understood and accepted that acetylcholine have an excitatory effect on the brain [102] while GABA is the main inhibitory neurotransmitter [103,104]. According to the physiological model we suggest, it becomes clear that the release of acetylcholine to the M1 leads to the activation of the pyramidal cells of the primary motor cortex thus increasing cortical

excitability while simultaneously this release activates certain GABAergic neurons of this area affecting cortical inhibition.

After forming our physiological model which illustrates this type of interconnection between cortical excitability and cortical inhibition, we reviewed the literature for any additional evidence of the effect of excitatory or inhibitory neuronal networks upon the pyramidal cells of M1. We found that Xiang at al. [105] in an animal experiment with rats, have demonstrated the existence of a selective cortical muscarinic disinhibition of the pyramidal cells. Specifically, Xiang' research has shown that acetylcholine hyperpolarizes a certain type of inhibitory interneurons, the fast spiking (FS) cells located in layer V of M1 which forms functional synapses on layer V pyramidal cells. As the axons of the FS inhibitory interneurones in layer V tend to be distributed more horizontally (intralaminar), their hyperpolarization results in disinhibition of their pyramidal cell targets of the same area. Thus, the activation of the cortical cholinergic system could reduce some forms of intralaminar inhibition. Together with direct muscarinic depolarisation of layer V pyramidal cells it could increase pyramid-pyramid recurrent excitation finally enhancing cortical excitability. This study outlined the important role of the cholinergic system upon the function of pyramidal cells through the regulation of the excitatory and inhibitory neuronal network further validating our hypothesis.

Taking into consideration the physiological model we have suggested concerning the interaction between the various cell types in the different layers of the primary motor area and given the direct connections that according to recent data [69] exist between the basal forebrain and the motor cortex, we applied the model of Orth–Rotwell to our experimental results. Therefore, we suggest that the disturbance in acetylcholine output observed in early Alzheimer's disease leads to a decrease in the excitability of the fast contacting pyramidal cells (PC) which are responsible for the production of MEP during TMS resulting to an increased motor threshold. Subsequently, the activation of the intercalated inhibitory GABAergic neurons which are directly affected by the firing of PC is also reduced. This results in less inhibition exerted by these GABAergic neurons onto the slow-conducting pyramidal cells which are responsible for the maintenance of the voluntary isometric

muscle contraction. The final outcome of the impaired inhibition on the slow-conducting PC is the appearance of various LEPs which fragment the valley of the silent period and increase its duration. Our study has shown that the oral administration of the acetylcholinesterase inhibitor donepezil caused normalization both in the active motor threshold and in the shape and duration of SP significantly reducing LEPs. In this way the key role of the cholinergic system becomes apparent as a regulating factor in the function of motor cortex in patients of Alzheimer's disease and explains the altered motor behaviour that these patients exhibit from the early stages.

CHAPTER 5.

FINAL CONCLUSIONS

Our study creates an original view of the motor function of patients in the early stages of Alzheimer's disease and proposes an explanation for the responsible regulatory mechanisms. Our TMS experiment shows an increase of the active motor threshold in early AD patients corresponding to a decreased cortical excitability when compared to normal individuals of the same age. It demonstrates a differentiation in the function of the primary motor cortex in AD from disease onset. This points to the fact that not only are the areas of the brain responsible for memory and cognition affected early in the process of the disease but the primary motor cortex is involved as well.

Investigating the mechanisms that are responsible for the decreased excitability of motor cortex in early AD patients, we hypothesized a key role of the cholinergic system in the regulation of cortical excitability considering the significance of the cholinergic hypothesis in the pathogenesis of Alzheimer's disease. The restoration of the active motor threshold to normal after the oral administration of the cholinesterase inhibitor donepezil argues in favor of a cholinergic regulation of cortical excitability in early AD. The absence of any traceable change in the active motor threshold in a group of early AD patients after receiving memantine which acts without implicating the cholinergic system reinforced the soundness of our initial hypothesis.

The assessment of the reaction time in early AD patients when given a simple visual stimulus confirmed from a different perspective the effect of Alzheimer's disease on the alertness of the motor system. The increased simple reaction time and simple movement time that these patients displayed when compared to normal patients are in accordance with the decreased excitability of the primary motor cortex addressed by our TMS study. A hypo-excitable motor cortex is unable to react quickly with a muscle contraction to a given visual stimulus.

The ‘normalization’ of reaction time after donepezil intake outlined the crucial role of acetylcholine in the ability of the motor cortex to be properly alert in order to react with the necessary speed every time that an implementation of a movement is required.

Recent studies have shown that the cholinergic deficit that exists in early AD is not caused by a cholinergic neuronal loss in the basal forebrain. A modulation of the synaptic cleft associated with a dysfunction of the cholinergic neurons and a loss of signaling by the nerve growth factor seems to be implicated. In the light of this scientific evidence our initial hypothesis about the functional role of the cholinergic system upon motor control in early AD patients became a reasonable argument.

Thus, we came to the conclusion that the disturbance in acetylcholine output in early AD, apart from being one of the main reasons for the deficit in cognitive function of the affected individuals, is also responsible for the alteration of their motor behaviour. Early Alzheimer’s disease causes a certain cholinergic dysfunction in the basal forebrain (BF) which affects the primary motor area (M1) directly through a connecting neuronal pathway; recent data reveal the existence of a direct connection between BF and M1. This cholinergic dysfunction results in the elevation of the active motor threshold manifesting itself in a decreased excitability of the primary motor cortex. The decreased excitability of the primary motor area is reflected on a clinical level as altered motor behaviour in the form of hypo-movement for the patients in early AD.

Observing the form of movement of the AD patients in more progressed stages as well, we concluded that the motor behaviour of the AD patients is stage dependent. Given the increased cortical excitability which is evident in advanced Alzheimer’s disease, in contrast to the decreased excitability of the early stages, we were able to establish a similarity between Alzheimer’s disease and amyotrophic lateral sclerosis. Though very different in pathophysiology, both neurodegenerative diseases affect the motor cortex following a pattern of stage dependent changes upon cortical excitability in terms of qualitative measures. The changes in the excitability of the motor cortex during the evolution of the neurodegeneration could be a common adaptive phenomenon in neurodegenerative diseases.

The measurement of the silent period provided a more holistic evaluation of the function of motor system in early AD. SP calculation, as an indicator of cortical inhibition, added a dynamic view upon physiology of a generated movement beyond the sole assessment of cortical excitability that the motor threshold reflects. The increase in duration of the silent period in the group of early AD patients was attributed to the fragmentation of SP valley by multiple late excitatory potentials due to cortical disinhibition. Given that silent period itself is a marker of late motor cortical inhibition in the postsynaptic level during voluntary muscle contraction, we concluded that an impairment of cortical inhibition is present in the early stages of Alzheimer's disease along with decreased cortical excitability. The restoration of the duration and shape of silent period of the early AD patients back to normal values after the intake of donepezil outlines the important role of the cholinergic system in the regulation of cortical inhibition during muscle contraction. The absence of any effect of memantine in the duration and shape of the silent period further reinforced the validity of our conclusion that the mechanisms which regulate cortical inhibition during early AD are acetylcholine dependent.

The common denominator between the decreased cortical excitability and the impaired cortical inhibition in the early AD patients is a disturbance in cholinergic output. Thus, we suggest a functional model which connects cortical excitability (aMT) with cortical inhibition (SP) under the regulation of the cholinergic system. The scientific work of Orth and Rothwell which argues that the spinal outflow that produces the MEP is also responsible for the generation of the SP provided us with a sound basis for doing so.

While TMS primarily activates the fast-conducting pyramidal neurons leading to the genesis of MEP, recurrent collaterals stemming from them, through the excitation of intercalated inhibitory neurons have an inhibitory effect on the slower-conducting pyramidal neurons. Given that slow-conducting pyramidal neurons are responsible for the maintenance of tonic voluntary muscle contraction, their inhibition leads to the occurrence of silent period. Neuroanatomy and animal experiments indicate that layer I of M1 is very rich in cholinergic fibers. The apical dendrites of pyramidal cells (PC) which are rich in cholinergic afferents are located in

layers II–III of M1. Also layers II–III of the motor cortex are very rich in GABAergic neurons, a specific type of which (the basket cells) form, via their axons, a connecting neuronal network with the apical dendrites of the PC located there.

We applied all the aforementioned to our experimental results in order to present our final proposal. So, we suggest that the disturbance in Acetycholine output from the cholinergic axons of layer I of M1 in early AD leads to a decrease in the excitability of the fast conducting pyramidal cells (PC) which are responsible for the production of MEP. This decreased excitability of the PC is illustrated as increased motor threshold. Subsequently, the excitability of the intercalated inhibitory GABAergic neurons which are activated by collaterals stemming from the fast conducting PC is reduced. Less inhibition is exerted by these GABAergic neurons onto the slow-conducting PC which are responsible for the maintenance of the voluntary isometric muscle contraction. As the muscle contraction is not efficaciously inhibited, various LEPs appear resulting in the fragmentation and prolongation of the SP valley being demonstrative of an impaired inhibition at a postsynaptic level. Donepezil intake appears to normalize both the active motor threshold and the shape and duration of the SP. This illustrates the interconnection that exists, according to our functional model, between decreased cortical excitability and decreased cortical inhibition in early AD with the cholinergic system as the regulating factor.

The complete model which our study proposes regarding the function of the motor cortex in Alzheimer's disease conforms to neuroanatomy, neurophysiology and previous suggestions by Brown and Pluck. It initiates a new way to view the motor behaviour of patients suffering by Alzheimer's disease. The actual changes in the motor behaviour of the affected individuals have a strong relationship with the existing cognitive deficit caused by AD. Through our study, motor behaviour is viewed in close relationship to cognition. A person moves his facial and body parts in response to the environmental stimuli based on his cognitive reserve. The motor cortex is no longer theorized as an isolated brain area but as a dynamic place; it interacts with the associative brain areas and the limbic system in order to generate a movement as the final expresser of a complete procedure including cognitive

processing. According to our study, the disturbance in the function of the cholinergic system is one of the main reasons for the altered motor behaviour of the patients in the initial stages of Alzheimer's disease as similarly it is responsible for the impairment of their more purely cognitive functions.

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