EXTRA Dementia and Geriatric Cognitive Disorders

Dement Geriatr Cogn Disord Extra 2014;4:457–464
DOI: 10.1159/000367841
Published online: November 21, 2014

1664–5464/14/0043–0457$39.50/0
www.karger.com/dee

Karger Open access

Original Research Article

An Investigation of the Late Excitatory Potentials in the Hand following Transcranial Magnetic Stimulation in Early Alzheimer's Disease

Christina Balla Alain Maertens de Noordhout Jean Louis Pepin

University Department of Neurology, CHR Citadelle, Liège, Belgium

Key Words
Transcranial magnetic stimulation · Alzheimer's disease

Abstract
Background: Recent neuroimaging studies in humans support the clinical observations that the motor cortex is affected early in the course of Alzheimer's disease (AD). ***Patients and Methods:*** We measured the silent period (SP) induced by transcranial magnetic stimulation in AD patients in the very early stage of the disease, and we explored whether and in which way the pharmacologic manipulation of the cholinergic system could modify it. ***Results:*** An increase in the duration of the SP was observed in AD patients in the early stage in comparison to controls. After 2 months of treatment with donepezil, the duration did not differ significantly from that of normal subjects. The results of our study show a fragmentation and an enlargement of the SP in the presence of multiple late excitatory potentials (LEPs) in early untreated AD patients. These LEPs were also modulated by donepezil. ***Conclusions:*** The results suggest an early functional impairment of cholinergic neurotransmission in AD. The disturbance in acetylcholine output in early AD leads to a decrease in excitability of the motor system.

Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder which does not spare the motor cortex. Experimental paradigms [1] and clinical studies [2–4] confirm the clinical observations about early involvement of motor behavior in AD patients. The main neurotransmitters implicated in the regulation of the motor cortex are acetylcholine (ACH) and glutamate as basically excitatory [5, 6] and GABA as the basic inhibitory neurotransmitter [7]. The cholinergic hypothesis about the central role of ACH in cognitive deterioration in AD in the

Jean Louis Pepin
University Department of Neurology, CHR Citadelle
Bd du 12ème de Ligne, 1
BE–4000 Liège (Belgium)
E-Mail jlpepin@chu.ulg.ac.be

light of new evidence in vitro [8, 9] and in vivo [10, 11] is enriched so as to suggest ACH as the main neurotransmitter regulating not only the cognitive but also the motor functions in AD patients already from the early stage.

Transcranial magnetic stimulation (TMS) has been widely used to assess the excitatory and inhibitory mechanisms which regulate the function of the motor cortex in AD [1, 12–14]. The effect of the cholinergic system on these inhibitory mechanisms has been extensively explored by TMS [15, 16]. A strong decrease in short-latency afferent inhibition which was reversed by acetylcholinesterase inhibitor intake was observed in various studies with AD patients [17]. A dominant role of ACH and a probable role of GABA as the intermediate neurotransmitter in motor control have been assumed, but a clear hypothesis concerning the underlying neuronal circuits has not yet been formed.

Among the neurophysiological parameters more often used in TMS, the silent period (SP) is considered as a direct indicator for cortical inhibition. It provides information about the underlying pathophysiological mechanisms, especially when measured in subjects taking pharmacological agents with an effect upon basic neurotransmitters [9, 16, 18]. The SP is defined as the interval of the cease of electromyographic activity which follows the production of a motor-evoked potential (MEP) in a muscle sustaining isometric voluntary contraction after the application of TMS in the primary motor area (M1). SP duration is the main parameter used to assess the cortical inhibition and is defined as the difference between the SP onset and the SP offset. However, the methods used to determine the onset and offset of the SP differ widely between studies. The SP onset has been defined as the onset of TMS, the MEP onset, the MEP offset, or when electromyography (EMG) drops below the volitional pre-TMS EMG level. The SP offset has been defined as the first return of any volitional EMG, the absolute return of EMG to the pre-TMS level, or when EMG no longer significantly differs from the pre-TMS EMG level [19, 20]. The evaluation of the whole SP duration is also difficult as sometimes, it can be temporarily interrupted by an electromyographic breakthrough of short duration and a low amplitude called 'late excitatory potential' (LEP), leading to a false, shorter SP. Several LEPs can scatter the SP into a lot of valleys until the final return of the full electromyographic activity. The occurrence of LEPs could be due to the activation of slow motor pathways or due to the activation of reflex pathways, but the most prominent hypothesis links them to cortical disinhibition [21].

Up until now, the SP has not been extensively studied in AD patients. The main reasons for this are the general problems arising with the calculation of the SP such as the considerable interindividual variation of its duration, the high degree of intraindividual asymmetry, the variable results when different examiners apply the TMS in the same subject [22], and the difficulty of an AD patient to collaborate.

In the present study, single-pulse TMS was applied in early AD patients before and after donepezil intake; the parameters of the SP were measured in order to explore the underlying cortical circuits.

Patients and Methods

Patients

Thirteen right-handed patients with AD (6 men, 7 women, mean age 72.9 ± 8.4 years) were studied. All patients were diagnosed with probable AD according to the criteria of the National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer Disease and Related Disorders Association. They were in an early stage of the disease according to the Clinical Dementia Rating Scale. The Mini Mental State Examination (MMSE) was evaluated in all patients. The mean MMSE score was 23.6 ± 2.4. Patients who exhibited

signs of other neurological diseases or those who were on medications with a possible effect on the cognitive function or the excitability of the nervous system were excluded. The neurological motor examination was normal. The patients were independent in the elementary activities of daily living even if some instrumental activities of daily living were impaired. All the patients underwent a brain MRI to exclude cerebral vascular lesions or any cause of reversible dementia.

All AD patients had the examination performed before the introduction of treatment with a cholinesterase inhibitor (10 mg donepezil per week once a day). The whole procedure was repeated after 2 months of treatment in all patients except one (patient 11) who did not reappear for the follow-up.

The age-matched (71.5 ± 8.4 years) control group consisted of 13 right-handed normal subjects (7 men, 6 women) with no history of neurological disease and a completely normal neurological examination.

All patients and all normal subjects were able to collaborate well during the electrophysiological procedure. They gave informed consent for the study which was approved by the local ethics committee.

Methods

The patients and normal subjects were seated comfortably on a chair in a quiet examination room. The EMG of the right abductor digiti minimi was recorded in a Nicolet Viking IV IES 405-1 EMG machine to assess the response to TMS.

TMS was used in accordance with the consensus guidelines [23]. It was applied by a Magstim 200 stimulator (Magstim Ltd., Whitland, UK) through a 9-cm diameter circular coil, located at the vertex area of the scalp. The center of the coil was measured on the line between the nasion and the union point for each patient to ensure the reproducibility of the procedure from the first session to the second one 2 months later. The circulating current was turning clockwise in order to elicit a response in the target muscle. The subjects were asked to perform a slight isometric voluntary contraction of the right abductor digiti minimi (around 10% of the maximum voluntary muscle contraction) during TMS, with an auditory feedback. This active cortical motor threshold, defined as the minimal intensity of cortical stimulation which produces MEPs of approximately 200 μV in 50% of consecutive trials during isometric contraction of the tested muscle, at about 20% of maximum voluntary contraction, was determined by lowering the stimulator output gradually from 60% of the maximal output. Once the active motor threshold (aMT) was determined, the intensity of the output of the magnetic stimulator was pushed to 150% of the threshold. The patients were asked to perform a voluntary contraction of the target muscle of about 50% of the maximal voluntary contraction assessed by amplitude of the EMG response. The base time of the recordings was 500 ms.

We have chosen to measure the SP 'valley' defining the onset of the SP by the MEP offset and the offset of the SP by the return of the electromyographic activity in an amplitude which no longer differed from the pre-EMG level and not by the first return of any volitional EMG. The duration of the SP was analyzed as well as the presence or absence of LEPs and, when present, their latency, amplitude and duration. The results were compared and statistically analyzed between groups by using unpaired Student's t test.

Results

The results are summarized in table 1.

Table 1. Comparison of results between groups

	LEP	SP duration, ms	LEP duration, ms	LEP amplitude, μV	LEP latency, ms	SP duration if LEP is present, ms	SP duration if LEP is absent, ms
Normal subjects							
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
AD patients before treatment							
1	yes	262	18	1,100	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	1,800	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
AD patients after treatment							
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	1,120	64	214	
8	no	68					68
9	yes	284	81	1,052	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

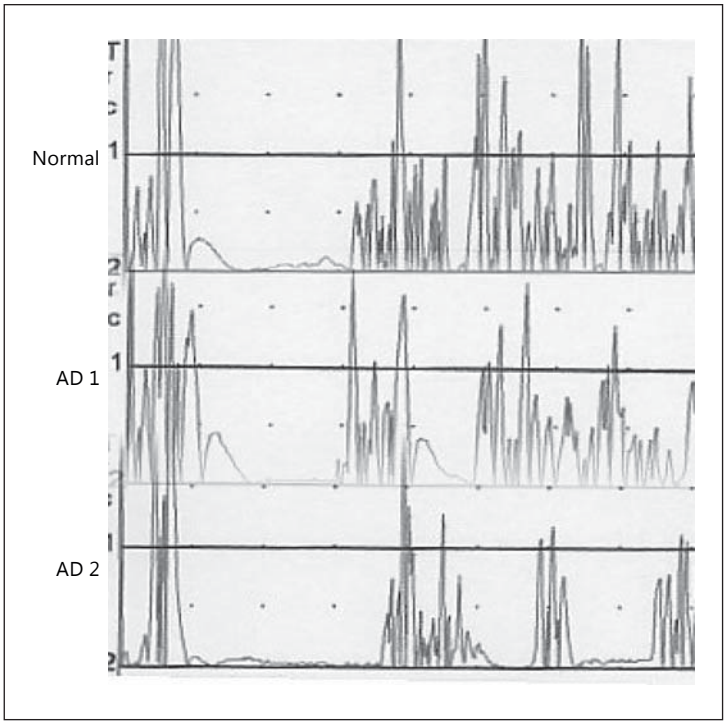

Fig. 1. Examples of LEPs in a normal subject and 2 untreated AD patients (base time: 50 ms/division; amplitude: 200 μV/division).

Normal Subjects

The mean ± SD aMT for normal subjects was 32.8 ± 7.7%. EMG following TMS generally showed the pattern of an MEP at a latency of around 20 ms followed by an SP of variable duration. LEPs were observed in 1/13 subjects (7%; patient L., 70 years old) with a latency of 67 ms, an amplitude of 487 μV and a duration of 111 ms. The SP duration for this subject was 229 ms. For the other normal subjects, the mean duration of the SP was 107.5 ± 42.2 ms. When the subject L. was included, the duration of the SP was 116.8 ± 52.6 ms.

AD before Treatment

Before treatment with donepezil, the mean aMT in the 13 AD patients was 37.6 ± 3.9%. In these patients, the mean duration of the SP was 186.4 ± 88.2 ms. It was significantly increased by comparison to normal subjects ($p < 0.001$).

In 9 out of 13 patients, LEPs were evident (69%), with a mean latency of 98.9 ± 41.5 ms, a mean amplitude of 815.7 ± 420.5 μV and a mean duration of 50.1 ± 22.8 ms. The SP valley was fragmented in these patients into multiple segments before the true return of full EMG activity (fig. 1). In the 9 patients with an LEP, the mean duration of the SP was 229.4 ± 66.3 ms, which differed significantly from the normal subjects ($p < 0.001$). In the 4 patients without an LEP, the mean duration of the SP was 89.5 ± 35.7 ms (not statistically significant compared to normal subjects).

AD after Treatment

Two months after treatment with donepezil, the mean aMT in the 12 AD patients was 33.5 ± 3.5%. The mean duration of the SP was 150.5 ± 85.0 ms. There was no significant statistical difference with the normal subjects or AD patients before treatment.

LEPs occurred in 5 out of 12 patients (42%), with a mean latency of 96.8 ± 29.2 ms, a mean amplitude of 860.4 ± 225.9 μV and a mean duration of 46.6 ± 21.9 ms. There was no

significant difference in latency, amplitude or duration of the LEPs between the patients before and after the treatment. In the 5 patients with an 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 an LEP before treatment. In the 7 patients without an LEP, the mean duration of the SP was 90.6 ± 28.6 ms, which was also not significant compared to the 4 patients without an LEP before treatment and to the normal subjects.

Discussion

In our study, the duration of the whole SP valley was significantly increased in untreated AD patients compared to normal subjects. After treatment with donepezil, the duration of the SP valley decreased. A significant increase in LEP occurrence causing a fragmentation of the SP valley was also observed in AD patients compared to normal subjects, with a trend to normalize when patients were treated with donepezil. The duration, the amplitude and the latency of LEPs in AD patients did not show any significant difference before and after treatment.

Combining those results, we could hypothesize that the increased duration of the whole SP valley in AD patients before treatment could be due to the presence of multiple LEPs. The presence of an LEP itself is indicative of cortical disinhibition [21]. The observed return of an SP valley to normal values along with the decrease in LEP occurrence after donepezil intake suggest a cholinergic control of the neuronal circuits controlling the genesis of the LEP and the duration and shape of the SP in early AD patients.

Based on Orth and Rothwell [24], and later supported by more studies [19, 20], there is a strong correlation between the production of MEP and SP occurrence. It is already known that TMS primarily activates the fast-conducting pyramidal neurons leading to the genesis of MEP. However, from experiments in cats [25] it is also known that recurrent collaterals of these fast-conducting neurons have an inhibitory effect on slower-conducting pyramidal neurons most probably by exciting intercalated inhibitory neurons. As slow-conducting pyramidal neurons are responsible for the maintenance of tonic voluntary muscle contraction, the inhibition of those neurons is presumed to be responsible for SP occurrence.

Neuroanatomy and animal experiments indicate that in the layer I of M1 there is a very rich presence of cholinergic fibers which lessens in layers II–III [25, 26]. In layers II–III, the apical dendrites of pyramidal cells (PC) are found, which have cholinergic afferents. A strong release of ACH from cholinergic axons located in layer I can therefore stimulate the PC by acting on their muscarinic receptors. In addition, the layers II–III of the motor cortex are very rich in GABAergic neurons [27, 28]. The basket which GABAergic neurons specifically form by their axons is a connecting network with the apical dendrites of PC located there.

The results of our study show a fragmentation and an enlargement of the SP in the presence of multiple LEPs in early untreated AD patients. Along with the neurophysiological model of Orth and Rothwell [24] we suggest that the disturbance in ACH output in early AD leads to a decrease in excitability of the PC. Subsequently, the excitability of the intercalated inhibitory GABAergic neurons is reduced. Less inhibition is exerted by the GABAergic neurons onto the slow-conducting PC responsible for the maintenance of the voluntary isometric contraction. As a final outcome, various LEPs appear which fragment the SP valley and increase its duration.

Donepezil intake tends to normalize the shape and duration of the SP with less LEP occurrence, which strongly supports the aforementioned hypothesis. The observation that in normal subjects LEPs are extremely rare is also in favor of our hypothesis of the central role of ACH in the regulation of the pathways controlling the corticospinal outflow of the primary motor cortex.

While LEP occurrence appears to be strongly influenced by the cholinergic system, the mean latency, duration and amplitude of LEPs does not differ in all 3 groups, suggesting a noncholinergic mechanism which determines those parameters. Moreover, ACH cannot completely inhibit LEP occurrence in all AD patients. In the cases where LEPs persist despite treatment, the SP has a similar duration to that of the untreated AD patients. Maybe this could be due to a lack of therapeutic efficacy of donepezil. However, the correlation between the clinical responsiveness to donepezil and LEP occurrence was not studied here. Also, the significance of the LEP occurrence in one normal subject is not clear. Further studies are needed to examine the utility of LEPs as potential biomarkers.

Disclosure Statement

The authors declare no conflicts of interest.

References

1 Khedr E, Ahmed M, Darwish E, Ali A: The relationship between motor cortex excitability and severity of Alzheimer's disease: a transcranial magnetic stimulation study. Neurophysiol Clin 2011;41:107–113.
2 Kobayashi K, Shimoda K, Higashima M, Nakano H, Miyazu K, Hayashi M, Tabata O, Koshino Y: Report of three cases of Alzheimer's disease with focal motor symptoms: clinical correlates of neuroimaging findings. World J Biol Psychiatry 2000;1:164–169.
3 Kearney F, Harwood R, Gladman J, Lincoln N, Masud T: The relationship between executive function and falls and gait abnormalities in older adults: a systematic review. Dement Geriatr Cogn Disord 2013;36:20–35.
4 Scarmeas N, Papadimitriou A, Dubois B, Sarazin M, Brandt J, Albert M, Marder K, Bell K, Honig LS, Wegesin D, Stem Y: Motor signs during the course of Alzheimer disease. Neurology 2004;63:975–982.
5 Pissiotto M, Higley M, Minneur W: Acetylcholine as a neuromodulator: cholinergic signaling shapes nervous system function and behaviour. Neuron 2012;76:116–129.
6 Meldrum BS: Glutamate as a neurotransmitter in the brain: review of physiology and pathology. J Nutr 2000;130:1007S–1015S.
7 McCormick DA: GABA as an inhibitory neurotransmitter in human cerebral cortex. J Neurophysiol 1989;62:1018–1027.
8 Schliebs R, Arendt T: The cholinergic system in aging and neuronal degeneration. Behav Brain Res 2011;221:555–563.
9 Schliebs R, Arendt T: The significance of the cholinergic system in the brain during aging and in Alzheimer's disease. J Neural Transm 2006;113:1625–1644.
10 Thiyagesh SN, Farrow TF, Parks RW, Accosta-Mesa H, Hunter MD, Young C, Wilkinson ID, Woodruff PW: Treatment effects of therapeutic cholinesterase inhibitors on visuospatial processing in Alzheimer's disease: a longitudinal functional MRI study. Dement Geriatr Cogn Disord 2010;29:176–188.
11 Vidoni E, Thomas G, Honea R, Loskutova N, Burns J: Evidence of altered corticomotor system connectivity in early-stage Alzheimer's disease. J Neurol Phys Ther 2012;36:8–16.
12 Pepin JL, Bogacz D, de Pasqua V, Delwaide PJ: Motor cortex inhibition is not impaired in patients with Alzheimer's disease: evidence from paired transcranial magnetic stimulation. J Neurol Sci 1999;170:119–123.
13 Di Lazzaro V, Oliviero A, Pilato F, Saturno E, Dileone M, Marra C, Ghirlanda S, Ranieri F, Gainotti G, Tonali P: Motor cortex hyperexcitability to transcranial magnetic stimulation in Alzheimer's disease. J Neurol Neurosurg Psychiatry 2004;75:555–559.
14 Pennisi G, Alagona G, Ferri R, Greco S, Santonocito D, Pappalardo A, Bella R: Motor cortex excitability in Alzheimer disease: one year follow-up study. Neurosci Lett 2002;329:293–296.
15 Di Lazzaro V, Oliviero A, Profice P, Pennisi MA, Di Giovanni S, Zito G, Tonali P, Rothwell JC: Muscarinic receptor blockade has differential effects on the excitability of intracortical circuits in the human motor cortex. Exp Brain Res 2000;135:455–461.
16 Di Lazzaro V, Oliviero A, Tonali PA, Marra C, Daniele A, Profice P, Saturno E, Pilato F, Masullo C, Rothwell JC: Noninvasive in vivo assessment of cholinergic cortical circuits in AD using transcranial magnetic stimulation. Neurology 2002;59:392–397.
17 Nardone R, Bergmann J, Kronbichler M, Kunz A, Klein S, Caleri F, Tezzon F, Ladurner G, Golaszewski S: Abnormal short latency afferent inhibition in early Alzheimer's disease: a transcranial magnetic demonstration. J Neural Transm 2008;115:1557–1562.

18 Nardone R, Golaszewski S, Ladurner G, Tezzon F, Trinka E: A review of transcranial magnetic stimulation in the in vivo functional evaluation of central cholinergic circuits in dementia. Dement Geriatr Cogn Disord 2011; 32:18–25.
19 Kimiskidis V, Papagiannopoulos Z, Sotirakoglou K, Kazis A, Kazis D, Mills K: Silent period to transcranial magnetic stimulation: construction and properties of stimulus-response curves in healthy volunteers. Exp Brain Res 2005;163:21–31.
20 Rabago C, Lancaster J, Narayana S, Zhang W, Fox P: Automated-parameterization of the motor evoked potential and cortical silent period induced by transcranial magnetic stimulation. Clin Neurophysiol 2009;120:1577–1587.
21 Wilson S, Thickbroom G, Mastaglia S: An investigation of the late excitatory potential in the hand following magnetic stimulation of the motor cortex. Electroencephalogr Clin Neurophysiol 1995;97:55–62.
22 Fritz C, Braune H, Pylatiuk C, Pohl M: Silent period following transcranial stimulation: a study of intra- and inter-examiner reliability. Electroencephalogr Clin Neurophysiol 1997;105:235–240.
23 Rossi S, Hallett M, Rossini P, Pascual-Leone A; Safety of TMS Consensus Group: Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Clin Neurophysiol 2009;120:2008–2039.
24 Orth M, Rothwell JC: The cortical silent period: intrinsic variability and relation to the waveform of the transcranial magnetic stimulation. Clin Neurophysiol 2004;115:1076–1082.
25 Phillips CG, Porter R: Corticospinal neurones. Their role in movement. Monogr Physiol Soc 1977;34:1–450.
26 Hess G, Donoghue J: Facilitation of long-term potentiation in layer II/III horizontal connections of rat motor cortex following layer I stimulation: route of effect and cholinergic contributions. Exp Brain Res 1999;127: 279–290.
27 Gentet L: Functional diversity of supragranular GABAergic neurons in the barrel cortex. Front Neural Circuits 2012;17:6–52.
28 Hendry S, House C, Jones E, Vaughn J: Synaptic organization of immunocytochemically identified GABA neurons in the monkey sensory-motor cortex. J Neurocytol 1983;12:639–660.