Int J Clin Exp Med 2014;7(12):5643-5649
www.ijcem.com /ISSN:1940-5901/IJCEM0002878

Original Article

Evaluation of spinal cord motor function in Alzheimer's disease using electrophysiological techniques indicates association of acetylcholine receptors with the disease

Li Yang*, Chunxia Li*, Xiuying Chen, Jie Wang, Shanshan Gao, Liling Yang, Yunxia Zhao, Hua Wang, Yifeng Du

*Department of Neurology, Shandong Provincial Hospital Affiliated to Shandong University, Jinan City, Shandong Province, P. R. China. *Equal contributors.*

Received September 29, 2014; Accepted December 8, 2014; Epub December 15, 2014; Published December 30, 2014

Abstract: Aims: This study is to evaluate the spinal cord motor function in patients with Alzheimer's disease (AD). Methods: In this study, 40 patients were diagnosed to have AD. The stages of dementia were evaluated using Mini Mental State Examination and Activity of Daily Living scale. According to the levels of movement disturbances, the patient group was further divided into dyskinesia subgroup (14 cases) and non-dyskinesia subgroup (26 cases). In addition, 45 age-matched healthy volunteers were included into the control group. Transcranial magnetic stimulation was used to evaluate motor function in the whole motor conduction pathway from motor cortex to target muscles. Electrophysiological studies were used to analyze the pyramidal tract and anterior horn neurons of the spinal cord. Results: The mean duration of F waves was prolonged, while no significant differences were found between AD patients and control subjects in parameters such as spinal cord motor conduction velocity, amplitude of motor-evoked potential, F-wave persistence, minimal latency of F-waves and maximal amplitude of F-waves. These data indicated that the excitability of the spinal cord was increased, and the number and function of pyramidal tract and anterior horn cells were integral. Conclusions: The primary mechanism of AD is probably associated with acetylcholine receptors that may participate in the formation of senile plaques and neurofibrillary tangles. These findings may provide new therapeutic targets for the treatment of AD.

Keywords: Alzheimer's disease, spinal cord, transcranial magnetic stimulation, F-waves, acetylcholine receptor

Introduction

Alzheimer's disease (AD), the most common type of dementia in the elderly, is a neurodegenerative disorder. It is characterized by progressive impairment of cognitive function in its early stage, which especially affects memory. Movement disturbances, such as narrow stride, bradykinesia and dyscoordinations, are developed in the late course of the disease. Currently, the cause of this disease is not clear, although it is possibly considered to be related to aging, heredity, environmental factors, etc. The neuropathology of AD is characterized by extracellular formation of amyloid β plaques, intracellular deposition of neurofibrillary tangles and neural loss, especially the loss of cholinergic neurons in specific cortical areas such as the basal forebrain, the hippocampus, and the associated parietal and frontal areas. However, it is still unclear what is the key factor leading to the pathogenesis of AD.

Recently, such pathological alterations were observed in the primary motor cortex [1] and spinal cord [2, 3] in autopsy cases, and were verified in transgenic mice [4, 5]. Therefore, more attentions should be paid on the spinal cord of patients with AD. There is no unified point of view on the pathogenesis of AD by now. Cholinergic system dysfunction is a very important hypothesis. Cholinergic receptor was considered to be closely related to cognition [6-10], playing an important role in the pathogenesis of AD. There were many aspects of cholinergic system dysfunction in the brains of AD patients, such as decrease of acetylcholine, low activity of choline synthetase, reduction of the number of cholinergic receptor, etc. For a large class of cholinergic cells, it is unknown whether anterior

Table 1. Demographic and clinical data of AD patient and control groups

	AD patients	Control
Age (years)	66.4 ± 4.9	66.8 ± 5.7
Gender (M/F)	23/17	25/20
Disease duration (months)	23.5 (11.1)	-
Education levels (years)	9.3 ± 4.8	9.6 ± 4.2
MMSE score	16.5 ± 8.1	28.4 ± 4.2
ADL score	29.2 ± 12	17.5 ± 5.6

Note: AD, Alzheimer's disease; MMSE, Mini Mental State Examination. Data are expressed as mean values ± standard deviations.

horn neurons of the spinal cord are also involved. Because the same pathological changes are found in the spinal cord as in the brain, it is worth investigating whether the motor conduction of spinal cord is affected. In the present study, spinal cord motor function in AD is evaluated using electrophysiological techniques.

Materials and methods

Subjects

In this study, 40 patients (23 females and 17 males; mean age, 66.4 years; age range, 55-82 years) were recruited into the patient group. The patients were diagnosed to have mild-to-severe AD according to the criteria of the National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer Disease and Related Disorders Association (NINCDS-ADRDA) [11]. The stages of dementia were evaluated by means of the Mini Mental State Examination (MMSE) [12] and Activity of Daily Living (ADL) scale [13]. According to the presence or absence of movement disturbances, the patient group was divided into two subgroups, dyskinesia group (14 cases) and non-dyskinesia group (26 cases). All patients showed diffuse brain atrophy according to computed tomography (CT) or magnetic resonance imaging (MRI). Patients with cerebral vascular disease, metabolic disturbance, epilepsy, metallic objects in the body, history of brain trauma, spinal cord diseases or radiculopathy were excluded. Routine nerve conduction examination was implemented to rule out peripheral neuropathy. None of the patients were treated with medicine or any other forms of medication. In addition, 45 age-matched healthy volunteers were included into the control group (25 females and 20 males; mean age, 66.8 years; age range 55-83 years; mean MMSE, 28.4) (**Table 1**). All subjects were right-handed and were able to understand and execute the tasks instructed during the whole test procedure. Written informed consent was obtained from all subjects. The study was approved by the Ethics Committee of Shandong University in accordance with the Declaration of Helsinki of the World Medical Association.

Transcranial magnetic stimulation and electromyogram

Transcranial magnetic stimulation, widely used for AD in recent years, is a non-invasive technique used to evaluate motor function in the whole motor conduction pathway from motor cortex to target muscles. At the time of study, the neurophysiologists who performed the tests were blinded to neuropsychological diagnosis. Magnetic stimulation was delivered using a Magpro Compact stimulator (Medtronic A/S, USA). A circular coil was held over the right motor cortex at the optimum scalp position to elicit motor-evoked potentials (MEP) in the contralateral abductor pollicis brevis (APB) muscles and abductor hallucis (AH) muscles. For the activation of APB muscles, the magnetic coil was placed in the center at 2 cm in front of C^{-3} or C^{-4} when 10-20 system was used. For the activation of AH muscles, the stimulating coil was placed at the vertex. Muscle responses were obtained via two surface electrodes with the active electrode over the belly and the reference electrode at the tendon of the muscle. The shortest onset latency and the largest peak-to-peak amplitude of MEP (MEPamp), obtained from 5-10 responses were selected and analyzed. The rest motor threshold (RMT) was also recorded and analyzed. A Dantec-Keypoint electromyogram instrument was used for electrophysiological studies. Electromyogram parameters included a bandpass of 20-1000 Hz and a recording time window of 200 ms.

F-waves

F-wave is an effective index reflecting the number and excitability of functional lower motor neurons. F-waves were evoked by 20 supramaximal stimuli (1 Hz) on the median and tibial nerve at the wrist or ankle bilaterally. The record

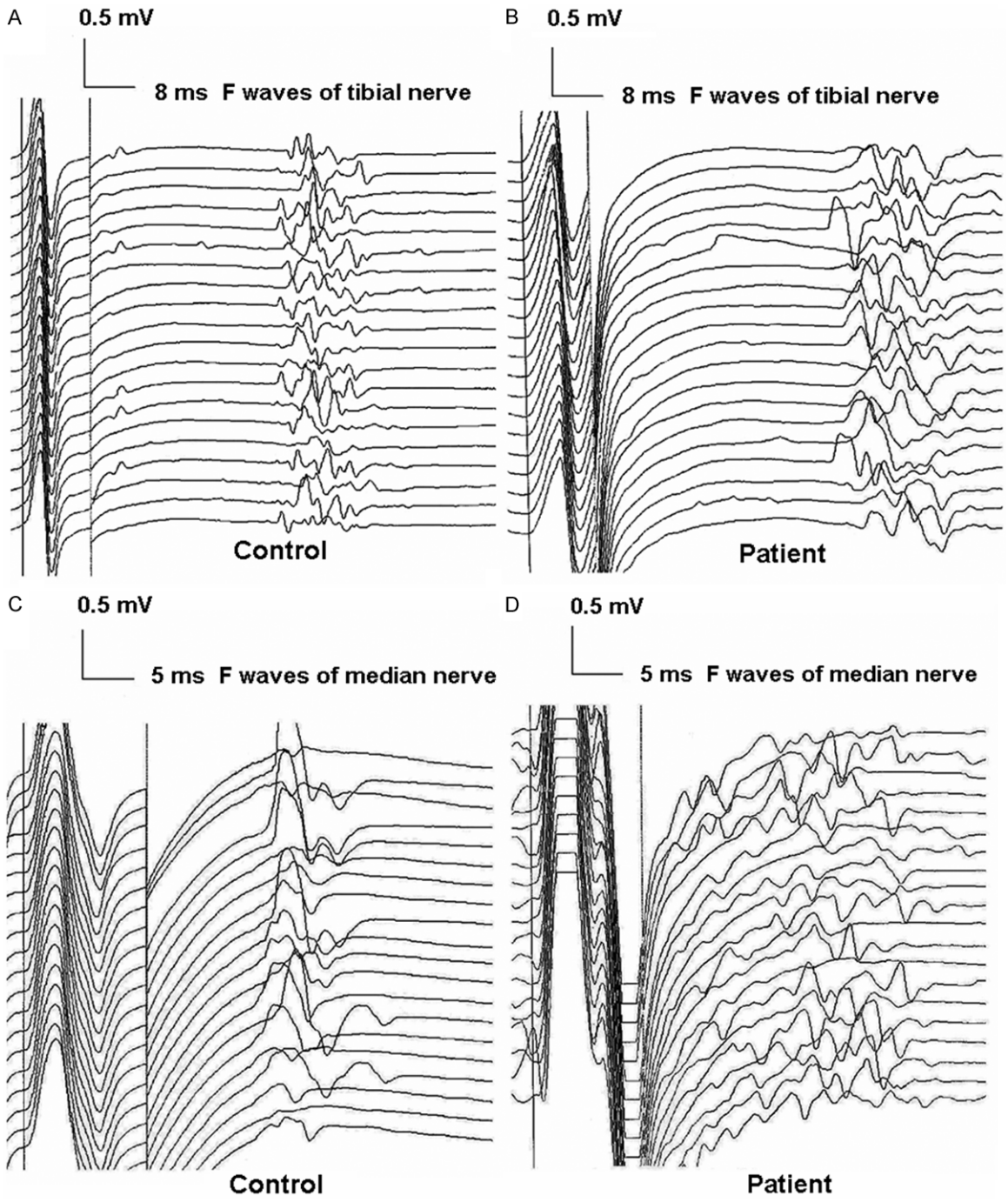

Figure 1. F waves for tibial and median nerves in control and patient groups. The mean duration of tibial and median nerve recorded on AH muscle and APB muscle was more extended in AD patient than in control subject. The horizontal bar indicates 8 ms and 5 ms in the figures of F waves for tibial and median nerves, respectively, and the vertical bar indicates 0.5 mV.

positions were the same as transcranial magnetic stimulation. F-wave persistence, minimum latency (Fmin), mean duration (Fdur, time from onset to the final return to baseline) and maximal amplitude (Famp, peak to peak amplitude) were analyzed, and Fmin values were corrected for height [Fmin values (ms)/height (m)]. F-wave duration can reflect the excitability of spinal cord motor neurons. In situations where the excitability of motor neuron pool increases,

Table 2. Electrophysiological data of dyskinesia subjects and non-dyskinesia subjects with AD

Measurements	Non-dyskinesia subjects (means ± SD)		Dyskinesia subjects (means ± SD)		P (t-test)	
	Median	Tibial	Median	Tibial	Median	Tibial
MMSE score	20.7 ± 6.0		12.1 ± 7.4		0.003	
SCMCV	56.65 ± 8.14		57.93 ± 6.67		0.668	
MEPamp	6.12 ± 3.14	2.33 ± 1.68	5.91 ± 3.48	2.18 ± 1.83	0.468	0.389
F-persistence	0.88 ± 0.08	0.91 ± 0.09	0.92 ± 0.09	0.93 ± 0.07	0.316	0.288
Fmin/H (ms/m)	17.89 ± 3.23	28.90 ± 5.05	18.11 ± 3.84	29.09 ± 5.11	0.673	0.588
Fdur (ms)	10.73 ± 2.99	19.01 ± 3.71	10.823 ± 3.622	18.11 ± 3.84	0.323	0.469
Famp	0.72 ± 0.56	0.68 ± 0.35	0.78 ± 0.55	0.66 ± 0.33	0.666	0.573
RMT (%MSO)	35.82 ± 8.90	52.32 ± 7.40	38.37 ± 8.80	50.53 ± 7.14	0.321	0.398

Note: MMSE, Mini Mental State Examination; SCMCV, spinal cord motor conduction velocity; MEPamp, amplitude of motor evoked potential; Fmin, minimum latency of F-waves; Fdur, duration of F-waves; Famp, amplitude of F-waves; RMT, resting motor threshold; MSO, maximum stimulator output.

a greater number of smaller and slow conduction motor units discharge. Since the conduction velocity of this new responsive group of neurons is more variable than that of larger ones, the emergent F wave has prolonged duration [14, 15].

Spinal cord motor conduction velocity (SCMCV)

SCMCV is used to reflect the integrity of the myelin sheath of pyramidal tract. It is calculated from the distance between the spinous processes of C-7 and T-12 divided by the spinal cord motor conduction time [16]. The formula is as follows:

SCMCV = spinal distance (C7-T12)/{[$L_{MC/AH}$-(F_t+M_t-1)/2]-[$L_{MC/APB}$-(F_m+M_m-1)/2]}, where $L_{MC/AH}$ represents latency from motor cortex to abductor hallucis muscle; $L_{MC/APB}$ represents latency from motor cortex to abductor pollicis brevis muscle; (F+M-1)/2 represents the peripheral conduction time (from spinal cord to muscle); t represents tibial nerve; m represents median nerve.

Statistical analyses

Statistical analysis of experimental data and comparisons of groups were performed using Student's t-test by SPSS v17.0 (IBM, USA). The relations between different variables were evaluated by means of non-parametric Spearman correlation. $P < 0.05$ was considered statistically significant.

Results

The excitability of cortex and spinal cord in AD patients is increased, but the motor neurons in cortex, corticospinal tract and anterior horn neurons in spinal cord are not involved even in dyskinesia subjects. To evaluate the function of spinal cord motor in Alzheimer's disease, electrophysiological techniques were employed. As there was no significant difference between the right and left hemispheres in any of the parameters studied, the right hemisphere was used in both patient and control groups. The mean Fdur recorded on APB and AH was more extended in AD patients than in control subjects (**Figure 1**; **Table 3**), but no significant difference was observed between dyskinesia subjects and non-dyskinesia subjects with AD (**Table 2**). The mean RMT for transcranial magnetic stimulation was significantly lower in AD patients than in the control subjects (**Table 3**), but no significant difference was observed between dyskinesia subjects and non-dyskinesia subjects with AD. In addition, no significant results were obtained for SCMCV between AD patients and control subjects, or between dyskinesia subjects and non-dyskinesia subjects with AD (**Table 2**). MMSE average scores obtained for the patients and control subjects were 16.5 ± 8.1 and 28.4 ± 4.2, respectively. The MMSE average scores of dyskinesia subjects were lower than that in non-dyskinesia subjects (12.1 ± 7.4 and 20.7 ± 6.0, respectively; $P = 0.003$) (**Table 2**), indicating that more severe patients were more prone to have movement disorders. The values of MEPamp, F-persistence, Fmin and Famp were not significantly different between the patient group and control group, or between dyskinesia subjects and non-dyskinesia subjects with AD. The Fdur and RMT values were not correlated significantly with the age of patients, the duration of the

Table 3. Electrophysiological data of AD patients and control subjects

Measurements	AD patients (means ± SD)		Control subjects (means ± SD)		P (t-test)	
	Median	Tibial	Median	Tibial	Median	Tibial
SCMCV	56.76 ± 7.21		58.55 ± 6.83		0.622	
MEPamp	5.99 ± 3.35	2.21 ± 1.72	5.39 ± 2.72	1.61 ± 1.29	0.533	0.272
F-persistence	0.90 ± 0.08	0.93 ± 0.08	0.92 ± 0.09	0.95 ± 0.07	0.417	0.429
Fmin/H (ms/m)	18.11 ± 3.84	29.09 ± 5.11	16.16 ± 2.90	28.64 ± 4.88	0.522	0.328
Fdur (ms)	10.82 ± 3.62	18.11 ± 3.84	9.273 ± 2.122	16.16 ± 2.90	0.032	0.013
Famp	0.78 ± 0.55	0.66 ± 0.33	0.86 ± 0.382	0.79 ± 0.70	0.460	0.361
RMT (%MSO)	38.10 ± 8.80	50.53 ± 7.14	44.5 ± 11.2	62.15 ± 12.41	0.026	0.000

Note: SCMCV, spinal cord motor conduction velocity; MEPamp, amplitude of motor evoked potential; Fmin, minimum latency of F-waves; Fdur, duration of F-waves; Famp, amplitude of F-waves; RMT, resting motor threshold; MSO, maximum stimulator output.

disease or the scores of MMSE. There was no significant correlation between Fdur and RMT (Spearman's r = 0.24). These data suggested that the excitability of cortex and spinal cord in AD patients was increased, but the motor neurons in cortex, corticospinal tract and anterior horn neurons in spinal cord were not involved even in dyskinesia subjects.

Discussion

In this study, the amplitudes of MEP, SCMCV and peripheral nerve conduction were not significantly different between patient group and control group. This meant that cortex motor neurons, corticospinal tract and anterior horn neurons in the spinal cord were normal in both number and function, even in patients with movement disturbances. The neurotransmitter of corticospinal tract is glutamic acid instead of acetylcholine, which may explain the protection of motor neurons in cortex and corticospinal tract. Since the main pathological change of AD is the loss of cholinergic neurons in central neuron system, it is interesting to know why the anterior horn neurons in spinal cord, as cholinergic neurons, can avoid injury. We interpret out data to suggest that this may be related to the difference between central and peripheral cholinergic receptors. Nicotinic acetylcholine receptors (nAChRs), a kind of ion channel receptors, include two types, peripheral nAChRs and central nAChRs. Peripheral nAChRs include five subtypes, α1, β1, γ, δ and ε, while central nAChRs include twelve subtypes, α2-10 and β2-4. Among these subtypes, α4β2 and α7 are the commonest in the brain. Neuron loss within certain areas of brain is probably related to subtypes of nAChRs in these regions. A pronounced loss of α4β2 nAChRs in the cerebral cortex has been found in autopsy studies of AD brain and by *in vivo* positron emission tomography imaging with 11C-nicotine in AD patients [17]. There are massive cholinergic neurons in basal forebrain that mainly project to the limbic system and the hippocampus, distributing large amount of nAChRs. Therefore, these regions are injured more severely. Although the anterior horn neurons in the spinal cord are cholinergic neurons, they are protected by their normal peripheral nAChRs in the postsynaptic membrane. There are no reports about the decrease of peripheral nAChRs in AD patients. Schmidt et al. [2] showed that neurofibrillary tangles are mostly located in the intermediate substance of spinal cord in autopsy cases. Only a few tangles are found in the anterior horn of spinal cord, but most of them appear in the microglia and astrocytes. The microglia and astrocytes are innervated by axon collaterals of anterior horn neurons, in which the neurotransmitter is acetylcholine. It can be inferred that neurofibrillary tangle formation is associated with acetylcholine receptor. It has been found that subtypes α4β2 and α7 of central nAChRs may promote the phosphorylation of tau protein. Other researchers demonstrated that α4β2 and α7 subtypes are easy to combine with β amyloid to stimulate the deposition of senile plaque [18-20], and the presence of nicotinic receptors sensitizes cells to the toxic actions of β-amyloid [21].

The results of the present indicated that the high excitability of motor cortex and anterior horn cells in the spinal cord might be related to

the damage of cholinergic system. The possible mechanism is that most of the interneurons in cerebral cortex and spinal cord are inhibitory neurons, using γ-aminobutyric acid or glycine as the neurotransmitter. If these interneurons lose control of acetylcholine, the inhibition to motor neurons of the cortex and anterior horn cells in the spinal cord may be reduced or absent, leading to high excitability of cortex and spinal cord. One study strongly suggested that functional activity and perhaps functional upregulation of α7-nAChR are necessary for production of Aβ-induced neural hyperexcitation and possibly AD [22]. The reason for movement disturbances may be the disregulation of the motor pathway, such as the change of inhibition or facilitation of intercortex or intracortex and the alteration of extrapyramidal system affected by the change of neurotransmitter.

In summary, the study of spinal cord motor function using electrophysiological techniques showed that the number and function of pyramidal tract and anterior horn cells were integral, and the excitability of spinal cord was increased. We interpret these data to suggest that AD is primarily associated with AChRs that may participate in the formation of senile plaques and neurofibrillary tangles. These findings may provide new therapeutic targets for the treatment of AD.

Acknowledgements

This work was supported by Shandong University and Shandong Provincial Hospital.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Yifeng Du, Department of Neurology, Shandong Provincial Hospital Affiliated to Shandong University, 324 Jingwuweiqi Road, Jinan City 250021, Shandong Province, P. R. China. Tel: +86-531-68776451; E-mail: alzheimer2014@163.com

References

[1] Suvà D, Favre I, Kraftsik R, Esteban M, Lobrinus A and Miklossy J. Primary motor cortex involvement in Alzheimer disease. J Neuropathol Exp Neurol 1999; 58: 1125-1134.

[2] Schmidt ML, Zhukareva V, Perl DP, Sheridan SK, Schuck T, Lee VM and Trojanowski JQ. Spinal Cord Neurofibrillary Pathology in Alzheimer Disease and Guam Parkinsonism-Dementia Complex. J Neuropathol Exp Neurol 2001; 60: 1075-1086.

[3] Saito Y and Murayama S. Expression of tau immunoreactivity in the spinal motor neurons of Alzheimer's disease. Neurology 2000; 55: 1727-1729.

[4] Seo JS, Leem YH, Lee KW, Kim SW, Lee JK and Han PL. Severe motor neuron degeneration in the spinal cord of the Tg2576 mouse model of Alzheimer disease. J Alzheimers Dis 2010; 21: 263-276.

[5] Spittaels K, Van den Haute C, Van Dorpe J, Bruynseels K, Vandezande K, Laenen I, Geerts H, Mercken M, Sciot R, Van Lommel A, Loos R and Van Leuven F. Prominent axonopathy in the brain and spinal cord of transgenic mice overexpressing fourrepeat human tau protein. Am J Pathol 1999; 155: 2153-2165.

[6] Albuquerque EX, Pereira EF, Alkondon M and Rogers SW. Mammalian nicotinic acetylcholine receptors: Fromstructure to function. Physiol Rev 2009; 89: 73-120.

[7] Taly A, Corringer PJ, Guedin D, Lestage P and Changeux JP. Nicotinic receptors: Allosteric transitions and thera-peutic targets in the nervous system. Nat Rev Drug Discov 2009; 8: 733-750.

[8] Thomsen MS, Hansen HH, Timmerman DB and Mikkelsen JD. Cognitive improvement by activation of alpha7 nicotinic acetylcholine receptors: From animal models to human pathophysiology. Curr Pharm Des 2010; 16: 323-343.

[9] Bao F, Wicklund L, Lacor PN, Klein WL, Nordberg A and Marutle A. Different beta-amyloid oligomer assemblies in Alzheimer brains correlate with age of diseaseonset and impaired cholinergic activity. Neurobiol Aging 2012; 33: 825.

[10] Okada H, Ouchi Y, Ogawa M, Futatsubashi M, Saito Y, Yoshikawa E, Terada T, Oboshi Y, Tsukada H, Ueki T, Watanabe M, Yamashita T and Magata Y. Alterations in a4b2 nicotinic receptors in cognitive decline in Alzheimer's aetiopathology. Brain 2013; 136: 3004-3017.

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

[12] Folstein MF, Folstein SE and McHugh PR. "Mini-mental state": A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975; 12: 189-198.

[13] Lawton MP and Brody EM. Assessment of older people: self-maintaining and instrumental

activities of daily living. Gerontologist 1969; 9: 179-186.
[14] Fisher MA. H reflexes and F waves Fundamentals, normal and abnormal patterns. Neurol Clin 2002; 20: 339-360.
[15] Milanov IG. F-wave for assessment of segmental motoneurone excitability. Electromyogr Clin Neurophysiol 1992; 32: 11-15.
[16] Chang CW and Lin SM. Predictability of surgical results of herniated disc-induced cervical myelopathy based on spinal cord motor conduction study. Neurosurg Rev 1999; 22: 107-111.
[17] Paterson D and Nordberg A. Neuronal nicotinic receptors in the human brain. Prog Neurobiol 2000; 61: 75-111.
[18] Wang HY, Lee DH, D'Andrea MR, Peterson PA, Shank RP and Reitz AB. beta-amyloid (1-42) binds to alpha 7 nicotinic acetylcholine receptor with high affinity-Implications for Alzheimer's disease pathology. J Biol Chem 2000; 275: 5626-5632.
[19] Nagele RG, D'Andrea MR, Anderson WJ and Wang HY. Intracellular accumulation of beta-amyloid (1-42) in neurons is facilitated by the alpha7 nicotinic acetylcholine receptor in Alzheimer's disease. Neuroscience 2002; 110: 199-211.
[20] Pettit DL, Shao Z and Yakel JL. beta-Amyloid (1-42) peptide directly modulates nicotinic receptors in the rat hippocampal slice. J Neurosci 2001; 21: RC120.
[21] Arora K, Alfulaij N, Higa JK, Panee J and Nichols RA. Nichols Impact of Sustained Exposure to β-Amyloid on Calcium Homeostasis and Neuronal Integrity in Model Nerve Cell System Expressing α4β2 Nicotinic Acetylcholine Receptors. J Biol Chem 2013; 288: 11175-11190.
[22] Liu Q, Xie X, Lukas RJ, St John PA and Wu J. A Novel Nicotinic Mechanism Underlies β-Amyloid-Induced Neuronal Hyperexcitation. J Neurosci 2013; 33: 7253-7263.