Study on variation trend of repetitive nerve stimulation waveform in amyotrophic lateral sclerosis

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Abstract

Background: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease involving both upper and lower motor neurons with no effective cure. Electrophysiological studies have found decremental responses during low-frequency repetitive nerve stimulation (RNS) except for diffused neurogenic activities. However, the difference between ALS and generalized myasthenia gravis (GMG) in terms of waveform features is unclear. In the current study, we explored the variation trend of the amplitudes curve between ALS and GMG with low-frequency, positive RNS, and the possible mechanism is discussed preliminarily.

Methods: A total of 85 ALS patients and 41 GMG patients were recruited. All patients were from Peking Union Medical College Hospital (PUMCH) between July 1, 2012 and February 28, 2015. RNS study included ulnar nerve, accessory nerve and facial nerve at 3 Hz and 5 Hz stimulation. The percentage reduction in the amplitude of the fourth or fifth wave from the first wave was calculated and compared with the normal values of our hospital. A 15% decrease in amplitude is defined as a decrease in amplitude.

Results: The decremental response at low-frequency RNS showed the abnormal rate of RNS decline was 54.1% (46/85) in the ALS group, and the results of different nerves were 54.1% (46/85) of the accessory nerve, 8.2% (7/85) of the ulnar nerve and 0% (0/85) of the facial nerve stimulation, respectively. In the GMG group, the abnormal rate of RNS decline was 100% (41/41) at low-frequency RNS of accessory nerves. However, there was a significant difference between the 2 groups in the amplitude after the sixth wave.

Conclusions: Both groups of patients are able to show a decreasing amplitude of low-frequency stimulation RNS, but the recovery trend after the sixth wave has significant variation. It implies the different pathogenesis of NMJ dysfunction of these 2 diseases.

Keywords: Amyotrophic lateral sclerosis; Generalized myasthenia gravis; Neuromuscular junction; Repetitive nerve stimulation

Introduction

Amyotrophic lateral sclerosis (ALS) is the most common type of motor neuron disease, and it was first described by French neurologist Jean-Martin Charcot in 1869. It is one of the multiple neurodegenerative disorders with the occult onset and a relentlessly progressive duration, mainly involving pyramidal cells in the cerebral cortex and the motor nucleus in the brainstem, as well as pyramidal tracts and anterior horn cells of the spinal cord. ALS has an annual incidence of 1.5 to 2.5 cases per 100,000 people, among whom 50% survive 30 months; approximately 10% can live 10 years or more.[1-4]

Recently, an increasing number of studies have discovered that apart from upper and lower motor neurons, ALS also involves the neuromuscular junction (NMJ) and leads to its dysfunction. Currently, the function of NMJ is mainly evaluated by repetitive nerve stimulation (RNS). In 1959, Mulder first discovered that ALS patients with muscle fatigable phenomena produced a decremental response during low-frequency RNS, which suggested that the NMJ was involved in ALS.[5] The decremental pattern in low-frequency RNS of ALS can be shown in multiple nerves (eg, the accessory nerve, axillary nerve, radial nerve, median nerve, ulnar nerve, and facial nerve); the accessory nerve, which dominates proximal muscles, shows the highest positive rate, while a decremental pattern in low-frequency RNS for the facial nerve is rarely seen.[6-12] Several investigators analyzed the relationship between low-frequency RNS results and the clinical features of ALS patients and found that the positive rate of the decremental pattern in low-frequency RNS was higher in the limb onset group than that in the bulbar onset group.[10] Additionally, the extent of the decrement in low-frequency RNS was more significant in the rapidly progressive group than in

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those for whom the disease developed slowly,[7,10] and patients with severe muscle atrophy were more liable to produce a decremental pattern in low-frequency RNS.[6] However, there were no dominant discrepancies between the low-frequency RNS results of different disease duration and the ALS function scale.[8-10] The mechanism underlying the decremental pattern in low-frequency RNS of ALS patients has not been clarified and requires further exploration. Furthermore, there has been no research thus far focusing on the regularity of the amplitude curve in ALS patients.

Myasthenia gravis (MG), a classical NMJ disorder, caused by the action of acetylcholine receptor antibody (AchR-ab) on the postsynaptic membrane blocking the transmission process of the NMJ. MG always exhibits a decremental response in low-frequency RNS, with the most obvious decrement in the 4th or 5th waves. For generalized MG (GMG), the sensitivity and specificity of the low-frequency RNS are 79% and 97%, respectively, while for ocular myasthenia, they are 29% and 94%.[13]

Though ALS and GMG have different mechanisms, they both exhibit a decremental pattern in low-frequency RNS. This study aimed to discover the differences in decremental patterns in low-frequency RNS between ALS and GMG.

Methods

Ethical approval

The retrospective study was conducted in accordance with the *Declaration of Helsinki* and was approved by the Ethics Committee of Peking Union Medical College Hospital (PUMCH, No. S-619). And the requirement for the written informed contents was waived due to the retrospective nature of the study.

Subjects

Eight-five ALS patients, 50 males and 35 females with mean age 54.03 ± 11.78 years, diagnosed as clinically definite ALS, clinically probable ALS and clinically probable ALS–laboratory-supported using the revised EI Escorial criteria.[14] The median duration of ALS patients from symptom onset was 13.94 ± 11.78 months. Forty-one with age-matched GMG patients, 24 males and 17 females were recruited. Their mean age was 53.69 ± 11.10 years, diagnosed according to clinical features, RNS, the titer of AchR-ab and responds to treatment. All patients were from PUMCH between July 1, 2012 and February 28, 2015. Exclusion criteria for ALS patients were as follows: a) ALS-like syndrome caused by other etiologies; b) comorbidities such as severe cervical spondylopathy, lumbar spondylopathy, syringomyelia, and so on; and c) suffering from neurological diseases such as a cerebral vascular disease that would interfere with the muscle strength evaluation. Exclusion criteria for GMG patients were as follows: a) taking anticholinesterase agents for worse disease conditions during the RNS test and b) comorbidities such as peripheral neuropathy and other diseases that influence nerve and muscle functions.

Nerve conduction and EMG studies

Routine nerve conduction studies included motor and sensory conduction studies of the median, ulnar, tibial, and peroneal nerves. Motor conduction parameters included measurements of peak to peak amplitude of CMAP, distal motor latency (DML) and motor conduction velocity (MCV). Measurements of peak to peak amplitude of sensory action potential and sensory conduction velocities were recorded in sensory conduction studies. Nerve conduction studies were compared with normal values of the neurophysiological laboratory of PUMCH. Needle EMG included spontaneous potentials, duration, and amplitudes during slight muscle contraction and phase and amplitude of raising potential during strongly muscle contraction.

RNS test

Low-frequency RNS was performed on the accessory nerve, ulnar nerve, and facial nerve, and recorded on the trapezius, abductor digiti minimi and orbicularis oculi. Stimulation frequencies included 3 Hz and 5 Hz with 3 to 5 s stimuli each time. The calculation method of decreasing amplitude was (1st CMAP amplitude 4th CMAP amplitude) × 100%/1st CMAP amplitude. A positive result of low-frequency RNS (denoted LFRNS-P) was defined as a decrement ≥15%, while a suspected positive result was defined as a decrement of 10% to 15%, according to the results of age and gender-matched normal controls from the neurophysiological laboratory of PUMCH.

All ALS patients and GMG patients had RNS tests. We also analyzed the variation trend of waveforms.

Statistical analysis

SPSS 17.0 software (SPSS Inc., Chicago, IL, USA) was applied for statistical analysis. Categorical variables were expressed as frequency or percentage (%), while continuous variables were expressed as the mean ± standard deviation (SD) and were evaluated with a normality test (One-Sample Kolmogorov-Smirnov test). In the analysis of significant differences between groups, categorical variables were compared with the χ^2 test. For continuous variables, differences between groups were assessed with Student's t test or the Mann-Whitney U test according to their distribution. In the correlation analysis, Pearson analysis was used for normal distribution and Spearman analysis was used for non-normal distribution. Clinical factors that influenced the low-frequency RNS results of ALS were accessed by logistic regression analysis. All P values were 2-tailed, and a $P < 0.05$ was considered statistically significant.

Results

Abnormal rates in both groups

Eight-five ALS patients with RNS results were enrolled in the present study. The abnormal rate of low-frequency RNS was 54.1% (46/85), but the abnormal rate is different at different nerve stimulation. The accessory nerve was 54.1% (46/85), the ulnar nerve was 8.2% (7/85) and the

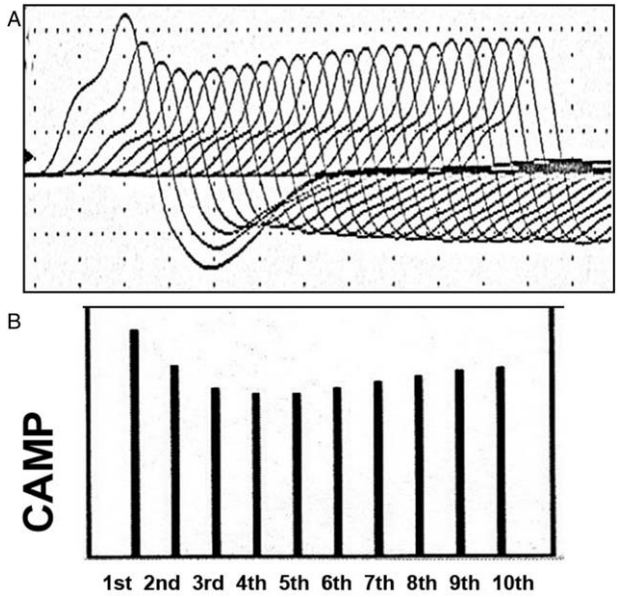

Figure 1: Descent pattern of low-frequency RNS in GMG patients. (A) and (B) are the results of the accessory nerve stimulation RNS in a GMG patient. There is an obvious decreasing amplitude at 5 Hz stimulation, but the fifth wave starts to rise significantly. CMAP: Compound muscle action potential; GMG: Generalized myasthenia gravis; RNS: Repetitive nerve stimulation.

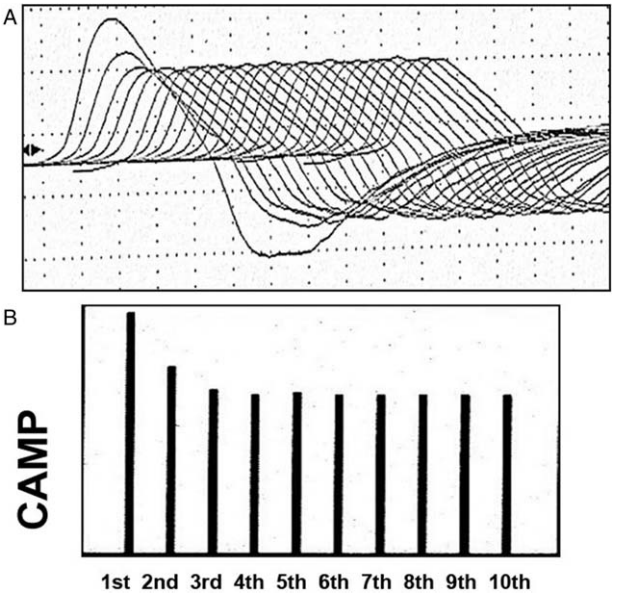

Figure 2: Descent pattern of low-frequency RNS in ALS patients. (A) and (B) are the results of the accessory nerve stimulation RNS in an ALS patient. There is an obvious decreasing amplitude at 5 Hz stimulation, but no obvious rebound after the fifth wave. ALS: Amyotrophic lateral sclerosis; CMAP: Compound muscle action potential; RNS: Repetitive nerve stimulation.

facial nerve was 0% (0/85), respectively. There were a significantly different among the 3 nerves RNS at the low-frequency ($P < 0.001$). The abnormal rate of RNS was 100% in 41 GMG patients, but the abnormal rate was different in different nerve stimulation, with 100% of accessory nerve, 43.9% of the ulnar nerve and 79.0% of the facial nerve.

Tendency of change in shape with decreasing amplitude

The results of RNS in all patients showed a trend of decreasing amplitude study in 46 ALS patients and 41 GMG patients. There was no significant different change in waveforms of RNS between 3 Hz and 5 Hz stimulation. The analysis of facial nerve RNS waveform changes was abandoned because facial nerve RNS did not decrease in patients with ALS.

In GMG patients, the decrement of low-frequency RNS usually drops to the nadir at 4th or 5th CMAP, and recovers significantly since the 6th CMAP [Figure 1]. However, it is found different waveform changes in ALS [Figure 2].

Based on the mean value and standard deviation of each wave's (1st to 10th) amplitude ratio [Table 1], the respective amplitude curves of the accessory nerve and ulnar nerve in 3 and 5 Hz frequencies are displayed in Figures 3 and 4.

In ALS patients, we found that the curves of RNS at 3 and 5 Hz stimulation did not show significant recovery after decreasing to the greatest extent at the 4th or 5th CMAP. In GMG patients, there were remarkable amplitudes recover after the 4th or 5th wave starts to recover comparing the changes of amplitude curve at the stimulation of an accessory nerve with the same frequency in GMG patients [Figure 3]. A similar phenomenon was discovered in the analysis of the ulnar nerve [Figure 4].

As shown in Figures 3 and 4, both the amplitude curves shaped like "L" were similar in the 2 low frequencies stimulation (3 and 5 Hz) for the same nerve (accessory nerve or ulnar nerve) in the ALS patients. The amplitude curves shaped like "U" was found in GMG patients at the low frequencies (3 and 5 Hz) at the accessory nerve and ulnar nerve.

The mean values of each (1st to 10th) amplitude ratio of the accessory nerve stimulation at 3 and 5 Hz in ALS patients and GMG patients were listed in Table 1. The lowest decremental response was found in 4th at 3 Hz stimulation and 5th at 5 Hz stimulation respectively in ALS patients. The amplitude of the 10th wave was slightly increased regardless of the 3 or 5 Hz stimulation comparing with 4th. The amplitude ratio of the 10th and that of the 6th was the same at 3 Hz stimulation and slightly increased at 5 Hz stimulation. It was consistent with shaped "L". In GMG patients, the lowest decremental response was found in 5th at either 3 and 5 Hz stimulation respectively. The amplitude ratio of the 10th wave was significantly elevated between the 3rd and 4th. It was consistent with shaped "U".

The result of ulnar nerve RNS is almost the same as that of accessory nerve. The mean values of each (1st to 10th) amplitude ratio at 3 Hz and 5 Hz ulnar nerve stimulation in ALS patients and GMG patients were listed in Table 1. The lowest decremental response was found in 5th at 3 Hz stimulation and 4th at 5 Hz stimulation respectively in ALS

Table 1: Mean value of amplitude ratio in accessory and ulnar nerve for RNS in ALS and GMG.

Disease	Parameters		Stimulations									
			1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
ALS	Accessory nerve	3 Hz	1.000	0.874	0.814	0.782	0.787	0.789	0.794	0.789	0.791	0.789
		5 Hz	1.000	0.884	0.809	0.779	0.771	0.777	0.783	0.784	0.784	0.786
	Ulnar nerve	3 Hz	1.000	0.878	0.825	0.813	0.808	0.815	0.813	0.813	0.820	0.818
		5 Hz	1.000	0.892	0.832	0.804	0.810	0.816	0.818	0.824	0.830	0.826
GMG	Accessory nerve	3 Hz	1.000	0.831	0.740	0.702	0.699	0.704	0.712	0.712	0.711	0.717
		5 Hz	1.000	0.829	0.723	0.677	0.664	0.668	0.673	0.680	0.687	0.689
	Ulnar nerve	3 Hz	1.000	0.847	0.764	0.734	0.737	0.755	0.765	0.775	0.779	0.787
		5 Hz	1.000	0.856	0.763	0.728	0.730	0.742	0.757	0.773	0.786	0.793

ALS: Amyotrophic lateral sclerosis; GMG: generalized myasthenia gravis; RNS: repetitive nerve stimulation.

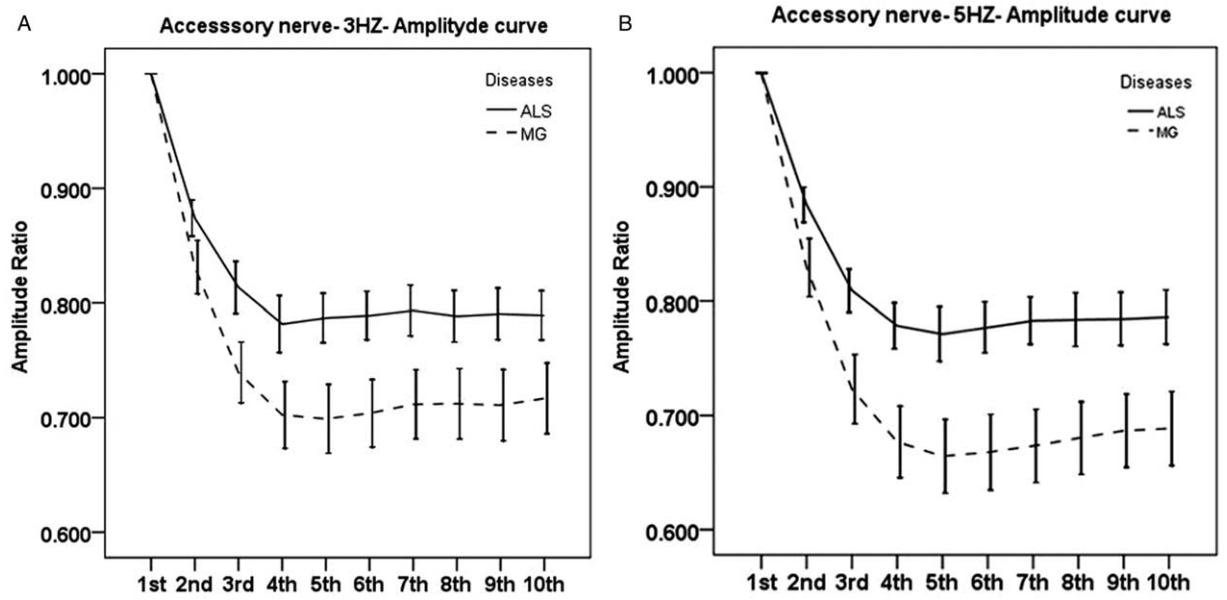

Figure 3: RNS in accessory nerve. There are remarkable decremental responses at either 3 Hz (A) and 5 Hz (B) accessory nerve stimulation RNS. The lowest was found in 4th and 5th. After the waves gradually pick up and the waveform looks like "U" in GMG patients. The waveform looks like "L" in ALS patients. ALS: Amyotrophic lateral sclerosis; GMG: Generalized myasthenia gravis; RNS: Repetitive nerve stimulation.

patients. The amplitude ratio of the 10th was slightly increased regardless of the 3 or 5 Hz stimulation comparing with 4th or 5th in ALS patients. The amplitude ratio of 10th was closed to the 6th at 3 Hz stimulation and closed to the 8th at 5 Hz stimulation. It was consistent with shaped "L". In GMG patients, the lowest decremental response was found in 4th at either 3 and 5 Hz stimulation respectively. The amplitude ratio of the 10th increased significantly between the 2nd and 3rd at 3 and 5 Hz stimulation. It was consistent with shaped "U".

The comparison of the amplitude ratio between the 2 waves at the same frequency was undertaken with a paired *t* test; the *P* values are listed in Figure 5. For ALS patients' accessory nerves [Figure 5A], the lowest CMAP appeared in the 4th one at 3 Hz, and there were no significant differences between the 4th and 5th, 6th, 8th, and 10th CMAP amplitude ratios. The lowest CMAP appeared in the 5th one at 5 Hz, and the 5th CMAP was significantly lower than the 4th and 7th–10th CMAPs. However, for both 3 and 5 Hz, there were no significant differences between either of them in the 6th–10th CMAPs, and the amplitudes of the 4th–10th CMAP were all less than that of the 3rd one. These characteristics indicated that for the accessory nerve in ALS, there was no significant recovery after the nadir of decrement. For ALS patients' ulnar nerve [Figure 5B], the lowest CMAP appeared in the 5th one at 3 Hz, and there were no significant differences between the 5th and 6th–10th CMAP. However, the lowest CMAP appeared in the 4th one at 5 Hz, and there were no significant differences between the 4th and 5th, 6th, 7th, and 10th CMAP amplitude ratios. At 3 and 5 Hz, there were almost no statistically significant differences in either frequency between the 6th and 10th CMAP, and there were no significant differences between the 5th–10th and 3rd CMAP. These characteristics suggested that for the ulnar nerve in ALS, there was also no significant recovery after the nadir of decrement.

The comparison of amplitude ratio between 2 waves at the same frequency was undertaken by a paired *t* test. The

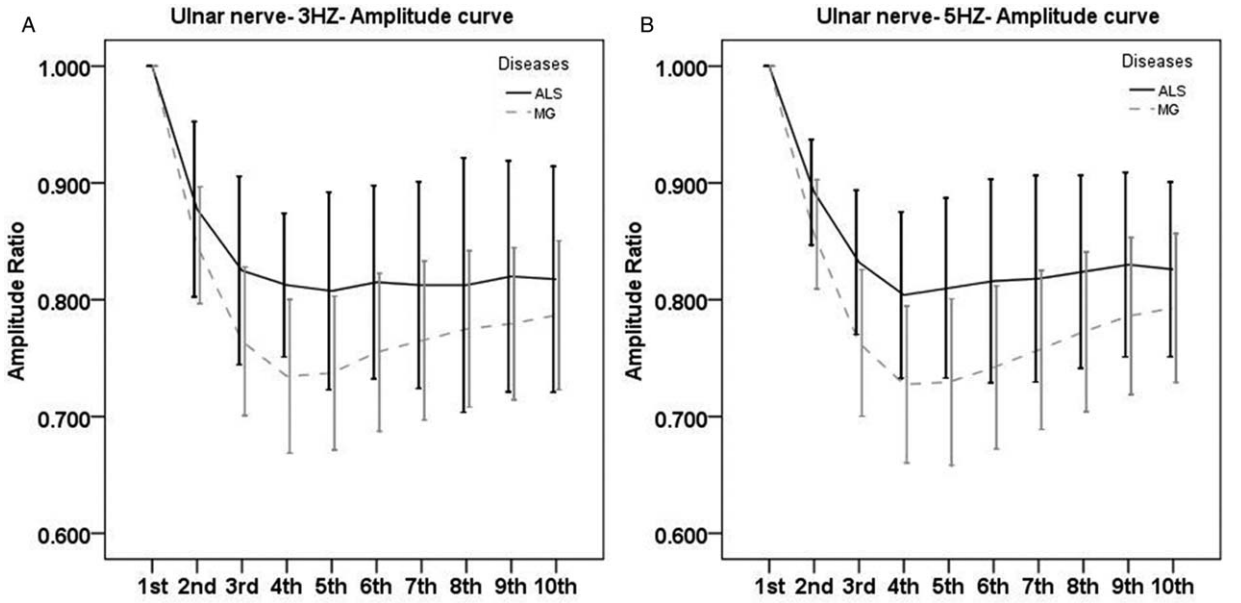

Figure 4: RNS in ulnar nerve. There are remarkable decremental responses at either 3 Hz (A) or 5 Hz (B) ulnar nerve stimulation RNS. The lowest was found in 4th and 5th. After the waves gradually pick up and the waveform looks like "U" in GMG patients. The waveform looks like "L" in ALS patients. ALS: Amyotrophic lateral sclerosis; GMG: Generalized myasthenia gravis; RNS: Repetitive nerve stimulation.

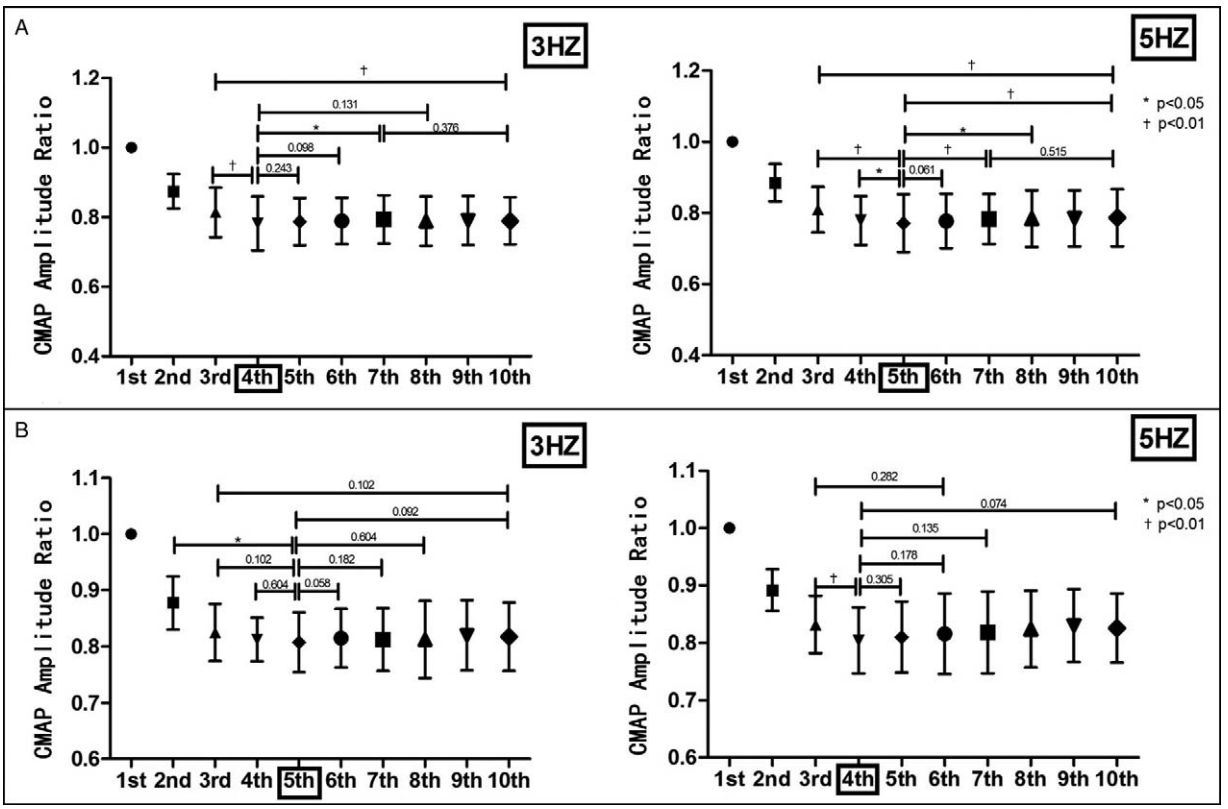

Figure 5: Amplitude ratio curve of ALS's accessory nerve and ulnar nerve. For ALS patients' accessory nerves (A) and ulnar nerve (B), the lowest CMAP appeared in the 4th or 5th one at 3 Hz or 5 Hz RNS, there was no significant recovery after the nadir of decrement. ALS: Amyotrophic lateral sclerosis; CMAP: Compound muscle action potential; GMG: Generalized myasthenia gravis; RNS: Repetitive nerve stimulation.

amplitude ratio curves, as well as some of the *P* values for the 2 paired CMAPs are shown in Figure 6. For GMG's accessory nerve [Figure 6A], the lowest CMAP appeared in the 5th one at 3 Hz, with no significant differences between the 5th and either the 4th or 6th CMAP amplitude. However, the 5th CMAP was lower than the 7th to 10th CMAPs and this result was statistically significant ($P < 0.01$). Additionally, the amplitudes of the 7th to 10th

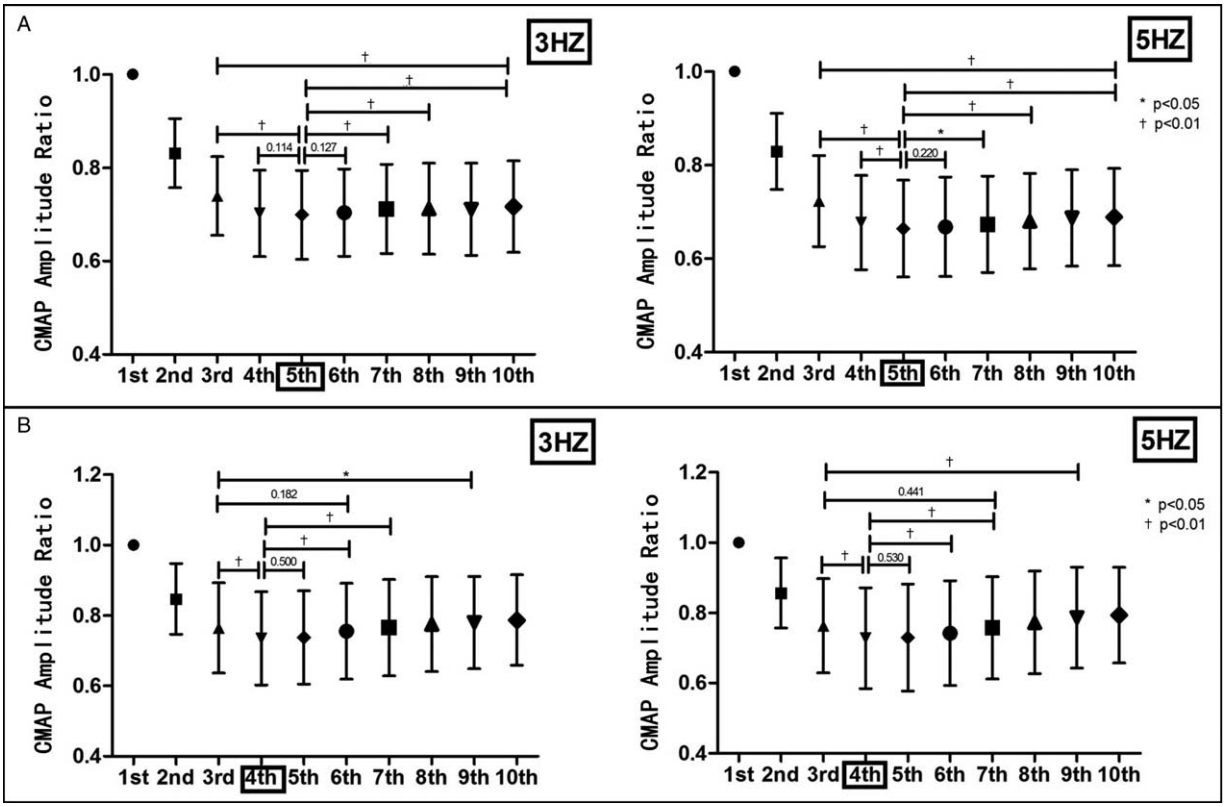

Figure 6: Amplitude ratio curve of GMG's accessory nerve, ulnar nerve. For GMG's accessory nerve (A) and ulnar nerve (B), the lowest CMAP emerged in the 4th or 5th one at low-frequency RNS, there was slow recovery after the nadir of decrement from the 6th or 7th CMAP. ALS: Amyotrophic lateral sclerosis; CMAP: Compound muscle action potential; GMG: Generalized myasthenia gravis; RNS: Repetitive nerve stimulation.

CMAP were higher than that of the 6th one ($P < 0.05$), slowly rebounding with all amplitudes lower than the 3rd ($P < 0.01$). Similarly, the lowest CMAP appeared in the 5th one at 5 Hz, and the 5th CMAP was lower than the 4th and 7th to 10th ones (with statistical significance). Furthermore, the 7th to 10th CMAP increased successively, and the amplitude ratios of the 4th to 10th CMAP were less than that of the 3rd one ($P < 0.01$). These characteristics indicated that for GMG's accessory nerve, there was slow recovery after the nadir of decrement; there was an obvious rebound from the 7th CMAP, though it was no higher than the 3rd one. For GMG's ulnar nerve [Figure 6B], the lowest CMAP emerged in the 4th one upon stimulation with both 3 and 5 Hz and with no significant difference between the 4th and 5th CMAP. Nevertheless, the 4th CMAP was significantly lower than the 6th to 10th ones ($P < 0.01$). The 6th to 10th CMAPs rose gradually, and the 8th to 10th CMAPs were higher than the 3rd one but lower than the 2nd one ($P < 0.05$). These results implied that for GMG's ulnar nerve, there was gradual recovery after the nadir of decrement in the 4th CMAP, with an apparent rebound from the 6th CMAP. The 8th to 10th CMAPs were higher than the 3rd one but lower than the 2nd one.

Correlation between RNS results and clinical features for ALS

Eighty-five ALS patients were divided into 2 groups according to the results of the RNS [Table 2]. Abnormal results of RNS were not correlated with age, gender, and course of the disease. However, there was a significant correlation with the onset site and the degree of limbs muscle weakness. Abnormal rates were higher in the limb onset group than that in the bulb onset group ($P < 0.05$).

Discussion

In the current study, we found that the decrement of low-frequency RNS in GMGs was typical "U-shaped", which presented as gradual recovery after the nadir of decrement. Nevertheless, there was no significant recovery after the nadir of decrement in ALS as "L-shaped". It is useful to differential diagnosis between GMG and ALS. The tendencies of variation in the decrement curve of low-frequency RNS between GMG and ALS indicated the different pathogenesis of NMJ dysfunction of these 2 diseases.

NMJ is the structure that is vital to achieving unidirectional impulse conduction between nerve and muscle and consists of the presynaptic region, synaptic cleft, and postsynaptic region. There are a large number of mitochondria, vesicles containing abundant acetylcholine (Ach) and presynaptic membrane in the presynaptic region. Each vesicle releases 5000 to 10,000 molecules of Ach in the form of quantum.[15] Ach combines with AchR located in the postsynaptic membrane, allowing the Na^+ channel to open and the muscle cell membrane to be depolarized to produce miniature endplate potential

Table 2: Correlation between RNS results and clinical features for ALS.

Parameters	ALS/RNS (+)	ALS/RNS (−)	Statistics	*P*
Frequency	46	39		
Gender (M/F)	26/20	24/15	0.219*	0.640
Age (years)	54.39±11.50	53.67±11.96	−0.284†	0.777
Duration (months)	14.80±8.96	13.08±8.63	−0.901†	0.370
Onset site				
Limb	32	17	5.833*	0.016
Bulb	14	22		
Tendon reflexes of upper limb				
Hyporeflexia	7	2	2.342*	0.310
Normal	17	15		
Hyperreflexia	22	22		
Muscle strength of upper limb proximal				
0–III	21	3	18.278*	0.001
IV–V	25	36		
Muscle strength of upper limb distal				
0–III	16	5	8.385*	0.015
IV–V	30	34		

* χ^2 value. † *t* value. ALS: Amyotrophic lateral sclerosis; RNS: repetitive nerve stimulation.

(MEPP). Abundant combinations of Ach and AchR happen simultaneously, and ample MEPPs then combine together to form end-plate potential (EPP). When EPP exceeds the threshold, also known as a safety factor, the muscle fiber action potential (MFAP) is generated and then leads to muscle contraction. The EPP is proportional to the amount of Ach binding to AchR.

MG is a typical NMJ disorder caused by immune mediation. Previous research has indicated that antibodies against AchR (AchR-Ab)[16] exist in approximately 80% to 90% of GMG. The MEPP, EPP and safety factor all consequently decline as the number of effective bindings between Ach and AchR decreases. When MG patients undergo low-frequency RNS, the EPP of the 1st CMAP is above the safety factor, MFAPs form normally, and no decrements of CMAP occur. As the number of stimulations increases, normal quanta depletion and the declined safety factor lead to lower EPPs produced by later stimulations, and no MFAP formation occurs. Thus, with the decrease in the number of MFAPs, the decrements of CMAP amplitude and area are observed as the CMAP decremental phenomenon. The recovery of calcium level in the presynaptic membrane requires approximately 100 ms, so low-frequency RNS has little effect on the recovery of calcium concentration. As the mobilized quanta replenish, immediate release quanta require 1 to 2 s; thus, for low-frequency RNS, the depleted quanta for immediate release are resupplied at approximately the 6th or 7th wave, and the number of MFAPs increases and recovers gradually. For this reason, MG patients gain significant recovery at roughly the 6th or 7th CMAP after the nadir of the decrement in low-frequency RNS, which is also named the "U-shape".

ALS is not a primary NMJ disorder such as MG or Lambert-Eaton syndrome, and the mechanism of NMJ dysfunction caused by ALS is very sophisticated. ALS is characterized by progressive denervation and chronic nerve regeneration. The anterior horn motor neurons of the spinal cord degenerate and are lost in ALS; thus, the corresponding denervation occurs in the muscle fibers dominated by these neurons, and NMJ structures are destroyed. Denys has implied that neuronal degeneration contributes to insufficient nutrition of nerve endings and the corresponding NMJ structure changes.[6] The surviving lower motor neurons around the degenerated neurons will dominate the muscle fibers with new denervation through collateral sprouting, and new NMJ structures form. With the sustained denervation and reinnervation in ALS, the survival neurons degenerate and necrosis occurs before the newly formed NMJ structures mature; meanwhile, other nearby survival motor neurons sprout collaterally to form other new NMJ structures. Along with the repeated cycles, the newly sprouted NMJ structures are always immature;[17] the presynaptic membrane, synaptic cleft, and postsynaptic membrane are all abnormal, and the safety factor of neuromuscular transmission descends. Additionally, the release and storage amount of Ach decline accordingly with the immature NMJ structure. Wollman conducted an intracellular record in the NMJ structure of ALS and found that MEPP, the Ach release amount, the number of immediate release quanta and the storage quanta to resupply all descend, but the possibility of Ach release in most synapses is regular.[18] In conclusion, the persistent denervation and reinnervation existing in ALS lead to involvement of the whole NMJ structure rather than a specific and local region such as the presynaptic region, synaptic cleft or postsynaptic membrane.

Some researchers have revealed that the NMJ involvement in ALS might be related to inflammation or an autoimmune reaction. Currently, the immune-mediated or inflammatory mechanisms mainly contain the following

aspects. The overexpression of *Bax* and *Bcl-2* genes in the postsynaptic membrane of the NMJ of ALS bring about the apoptosis of correspondingly allocated muscle fibers.[19] Some studies reported that there are various antibodies against VGCC, AchR, and Lrp4, which lead to neuromuscular transmission disorder.[20-24] Furthermore, a high level of some inflammatory cytokines (IL-8, IL-15, TNF-α, and others) might be connected to NMJ dysfunction.[23] Several studies have confirmed that microRNAs play key roles in generation, development, and repair after injury to the NMJ structure.[24] For example, Gregorio found that in ALS mouse models, the miR-206 mutation weakens reinnervation and further promotes the destruction of NMJs.[25] Nevertheless, the concrete signal pathway of miRNAs in NMJ involvement in ALS is not clear, nor is the explicit region of NMJ affected by miRNAs. In summary, the mechanism of NMJ involvement in ALS is highly sophisticated and is unlike MG or Lambert-Eaton syndrome, which involves particular regions of the NMJ structure.

The mechanism in ALS is probably related to the involvement of the whole NMJ structure. Consequently, in the present study, there was no significant recovery after the nadir of the decrement (U-shape) for ALS like there was in the GMGs in the low-frequency RNS in all tested nerves. The present study showed that the positive rate of low-frequency RNS in ALS is 54.1%. The positive rate for the accessory nerve governing proximal muscles was significantly higher than that for the ulnar nerve governing distal muscles, which was consistent with previous research and indicated that the safety factor of distal muscles is relatively high.[8,10,11] A correlation analysis between low-frequency RNS and clinical characteristics of ALS was conducted in this study. No significant correlation between a low-frequency, positive RNS rate, and gender, age or disease duration was observed, but the positive rates were higher in the limb onset group than those in the bulbar onset group, which agreed with prior studies.[8-10,12] This is a rare study to report that the positive rate of low-frequency RNS was not different in the tendon reflexes of the upper limb, but was significantly higher in those with muscle strength of 0–III.

The number of ALS patients in the present study is relatively small compared to other neurological diseases. The incidence of ALS disease is about 1–3/100,000, so it is difficult to study patients with large sample size. The future study of multi-center including larger samples is needed. On the other hand, various factors in ALS procession can influence the result of RNS, like the staging. However, there is no uniform staging method be used worldwide to date. Therefore, there is still a need for more detailed stratification research in the future.

It is of profound significance to compare the patterns of low-frequency RNS decrement curves between ALS and GMG. The finding that there was a significant difference in variation tendency of the decrement curve further implied different pathogenesis of NMJ dysfunction in these 2 diseases. If RNS can be determined many times after diagnosis, it might be more valuable to explore the mechanism and study the clinical relationship.

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Conflicts of interest

None.

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