

# Low Frequency-Repetitive Nerve Stimulation and Factors Affecting Decremental Response in Amyotrophic Lateral Sclerosis: A Retrospective Study of 449 Cases

**Jinghong Zhang**

Chinese PLA General Hospital

**Hongfen Wang**

Chinese PLA General Hospital

**Fei Yang**

Chinese PLA General Hospital

**Zhengqing He**

Chinese PLA General Hospital

**Fang Cui**

Chinese PLA General Hospital

**Mao Li**

Chinese PLA General Hospital

**Bo Sun**

Chinese PLA General Hospital

**Xusheng Huang** (✉ [lewish301@sina.com](mailto:lewish301@sina.com))

Chinese PLA General Hospital

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## Research Article

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# Abstract

A number of studies have demonstrated that decremental response to low frequency repetitive nerve stimulation (LF-RNS) is frequently observed in amyotrophic lateral sclerosis (ALS). However, due to the small sample size involved in previous studies, large discrepancies exist about the positivity rates of LF-RNS tests and factors affecting decremental response. This retrospective study of 449 cases, the largest sample size ever reported, shows that the overall positivity rate of LF-RNS is 49.9%. 3Hz RNS delivered to the accessory nerve has the best sensitivity and highest positivity rate. It obviously increases in response to upper limb onset, disease progression rate < 0.5 score/month, definite ALS and electromyography positive(EMG(+)) in sternocleidomastoid muscle. There is a linear increase in the decrement percentage of CMAP amplitude at 3Hz RNS delivered to the accessory nerve in response to longer disease duration, longer MUP duration and greater MUP duration increment percentage. These findings substantially advance the understanding of RNS results in ALS patients and effectively instruct clinical application.

## 1. Introduction

Amyotrophic lateral sclerosis (ALS), a fatal motor neuron disease, causes progressive muscle atrophy and weakness in bulbar, limbs and trunks. It also affects sensory system, autonomic nerve system and senior cortex functions, leading to non-motor symptoms such as paresthesias, pain, cognitive impairment, affective disturbance and behavioral disorders (Tao et al., 2018). The incidence of ALS ranges from 1.5 to 2.4 out of 100,000 individuals per year, and its prevalence approximately from 4.0 to 7.9 out of 100,000, and about 10% of the patients are familial while the rest 90% are sporadic (Chiò et al., 2013). Since Mulder et al (1959) first detected the decrement amplitude of compound muscle action potential(CMAP) when they administered low-frequency RNS(LF-RNS) to ALS patients with muscle fatigue, more and more studies have confirmed the presence of the decrement, which suggests that neuromuscular junction(NMJ) dysfunction is involved in ALS (Oral et al., 2017). However, due to the inconsistency of the instruments and cut-off values in different laboratories as well as the limited number of the samples involved in previous studies (Sun et al., 2018; Tomoko et al., 2011; Zheng et al., 2017a; Fu et al., 2019; Wang et al., 2017; Alanazy et al., 2017; Zheng et al., 2017b; Hatanaka et al., 2017; Hu et al., 2018), the consequences caused by both random errors and systemic errors have been unavoidable, which have resulted in disparities in the reported conclusions of previous studies and their alleged clinical significance.

Therefore, we collected the records of 449 ALS cases, the largest sample size that has ever been reported, from the Neurology Department of the First Medical Center, Chinese PLA General Hospital and performed a retrospective study of RNS involved in the cases with an aim to determine the positivity rate at LF-RNS, the best stimulus position and frequency, and to quantify the relationship of decremental CMAP amplitudes to clinical indicators and motor unit potential (MUP) duration, so as to substantially advance the understanding of LF-RNS results in ALS patients and effectively instruct clinical application.

## 2. Methods

The subjects of the study were 449 patients diagnosed with ALS based on the revised El Escorial (Brook et al., 2000) at Neurology Department of the First Medical Center, Chinese PLA General Hospital from June 2016 to September 2020 and their clinical and neuroelectrophysiological data were collected with certainty that none of

the subjects had other NMJ diseases. This study was approved by the Medical Ethics Committee of Chinese PLA General Hospital. All enrolled subjects signed informed consents by themselves or their legal guardians.

All the patients were classified into four diagnostic levels (clinically definite, clinically probable, clinically probable laboratory-supported and clinically possible) according to the revised El Escorial criteria. Their data included gender, age, disease duration, onset site, the results of needle electromyography (EMG) and LF-RNS, etc. The patients were scored according to the scales of ALSFRS-R (Cedarbaum et al., 1999), and the rate of disease progression ( $\Delta FS$ ) was calculated by the following formula:  $\Delta FS = [48 - \text{ALSFRS-R score}] / \text{duration (month)}$  (Labra et al., 2016). The increasing values of  $\Delta FS$  indicate the faster rates of the disease progression.

The electrodiagnostic studies, including RNS and EMG, were performed on a Keypoint workstation(31A06, Alpine Biomed ApS, Denmark). Skin temperature over the examined muscle was maintained at 32°C or above throughout the entire measurement. Surface electrodes were used to record the belly-tendon compound muscle action potential (CMAP). LF-RNS was performed in the following muscles: deltoid for the axillary nerve ( $n = 65$ ), trapezius for the accessory nerve ( $n = 446$ ), abductor digiti minimi (ADM) for the ulnar nerve( $n = 308$ ), tibialis anterior(TIB) for the common peroneal nerve( $n = 73$ ). A low frequency of 3Hz train of the 10 stimuli was delivered to the nerves and recorded. The peak-to-peak CMAP amplitude decrement was measured by the decremental percentage of the forth CMAP as compared to the first CMAP amplitude. Based on the conventional criterion, a decremental response of 10% or greater in 1Hz or 3Hz were considered positive, in accordance with the suggestions of the American Academy of Emergency Medicine Quality Assurance Committee. With reference to the normative values as set up by EMG laboratory of Peking Union Medical College Hospital, MUP mean duration increment percentage was calculated by the following formula: mean duration increment percentage (%) = (measured mean duration-normal mean duration)/normal mean duration. Measured mean duration indicates the mean value of 20 MUP durations. According to our EMG department criteria, MUP duration exceeding 20%, with or without abnormal spontaneous potential, is considered as chronic denervation potential, i.e. EMG positive result.

MS-Excel 2016 and MATLABR2019b software (multifunction, trial free version) were employed for statistical analysis. Measurement statistics satisfying the normal distribution was expressed as mean  $\pm$  SD, while non-normal distribution data was expressed as mean  $\pm$  SD and median. Enumeration data were presented as the number of cases and percentage. Chi-square test was used for comparison between different enumerative groups. Least squares method and F-test were used for simple linear regression.  $P < 0.05$  was considered statistically significant.

## 3. Results

### 3.1 Clinical records of the enrolled patients

The present study involves 449 patients with 267 males and 182 females at the ratio of 1.47:1, whose ages are between 26 and 86 years old in a normal distribution (average age at  $54.4 \pm 10.3$  years old). The disease onset of 185 patients (41.2%) are from upper limbs, 162(36.1%) from lower limbs and 102(22.7%) from bulbar, with the former two types of samples occupy the majority, whereas there are no cases begins with weakness in respiratory muscles. 180 patients are assessed by ALSFRS-R, scoring 14–46(average:  $8.5 \pm 5.2$ ; median: 40) so that the progression rate ranges from 0.07 to 7.5 score per month (average:  $0.95 \pm 0.97$ ; median: 0.67).

### 3.2 LF-RNS positivity rate

The distributions of decremental responses on four detected nerves are summarized in Table 1. Results show: the positivity rate of 3Hz is significantly higher than that of 1Hz; 3Hz RNS on the accessory nerve, followed by that on the axillary nerve (40.0%), has the highest positivity rate (47.3%) and it covers all positive cases stimulated by 1Hz. The positivity rates of both common peroneal nerve and ulnar nerve are lower than 8%. As it is, the accessory nerve is the best ideal site for detection and 3Hz is the best ideal frequency.

Table 1  
The positivity rate of LF-RNS in ALS patients

Tested nerves	1Hz			3Hz		
	Cases	ADR(%)	RNS (+, %)	Cases	ADR(%)	RNS (+, %)
Accessory nerve	431	-57 ~ 8	25.1 (108)	446	-55 ~ 18	47.3 (211)
Axillary nerve	65	-29 ~ 14	13.8 (9)	65	-37 ~ 16	40.0 (26)
Common peroneal nerve	73	-12 ~ 5	1.4 (1)	79	-14 ~ 9	3.8 (3)
Ulnar nerve	308	-23 ~ 10	2.9 (9)	332	-30 ~ 8	7.5 (25)

Note: The total were 449 cases; positive case numbers are given in parentheses; ADR indicates amplitude decline range.

Statistic analysis also shows the distributions of positivity rates at different diagnostic levels (Table 2). Overall, the positivity rate of 3Hz RNS test for all diagnostic levels (n = 449) in at least one muscles is 49.9% (n = 224), and that of clinically definite ALS (n = 364) at 3Hz RNS reaches 52.5%, both of which are significantly higher than that in other three diagnostic levels (n = 85, positivity rate = 38.8%) (Chi-square, p = 0.05).

Table 2  
The differences in positivity rates of 3Hz RNS between definite ALS and other three diagnostic levels

Clinic diagnostic levels	positivity rate (%) on the accessory nerve	P value at $\chi^2$ testing	Positivity rate (%) on at least one nerve	P value at $\chi^2$ testing
definite ALS	49.4 (180/364)	0.015	52.5 (191/364)	0.005
other three diagnostic levels	37.6 (32/85)		38.8 (33/85)	

Notes: other three diagnostic levels include clinically probable, clinically probable laboratory-supported and clinically possible

Besides, the range of CMAP amplitude decrement at 3Hz RNS delivered to the accessory nerve among 446 cases is -55 ~ 18% (Table 1), which include 26.4% of the cases with a decremental range exceeding 15%, 15.4% exceeding 20%, 7.6% exceeding 25% and 4.0% exceeding 30% (Table 3).

Table 3

The number of cases in certain decremental/incremental ranges of CMAP amplitude at 3Hz RNS on the accessory nerve

Items	Decremental range(%)							Incremental range (%)
	≥ 30	30 ~ 25	25 ~ 20	20 ~ 15	15 ~ 10	10 ~ 5	5 ~ 0	
Case number ( <i>n</i> )	18	16	35	49	93	124	80	31
Proportion (%)	4.0	3.6	7.8	11.0	20.9	27.8	17.9	7.0
Cumulative proportion (%)	4.0	7.6	15.4	26.4	47.3	75.1	93.0	100.0

### 3.3 The effect of clinical indicators on positivity rate of 3Hz RNS test delivered to the accessory nerve

Each clinical indicator and its relationship with positivity rate of 3Hz RNS test are summarized in Table 4. No substantial difference is found in either between male and female patients, or among patients with an onset age of  $\geq 60$  and those  $< 60$ , nor is any difference found in other age brackets measured. No substantial difference is found among patients with ALSFRS-R at initial visit  $\geq 40$  and those  $< 40$ , nor was any difference found in other ALSFRS-R score groups.

In contrast, the 3Hz RNS positivity rate of the patients with disease duration exceeding 6 months at initial visit ( $n = 339$ ) is significantly higher than that of those within 6 months ( $n = 107$ ). The 3Hz RNS positivity rate of the patients with upper limb onset ( $n = 188$ , positivity rate = 60.6%) is significantly higher than that of those with lower limb onset ( $n = 158$ , positivity rate = 32.9%) or bulbar onset ( $n = 100$ , positivity rate = 45.0%). The 3Hz RNS positivity rate of the patients with  $\Delta\text{FS} < 0.5$  score/month ( $n = 56.5\%$ ) is significantly higher than that of those with  $\Delta\text{FS} > 0.5$  score ( $n = 88$ , positivity rate = 40.2%). The positivity rate of definite ALS ( $n = 362$ , positivity rate = 49.4%) is significantly higher than that of patients at other three diagnostic levels ( $n = 84$ , positivity rate = 37.6%). The positivity rate of patients with EMG (+) in sternocleidomastoid muscle ( $n = 183$ , positivity rate = 60.7%) is significantly higher than that of patients with EMG (-) ( $n = 141$ , positivity rate = 30.5%). In short, the positivity rate of 3Hz RNS delivered to accessory nerve obviously increases in response to disease duration  $> 6$  months, upper limb onset,  $\Delta\text{FS} < 0.5$  score/month, definite ALS and EMG(+) in sternocleidomastoid muscle.

Table 4

The effect of clinical indicators on the positivity rate of 3Hz RNS delivered to the accessory nerve

Clinical indicators		Cases (n)	RNS+ (%)	P value	Clinical indicators		Cases (n)	RNS+ (%)	P value
gender	male	265	45.7 (121)	0.399	ALSFRS- R(score)	< 40	88	45.5 (40)	0.762
	female	181	49.7 (90)			≥ 40	88	47.7 (42)	
Age at onset (year)	< 60	306	48.0 (147)	0.648	ΔFS	< 0.5	69	56.5(39)	0.034
	≥ 60	140	45.7 (64)			≥ 0.5	107	40.2 (43)	
					(score/month)				
Disease duration (month)	≤ 6	107	34.6 (37)	0.003	Diagnostic level	Definite	362	49.4 (179)	0.049
	> 6	339	51.3 (174)			Others *	84	37.6 (32)	
Onset site	Upper limb (S1)	188	60.6 (114)	S1S2: 0.000	Sternocleido -mastoid muscle	EMG (+)	183	60.7 (111)	0.000
	Lower limb (S2)	158	32.9 (52)	S1S3: 0.011		EMG (-)	141	30.5 (43)	
	Bulbar (S3)	100	45.0 (45)	S2S3: 0.508		-	-		
Note: 446 cases underwent 3Hz RNS delivered to accessory nerve. Chi square was used for above statistic analysis. Others include probable ALS, laboratory supported probable ALS and possible ALS.									

### 3.4 The relationship between decremental response at 3Hz RNS delivered to accessory nerve and disease duration at initial visit

Scatter plot is used to display disease duration and the decremental percentage of CMAP amplitude at 3Hz RNS test (Fig. 1). A linear regression analysis indicates that the decremental response is linearly associated with disease duration ( $Y = 8.890 - 0.126X$ ,  $F = 12.8$ ,  $P = 0.000$ ).

### 3.5 The relationship of decremental response at 3Hz RNS delivered to accessory nerve respectively to MUP duration and to duration increment percentage in sternocleidomastoid muscle

324 patients underwent 3Hz RNS test on the accessory nerve along with needle EMG in the sternocleidomastoid muscle. Scatter plots are used to display the correlation between the decremental range of CMAP amplitude and MUP duration or the increment percentage of MUP duration (Fig. 2). Both MUP duration and duration increment

percentage have significantly negative linear correlations with decremental response of 3Hz RNS delivered to the accessory nerve, suggesting that the more serious neurogenic damage is detected by EMG, the more obvious will be the decremental response. Besides, as can be seen from the regression model, MUP duration is more responsive than the duration increment percentage to the decremental response.

## 4. Discussion

RNS has been widely applied in the evaluation of NMJ functions. A number of studies have demonstrated that decremental responses are frequently observed in ALS, beginning with the first description from Mulder et al. (1959). However, the results of RNS test and its implications in clinical applications and pathogenic mechanism have not yet been fully elucidated (Alanazy et al., 2017; Fischer et al., 2004; Hegedus et al., 2007; Miyaji et al., 2018; Clark et al., 2016; Iwai et al., 2016; Wainger et al., 2014; Mukhutdinova et al., 2018). So we have conducted a retrospective analysis based on the largest sample size ever to our knowledge, in order to quantify the results of RNS test and find out the respective relationship of CMAP decremental response either to clinical manifestations or to EMG results, so as to substantially advance the understanding of RNS results in ALS patients, effectively instruct clinical application, supply a clue to the establishment of a more reasonable exclusion criterion and enlighten the exploration of pathogenic mechanisms from the perspective of clinical neuroelectrophysiology.

In previous studies, due to the differences in the races, the inconsistency of the instruments and cut-off values in different laboratories as well as the limited number of the samples involved in the test, a large discrepancy exists about the positivity rates in RNS tests, ranging from 29–83% (Sun et al., 2018; Tomoko et al., 2011; Zheng et al., 2017a; Fu et al., 2019; Wang et al., 2017; Alanazy et al., 2017; Zheng et al., 2017b; Hatanaka et al., 2017; Hu et al., 2018). To minimize the effect of potential systemic and random errors, we collected and analyzed an adequately large sample size of case data to improve the generalizability of the results. The present study shows that 3Hz RNS delivered to the accessory nerve has the highest diagnostic sensitivity. The overall positivity rate of 3Hz RNS (decremental response exceeding 10%) for the 449 patients is 49.9%, while that for the 364 clinical definite ALS patients reaches 52.5%. As for the range of decremental CMAP amplitude at RNS test, very few studies have reported the exact values of the range in addition to mean and standard deviation (Sun et al., 2018; Tomoko et al., 2011; Zheng et al., 2017a; Hu et al., 2018). The largest decrement of CMAP amplitude that has ever been reported was 50% in our previous research (Sun et al., 2018) while this study shows that it can reach 55% (Table 1).

According to the Electrophysiological Diagnostic Criteria for ALS revised in 2000 by the World Federation of Neurology Research Group on Motor Neuron Diseases, a significant decrement in CMAP, i.e. when it's exceeding > 20% in RNS, could be a criterion to exclude ALS (Brook et al., 2000). In Li et al (2020) studies, a significant decrement ( $\geq 20\%$ ) in at least one muscle was observed in 11.3% of the ALS patients, while decrements ( $\geq 10\%$ ) in at least one muscle was observed in 41.3%. However, our study of the 449 cases shows that in 15.5%, 7.6% and 4.0% of the cases RNS decremental responses exceed 20%, 25% and 30% respectively. As far as we can see, the implementation of the current Electrophysiological Diagnostic Criteria will result in the misdiagnosis of more than 15.5% of ALS patients. Then, what percentage of RNS decremental response can serve as a criterion to exclude ALS still warrants further investigations.

In addition to RNS test results in ALS patients mentioned above, the results of our statistical analysis also provide robust evidence for the correlations between RNS decremental responses and ALS clinical manifestations. For the first time, a perfect negative linear correlation between disease duration and 3Hz RNS decremental responses is

established by regression analysis and a significantly higher positivity rate in patients with  $\Delta FS < 0.5$  score/month ( $n = 88$ ) is shown by Chi square. As can be drawn from the  $\Delta FS$  formula, the longer the disease duration is, the lower the  $\Delta FS$  will be. Taking the  $\Delta FS$  formula and the above two results in account, our study indicates that the NMJ dysfunction is deteriorating as the disease progresses, especially after 6 months.

As for the relationship between RNS decremental responses and EMG results, we are the first to establish a regression model (Fig. 2) and compare the sensitivities of two indicators (i.e. MUP duration and duration increment percentage) to RNS test. Our study shows that MUP duration rather than the range of duration increment is more suitable to be used in future studies. The regression model in Fig. 2 confirms the correlation between the impairment of motor neuron and NMJ dysfunction from the perspective of clinical neuroelectrophysiology for the first time. By two intersecting lines, namely  $x = 20$  and  $y = -10$ , the coordinate plane is divided into four quadrant (Fig. 2b). It's worth noting that scatters in the third quadrant satisfying the requirement of RNS(+) and EMG(-) seems to indicate that in some cases, RNS test is more sensitive than EMG and NMJ dysfunction is prior to the loss of motor neuron. Whether this electrophysiological phenomenon can serve as electrophysiological evidence for "dying-back" hypothesis (Michal et al., 2011) still needs further investigations.

This study is not free of limitations. Firstly, the positivity rates of clinically probable cases ( $n = 46$ ), clinically probable laboratory-supported cases ( $n = 20$ ) and clinically possible cases ( $n = 18$ ) are 30.4%, 35.0% and 61.1%, respectively. However, the sample size of the cases at the three levels is relatively small in comparison with that of definite ALS. The difference in positivity rate among these three diagnostic levels has very little meaning. On the one hand, our neurology department is one of the best national medical research institution which mostly receives patients suffering rare and serious diseases and most patients at the early stage of ALS just see a doctor near their hometown as their muscle weakness is not obvious. On the other hand, people are not well informed of ALS knowledge, which makes patients at early stage likely to be missed or even misdiagnosed. That's why we think multicenter retrospective studies are needed in the future. Secondly, present statistic analysis shows the positivity rate of 3Hz RNS test delivered to the accessory nerve in upper-limb-onset group is 60.6%, leading all the others and followed by bulbar group (45.0%) and lower limb group (32.9%), which is in line with some previous study (Sun et al., 2018; Hatanaka et al., 2017; Hu et al., 2018; Miyaji et al., 2018; Clark et al., 2016). The probable reason for this obviously high positivity rate in the upper-limb-onset group may be ALS's preference for spreading around its onset site. However, as to whether the positivity rate on the common peroneal nerve at 3Hz RNS might be higher in patients with lower-limb-onset than that in patients with other onset sites, our statistic analysis can hardly lead to a conclusion because of the low positivity rate (3.8%) of RNS test applied to the common peroneal nerve and the lack of positive cases ( $n = 3$ ). Thirdly, as can be seen from the Fig. 2b, some cases are found with RNS (+) and EMG (-). So some questions remain unanswered. What's the proportion of these cases in ALS population? Is disease duration of these cases shorter than others? How is their diagnostic levels? To answer them, we'll keep on collecting data and trying to illuminate these questions in future study.

In conclusion, this is a retrospective study based on 449 cases, with the largest sample size ever reported. It discovers that the overall positivity rate of RNS test is 49.9%. CMAP decremental response at 3Hz RNS applied to the accessory nerve has the best sensitivity and highest positivity rate (52.5% in definite ALS). It obviously increases in response to upper limb onset,  $\Delta FS < 0.5$  score/month, definite ALS and EMG(+) in sternocleidomastoid muscle. Perfect negative linear correlations exist between either disease duration or MUP duration and decremental percentage of CMAP amplitude. The regression models indicate that the neuromuscular junction

(NMJ) dysfunction is deteriorating as the disease progresses; the more serious neurogenic damage is detected by EMG, the more obvious will be the decremental response. Cases which are found with RNS (+) and EMG (-) may enlighten the pathogenic mechanisms from the perspective of clinical neuroelectrophysiology.

## **Declarations**

### **Conflict of interest:**

There are no conflicts of interest to declare.

### **Ethical publication statement**

The patient has provided consent for the use of the clinical information. This study has been approved by the Chinese PLA General Hospital Ethical Review Committee and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

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### **Disclosure statement**

The authors disclose no conflicts of interest.

### **CRediT authorship contribution statement**

Jinghong Zhang had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Xusheng Huang and Jinghong Zhang contributed equally to this work.

Concept and design: All authors.

Acquisition, analysis, or interpretation of data: Jinghong Zhang and Xusheng Huang

Drafting of the manuscript: Jinghong Zhang and Xusheng Huang

Critical revision of the manuscript for important intellectual content: All authors.

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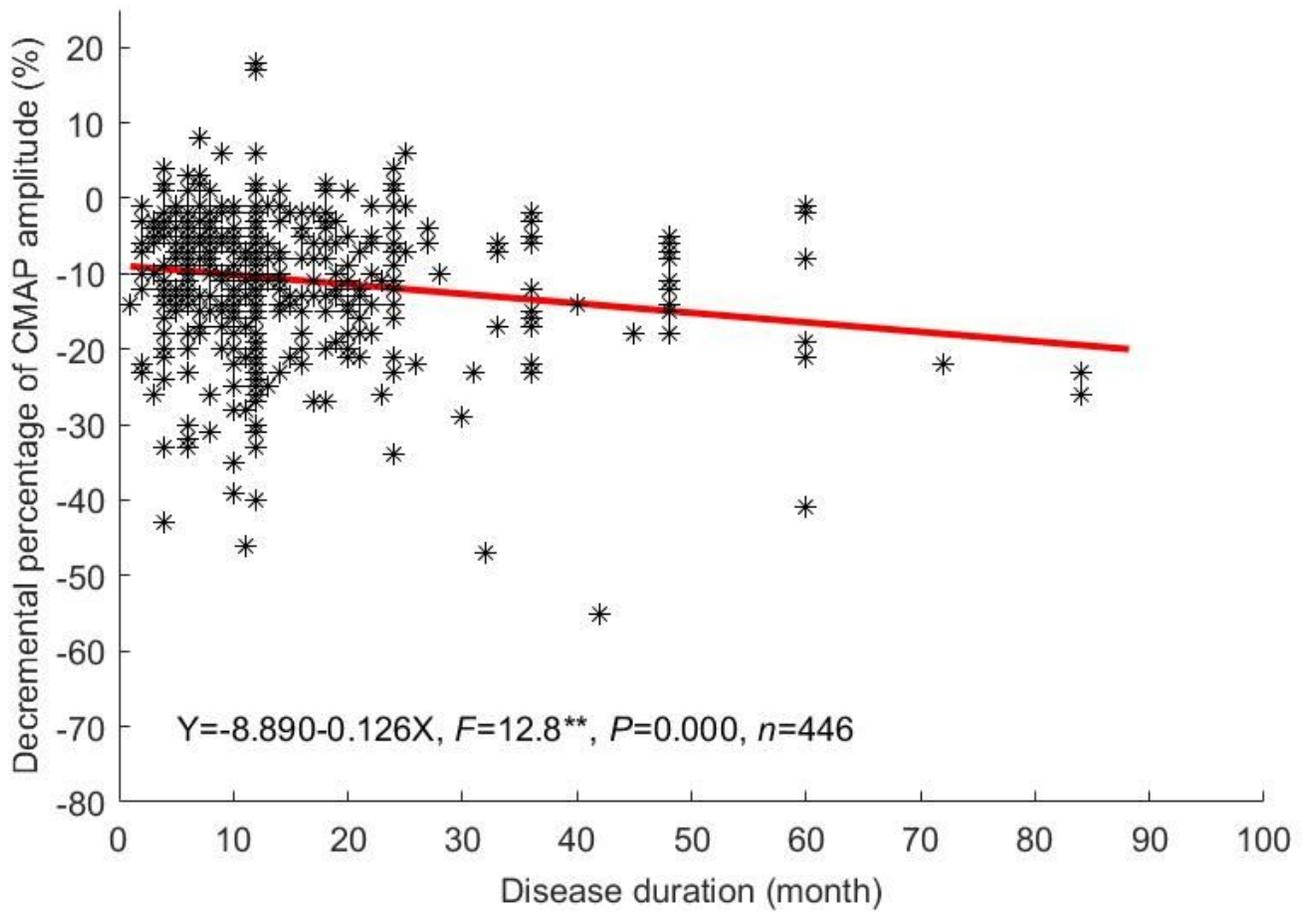
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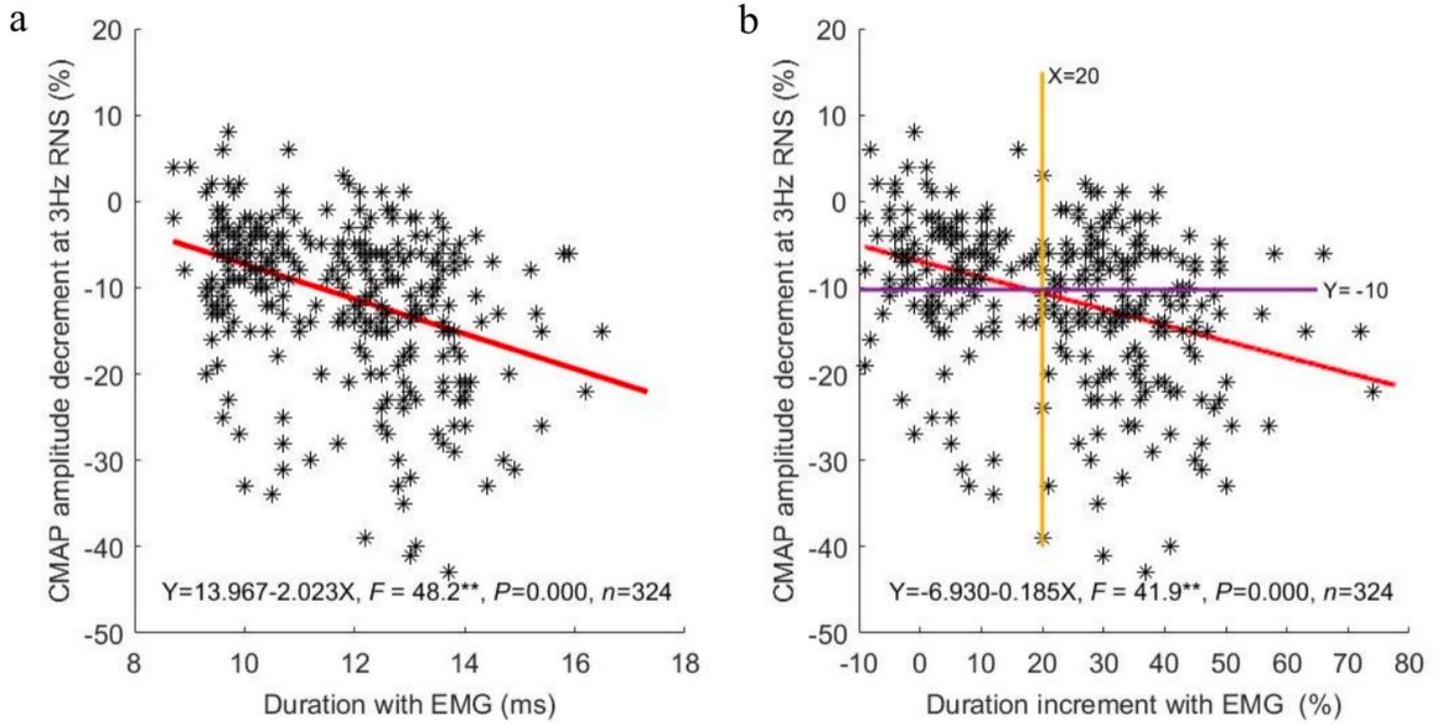
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## Figures



**Figure 1**

The relationship between disease duration and the decremental amplitude of CMAP at 3HzRNS delivered to accessory nerve



**Figure 2**

The correlations between the decremental response by accessory nerve at 3HzRNS and MUP