Correlation between Spike Potential and Field Potential in the Motor Cortex of Rats with Parkinson's Disease

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ABSTRACT

Correlation between the spike discharge and rhythm of local field potential (LFP) of neurons provides clues on the information coded by the nerves. In this study, spike and LFP in the motor cortex (M1) of rats with Parkinson's diseases (PD) at the inattentive rest and during walking along a ladder were recorded using multi-channel neuronal recording system (Plexon). Neuronal classification was first performed for the nuclei of M1. Then for different types of neurons, the spike-LFP correlation was analyzed in the M1 using three indicators, namely, coherence value, phase locking and spike-field coherence (SFC). Results: 1. Based on electrophysiological characteristics, the nuclei neurons in M1 were classified into two types respectively; 2. There was no significant difference in spike-LFP correlation in PD rats compared with the controls when at the inattentive rest; 3. There were different changes in the spike-LFP relationship for the PD rats compared with the controls when walking the ladders. The intensity of spike-LFP correlation decreased for type A neurons in M1, while it increased for type B neurons in M1.

Key Words: Parkinson's Disease, Motor Cortex, Coherence

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Introduction

Multi-channel neuronal recording system can record the neuro-electric activities of the brain area in awake animals, namely, spike and local field potential (LFP). Spike refers to action potential of a single neuron and reflects spontaneous discharge of neurons. Spike is the basis of information transmission along the nerves (György *et al.,* 2004). LFP is captured around the electrode tip as the sum of action potentials and inhibitory postsynaptic potentials of several neurons. LFP reflects the overall activity of local neurons (Rutishauser *et al.*, 2010). LFP has become increasingly important in the study on information coded by the nerves.

LFP are a type of vibration signal that resembles electroencephalogram (EEG) and consists of several rhythmic waves. The rhythmic waves can be divided into five frequency ranges: δ wave (1-4Hz), θ wave (4-7Hz), α wave (7-12Hz), β wave (12-30Hz) and γ wave (30-70Hz). Exploring the correlation between spike and LFP is one of the hot spots in neuroscience. The spike-LFP correlation reflects the information coded by neurons. For example, Benchenane's study indicated an enhanced correlation between θ oscillations of LFP and spike in the hippocampus

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and prefrontal neural circuit (Benchenane *et al*. 2010). Brazhnik's research reported an enhanced spike-LFP phase locking in substantia nigra in primary motor cortex and basal ganglia of PD rats (Brazhnik *et al*., 2012). PD usually presents as rest tremor, myotonia, and gait abnormalities (Vergara *et al*., 2003). Electrophysiologically, PD patients or animal models exhibit abnormal changes in the frequency of electric activities along with abnormal discharge and increased synchrony (Ellens *et al*., 2013). All signals will project into the motor cortex (M1), which is the highest region of the brain that controls voluntary movement. The discharge pattern of spikes and rhythm changes of LFP indicate the importance of neuronal cluster activities in motor information coding.

We used a multi-channel neuronal recording system to collect the synchronous spike and LFP signals in M1 of PD rats and controls. M1 neurons were first classified using Neuroexplore and offline sorter 4.0 software. Then for each type of neurons, the spike-LFP correlation was analyzed statistically with three indicators, namely, coherence value, phase locking and SFC. According to the above results, phase locking was identified between neuronal discharge and rhythmic oscillations in LFP and the intensity of correlation was determined.

Methods

Animal experiments

Adult male Wistar rats (weighting about 270-310g) were purchased from Animal Laboratory Center of Shandong University. The rat model of PD was established by intraperitoneal injection of 4% chloral hydrate (400mg/kg). The coordinates of medial forebrain bundle (MFB) were determined (Paxions *et al*., 2007): (2.16±0.1) mm behind the anterior fontanelle, (2.1±0.1) mm beside the midline, and (8.40, 8.65±0.1) mm below the cranial surface. A microsyringe was used to inject 6-hydroxydopamine (4 ug/ul) dissolved in normal saline containing 0.2% vitamin C. At two weeks after construction of PD rat models, apomorphine hydrochloride (0.01%, 0.5mg/kg) was injected to the cervical muscles of rats. PD models were considered to be successfully built if the rats rotated towards the healthy side and the average rotational speed within 30min was above 7r/min. Rats were randomly divided into control group and experimental group. A multi-channel microelectrode array was prepared. The recording electrode was made of nickel-cadmium alloy wires with a diameter of 25μm. The microelectrode array was implanted to M1 (2.16±0.1) mm behind the anterior fontanelle, (2.1±0.1) mm beside the midline, and (8.40, 8.65±0.1) mm below the cranial surface. The electrode was immobilized to the skull with dental cement.

Signal collection

Spike and LFP signals were collected using the Omniplex 16-channel recording system (Plexon). This system had a built-in preamplifier for filtering and simultaneous recording of spike and LFP signals. Spike discharge series were collected by the threshold method. Only signals with a signal-to-noise ratio (SNR) above 2.0 would be recorded. The filtering range was 300-8000Hz and the sampling frequency was 40KHz. The filtering range for LFP was 0.5-200Hz and the sampling frequency was 1KHz. The system was configured with a 50Hz trapped wave to eliminate power line interference. Finally, the original data were stored in the hard disc for an off-line analysis.

Data analysis

Neuronal classification

Neurons were classified based on the different characteristics between recorded spike waveforms. Classification was done using Offline sorter 4.0 software (Plexon). Principle component analysis and K-means clustering method were conducted, and neurons were classified based on the amplitude, bandwidth and time course of action potential along with interspike internal (ISI), discharge frequency and discharge pattern.

Coherence analysis

(1) Coherence value

Coherence value is an indicator of spike-LFP correlation of neurons in frequency-domain analysis. Spike is considered as a point process, and LFP is a type of continuous signal in the time domain. In signal processing, multitaper method was used to estimate the spectrum for the time series. Correlation between the discrete stochastic process x(n) and continuous stochastic process y(t) is defined below (Mattijs *et al*., 2009):

$$C_{xy}(f) = \frac{S_{xy}(f)}{\sqrt{S_{xx}(f)S_{yy(}(f)}}$$

Where $S_{xx}(f)$ and $S_{yy}(f)$ are auto power spectrum; $S_{xy}(f)$ is a cross power spectrum.

$$S_{xx}(f)=\frac{1}{M}\sum_{m=1}^{M}\sum_{n=1}^{M}x(n)h_m(n)e^{-2\pi jfn}$$

$$S_{yy}(f)=\int y(t)h(t)e^{-2\pi jfn}dt$$

$hm(n)$ and $h(t)$ denote taper.

To analyze the data, the signals were input into chronux 2.0 (http://chronux.org) and analyzed by using the coherence function. The correlation value ranged between 0 and 1, with 0 indicating no coherence at all and 1 absolute coherence (Fries *et al*., 2001).

(2) Phase locking

To determine spike-LFP phase locking, bandpass filtering was first performed for the LFP to obtain LFP in five frequency ranges. For an LFP signal x(t), z(t)=x(t)+iHx(t), where the imaginary part is the Hilbert transform of x(t). At any moment, the tangent values of the instantaneous phase angle of z(t) was Hx(t)/x(t). Through Hilbert transform, the phase shift was done for all frequency components by 90°, while the amplitude of the spectrum remained unchanged.

For the single neuron spike train, its discharge phase corresponding to LFP was estimated. The circumferential probability distribution was calculated for the discharge phase of this neuron with a 10° phase class interval. Thus the histogram of circumferential distribution of the phase and the average orientation angle were obtained. The spike-LFP phase locking of the neuron was represented in an average direction.

The intensity of spike-LFP phase locking of the neuron was estimated by using the mean resultant vector. Any discharge phase α_i was expressed with a complex number $r_i=\cos\alpha_i+i\sin\alpha_i$. The mean resultant vector was defined as $\bar{r}=|\frac{1}{N}\sum_i r_i|$, where the value range of $\bar{r}$ is 0~1, indicating the synchrony between spike and LFP (Wen *et al*., 2013) The larger the value, the greater the concentration of the sampling data in the average direction. When the value of $\bar{r}$ was 1, complete synchrony was indicated.

Finally, spike-LFP phase locking was tested using Rayleigh's method. The data were considered valid when P<0.05 and eligible for statistical analysis; the spikes were not phase locked to the frequencies of LFP when P≥0.05 and the data were removed.

(3) SFC

Spike train and LFP were recorded simultaneously. Setting the discharge time of each spike as the center, the LFP signals in the preceding and following intervals were taken. All extracted signals were calculated and superimposed and the average was taken as STA (spike-triggered average). The LFP extracted at the discharge time of the i-th spike was $x_i(t)$, then $\mathrm{STA}=\frac{1}{N}\sum_{i=1}^{N}x_i(t)$. Multitaper method was used to estimate the spectrum of STA to obtain *f*STA.

For the constructed STA, original LFP was defined as $X_i(t)$. Multitaper method was first applied to $X_i(t)$, and then an average was taken of all power spectra to obtain STP (spike-triggered power).

SFC was defined as a function of frequency f (Halje *et al.,* 2012). The value range was 0%-100%. SFC is given by (Fries *et al*., 2001):

$$SFC(f)=\frac{fSTA}{STP}\times100\%=\frac{|F\{STA(t)\}|^2}{\frac{1}{N}\sum_{i=1}^{N}|F\{X_i(t)\}|^2}$$

Where *F* is multitaper spectral density estimation.

SFC is the percentage of the power of LFP rhythmic signals phase locked to the spikes to the total power of original LFP. The larger the percentage, the stronger the spike-LFP correlation in terms of power (Rutishauser *et al*., 2010).

STA can be used to extract the LFP signals phase locked to the spikes. However, if the t for STA sampling is too long, the calculation load will be very high; but if it is too short, some signals may be lost. Thus, *t* should contain at least one complete cycle of oscillations (500ms in experiments).

Results

Classification of M1 neurons

Fig. 1-A shows the spike continuous diagram of two types of neurons. The signal-to-noise ratio was lower with type A neurons, which were interneurons with a higher discharge rate and uniform discharge. The SNR was higher with type B neurons, which were pyramidal neurons with burst discharge. As shown in Fig.1-B, the time

course between peak and trough was shorter for interneurons and longer for pyramidal neurons. Fig.1- C is the two-dimensional clustering analysis diagram of interneurons and pyramidal neurons. Fig. 1-D shows the histograms of discharge interval for the two types of neurons. On the histogram for the interneurons, the interval between two adjacent discharges on the left was shorter, which corresponded to a higher discharge rate. On the histogram for the pyramidal neurons, the interval between two adjacent discharges on the right was longer, which corresponded to a lower discharge rate.

Changes in spike-LFP correlation of M1 neurons in PD rats

To analyze the changes of M1 neurons in PD rats, three indicators were used, namely, coherence value, phase locking and SFC.

(1) Correlation analysis in awake rats at rest

As shown by the statistical analyses, there were no significant differences in spike-LFP correlations of two types of neurons between the control rats and PD rats at rest in any frequency range (Fig. 2).

(2) Correlation analysis in rats during ladder climbing

Rat ladder was prepared by our research team with a total length of 1m and wooden sticks (3mm in diameter) were inserted at an interval of 1cm. The rats started at one end of the ladder and they were rewarded with food after climbing to the other end of the ladder along the wooden sticks.

All indicators of correlation changed to varying extent for the two types of neurons during ladder climbing. For type A neurons (Fig. 3-A1), the coherence value in PD rats decreased significantly in β oscillations compared with the control group. Fig. 3-A2 describes the variation trend of coherence value with frequency, from which the differences in β oscillations were more prominent. As to phase locking, the length of mean phase angle decreased significantly in β oscillations in PD rats compared with the control group (Fig3.-A3). SFC decreased considerably in the α oscillations in PD rats compared with the control group (Fig.3-A5). The above results indicated that the intensity of spike-LFP correlation was weakened considerably in type A neurons of M1 for PD rats during ladder climbing compared with the control group.

For type B neurons, no significant differences were observed in coherence value and phase locking between the PD rats and the control group. However, SFC was significantly higher in the β oscillations in PD rats compared with the control group, which indicated an enhanced synchrony between spike and LFP for type B neurons.

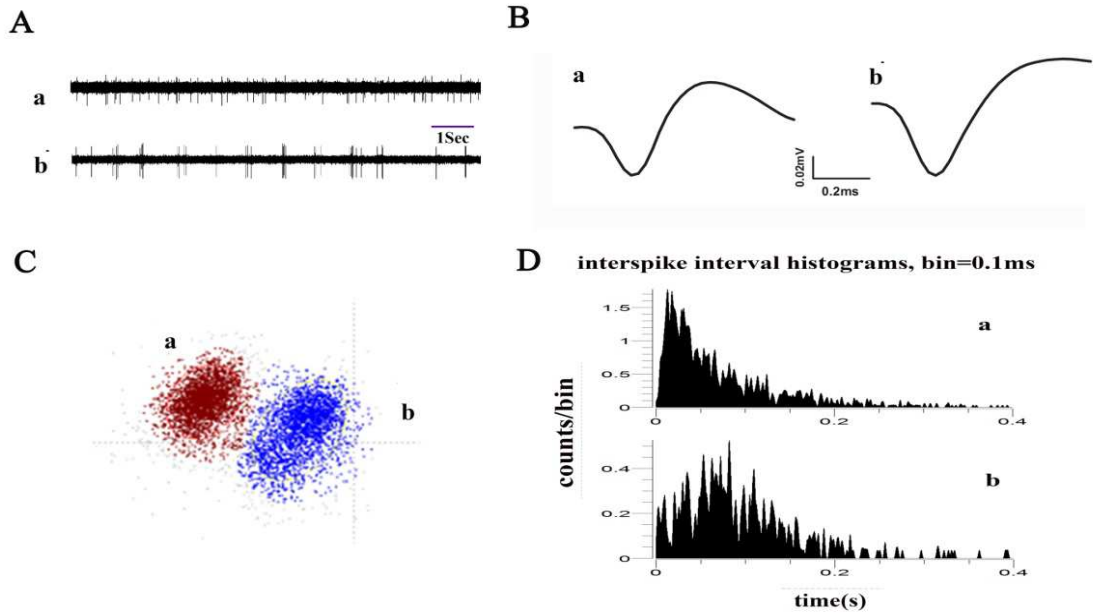

Figure 1. Two types of neurons in M1. (A) spike continuous grahic neuronal discharge (B) oscillogram of single cell discharge (C) two-dimensional clustering analysis graphic of two types of neurons (D) ISI histogra.

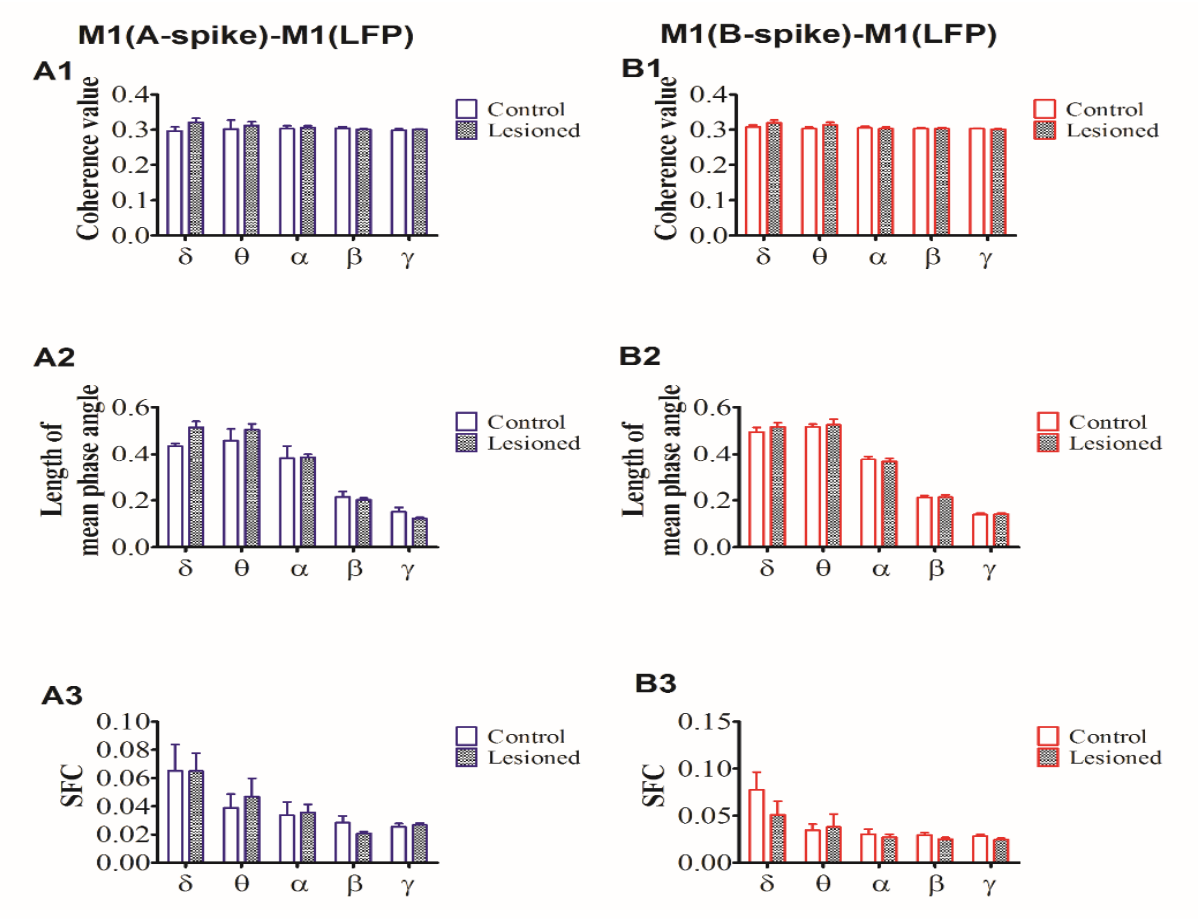

Figure 2. Spike-LFP correlations of two types of neurons between the control rats and lesioned rats at rest. (A1 B1) coherence value of two types of neurons (A2 B2) mean resultant vector. (A3 B3) SFC of two types of neurons.

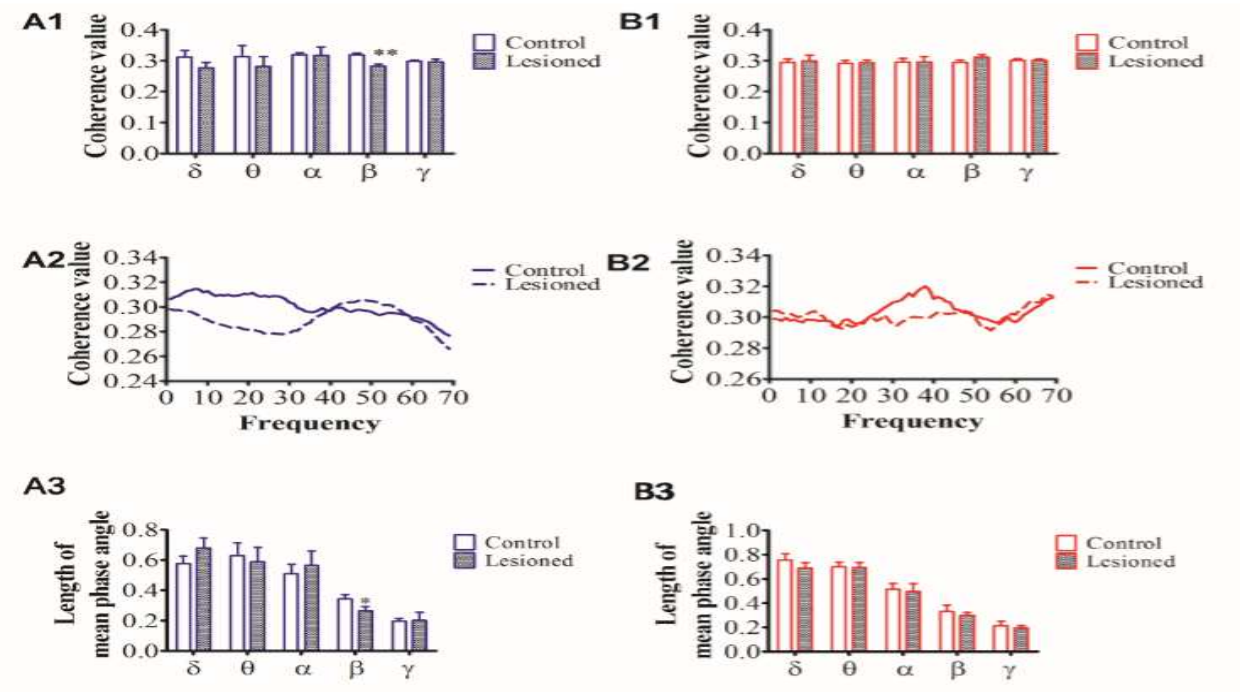

Figure3 (a). Spike-LFP correlations of two types of neurons between the control rats and lesioned rats during ladder climbing (A1 B1) coherence value of two types of neurons (A2 B2) diagram of correlation value changing with frequency (A3 B3) mean resultant vector.

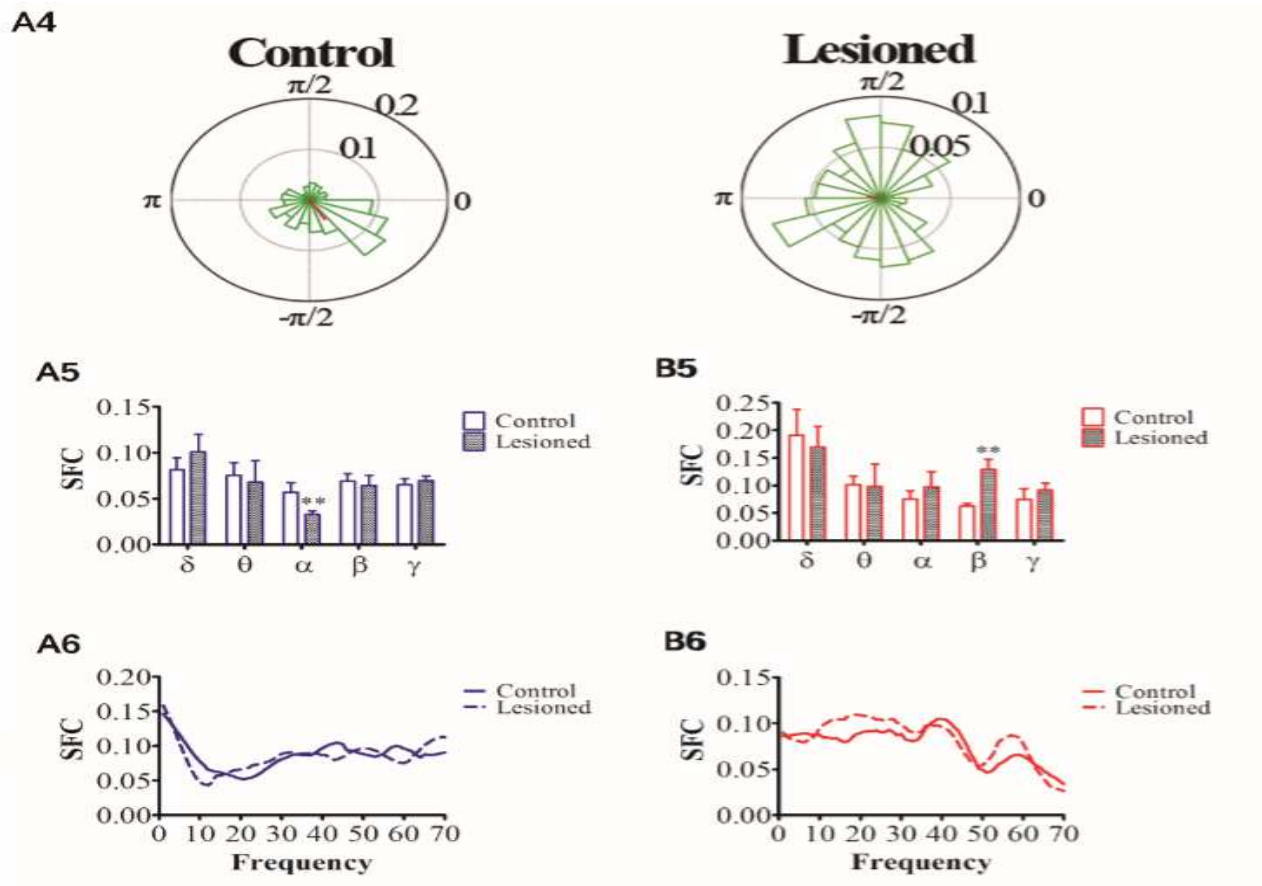

Figure3 (b). (A4) Phase rose plot with statistical difference frequency in M1-A, the red line indicates the angle orientation and the length of phase-locking (A5 B5) SFC of two types of neurons (A6 B6) diagram of SFC changing with frequency.

Discussion

Studies have observed different types of neurons in the discharge times of spiking activity show relationships to the various LFP rhythms. For Motor cortex is the highest region of the central venous system that controls voluntary movement, it plays a very important role in the cortex-basal ganglia-thalamus-cortex circuit (Ellens *et al.,* 2013), so the study of analyzing the synchrony between spikes and LFP in M1 neurons of PD rats can help clarify the neural mechanism. In this paper, spike activity and local field potentials in M1 of the rat model of Parkinson's disease were recorded synchronously using a multi-channel neuronal recording system. Before determining the correlation between spike and LFP, the nuclei neurons of M1 should be first classified as accurately as possible. In experiments, we used Offline sorter 4.0 software provided by Plexon to analysis. Based on several discharge parameters of spikes, neurons were classified into type M1-A and M1-B (Merchant *et al.*, 2012). Three indicators, namely, coherence value, phase locking and SFC, were used to characterize the spike-LFP coherence under MATLAB and Chronux. The average phase angle orientation, mean vector length and intensity of phase locking were calculated. In comparison with the three indicators, SFC has the best sensitivity when evaluating whether it is relevant. As shown by the statistical analyses of the correlation, there were significant differences in spike-LFP coherence between PD group and control group during a locomotor state of walking along a ladder. In some specific frequency bands, the spike-LFP relationship of type A neurons in M1 was decreased while that of type B neurons in M1 was enhanced. This is suggested that the information coding function may have abnormal changes at M1 in PD rats.

This paper indicates that a variety of evaluation indicators of correlation of one nucleus are also suitable for studying the correlation evaluation between the nuclei group. So, these can be subsequently applied to the correlation analyses of other nuclei in PD rats to better understand the mechanisms of PD and the pattern of information coding of the brain.

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