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The Optimal Electrode Distance in Recording Compound Nerve Action Potentials to Study Traffic Nerve Functional Injury Mechanism

Assoc. Prof. **Shengxiong Liu, *Ph.D.*,** [a, #, *] **, Shanshan Pu, *B.E.*** [a, #] **, Junhong Xiang, *B.E.*** [a, #] **,**
Sn. Engr.*, Daiqin Tao, *Ph.D. [b, c, *]

# These authors contributed to the work equally and should be regarded as co-first authors: Shengxiong Liu, Shanshan Pu, Junhong Xiang.

[a] Department of Biomedical Engineering, School of Pharmacy and Bioengineering, Chongqing University of Technology, Chongqing 400054, China;
[b] Institute for Forensic Science, Public Security Bureau of Chongqing, Chongqing 400707, China;
[c] Chongqing Municipal Key Laboratory of Forensic Evidence Technology, Chongqing 400707, China.

* CO-CORRESPONDING AUTHORS.
Sn. Engr., Daiqin Tao, *Ph.D.* (E-mail: taodaiqin@hotmail.com; Tel: +86-023-63964932; Fax: +86-023-63964998);
Assoc. Prof. Shengxiong Liu, *Ph.D.* (E-mail: 89484883@qq.com; Tel: +86-0-18702362625).

ABSTRACT The compound nerve action potential and its conductivity characteristics can be used to study the traffic brain functional injury mechanism. The recording of compound nerve action potentials is greatly affected by the distance between the electrode tips. In order to study the optimum parameters for these recordings in the bullfrog sciatic nerve trunk, compound nerve action potential was measured at different electrode spacing. The threshold and super stimulation intensity of compound nerve action potential were first decreased slowly and then gradually increased as the distance between the tips of the two stimulation electrodes was increased. When the distance between exciting point and recording point was gradually increased, the amplitude and area under the curve were decreased, but the action potential duration was increased. The amplitude, action potential duration and area under the curve had the tendency to improve with the distance between two recording electrode tips increasing from 5 mm to 20 mm. When that spacing was larger than 20 mm, the amplitude was relatively stable. The distance of two stimulating electrodes is 5mm, the distance between stimulating and measuring electrodes is 10mm and the distance of two measuring electrodes is 20mm, which is the most suitable distance for compound nerve action potentials collecting on the bullfrog sciatic nerve trunk.

KEY WORDS Compound nerve action potential; Traffic nerve functional injury; Amplitude; Area under the curve; Action potential duration

1. INTRODUCTION

In the study of traffic brain injury, the compound nerve action potential and its conductivity characteristics were often adopted especially to help understanding the nerve functional injury mechanism. The compound nerve action potential is the sum of the potentials produced by various nerve fibers as the nerve trunk is stimulated. It was applied to the evaluation of nerve injury by some scholars [1-4]. The amplitude and area under the curve are correlated with myelinated fiber numbers [5-7]. The action potential duration is associated with synchronous excitement extent of nerve fiber [8]. The lower of synchronous excitement extent of nerve fiber is, the lower of amplitude is, and the wider of action potential duration is. The nerve structure and the injury degree of nerve function could be analyzed by measuring compound nerve action potential.

Compound nerve action potentials have been recorded in amphibians and mammals, including bullfrogs [9, 10], rats [5], dogs [6], and humans [11]. And similar waveforms have been recorded both in vitro [12] or in vivo [13]. Due to different recording methods, different electrode positions, different distances between bipolar electrodes or stimulation intensity, there may be large differences in compound nerve action potentials measured in the same nerve trunk of the same individual [17]. In the isolated bullfrog sciatic nerve trunk, Dalkilic et al. [14] revealed that the amplitude and the action potential duration will change significantly when the distance

between the measuring electrode spacing gradually increases. Kline et al. [15] argued that the most suitable distance of bipolar recording electrodes between 3 and 5 mm. The amplitude becomes gradually smaller as the distance between stimulus and measurement points increases (from 10 to 20, 30, 40, 50 mm) [16]. The most ideal data model for compound nerve action potential measuring in rat median nerve, found in some other studies, were a distance of 5 mm between two stimulating electrodes and a distance of 10 mm between measuring and stimulating points and a distance of 5 mm between two measuring electrodes [17]. But there may be a problem that they were not able to test longer distances.

In recent years, it is common to study compound nerve action potentials or to study the effects of chemical substances on compound nerve action potentials by using bullfrog [10, 18-20]. But, as far as we know, there is no electrode spacing standard for measuring the compound nerve action potential on the bullfrog sciatic nerve trunk., that is the reason why compound nerve action potential measurement results were different in the same nerve of same species. This paper explored the influence of electrode distance on compound neural action potentials and explained the reasons, and gave the optimal distance between electrode distance.

2. MATERIALS AND METHODS

2.1 Materials

Fifteen bullfrogs(100～110g) were provided for the experiment. We tried our best to reduce the number of animals used and their suffering.

2.2 Methods

2.2.1 Sciatic nerve trunk preparation

According to the method introduced by Wang Limin [21], each bullfrog sciatic nerve specimen was prepared. It should be noted that the nerve trunk was dissected as long as possible, and nerve damage ought to be minimized during the process. In order to ensure the activity of the nerve specimen, the nerve specimen should be immersed in Ringer's solution for 20 minutes after dissection.

2.2.2 Neurophysiological measurement

Compound nerve action potentials were recorded by biomedical signal acquisition system. The waveform of compound nerve action potential sampling frequency was 10000 Hz, stimulating frequency 5 Hz, stimulus square wave duration 0.1 ms, and the filtration was lower than 1000 Hz.

The optimal distance of electrodes was explored by recording compound nerve action potentials at different distances between electrodes.

In the first case, the distance between two stimulating points was 2, 4, 6, 8 or 10 mm, the distance between two recording electrodes was maintained at 20 mm, and the distance between recording and stimulating electrodes was maintained at 15 mm. In the latter case, the distance increased from 10 to 15, 20, 25, 30, 35 mm between stimulating and recording sites, the stimulating electrode spacing and measuring electrode spacing were maintained at 5 mm and 20 mm respectively. In the third case, compound nerve action potential was measured with distances of 5, 10, 15, 20, 25, 30, 35 mm between two measuring electrodes, meanwhile the stimulating electrode spacing was fixed at 5 mm and the distance between stimulating and measuring electrodes was maintained at 15 mm. The compound nerve action potential was measured by orthodromic recording (Fig.1).

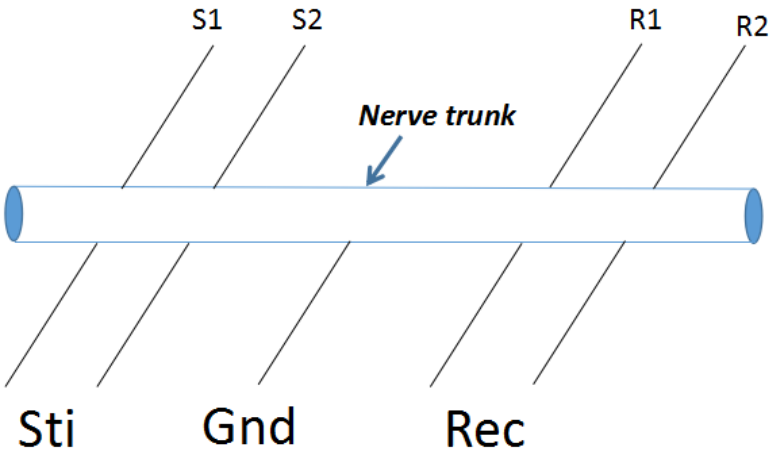

Fig. 1 The methods to compound nerve action potential measuring(S1 and S2 are stimulating electrodes, R1 and R2 are measuring electrodes.)

2.2.3 Statistical analysis

Spss26.0 was used for data analysis and the data were presented as mean ± SEM. One-way ANOVA with Least-Significant Different test was used for comparisons of threshold intensity, super stimulation intensity , amplitude, action potential duration and area under the curve between different experimental groups. The data was considered statistically significant when $P < 0.05$.

3. RESULTS

The threshold intensity(THI) and super stimulation intensity(SSI) of compound nerve action potential were first decreased slowly and then gradually increased as the stimulating electrode spacing was increased from 2 mm to 10 mm($P < 0.05$, Table 1).

When the distances between stimulating sites and measuring sites were gradually increased, the first peak amplitude(FPA), peak peak amplitude(PPA), and area under the curve(AUC) gradually decreased($P < 0.05$), while the action potential duration was increased($P < 0.05$, Table 2).

The FPA and PPA were dramatically increased when the spacing of two measuring electrodes changed from 5 to 20 mm($P < 0.05$), meanwhile the FPA and PPA were no obvious change when the measuring electrode spacing increased from 20 mm to 35 mm($P > 0.05$). As the measuring electrodes spacing increased from 5 mm to 35 mm, considerably greater AUC and APD were observed($P < 0.05$, Table 3).

Table 1 Effects of different electrode spacing on threshold intensity and super stimulus intensity

Variable	2.0mm	4.0mm	6.0mm	8.0mm	10.0mm	F	P
THI（V）	0.235±0.009	0.183±0.006	0.187±0.009	0.227±0.006	0.245±0.009	13.172	0.000
SSI（V）	0.412±0.020	0.327±0.013	0.323±0.01	0.368±0.009	0.406±0.011	10.215	0.000

Table 2 Effects of different spacing between stimulating and measuring electrodes on waveform

Variable	FPA（mv）	PPA（mv）	APD（ms）	AUC（mvms）
10mm	2.29±0.16	3.89±0.30	1.12±0.03	3.30±0.22
15mm	2.13±0.15	3.61±0.28	1.16±0.03	3.13±0.23
20mm	1.95±0.13	3.30±0.25	1.23±0.06	2.89±0.20
25mm	1.76±0.10	2.97±0.19	1.29±0.07	2.61±0.16
30mm	1.66±0.11	2.77±0.17	1.38±0.05	2.42±0.16
35mm	1.59±0.12	2.64±0.19	1.48±0.07	2.36±0.17
F	4.451	4.457	6.398	4.056
P	0.002	0.002	0.000	0.003

Table 3 Effects of different spacing between two recording electrodes on waveform

Variable	FPA（mv）	PPA（mv）	APD（ms）	AUC（mvms）
5mm	0.74±0.04	1.17±0.07	0.95±0.02	0.86±0.06
10mm	1.28±0.08	2.02±0.15	1.03±0.02	1.60±0.13
15mm	1.68±0.09	2.70±0.17	1.06±0.02	2.20±0.15
20mm	1.96±0.10	3.25±0.19	1.11±0.02	2.76±0.17
25mm	2.11±0.11	3.66±0.22	1.16±0.03	3.28±0.20
30mm	2.11±0.12	3.79±0.22	1.24±0.03	3.56±0.21
35mm	2.15±0.16	3.92±0.23	1.27±0.03	3.83±0.23
F	29.905	30.571	23.879	39.220
P	0.000	0.000	0.000	0.000

4. DISCUSSION

Neuroelectrophysiology is an important method to explore nerve function, with a long history of research [22, 23]. Compound nerve action potential recording, a classic and mature electrophysiological method, is a useful tool for assessing peripheral nerve diseases and nerve repair. Compound nerve action potential has a unique diagnostic value for the analysis of nerve function and structure[1-4, 22, 24].

Even if the same nerve of the bullfrog was measured, the waveform of the compound nerve action potential may be significantly different. Therefore, exploring the influence of electrode spacing on the waveform of compound nerve action potentials and finding the most ideal electrode spacing is of great value for experimental research.

In our study, when the distance between two stimulating electrodes was changed from 2 mm to 4mm and 6 mm, the threshold intensity and super stimulation intensity of compound nerve action potentials were gradually decreased, that was consistent with the conclusions of some scholars [17]. But, when the distance was changed from 6 mm to 8 mm and 10 mm, the threshold and super stimulation intensity were gradually increased (Fig.2). Other studies showed that the width between the two poles of the stimulating electrodes affected the waveform of the action potential [25]. The multiphase wave is more obvious with the increase of the stimulation electrode spacing. In other words, the optimal distance between two stimulating electrodes should be 4～6 mm.

Our research results demonstrated that the compound nerve action potential amplitude and the area under the curve decreased, while the action potential duration increased gradually when the distances between the stimulating or measuring points were increased from 10 to 35 mm(Fig.3, Fig.4). In the process of action potential transmission, the compound nerve action potential amplitude decreased due to the decrease of nerve fiber's number. Another reason for the decrease of amplitude is related to the dispersion of action potential velocity[8, 16]

When the measuring electrode spacing changed from 5 to 20 mm, the amplitude, action potential duration, and area under the curve were gradually increased. It was tough to record completely the waveform of compound nerve action potential when the measuring electrode spacing was less than 20 mm, that why if the distance was too short, both electrodes would be placed on the activation area of the nerve trunk and influenced by each other. Actually, biphasic action potential is the potential difference between two recording electrodes. That means the recording electrode spacing needs to be longer than the wavelength of the composite action potential. A problem in other studies was that the median nerve in rat was not long enough to be used, they were not able to test distances between the recording electrodes greater than 5 mm, hence they may not have recorded complete action potential waveforms [17]. When the spacing between the measuring electrodes changed from 20 to 35 mm, the amplitude was not found statistically significant effect, but the action potential duration and area under the curve were gradually increased (Fig.5, Fig.6).

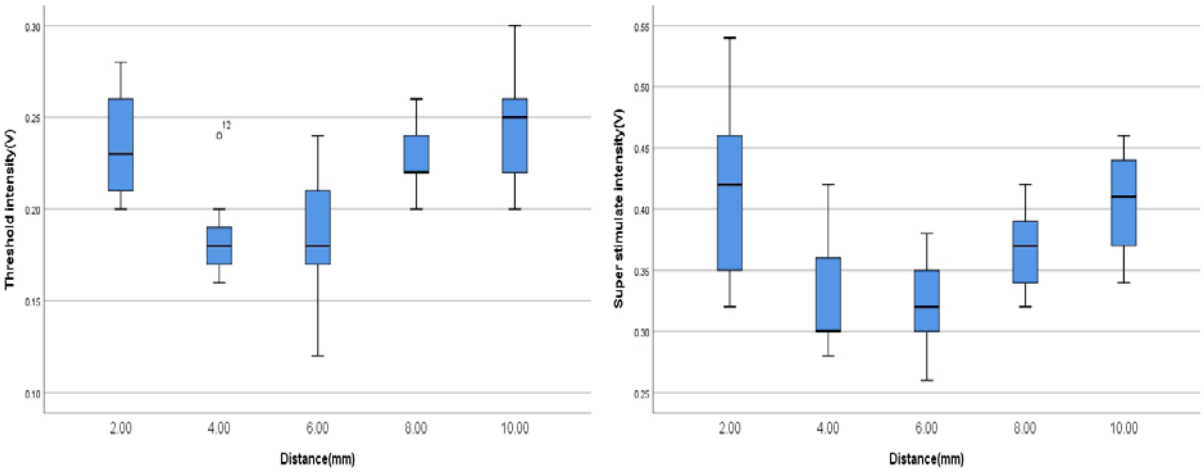

Fig. 2 Effects of different spacing between stimulating electrodes on threshold intensity and super stimulation intensity

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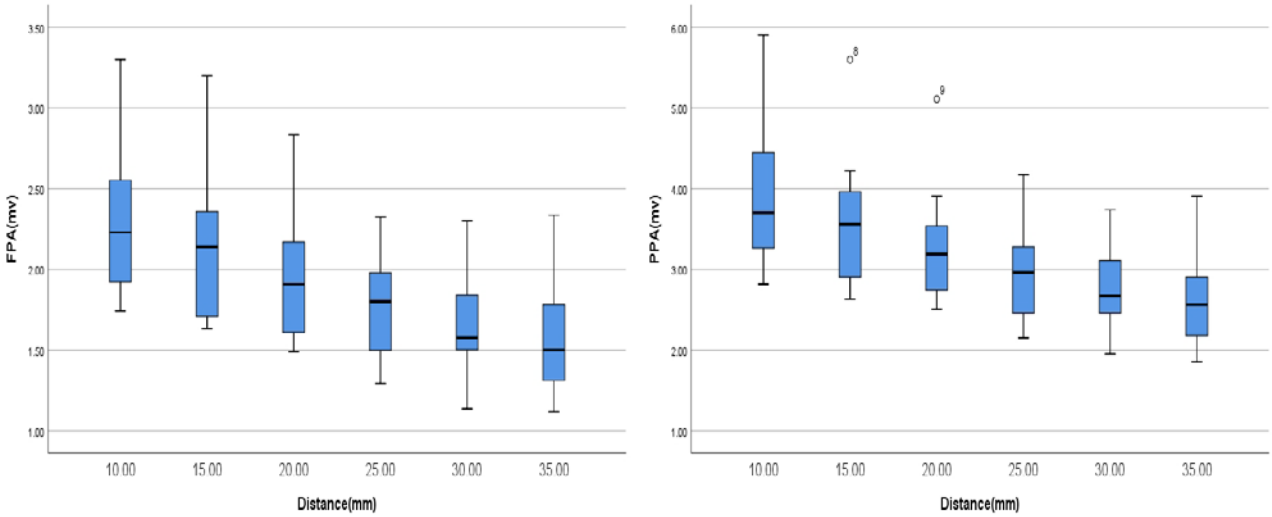

Fig. 3 Effects of different distances between exciting and measuring electrodes on the amplitude

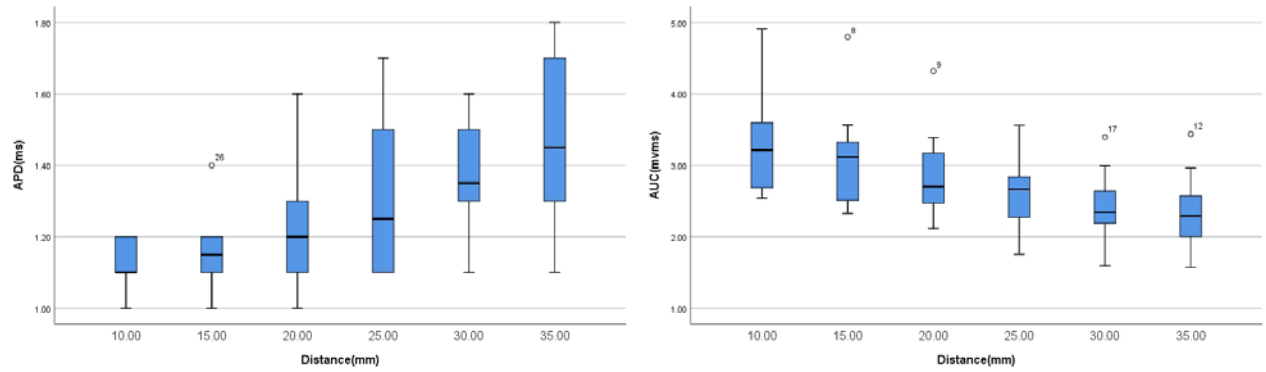

Fig. 4 Effects of different distances between exciting and measuring electrodes on the APD and AUC

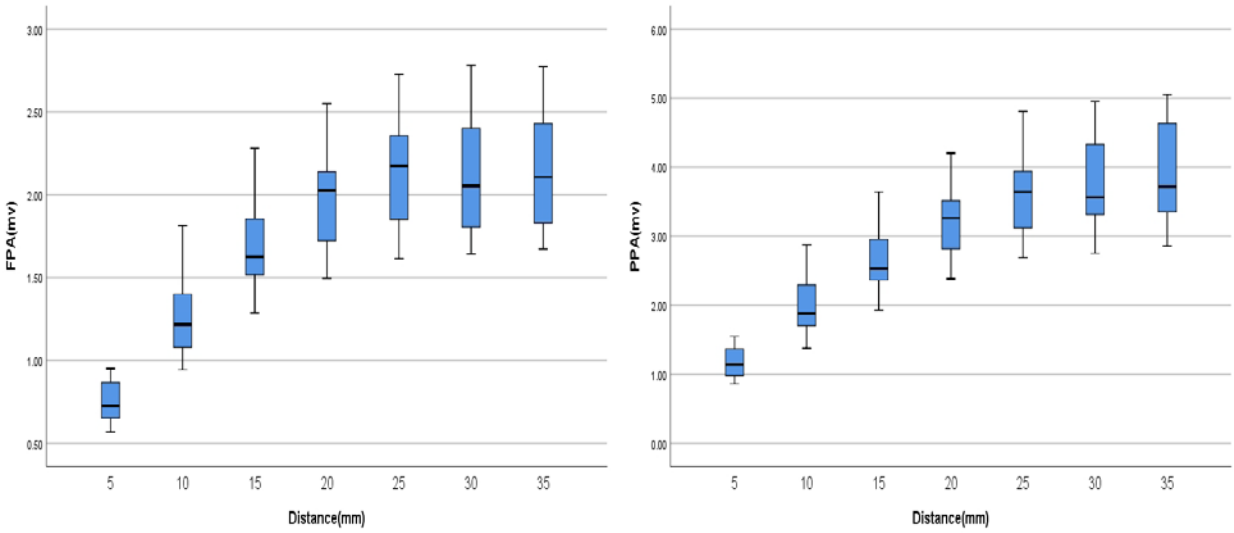

Fig. 5 Effects of different distances between measuring electrodes on the amplitude

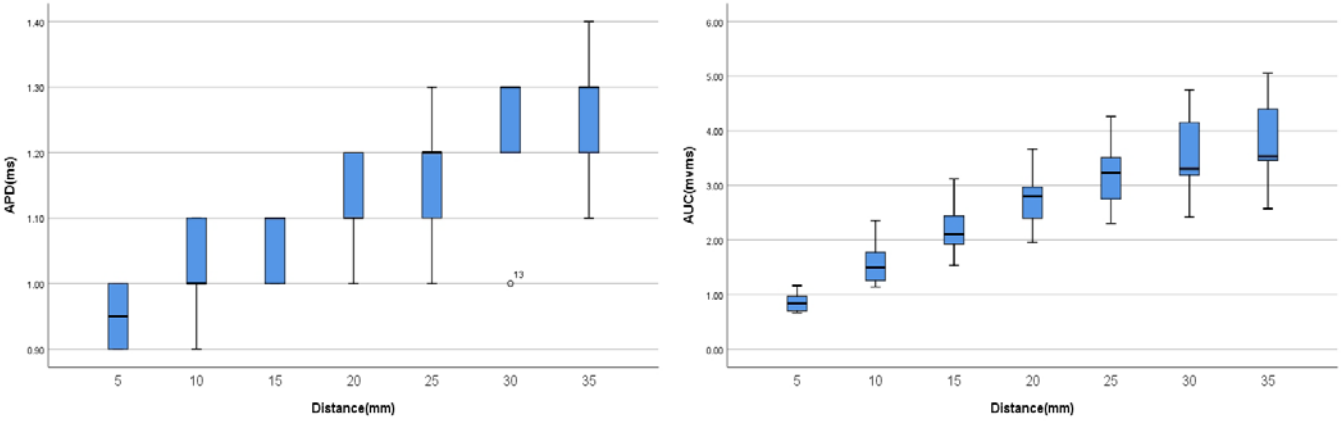

Fig. 6 Effects of different distances between measuring electrodes on the APD and AUC

The nerve trunk is composed of different diameters and types of nerve fibers, so the conduction velocity and wavelength of action potential are different from each nerve fiber. Our results further showed that the action potential synchronization of different nerve fibers decreased, while the action potential duration become longer when the distance between stimulating and recording electrodes or recording electrodes got longer. Therefore, in order to reduce the influence of the electrode spacing on the waveform and record complete information of the compound nerve action potential as much as possible, a distance of 5 mm between two stimulating electrodes, a distance of 10 mm between stimulating and measuring electrodes and a distance of 20 mm between two measuring electrodes were revealed to be optimum for compound nerve action potential measuring in the bullfrog sciatic nerve trunk.

The optimal parameters revealed in the study can provide experimental references and data support for accurate recordings of compound nerve action potential of bullfrog sciatic nerve. It could be used in related research fields (evaluation of nerve functional injury severities and healing effects after traffic accidents, evaluation of the results of drugs or chemicals in nerves and so on).

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Conflicts of interests

None declared.