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Compensation for Injury Potential by Electrical Stimulation after Acute Spinal Cord Injury in Rat *

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Abstract—**Injury potential, a direct current potential difference between normal section and the site of injury, is a significant index of spinal cord injury. However, its importance has been ignored in the studies of spinal cord electrophysiology and electrical stimulation (ES). In this paper, compensation for injury potential is used as a criterion to adjust the intensity of stimulation. Injury potential is modulated to slightly larger than 0 mV for 15, 30 and 45 minutes immediately after injury by placing the anodes at the site of injury and the cathodes at the rostral and caudal section. Injury potentials of all rats were recorded for statistical analysis. Results show that the injury potentials acquired after ES are higher than those measured from rats without stimulation and much lower than the initial amplitude. It is also observed that the stimulating voltage to keep injury potential be 0 remain the same. This phenomenon suggests that repair of membrane might occur during the period of stimulation. It is also suggested that a constant voltage stimulation can be applied to compensate for injury potential.**

I. Introduction

An injury potential, first discovered by Galvani in late 18th century, is a direct current (dc) potential gradient between intact section and injured site[1]. It was induced by current flowing into and around an injured nerve. Its discovery predated the discovery of the better known action potential, which is a rapid, self-regenerating voltage change localized across the cell membrane. Its appearance is an important indicator of changes in the microenvironment of damaged spinal cord. However, its importance has not been recognized until Borgens *et al.* investigated the injury currents after spinal cord injury (SCI) and suggested that compensation of the injury currents by applied electric field might enhance axonal regeneration in lamprey larvae[2, 3]. Since then, injury potential after SCI was investigated in rat, cat and guinea pig[4-7]. All these studies proved that the formation of injury potential occurred immediately after injury. The measured potentials, as a function of time, were fit with a logarithmic function[5, 8]. Moreover, the initial amplitude of injury potential is positively related to the grade of injury[8].

Because injury potential is formed by the movement of extracellular ions, ions such as Ca^{2+} and Na^{+} are carefully studied as well as injury potential. In the injury currents, about half of the injury current consists of Na^{+} and much of the rest may consist of Ca^{2+}[2], which is the major initiator of secondary injury process. So it is necessary to prevent the influx of the free calcium after injury immediately. One approach is using applied electric field to modulate the movement of extracellular and intracellular Ca^{2+} as Borgens proposed[9]. Strautman's in vitro study also showed that the movement of Ca^{2+} was greatly reduced by an externally applied electrical field with the cathode distal to the lesion and was increased by an applied field of the opposite polarity[10]. But the intensity of stimulation was not recommended in these papers, not to mention the combination of injury potential and applied electric field. In vivo experiments of ES concentrated on dc stimulation and oscillating field stimulation which could induce regeneration of injured axons after SCI[11-13]. The oscillating field stimulator (OFS) has been used in a phase 1 clinical trial[14]. Results show that all the patients attending the phase 1 trial acquire certain improvement in sensation. Unfortunately, the success of OFS in treating spinal injury is not based on in vitro experiments and considerable research will be required if the conditions of field application in mammalian SCI are to be optimized[15, 16]. Although some researchers have tried to explain the mechanism in early studies by assuming that the applied electric field canceled the injury currents, the following work of OFS, which alternate the field of stimulation every 15 minutes, cannot meet this assumption, because the oscillating field stimulation was delivered several hours after injury when the amplitude of injury potential almost disappeared. So it is not appropriate to consider compensation for injury currents as a mechanism of the therapeutic effect of OFS. Moreover, under the condition of lack of mechanism, the intensity of stimulation cannot be determined and the development of OFS is also restricted.

In order to seek the mechanism of regeneration of injured axons by ES and to establish the method of choosing proper stimulating parameters, we use injury potential as an index to choose the intensity of ES. We hypothesize that the injury potential as well as injury current can be canceled by dc stimulation by placing the anodes at the site of injury and cathodes at the rostral and caudal parts to prevent influx of cations such as Ca^{2+}. Experiments are conducted to verify the assumption and to establish a method of choosing proper stimulating parameters.

II. Materials and Methods

A. Experimental Animals and Groups

A total of 32 adult female rats weighing between 200 g and 250 g were used in this experiments. The animals of SCI were divided into four groups and each group contains 8 rats. Rats in the control group received no ES. Rats in 15 min ES group,

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30 min ES group and 45 min ES group received 15 minutes', 30 minutes' and 45 minutes' electrical stimulation for compensation for injury potential after SCI respectively.

B. The Electrical Stimulator and Injury Potential Measuring System

Figure 1 shows the proposed electrical schematic of the stimulator in compensation for injury potential. A 100 Ω potentiometer, R_0, and two 1 kΩ resistors, R_1 and R_2, are series connected between the two terminals of two series connected 9 V batteries. The electric potential of the central terminal of the potentiometer is amplified by a noninverting amplifier, which contains a 1 kΩ resistors, R_3, a 20 kΩ variable resistor, R_4, and a two-channel operational amplifier, LM 358. The amplification factor of the noninverting amplifier, which is determined by the value of $1+R_4/R_3$, can be adjusted by varying the resistance of R_4. The voltage follower, which has a large input impedance, is used to eliminate the influence of the impedance caused by tissue between electrodes on the former noninverting amplifier. The voltage between the outputs of the voltage follower is connected to the stimulating electrode, while the reference electrode is connected to "ground".

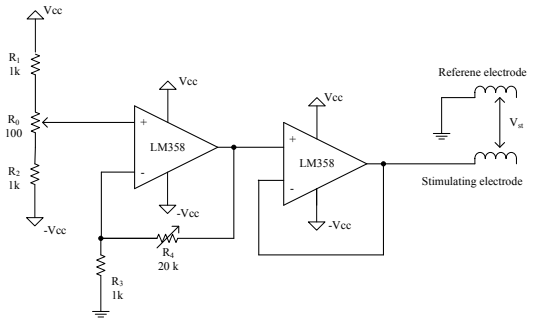


Figure 1. The circuit diagram of the stimulator used in compensation for injury potential.

Two stimulators are used for compensation for both rostral and caudal injury potential and the "ground" of them are connected to each other. Both reference electrodes of the stimulators will be placed besides the site of injury to make the potential of the site of injury the same. The amplitude of stimulating voltage can be adjusted by changing the resistance of both R_0 and R_4. Electrodes with helix profile are fashioned from 0.2 mm diameter Pt-Ir (90/10) wire and connected to the circuit through copper wires.

The injury potentials are measured by glass electrodes as a function of time. The glass electrode contains an upper and a lower glass tubes. The upper tube, which contains a calomel electrode, is filled with 3 M KCl solution and the lower tube with 0.9% saline. The tip of the lower tube is plugged by a small bulk made of porous ceramic. Two solutions in upper and lower tubes are separated by agar. The calomel electrodes are connected to voltmeters through conducting wires.

C. Experiment Procedure

All rats were anesthetized with an intraperitoneal injection of 2% Pelltobarbitalum Natricum (0.2 ml/100 g). The spinal cord was exposed by two small laminectomies placed two vertebral segments rostral and two vertebral segments caudal to a centrally located laminectomy (10th thoracic vertebrae). Two stimulators of the same kind were used for each rat in ES groups. The electrodes were secured to the paravertebral musculature with silk suture in such a way that the tips of the electrodes did not touch the spinal cord. Two anodes were placed beside the central laminectomy, whereas the two cathodes were placed besides the rostral and caudal laminectomies respectively. No electrodes were used in control group. Figure 2 shows the positions of the stimulating electrodes in experiment.

Rat models of SCI were built referring to the spinal cord contusion method of Allen[17]. A 10 g weight dropped 50 mm to impact onto an organic glass impounder centered on the cord at T10. Initial injury potentials were measured immediately after injury. Three measuring electrodes were put gently on the cord at the positions of laminectomies as shown in figure 2. The central glass electrode was connected to both positive inputs of the voltmeters while the other two electrodes were connected to the two negative inputs respectively. Then ES was applied as figure 2 shows. In ES groups, rostral and caudal stimulating voltages were adjusted to make both the rostral and caudal injury potential be 0.1~1 mV larger than 0 mV. The stimulating voltages were adjusted and recorded every 10 minutes in 30 min ES group or every 15 minutes in 45 min ES group. The stimulating voltages in 15 min ES group was not altered. After ES stopped, injury potentials were recorded every 15 minutes until 4 hours after injury. Rats in control group receive no stimulation and injury potentials were recorded every 15 minutes from immediately after injury to 4 hours post injury. The potentials differences detected rostral and caudal to the lesion are recorded as rostral injury potential and caudal injury potential.

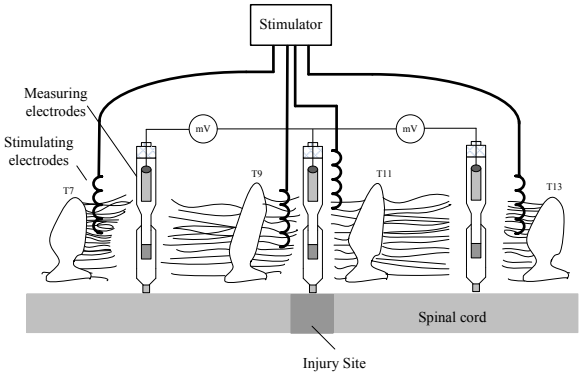


Figure 2. Positions of stimulating electrodes and measuring electrodes in the experiment of compensation for injury potential.

D. Statistics

All statistic data were analyzed using SPSS 15.0. Injury potentials were all normalized, in other words, they were described as the ratio of the injury potential at each moment to the injury potential at the initial time of the same rat. Injury potentials and stimulating voltages were compared between each ES group and control group using two-way ANOVA. One factor is group (control group or ES group) and the other factor is time. In each measuring time point, stimulating voltages and injury potentials measured at the same site are compared between each ES group and control group using

two-sample *t* test. Rostral and caudal stimulating voltages at the same time point were compared using two-sample *t* test. Rostral and caudal stimulating voltages in any two different time courses of the same group were also compared using two-sample *t* test. In all the statistical analysis, $p<0.05$ means that there has a significant difference.

III. Results

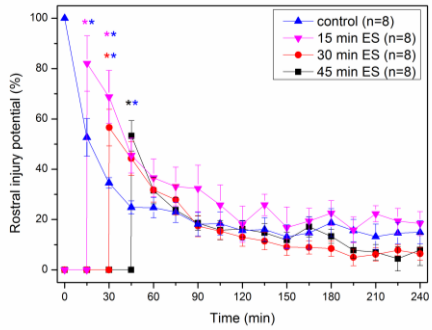
All the amplitudes of injury potential are smaller than zero, or in other words, electric potential at the injury site at each moment is lower than that at the normal site. The absolute value of injury potential declined rapidly at the early stage of injury, and the downtrends are flatter after 1 hour. The averaged initial amplitude of rostral and caudal injury potential is -17.4 mV and -20.8 mV respectively. At the final period of measurement, the injury potential still remained at several millivolts.

Figure 3 illustrates the rostral and caudal injury potentials detected immediately after ES in each ES group and those measured immediately after injury in control group. The injury potentials during ES are slightly larger than zero as a result of compensation for injury potential in each ES group. When the ES is finished, the formation of injury potential restarts. Although two-way ANOVA results shows that there is no significant difference in injury potentials between control group and each ES group after ES ($p>0.05$), there are significant differences between injury potential immediately after ES and those at the same moment in control group ($p<0.05$), as shown in Fig. 3 by asterisks of different colors. These injury potentials measured immediately after ES are larger than those at the same moment in control group, but much less than 100%, the initial amplitude. There is no difference of injury potential between each ES group and control group.

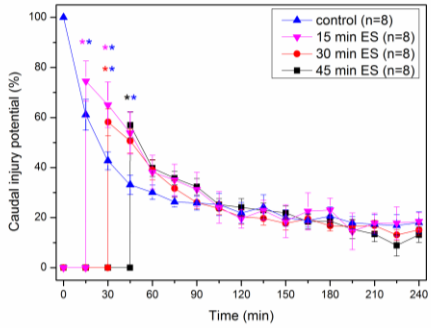
Figure 4 shows the rostral and caudal stimulating voltages at different periods of stimulation in 30 min ES group. The mean stimulating voltage is about 2.7 V. Two-way ANOVA results show that there are no significant differences between rostral and caudal stimulating voltages ($p>0.05$). When comparison is done inside the same group, there are no significant differences between any two periods in both rostral and caudal stimulating voltages ($p>0.05$). Similar results can also be acquired in 45 min ES group.

IV. Discussion

This study was undertaken to determine how injury potential, or injury current, could be canceled by applied electric field. When the axonal membrane is destroyed in SCI, a large amount of extracellular cations, such as Na^+ and Ca^{2+}, flow from normal extracellular sites to the intracellular space through the site of injury, leading to the disappearance of normal resting membrane potential and the formation of an injury current as well as injury potential. Apparently, the electric potential at the injury site is smaller than that at the normal site and this can be proved by the negative amplitudes of injury potentials measured in our experiment. If the electric potentials of intact sites, which are the same, are regarded as "ground", the initial amplitude of injury potential is about -20 mV. As time increases, the absolute value of injury potential decreases logarithmically. After 60 minutes of injury, the amplitude of injury potential reduces to only several millivolts. It is similar to the early results of measurements[4, 5].



(a)



(b)

Figure 3. Injury potential measured after SCI in control group and ES groups. According to the meaning of colors in legend, the two asterisks with different colors demonstrate significant difference between two groups with corresponding colors ($p<0.05$). (a) rostral injury potential in two groups. (b) caudal injury potential in two groups.

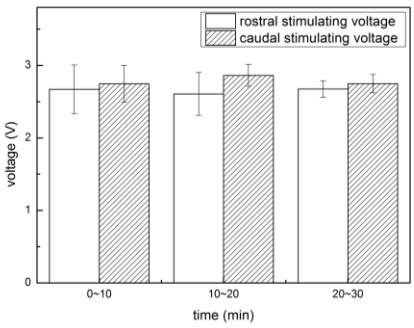


Figure 4. Rostral stimulating voltages in different period of stimulation in 30 min ES group

Injury contributes to the influx of extracellular Ca^{2+} into intracellular space which is sufficient to prevent membrane sealing and may, in contrast, contribute to axonal dieback and "secondary" axonal disruption[18, 19]. In this paper, a method of compensation for injury potential by ES is proposed as a method of selecting stimulation parameters to prevent the influx of extracellular cations. In order to avoid influx of cations, two kinds of forces which are imposed on

extracellular cations should be eliminated. On the one hand, the normal resting membrane potential disappears and an electric field with an inward direction exists across the broken membrane. Although extracellular cations will be driven into the cell by this electric field, compensation for injury potential may cancel the electric force. On the other hand, because the cations with higher extracellular concentrations, such as calcium and sodium, will flow into the intracellular space along the concentration gradient, the stimulating voltage is set to be a little higher than that compensates for injury potential to form a weak electric field with a direction of off the injury site. Therefore, a clear criterion is suggested by this paper while other studies provide only effects of ES without illustrating why the stimulating intensity is chosen.

In addition, we found that there is no significant difference between rostral and caudal stimulating voltages in the same period ($p>0.05$). This suggests that it is advisable to use only one stimulator with the same rostral and caudal stimulating voltages. As a result, the rostral and caudal injury potentials can both be modulated to slightly larger than 0 mV at the same time. There are also no significant differences in stimulating voltages between any two periods in either rostral or caudal stimulating voltages ($p>0.05$). So it is possible to apply a constant voltage during the whole course of stimulation. However, long time dc stimulation through metal electrodes, which were used in our experiment, may cause tissue damage due to the accumulation of charges on the surface of electrodes, so electrodes made of conducting polymer are preferred in future work.

According to our hypothesis, compensation for injury potential may prevent the influx of extracellular cations after injury. So when the ES ceases, the formation of injury potential will restart and its initial amplitude should be equal to that before ES. But in our experiment, the injury potential measured immediately after ES was much lower than the initial amplitude and higher than the injury potential in rats without stimulation at that moment ($p<0.05$). There may be several reasons. First, according to our early conclusion that the grade of injury was positively related to the initial amplitude of injury potential, the explanation of this contradiction is certain self-repair of the injured axonal membrane. So when the formation of injury potential restarts, the grade of injury has already been reduced, so does the influx of extracellular Ca^{2+} and Na^{+}. This is important for clinical use. Second, it is impossible to prevent all the cations from influx and a small amount of cations will flow into the cell during ES, but this quantity of influx is not enough to induce a reduction of injury potential as much as 40%. Third, because the normal intracellular concentrations of some cations, such as potassium, is higher than extracellular concentration, the anode at the injury site also prevent them from flowing out of the cell. However, the last two reasons are trivial compared with the first for the reduction is so great in our experiment. In other words, self-repair of the membrane dominate during the period of ES. Although it is deduced that the stimulating strategy in this paper may prove self-repair and reduction of calcium influx, the direct evidences of membrane resealing and the flowing direction of Ca^{2+} are not provided. So a lot of work, like calcium imaging and measurement of compound action potential, will be done to validate the assumption in the future , then a new kind of stimulator may come into being to help the patients of SCI.

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