fMRI evaluation of innovative implantable electrode for peripheral nerve stimulation animal model

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Introduction

Electrical stimulation of peripheral nerves has been widely used in various studies, especially those on small animals such as rats. Survival experiments are also widely used to study the progress following treatment of injuries associated with the neuronal system. Many of these animals have to be trained to cooperate before the experiment, which makes the animal more valuable. The surgical procedure of attaching the electrode to the nerve trunk damages the nerve despite how carefully the operation is performed. Scar and proliferated tissue also cause damage to the nerve. This kind of damage accumulates with each operation an animal receives and introduces non-neglectable bias to the research result. In order to avoid this bias, animals are divided into several groups. Each group, thus, becomes non-survival. This means that the research expenses increase dramatically, and, at the same time, statistically less convincing. Our research group encountered this problem and, therefore, has successfully designed an innovative implantable electrode, which has been tested with fMRI to evaluate its long-term effect on the nerve. This study provides researchers a reliable tool to carry out peripheral-nerve electrical stimulation during small-animal survival studies.

Methods

Animal preparation: Twelve Sprague-Dawley rats were divided equally into two groups. For all of the animals, the right brachial plexus was exposed. Stainless-steel electrodes were attached to the nerve trunk. In group 1, a self-designed electrode was buried under the skin (see Fig. 1). In group 2, a normal electrode was placed in the same manner as described Ref. 1.. All of the rats were put into the fMRI scanner. BOLD fMRI study was used as a tool to evaluate the nerve function. After the scan, the skin incisions of rats in group 1 were closed and the electrodes buried subcutaneously. In group 2, the electrodes were carefully removed. All of the rats were allowed to recover from anesthesia and were monitored for 2 h prior to being returned to the animal facility. During the three days following surgery, 0.1 ml/100 g of buprenorphine hydrochloride was administered intramuscularly twice a day. The rats' weight, food consumption, posture, behavior, and overall appearance were monitored daily throughout the study. The rats remained active and did not experience any significant weight loss or self-mutilation. Two weeks after the initial scan, all of the rats were again tested. A 5-mm skin incision was made on the experimental side of the rats in group 1. The end of the buried electrode was exposed and connected to the stimulator. In group 2, the right median nerve was exposed and a traditional electrode was attached to the nerve trunk. All of the rats were put into the scanner, and the same fMRI scan was performed. The whole procedure was performed every 2 weeks. Eight weeks after the initial surgery, all of the rats were euthanized. **Anesthesia:** Isoflurane (1.4%) was administered during the surgical portion of the procedure. Once the rat was transferred to the scanner, the isoflurane was turned off. A continuous infusion of Domitor (0.1 mg/kg/hr) was used during the fMRI acquisition. **MRI parameters:** Gradient echo scans (single shot EPI, TE = 18.4 ms, TR = 2 s, matrix 128 x 128, FOV = 3.5 cm, number of repetitions = 110, 10 contiguous 1 mm scans) were acquired on a 9.4T/30 cm Bruker MRI scanner. **Data analysis:** Two sets of gradient echo images were acquired for each stimulation protocol. The EPI scans were registered to an ideal anatomy. The images for each nerve and stimulation protocol were averaged. The averaged data for each nerve and stimulation level were then masked (3dAutomask) using AFNI. Activation was determined by an F test (3dDeconvolve) with a P-value threshold of 0.005 using AFNI.

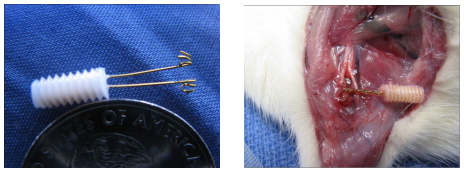

Fig. 1. Innovative 150-um diameter stainless-steel implantable electrode with a total length of 17 mm. The right figure shows the incision 8 weeks following surgery; the new electrode remains in contact with the nerve trunk without causing obvious nerve damage.

Results

Figure 2 shows comparisons of nerve stimulation results between the new implantable electrode and the traditional non-implantable electrode. Fig 2.1 shows normal median nerve stimulation results using the non-implantable electrode on a non-survival animal.[1]. It can be considered the normal control. Comparing Figs. 2.2 and 2.3, which both show results 4 weeks following the initial operation, the brain representation area of the median nerve in both groups diminished significantly. However, functional loss of the rats using our implantable electrode is significantly better than those using the traditional electrode. Eight weeks later (shown in Figs. 2.4 and 2.5), distinct activation can still be found in the new electrode group. No statistical significance can be found when comparing the results 8 and 4 weeks following surgery, which means the nerve remains the same during this period. In group 2, the accumulation of scar and proliferated tissue causes tremendous damage to the nerve trunk, which blocks signal transfer and makes the whole brain "silent" during nerve stimulation. Figure 2.6 shows the statistical analysis of the number of voxels across scans. Although brain response to nerve stimulation inevitably decreases after each procedure, the innovative implantable electrode does significantly less damage to the nerve when a survival experiment is conducted.

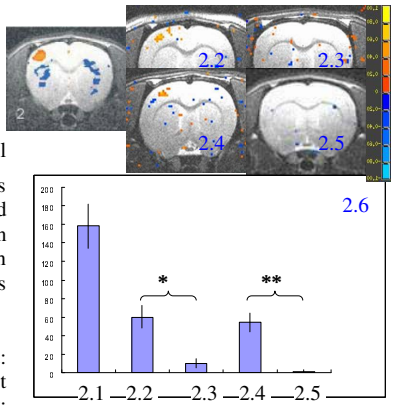

Fig 2. fMRI evaluation of implantable electrode. 2.1 Normal median nerve activation. 2.2 Median nerve activation at 4 weeks using implantable electrode. 2.3 Median nerve activation at 4 weeks using traditional electrode. 2.4 Median nerve activation at 8 weeks using implantable electrode. 2.5 Median nerve activation at 8 weeks using traditional electrode. 2.6 Voxel counting of different scans. (*)$p<0.05$ (**)$p<0.01$. Note that the significant difference happens at 8 weeks, and also note the comparable result of group 1 at 4 and 8 weeks after the initial surgery.

Conclusion and Discussion

In any survival study using peripheral nerve stimulation, there are four factors that might affect the experimental result: first, whether or not the electrode can keep good contact with the nerve after a long period of time; second, whether or not the electrode can remain stable during the procedure in order to avoid extra nerve injury that is caused by movement; third, whether or not the electrode is small enough to minimize the "reject" reaction; and, lastly, whether or not the implanted electrode can interfere with the nerve responses of both the peripheral and central nervous system (PNS; CNS). Our implantable electrode shows excellent results in all four aspects and significantly lowers the cost to carry out survival studies involving peripheral nerve stimulation. In fact, the heavy scar and proliferated tissue caused by reiterative operation damage not only the target nerve itself, but also the whole nerve bundle like brachial plexus in our experiment . According to our analysis, the forepaw signal of group 2 also significantly decreased compared to group 1 4 weeks following the initial surgery (data not shown). This study provides a reliable tool for researchers performing survival studies involving the PNS and CNS. The implantable electrode can both lower research expenses and simplify the research process, and also can be potentially used on different types of animals in both fMRI and electrophysiology studies.

Reference

1. Cho YR, et al. Refining the sensory and motor ratunculus of the rat upper extremity using fMRI and direct nerve stimulation. Magn Reson Med 2007;58:901-909

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