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Research report

Cortical electrical stimulation with varied low frequencies promotes functional recovery and brain remodeling in a rat model of ischemia

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ABSTRACT

In this study, we investigated whether fully implantable CES with low current density and varying low-frequency burst impulse train enhances functional recovery and promotes brain remodeling in both the ipsilesional and contralesional cortex. Adult rats received occlusion of the right middle cerebral artery for 120 min. One week after ischemia, electrodes were implanted to rats with CES lasting 2 weeks followed by 4-week observation period. After 2-week stimulation and 4-week observation period, body weight (BW) of the rats in CES group was higher than that in no stimulation (NS) group. Limb placement test, foot-fault test and beam walking test demonstrate that CES significantly enhanced functional recovery. Immunohistochemical study has shown that CES enhanced angiogenesis and dendritic sprouting, and suppressed inflammatory response in the ischemic cortex. CES also promoted dendritic sprouting and suppressed inflammatory response in the contralesional cortex. These results suggest the stimulation protocol is safe, and greatly improves functional recovery and brain remodeling in the 4 weeks following 2 weeks stimulation.

1. Introduction

In recent years, implanted electrical neural stimulation devices have received widespread attention for treating neurological disorders. For example, deep brain stimulation (DBS) has been successfully applied in clinic to date. DBS has become a recognized therapy technique for patients with Parkinson's disease and has potentially promising applications against a wide range of neurological diseases (Deniau et al., 2010). The success of DBS inspired the development of other forms of electrical brain stimulation, such as cortical electrical stimulation (CES) for patients suffering from cerebral ischemia.

Many researchers have devoted their efforts to exploring the feasibility of CES. Human and animal studies showed that CES concurrent with rehabilitation training can enhance neuroplasticity and improve motor performance after stroke (Boychuk et al., 2011; Adkins et al., 2008; Levy et al., 2008; Brown et al., 2008; Harvey and Nudo, 2007; Adkins et al., 2006; Plautz et al., 2003; Kleim et al., 2003). It was reported that implanted cortical stimulation of the peri-infarct cortex promoted the sprouting of dendrites (Zhou et al., 2010) and exerted an antiapoptotic effect through phosphoinositide 3-kinase (PI3K)/Akt signaling pathways in the stroke rat model (Baba et al., 2009). Cortical stimulation of the forelimb representation in the ischemic cortex, concurrent with task-specific practice, increased neuronal density (Adkins et al., 2006), promoted synaptogenesis in layer V of the perilesional cortex (Adkins et al., 2008) and expanded the areas of forelimb representation (Kleim et al., 2003). Two pilot clinical trials supported the safety and efficacy of invasive cortical stimulation for improving upper limb function in patients with stroke (Levy et al., 2008; Brown et al., 2008; Huang et al., 2008). However, a large-scale clinical study (EVEREST study) did not show a significant effect of cortical stimulation in stroke survivors, possibly due to patient characteristics, stimulation protocol or the design of devices (Plow et al., 2009).

Stimulation location and device design could significantly influence the outcome of electrical neural stimulation. DBS, which employs high frequency stimulation (120–180 Hz) and places the electrodes into the subthalamic nucleus, could produce a lesion-like effect for the treatment of Parkinson's disease (Awan et al., 2009). In addition to movement disorders, promising results have been shown in epilepsy (Boon et al., 2007; Andrade et al., 2006) and psychiatric diseases (Mallet et al., 2008; Shahed et al., 2007), which have different targets from Parkinson's disease. CES can provide a relatively focused low intensity electrical stimulation and can selectively excite the target neural population. However, it was suggested that different neurons in the cortex vary in size, shape,

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location and orientation (Wongsarnpigoon and Grill, 2008, 2012). Thus, questions regarding better placement and optimal protocol of stimulation are still open for future study.

One proposed mechanism of electrical brain stimulation is to increase the neural activity of the perilesional hemisphere and/or decrease the neural activity of the contralesional hemisphere (Alonso-Alonso et al., 2007). Electrical stimulation affects the neural activity by driving current across neuronal membranes and triggering their responses (Histed et al., 2009), which could be influenced by stimulation models (bipolar or monopolar) and stimulation programs, including stimulation period, position of the electrode, stimulation frequency and charge density per phase (Plow et al., 2009; Harvey and Stinear, 2010; Hummel et al., 2008). However, in the majority of previous studies, stimulation parameters and experimental devices were not discussed in detail. In fact, we believe that the stimulation protocol can significantly affect the outcome of CES. We previously developed a fully implantable electrical stimulation for delivering stimulation to the peri-infarct cortex in order to investigate activity-related plasticity in a stroke rat model with a new stimulation protocol. The protocol consisted of varied low frequency pulse bursts with low intensity (Zhou et al., 2010). This study suggests that the long lasting low-charge-density electrical stimulation of the peri-infarct cortex could enable the maintenance of basic neuron activities, which may promote dendritic plasticity. Also, the study demonstrated the safety and promising benefits of CES with the particular set of stimulus parameters and experimental devices in detail. However, that study only evaluated the neurorestorative effects of CES in a short period of time (16 days) because of the limitations of the experimental stimulator. The other limitation was that there were not enough histochemical markers being used in evaluating the effects of CES on neural plasticity with the new stimulation protocol.

In this study, using the fully implanted cortical electrical stimulator with low-charge-density electrical stimulation and varying low frequency pulse bursts for stimulating the peri-infarct cortex after middle cerebral artery occlusion (MCAO), we look at whether it can enhance functional recovery and brain remodeling in a longer observation period even after stopping the stimulation after a short period. Based on the previous method, we evaluated the neurorestorative effects of CES with behavioral tests during 2 weeks stimulation and 4 weeks observation period. Meanwhile, we increased the stimulation timing from 1 h per day to 2 h per day compared with the previous study (Zhou et al., 2010). Moreover, we used a comprehensive immunohistochemical evaluation of brain remodeling in both hemispheres, in order to characterize how endogenous brain responses are modulated by CES with the new stimulation protocol.

2. Methods

2.1. Experimental animals

Twenty-three adult male Sprague-Dawley rats, weighing 250–300 g, were used in this study. Rats were group housed (five animals per cage) under standard laboratory conditions (12/12-h light/dark cycle, 24.5–25 °C, humidity 50–55%), and allowed free access to food and water during this study. All experimentation was conducted between 08:00 and 17:00 h. All procedures involving rats used in this study strictly adhered to the Guidelines for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication, revised 1996) and approved by the Ethics Committee for Animal Research, Wuhan University, China.

Body weight (BW) was recorded everyday. MCAO was induced using a method of intraluminal vascular occlusion. Two days after MCAO, magnetic resonance imaging (MRI) was used to confirm the cortical ischemia and to guide electrode placement. Rats with lesions restricted to the caudoputamen (Fig. 1A) were excluded, while rats (n = 23) with lesions involving both caudoputamen and cortex (Fig. 1B) were randomly divided into the CES group (n = 13) and no stimulation (NS) group (n = 10). One week after MCAO, we used cortical electrodes to deliver electrical stimulation for 2 h daily over 2 weeks to the rats with injury and implant plus stimulation (CES group) but not rats with injury and implant only (NS group).

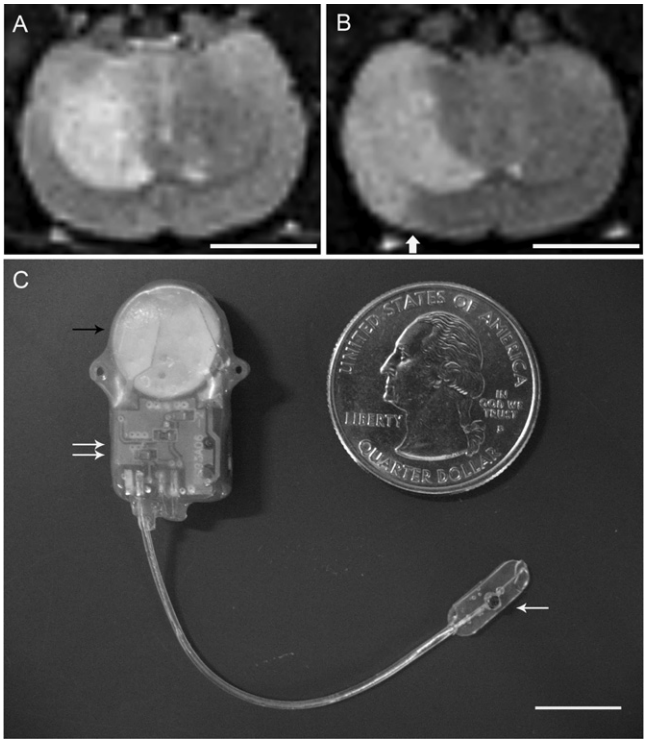

Fig. 1. Representative T_2-weighted MR images and fully implantable device. There were two lesion types on T_2-weighted MRI after 120 min of MCAO. (A) Lesion restricted to the caudoputamen which was the type excluded in the experiment. Scale bar: 7.5 mm. (B) Lesion additionally involving the cortex which was the type chosen for the experiment. White arrow shows the location of the electrode. Scale bar: 7.5 mm. (C) A fully implantable device consists of a stimulator (double white arrows) and a pair of electrodes. The black arrow represents a lithium cell and the white arrow represents a stimulating platinum electrode (d = 1.5 mm). Scale bar: 10 mm.

2.2. MCAO

Transient MCAO was induced using a method of intraluminal vascular occlusion, as described previously (Zhou et al., 2010). Rats were anaesthetized with pentobarbital sodium (50 mg/kg ip; Westang Biotechnology, Inc., Shanghai, China). A 3-0 monofilament nylon suture was gently advanced from the right external carotid artery into the lumen of the right internal carotid artery until it blocked the origin of the right middle cerebral artery. The suture was left in place for 120 min and then withdrawn. Body temperature was monitored and controlled at 37 °C during the surgical procedure using a heating pad in conjunction with a rectal probe.

2.3. MRI studies and measurement of infarct volume

MRI was used to verify the anatomical location of stroke and identify the placement of the surface electrode over the peri-infarct cortex, as well as analyze lesion volume. Two days after MCAO, all the MRI experiments were performed on a 4.7-T BioSpec animal scanner with a 30-cm horizontal bore magnet on the rats which were anaesthetized with pentobarbital sodium (50 mg/kg ip). The electrode was within the range between 0.3 mm anterior and 1.3 mm posterior to the bregma, overlying the ischemic boundary of the cortex. The distance of the ischemic boundary to midline of hemispheres was measured by MRI (Zhou et al., 2010). Nine contiguous coronal T_2 weighted images (T_2-WI) were acquired with the following parameters: T_R/T_E = 3000/20 ms, 6 echoes, FOV = 3.5 cm × 3.5 cm, slice thickness = 0.8 mm, interslice distance = 1.6 mm, matrix = 128 × 128, NA = 1. The ischemic lesion area was evaluated from T_2-WI based on the previously described method (Neumann-Haefelin et al., 2000). For each slice, the areas of ischemic lesion in T_2-WI have higher intensity. Infarct volumes were calculated by summing the ischemic lesion area and multiplying the slice thickness plus interslice distance (Neumann-Haefelin et al., 2000).

2.4. Electrode implantation and stimulation protocol

One week after MCAO when the rats have somewhat recovered from the last surgery, both the CES and NS groups received the same electrode implantation, only the CES group received 2-week stimulation. Briefly, rats were anaesthetized with pentobarbital sodium (50 mg/kg ip). We made a craniotomy over the peri-infarct