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LETTER TO THE EDITOR

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Gene Transfer into the Both Sides of Postnatal Cerebral Cortex Using Tweezer Electrode

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In vivo electroporation works as highly efficient nonviral methods to transfer foreign genes into mouse brain [1–3]. Nevertheless, an important limitation of the present *in vivo* electroporation is that the transfection is unidirectional, and only one side of cerebral cortex can be electroporated [4,5], which may limit its application in gene therapy studies [6,7]. Thus, it is necessary to improve the one side electroporation method into bilateral electroporation.

Recently, foreign gene was successfully transferred into the both sides of fetal cortex using a triple-electrode, which may facilitate gene therapy studies on brain disorders [8–10]. However, there is no efficient electroporation method to introduce foreign gene into the both sides of postnatal cerebral cortex until now. Here, we successfully introduced foreign gene into both sides of postnatal cerebral cortex using tweezer electrode by optimizing the time interval between the twice *in vivo* electroporation. Briefly, the Pregnant ICR strain mice were purchased from Experimental Center of Peking University,and the newborn pups (within 12 h of birth, and the gender was selected randomly) were anesthetized with diethyl ether properly and then were injected 1.5 μL DNA solution (5 μg/μL in normal saline containing 100 μg/mL Fast Green) into one lateral ventricle using mouth-controlled microinjection system. Immediately after injection, the pups were electroporated with tweezer-type electrodes (7 mm diameter; BTX, Holliston, MA, USA). Anode was placed on the injected sides, and five electric pulse at 90 V was delivered to the operated head, with 50 ms duration and 1 second interval. Next, with different time interval (0, 6, and 24 h), the same volume of DNA solution was injected into the other lateral ventricle and then the same number of pulses was delivered in the reverse direction. Next, the successfully operated pups were placed on a heat pad until they recovered full mobility and subsequently returned to their mother. The results demonstrated that the foreign gene (GFP) was successfully introduced into the both sides of postnatal cerebral cortex (Figure 1A). Analysis on frozen sections showed that GFP positive signal was mainly located at ventricular zone and subventricular zone, while less GFP positive signal was found in the upper cortical plate (Figure 1B,C). Further investigation showed that performing twice electroporation in reverse direction with 6 and 24 h interval could get ideal survival rate and bilateral GFP positive rate (Table 1), however by comparing the difference of fluorescence between the both sides of cortex; it was found that 6-h interval was the most feasible condition for bilateral electroporation (Figure 1D). To discuss the possibility of gene therapy using this method, the persistence of foreign gene expression was investigated. As shown in Figure 2A,B, after bilateral electroporation with GFP-N1 plasmid (10 μg/μL), bilateral GFP positive signal could be found even 15 days after the bilateral electroporation, although the GFP was mainly expressed in cells located at ventricular zone.

In summary, we improved the previous electroporation method and promote it to be a useful tool to transfer foreign gene into both sides of postnatal cortex, and it may facilitates to discuss the possibility of gene therapy on some developmental brain disorders.

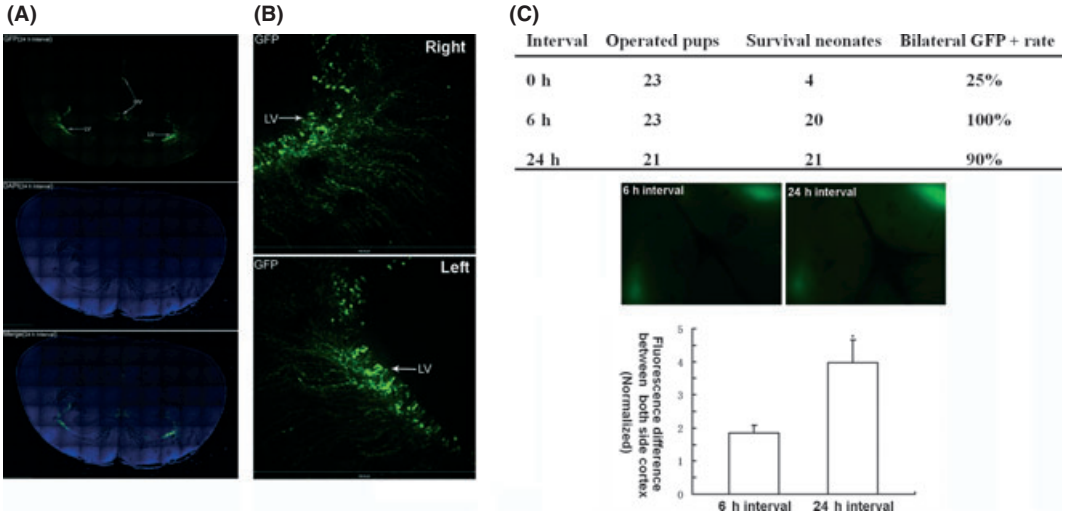

Figure 1 pEGFP-N1 was introduced into the both sides of cerebral cortex of neonatal mouse brain successfully. (**A**) The operated brain was observed with fluorescent stereo microscope 24 h after bilateral electroporation, and pEGFP-N1 was expressed in both sides of cortex. (**B**, **C**) The EGFP positive brains were sliced and confocal analysis was performed, and the strong GFP positive signal was found both in the right and left cortex. (**D**) Optimization of the Conditions for Bilateral Electroporation. (a) The effect of Different Interval on bilateral transfection efficiency; (b) The difference of fluorescence intensity between the both sides of cortex at 6-h interval was smaller than that of 24-h interval, $P < 0.05$. Results were means + SEM (n = 10).

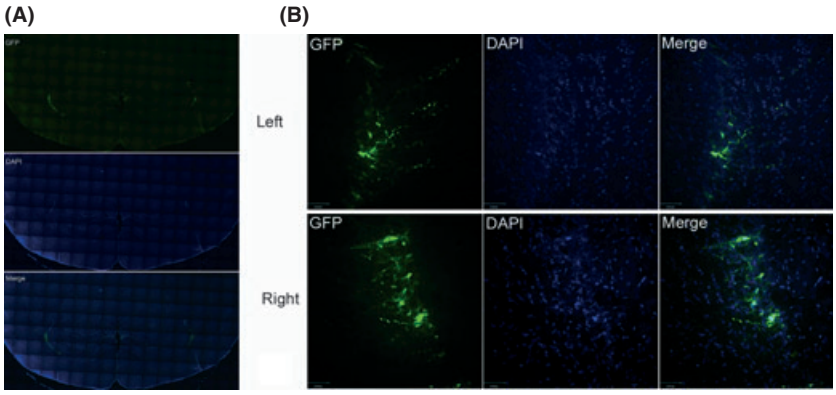

Figure 2 Foreign gene can be persisted for long time. (**A**) Overall view of frozen sections showed bilateral GFP positive on P15 sections. The nucleus was stained with DAPI. (**B**) Enlarged GFP positive area, including right and left cortex, and GFP positive signal was mainly located at VZ. Scale bars = 36 μm.

Acknowledgments

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Conflict of Interest

The authors declare no conflict of interest.

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