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Three puncture sites used for *in utero* electroporation show no significantly different negative impacts during gene transfer into the embryonic mouse brain

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HIGHLIGHTS

- Micropipettes affect the death rate of embryos during *in utero* electroporation.
- The three puncture sites do not affect the death rate of embryos, GFP-positive rate and embryonic cortical surface area.
- Difference about cell differentiation, proliferation, migration and apoptosis do not exist in the three puncture sites.

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ABSTRACT

Although various ways to manipulate genes *in vivo* exist, *in utero* electroporation is a widely used technique, especially in the field of neural development due to its many advantages. In this study, we focused on direct comparison between three puncture sites during *in utero* electroporation on the death rate of embryos, the thickness and the area of cortex, cell differentiation, cell proliferation, cell migration and cell apoptosis. We found no statistical significant differences between the three puncture methods in the death rate of embryos, the thickness and the area of cortex, cell differentiation, cell proliferation, cell migration and cell apoptosis.

1. Introduction

As many new genes are identified by genome projects, the study of the function and the network activity of these genes *in vivo* becomes a key problem. There are multiple ways to manipulate genes *in vivo*. Transgenic and gene targeting techniques have the ability to change specific genes that are then stably transmitted to the next generation [7]. Recombinant viruses and biolistic gene guns have been used to deliver genes to *in vivo* tissues. However, these methods have certain limits. For example, the process of producing transgenic, gene-targeted mice and recombinant viruses is time-consuming and strenuous. Moreover, it is difficult to express the gene of interest precisely at a specified time and location, as the transcriptional regulation of genes is complicated. Thus, *in utero* electroporation, as a quick and easy method, has been favored by more and more researchers, and the use of this technique will greatly promote the understanding of gene function and networks in the brain.

For electroporation, after introducing the appropriate vectors into the ventricle of embryonic mouse brain, an electric field is applied to the proper extra-uterine position, and the vectors move toward the positive electrode and then to the targeted specific regions. We can study the migration and differentiation of the cells to analyze the functions of genes. Compared with other DNA transfer methods, *in utero* electroporation method has several advantages: it is a highly efficient, spatially and temporally specific technique and can be used to transfect multiple genes into one cell [14]. Furthermore, *in utero* electroporation can be used similarly to mitotic birth dating. Following this principle, different

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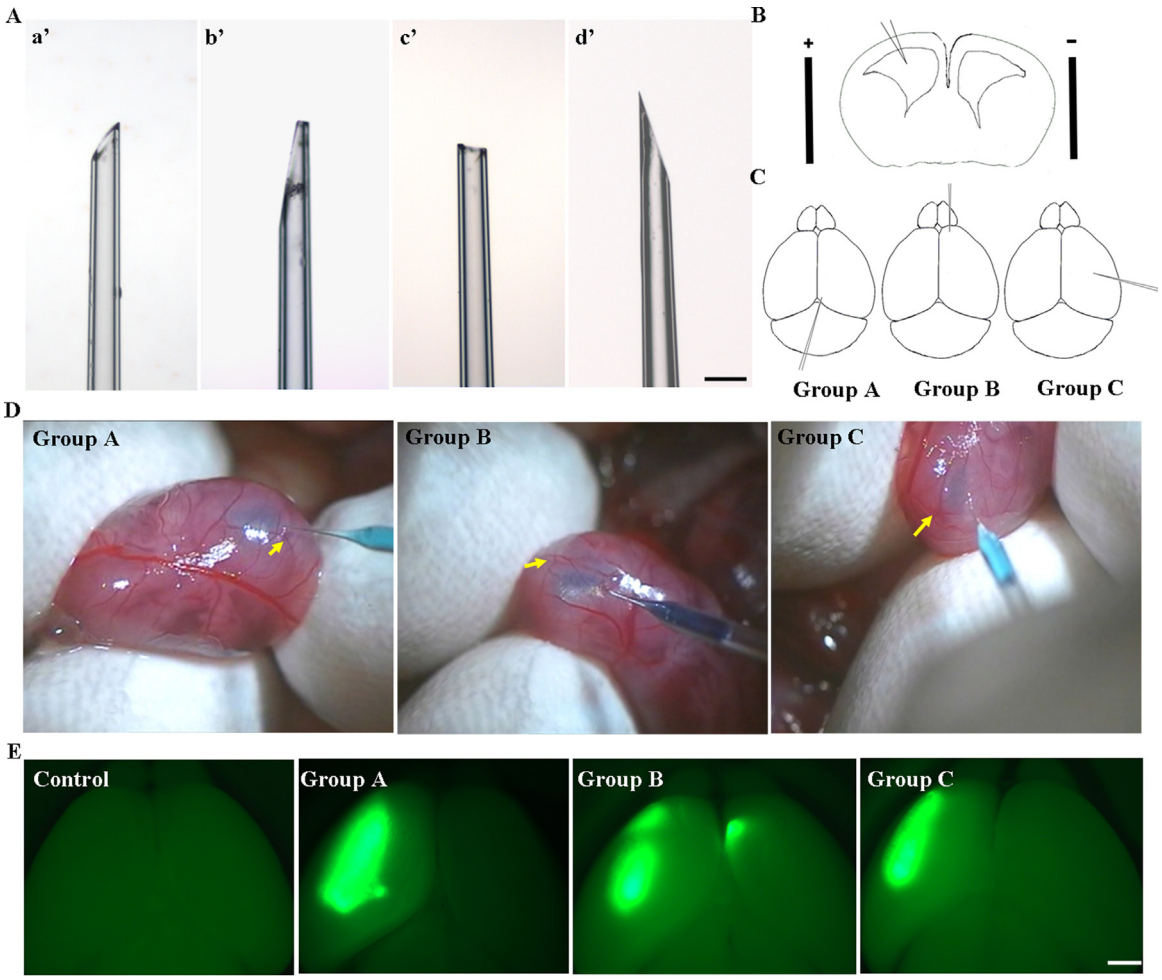

Fig. 1. Preparation of the micropipette and effects of different injection positions in *in vivo* electroporation. (A) Preparation of the micropipette for *in vivo* electroporation. a', The angle is too great. b', The pipette has no tip. c', The pipette has no angle. d', The ideal needlepoint should meet the following requirements: the diameter should be between 40 and 60 μm, and the angle should be 15–30°. Scale bars, 50 μm. (B) Schematic of electroporation protocol: DNA vectors were injected into the ventricle and were introduced into the adjacent neuroepithelial cells, which were directed toward the positive electrode. The positive electrode was aligned outside the brain as shown. Schematic representation of different injection positions (C) and the actual operation (D). Yellow arrows indicate posterior fontanelle regions. (E) The location of GFP expression after different injection method. There was no significant difference by florescent microscopy. Scale bars, 500 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

electroporation times can lead to expression in specific cell types [8]. The *in utero* electroporation method has also been used to study the gain of function and loss of function of some genes [4,17]. The application of this method contributes to illuminate the molecular mechanisms through identification and characterization of the mislocalization of neurons or other cells in brain development and pathophysiology [5,16]. Therefore, *in utero* electroporation is playing an increasingly important role in neuroscience. Scientific experiments require following a strict protocol, and small details determine success or failure. For electroporation, essential details include the following: tip diameter of the micropipettes, the puncture site, the voltage and pulse duration of the electric pulse, the clip position of the paddle electrodes, the operation time, DNA extraction, and the concentration and quality of vectors. All these elements will influence the results to a certain extent.

Although the embryonic head can be seen through the uterine wall, it is still difficult to inject DNA precisely into the region of interest, particularly into the diencephalon and ventral parts of the embryonic brain. The selection of puncture sites is a key event. Common puncture sites include the following: injection near the posterior fontanel, at about 1 mm; injection along the anteroposterior axis into the cephalic ventricle; and injection near the midpoint between the anterior fontanel and posterior fontanel, at about 3 mm. Different laboratories have their own methods. Beginners struggle with how to choose the injection position. This article studies the different influences on cortical development when injection is performed at different sites.

2. Materials and methods

2.1. Animals

We used CD1 (ICR) mouse, which was purchased from the Vital River Company (Beijing, China). All animal handling protocols were approved by the local Institutional Animal Care Committee.

2.2. Plasmid

The plasmid contained a GFP/DsRed reporter gene that is downstream of the CAG promoter (Addgene 11150, Addgene 11151). The plasmids were purified using the EndoFree Plasmid Kit (12362, Qiagen).

2.3. In utero electroporation

The *in utero* electroporation was performed with an electroporator (ECM830, BTX) using five pulses of 45 V with 1 s intervals