Video Article

Targeting of Deep Brain Structures with Microinjections for Delivery of Drugs, Viral Vectors, or Cell Transplants

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Abstract

Microinjections into the brain parenchyma are important procedures to deliver drugs, viral vectors or cell transplants. The brain lesion that an injecting needle produces during its trajectory is a major concern especially in the mouse brain for not only the brain is small but also sometimes multiple injections are needed. We show here a method to produce glass capillary needles with a 50-µm lumen which significantly reduces the brain damage and allows a precise targeting into the rodent brain. This method allows a delivery of small volumes (from 20 to 100 nl), reduces bleeding risks, and minimizes passive diffusion of drugs into the brain parenchyma. By using different size of capillary glass tubes, or changing the needle lumen, several types of substances and cells can be injected. Microinjections with a glass capillary tube represent a significant improvement in injection techniques and deep brain targeting with minimal collateral damage in small rodents.

Video Link

The video component of this article can be found at https://www.jove.com/video/2082/

Protocol

1. Make glass needles before mouse surgery:
    a. Put the capillary glass tube in a micropipette puller.
    b. Heat the middle of the glass tube to soften the glass tube in the localized area.
    c. Stretch the glass tube along its longitudinal axis by an initial distance sufficient to cause a reduction in the diameter of the glass tube in the localized area.
    d. Keep stretching the glass tube until it breaks. In this way, two identical single barrel needles are obtained.
2. Put the glass needle in a microforge. A 30° angle is enough to bevel the tip.
3. Check under the microscope that each needle has the correct inner diameter. For most of the hydrophilic drugs a 30-50 µm inner diameter is ideal (figure 1).
4. Fill up the needle with mineral oil from the widest end of glass needle. Let that mineral oil (MSDS, Cat. M7700) enters by capillarity until it reaches ~50% of the length of the capillary tube. Put high-vacuum grease (Dow Corning, Cat. 05054-AB) around the plunger to seal the widest end of the needle.
5. Insert the plunger by the widest end of the needle and push gently mineral oil until a small drop of oil is seen going out of the tip of the glass needle.
6. Secure the glass needle in the holder of the microinjector.
7. Anesthetize the mouse with 2.5% Avertin (2,2,2-tribromoethanol + tert-amyl alcohol, 1:1 w/v). Dosis 25-30µL per gram intraperitoneal.
8. Put a heater pad on the stereotactic device to keep animal's body temperature at 37°C.
9. Place and secure the mouse head in the stereotactic device using ear bars and a teeth holder.
10. Clean the mouse head with 0.1% Chlorhexidine gluconate solution, followed by 70% Ethanol and 0.1% Chlorhexidine gluconate for a second time.
11. Incise the skin with a surgical blade # 15 from mouse ears to the Lamba skull line.
12. Polish the skull with a cotton swap to dry it out and pull skin out of the surgical field.
13. Use the tip of the glass needle to point at the vertex of the Bregma suture. There you have to set the initial position of injector (the coordinate "zero").
14. Set the point to be drilled by moving the "X" and "Y" axis of stereotactic device over the skull.
15. Drill very carefully holes at the selected coordinate. Microdrills and other metal tools were previously sterilized at 250°C for 60 seconds with a dry glass bead sterilizer (Cole Palmer, Cat. No. EW-10779-00).
16. With fine forceps remove carefully the deepest layer of bone and break the duramatter membrane (you will see some cerebral spinal fluid leaking out).
17. Confirm that needle can go through drilled holes.

18. Pipette 1 µl of the drug you want to inject and put it on a small piece of parafilm.
19. Put the parafilm onto the skull.
20. Suck up the drug by spinning the microinjector wheel, while you check under the microscope that the fluid is going up into the glass needle.
21. Remove the parafilm and re-set the zero coordinate.
22. Move the needle to the selected coordinates, down the holder until the tip of glass needle gently touch the brain surface and set the "Z" coordinate at zero.
23. Introduce the glass needle into the brain parenchyma at selected depth.
24. Inject the drug slowly at rate of ~1 nl/ sec.
25. When desire volume is injected, let the drug diffuse into the parenchyma for 2 min. Then proceed to remove the needle smoothly and slowly.
26. Glue the surgical wound and remove the animal from the stereotactic device and put the animal in a pre-warmed cage containing a clean bed for anesthesia recovery.
27. To provide post-operative analgesia all animals received 5 mg/kg subcutaneous Ketorolac every 12-hour for 24 h.

Representative Results:

When following this protocol, a very precise injection is obtained and a very narrow needle track minimizes brain injury. As a representative result of this method, we injected lysophosphatidyl choline (lysolecithin) into the corpus callosum that produces demyelination of white matter tracts 1-4. To minimize the brain injury produced by the glass needle, we injected only 20 nl of lysolecithin into the corpus callosum, but if required higher volumes as much as 200 nl can be injected with the same method. Demyelination is detected by the absent of myelin basic protein expression in the white matter tracts (Figure 2).

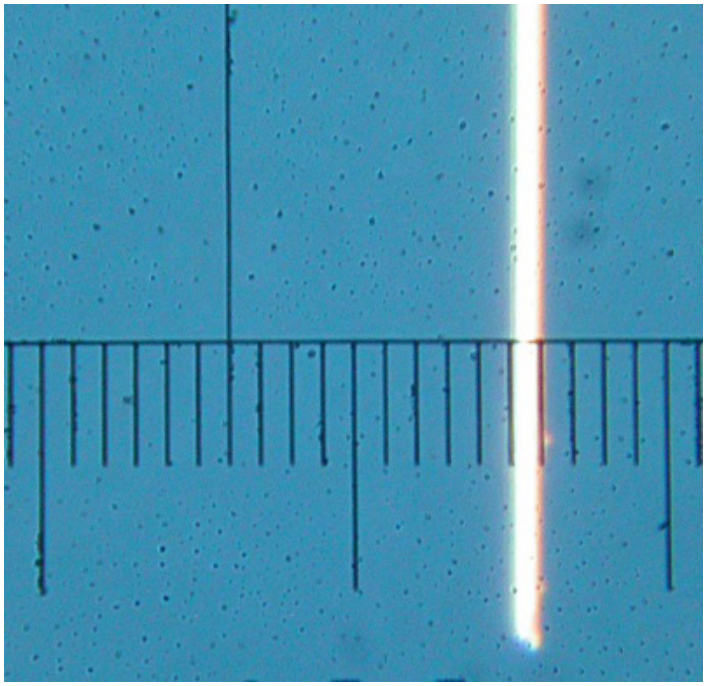

Figure 1. A glass needle with a 50-µm diameter. Distance between two short ticks in the scale represents a 50-µm length.

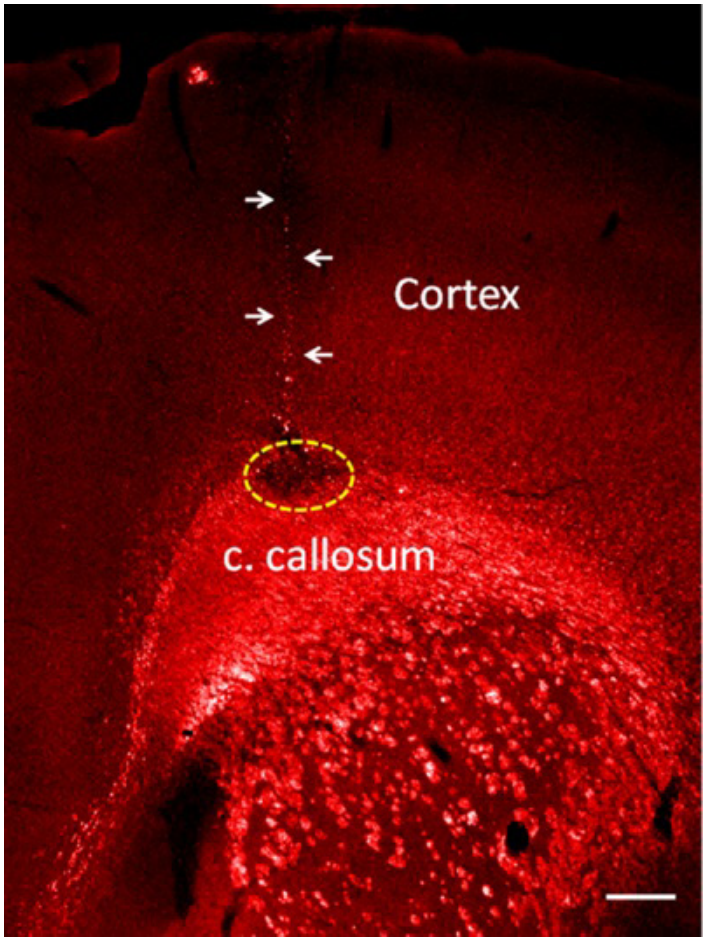

Figure 2. Lysolecithin injection in the corpus callosum. Demyelination is shown as a no myelin basic protein expression (dotted area). Note the small size of glass needle tract (arrows). Bar = 100 µm

Discussion

The method showed in this video is very useful to deliver most of the drugs or viral vectors into very precise places into the brain. Some of the main advantages of this technique are the reliability of the targeting point, the accuracy of injections and the small size of brain lesion and tract damage[1, 2, 5, 6]. Once the technique is standardized the range of mistargeting should 50 µm or less[1, 2]. Cell transplants can also be done by using wider, 100-150 µm[7-9], needles. Therefore efficient cell delivery can be deposited into very specific lesion minimizing collateral damage induced by cell transplant. There are three crucial steps in this technique:

1. We always recommend scratching softly the skull with an insulin needle to label the selected coordinates before drilling.
2. Introduce the glass needle with a quick movement to rapidly penetrate the brain surface.
3. Once the optimal depth is reached you should introduce the needle 0.1 mm deeper into the brain parenchyma to make a "canal" that let drug to freely diffuse into the brain.

Disclosures

No conflicts of interest declared.

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