

Retrograde labeling

Stereotaxic injections were made into the dorsolateral striatum at coordinates 0.0 mm posterior, 2.0 mm lateral, and 2.5 mm ventral to bregma. Mice were deeply anesthetized by inhaled isoflurane. A small craniotomy was opened over the left or right parietal area 2.0 mm posterior and 3.5 mm lateral to bregma. A 5 μ L capacity borosilicate glass pipette (VWR, pulled to a long, shallow taper >1 cm with a tip diameter of 30 μ m, Narishige) was advanced into 3.5 mm into the dorsolateral striatum at an angle $\sim 17^\circ$ off the sagittal plane and $\sim 48^\circ$ off the horizontal plane. A small volume (~ 25 nL) of undiluted solution with red fluorescent RetroBeads (Lumafluor) (2) was pressure-injected (Picospritzer III, Parker Hannifin). The pipette was kept in place for several minutes prior to extracting it. Acute brain slice experiments were performed 1-7 days later and inspected to confirm accurate placement of the dorsoalateral striatal injection. These injections gave a characteristic distribution of labeling in the contralateral motor-frontal cortex consistent with the known anatomy of the crossed corticostriatal projection (3). In contrast, injections in adjacent striatal zones resulted in different labeling patterns, indicating that the labeling we observed was specific to the striatal injection location and not due to nonspecific labeling along the track of the injection pipette.

We inject flunixin (SQ, 1.1mg/kg) and, use Vet glue (super glue) to close the wound. They heal beautifully.

The beads come from a company called Lumafluor.

http://www.lumafluor.com/Order_Now.php

They have no part number, you just email them info@lumafluor.com (his name is Ryan) and ask for an order of green or red retrobeads, we have better labeling with the IX version of the beads, so I recommend using them. And if you only need one color, red is much better because it doesn't compete with the slice background fluorescence.

The glass we use comes from VWR, part number 53432-706. It's calibrated so you can figure out how many mm per uL of beads it uses, which gives you control over the volume of beads you inject.

The hard part for us was getting the injection pipette to be in exact alignment with the arm of the stereotax. We use a couple of pipette holders and a homemade adapter to get the job done.

Our coordinates are optimized for p 21-27 animals. When I was working out the coordinates, I injected a 1% fast-green in ACSF solution, which gives a nice bright blue spot if you then slice the animal immediately. You can try the coordinates I listed above with some fast-green and then check if it's in the right spot. If not, you can make adjustments either to the angle, or the injection site and dial it in perfectly before you go using the expensive beads.