



Stereotaxic manipulation for neuroscience research

Application: Stereotaxic micromanipulation in a rodent brain



Stereotaxic brain surgery makes use of a 3-dimensional coordinate system to position a tool, which could be an electrode or an injection needle, at a well-defined brain region.

The coordinates are typically taken from a brain atlas for the species of interest and dialed into the three manipulator arm coordinates of a stereotaxic frame.

Stereotaxic gene delivery injects a vector, typically a virus carrying a modified gene, into a brain region of interest. The genetic information is delivered by the vector to the brain cells altering them genetically.

This can introduce new ion channels (Optogenetics, Chemogenetics), change the function of the neurons or cause malfunction in a disease model.



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WHO

Research Disciplines

- Animal Physiologists
- Cellular Neuroscientists
- Research and Developments
- Biotechnicians
- Chemists

Benefits of Drug Delivery System

A variety of WPI products can be used for this drug delivery application, including the digital stereotaxic frame, animal temperature controller and microliter pump which can be mounted directly on the stereotaxic frame.

- MTM-3 digital stereotaxic frame reduces the errors from brain map calculations and Vernier scale readings. You may automate tasks like drilling skull openings and making injections. The MTM-3 offers flexibility in positioning. The reference point (like bregma) can be zeroed, and all coordinates may be entered directly from the atlas. Or, use WPI's motorized MTM frame to directly set the coordinates in absolute values or relative to a selected reference for automatic positioning.
- ATC2000 Animal Temperature controller automatically adjusts heating parameters to compensate for the size of the subject.
- UMP3 UltraMicroPump is a versatile microsyringe pump injector that uses micro syringes to deliver microliter and nanoliter volumes.



WHY IT WORKS

Benefits of using the Drug Delivery System

Unlike work with cultured cells, *in vivo*-infected neurons maintain all their native properties except for those introduced by the genetic manipulation. They also maintain their neural network. Reasons for using a drug delivery system like this include:

- Genetic manipulations are easy to introduce, and data may be collected in a relatively short period of time.
- Researchers control exactly what area of the brain is targeted for genetic manipulation.
- Because the results are localized, gene function experiments which would have traditionally been lethal or would have impacted large brain regions or the entire brain can now be safely conducted on a localized region.



The following WPI equipment will work well for this application:

MTM-3 Motorized Stereotaxic Frame

- Non-rupture Ear Bars (WPI #502235)

UMP3 UltraMicroPump

- NanoFil syringe (WPI #NANOFIL or NANOFIL-100)
- Bevelled needle (WPI #NF33BV-2)

Basic Anesthesia System (WPI #EZ-B800)

- Anaesthesia mask (WPI # 502063-xGM)

ATC2000 Animal Temperature Controller

- Rectal Temperature Probe (WPI #RET-2 or RET-3)
- Heating Plates for use with the ATC2000 (WPI #61800, 61830 or 61840)

Surgical Instruments

- Scalpel handle #3 (WPI #500236)
- Standard scalpel blade #10 (WPI #500239)
- Dumont™ tweezers #5 (WPI #500233)
- Graefe forceps, curved (WPI #14141)

Other Equipment and Supplies

- Autoclave (WPI #504188)
- Rapicide® OPA/28 Disinfectant/Sterilant (WPI #504611)
- Stereo Microscope (WPI #PZMIII-AAC or PZMIII-BS)
- Microdrill (WPI #503598/503599)
- Clamp for Microdrill (WPI #502237)



BE SURE TO FOLLOW ALL SAFETY PRECAUTIONS AS DESIGNATED BY YOUR LABORATORY WHEN YOU ARE WORKING WITH VIRUSES.

1. Sterilize all surgical instruments and tools, and disinfect the surgical area.
2. Anesthetize the subject.
3. Shave the skull and clean the fur with 70% ethanol.
4. Secure the subject in the stereotaxic frame. Use ONLY non-rupture ear bars with a wide tip to avoid piercing the ear drum. Be sure to apply a corneal lubricant to protect the eyes. Properly position the tooth bar and nose clamp (if using) to securely hold the head in position.
5. Using a dissecting microscope like the PZMIII, make a small incision with a surgical scissors (WPI # 500046) or a scalpel. Using a surgical hook (WPI # 501846 or 501899), to keep the area accessible. Clean the bregma and lambda regions of the skull with a small bone scraper.
Keep the skull moist with sterile PBS solution or saline solution.
6. Use the bregma and lambda points on the animal's skull to properly target the specific brain region of interest. Horizontally level the animal's head using the dorsal-ventral position of the nose clamp to set the z-position of lambda and bregma.

Setting the position with WPI's MTM-3 is easy, because the reference point can be zeroed and all coordinates can be entered directly from the atlas. Or, use WPI's motorized MTM frame to directly set the coordinates in absolute values or relative to a selected reference for automatic positioning.

Stereotaxic Micromanipulation

Stereotaxic micromanipulation in a rodent brain

PROCEDURE

7. Set bregma as the reference location on the MTM-3 stereotaxic frame controller. Using a brain atlas locate the target coordinates.
8. Use the 503598/503599 Microdrill mounted on the MTM-3 frame (using a 502237 clamp) to thin a 1mm x 1mm area around the target coordinates. Do not drill through the bone. Stop when the bone is thin. Blood vessels in the dura become visible as the bone thins out.
9. Use a 27 g dissecting needle like WPI # 500458 to gently pierce the edges of the craniotomy. Work around the perimeter to perforate the edges. Carefully, use the needle to invert the thinned bone and fine forceps to remove it. Remember to wet the surface with PBSW.
10. Mount the UltraMicroPump on the MTM-3 stereotaxic frame. If you use the MTM-6 Dual arm stereotaxic frame, you can mount the Microdrill on one arm and the UltraMicroPump on the other. Set up the NanoFil syringe with a NF33BV-2 33 gauge beveled needle. Load the syringe with the injection solution.
11. Locate the tip of the beveled needle at bregma. Enter the proper coordinates (Anterior/Posterior, Dorsal/Ventral, and Medial/Lateral) for the injection site into the MTM-3 controller. The needle tip should barely touch the dura. Slowly lower the tip to penetrate the dura. If the dura is too tough to penetrate, you may pierce it with a dissecting needle held at a flat angle. After penetration, command the MTM-3 controller to position the needle at the proper depth, moving slowly.
12. Using the controller for the UMP3 UltraMicroPump, inject the appropriate aliquot of the viral solution.
13. Wait 2-3 minutes. Then, slowly withdraw the needle.
14. Clean the injection site. If the site is larger than 1 x 1mm, place a thin slice of bone wax over the skull. Then, suture the skin and apply a topical antibiotic ointment. Inject a local anesthetic like lidocaine near the wound. Inject a sterile PBS solution subcutaneously to prevent the subject from dehydration after surgery.
15. The subject must be kept warm until it fully recovers. At that time, an analgesic like buprenorphine may be administered. Return the animal to a single cage and monitor it for at least a week.
16. Depending on the experiment, recombinant in vivo expression from the rAAV or lentiviral vectors varies from a few days to a few months.

References

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