User Manual

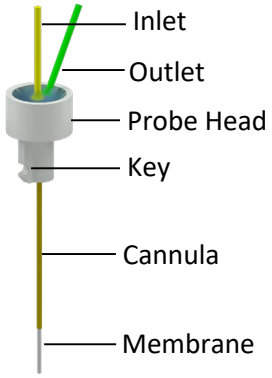
Brain "BR" Microdialysis Probes
Intracerebral Guides for Brain "BR" Probes

Brain "BR" Probes

Introduction

Brain "BR" Microdialysis Probes are pin-style probes. Pin-style probes are most commonly used for studies in the brain or spinal cord. The dialysis membrane is located at the distal end of a supporting cannula. Flow goes in and out one end of an otherwise sealed membrane cylinder. Inlet-outlet connections are made at the "head" or proximal end of the supporting cannula.

Brain "BR" Microdialysis Probe



BR probes may be implanted directly into the brain or may be placed in a separate guide cannula as described further in this manual.

Specifications

- Polyacrylonitrile (PAN) membrane, 320µm OD, 220µm ID
- MWCO 30KDa
- Standard membrane lengths of 2mm and 4mm
- Probe Cannula diameter- 510µm, length 15 mm, length when inserted in BASi Guide Cannula 10mm
- Head diameter 6.4mm
- Inlet/Outlet tubing diameter 635µm OD, 254µm ID
- Custom BR Probes available upon request

Benefits

- Measure target tissue concentration of compounds, neurotransmitters and other small molecules.
- Probes (and guides) are MRI compatible, as long as the dummy stylet is removed.
- Use of Guide Cannula makes it possible to recover animal and re-establish the blood brain barrier possible before insertion of the probe.

Molecular Weight

Under conditions of equilibrium dialysis, the molecular weight cutoff of the membrane on BASi BR probes is 30KDa. To test whether your analyte is an appropriate candidate for microdialysis using BR probes, it is recommended to perform an in vitro recovery test prior to in-vivo studies. For higher MWCO or for studies using lipophilic molecules, see our Open Flow Microperfusion Probes.

Limitations

- In brain tissue, the upper limit for continuous sampling is three to four days. After, gliosis around the probe and protein build-up on the probe being to limit diffusion into the probe.
- PAN membrane has a slight negative charge, so it will not be compatible with all compounds. For an alternative membrane materials, see our cellulosic MBR-10 Guides and Probes.

Sterilization

Probes are not sterile and cannot be sterilized in their shipping package. BR probes can be sterilized using gas sterilization (ethylene oxide) or plasma sterilization (hydrogen peroxide).

To sterilize:

1. Remove the paper seal and transfer the probe tray to an appropriate sterilizing bag.

2. BR probes are packaged with a small plastic vial protecting the dialysis membrane and probe cannula. The tube should be removed to expose the probe to the sterilizing gas. Save the tube so that you can use it for later flushing procedures. It may be helpful to prop the probe cannula with a small piece of sponge from the package. This will prevent the membrane from touching the probe tray during sterilization.

3. Once wetted, probes must stay wet to remain viable. Since EtO impacts the membrane lock, this means the probes must be used as soon as possible after outgassing to ensure that the probe remains viable.

4. Take care to keep the dialysis membrane tip away from any other surface.

General Storage Conditions

The probe packaging provides protection from both light and moisture. However, it is not completely impermeable to either. Please store your probes under standard laboratory conditions, avoiding extremes of temperature and humidity.

Preparing Microdialysis Probes

The membrane of a microdialysis probe is filled with microscopic pores. During a microdialysis experiment, the analyte diffuses through these pores into the probe. The pores of new probes are filled with glycerol, which keeps the pores of the membrane open. The glycerol lock must be flushed out prior to use; failure to properly flush the glycerol can affect recovery or interfere with sample analysis.

Preparing Brain "BR" Probes involves the following steps:

1. **Wetting (new probes only)**

2. **Eliminate trapped air in the probe and connecting tubing.**

3. **Check for leaks.**

After the initial flushing of a new probe, the glycerol is displaced and the pores are held open solely by the washing solution. ONCE ITE HAS BEEN WETTED, the probe membrane is susceptible to drying damage. If a wetted probe is allowed to dry, the membrane pores will close and the probe will be irreversibly damaged. Keep a flushed probe constantly wet (either by placing in solution or by perfusing solution through it) to prevent damage.

1. Fill a gas-tight syringe (MDN-0100) with filtered perfusion fluid (BASi recommends artificial cerebrospinal fluid; aCSF, MD-2400). Make sure that the fluid is at ambient temperature and not cold. Mount the syringe in a syringe pump.

2. Open a probe package and remove the plastic vial containing the probe. If using the BASi Calibration Station (MD-1522), remove the probe from the vial by holding the white probe head (not the cannula) and gently pulling the probe straight out of the vial. The probe clamp in the BASi Calibration Station (MD-1522) has a U-shaped cavity. The U-shaped cavity has a step or shelf within it. Insert the probe into the clamp's U-shaped cavity. The U-shaped cavity has a step or shelf within it. Insert the probe into the clamp's U-shaped cavity so that the upper larger diameter part of the probe head rests on the shelf in the cavity. Orient the probe so that the "key" on the lower part of the probe head faces toward the open end of the U. Turn the clamp knob nearest the probe to tighten; the probe should self-align in the clamp. Use of the Calibration Station greatly facilitates probe preparation. If not using the Calibration Station, leave the probe in the vial; this will protect the probe until ready for use.

3. Before adding any tubing, start a syringe pump at a flow rate of 20uL/min and make sure that liquid leaves the tip of the syringe needle.

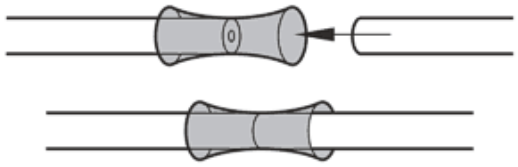
4. Flanged tubing connectors (MD-1510 expandable connectors, or MD-1516 silicone connectors) and either FEP Teflon (MF-5164) or PEEK (MF-5366) tubing should be used for all connections. Both types of tubing have a swept volume of 1.2µL per 10cm. The low volume reduces the sample mixing in the tubing,

improves the temporal resolution of the sample, and, for reactive analytes, allows you to collect the sample as soon as possible to minimize sample degradation.

5. Cut the desired lengths of inlet and outlet tubing to connect the probe to the syringe pump and the point of collection.

> **Do not use scissors to cut tubing! Scissors may collapse the tubing. Use only a sharp razor blade!**

6. Connect the inlet tubing to the gas-tight syringe and observe flow. Once flow is established, reduce the pump flow rate to 5uL/min or below and connect the tubing to the inlet of the probe (yellow). Be sure to make zero-dead-volume joints between pieces of tubing and/or cannulae.



7. To aid in the dissolution of the glycerol in the membrane pores, fill a microfuge vial with distilled water or perfusion solution, insert it in the Calibration station, and lower the membrane end of the clamped probe into the vial. Soak the probe in this solution for at least 10 min.

8. Flush the probe with perfusion fluid to wash out residual glycerol and air. BASi recommends a flow rate of 2uL/min for 30 minutes.

9. Confirm that there are not any air bubbles in the probe. This can be done with a magnifying lens or microscope. A bubble will often be elongated and fill part of the side of the membrane lumen. Look for telltale air-water meniscus.

NOTE: You should continue to pump liquid through the probe while examining it. Otherwise, the pores in the membrane may dry and close. ALWAYS pump liquid through the probe any time the membrane is not immersed in solution.

If an air bubble is observed, increase the pump flow-rate to 10uL/min for 1-2 minutes. This increased flow rate will typically dislodge the air bubble and make the probe ready for use.

10. When the membrane lumen is free of air, and fluid flow from the probe outlet (green) is established, attach the outlet tubing with tubing connectors, and once again observe for fluid flow before proceeding. Before attaching the outlet tubing, make sure that the flow rate is at or below 5uL/min; this will reduce the risk of damage to the probe.

11. Set the pump to the desired flow rate for your study (usually 1uL/min) and check for leaks at all tubing connectors, syringe seals, etc. Tighten or repair leaks if necessary. The probe is now ready for use.

12. For an anesthetized animal study, the probe can be directly inserted in the animal while on the stereotaxic frame.

13. For awake animal studies, the probe will be inserted into the guide cannula (see below for guidance). It is important to remember the following:

    a. Probes are delicate. Exercise extreme care whenever handling a probe. Hold the animal firmly when inserting the probe into a guide cannula. In order to minimize probe damage, it is recommended to either anesthetize the animal for insertion or to enlist the assistance of a second technician; one to hold the animal and one to insert the probe.

b. During connection of the tubing inlet and outlet, take care to plumb the lines appropriately for your system. For example, when using a swivel, add a secondary step to connect the inlet and outlet lines to the inlet and outlet of the swivel. When using the Raturn Movement Responsive System, ensure that the inlet and outlet tubing is run through the center hole of the balance arm before attaching the tubing connectors.

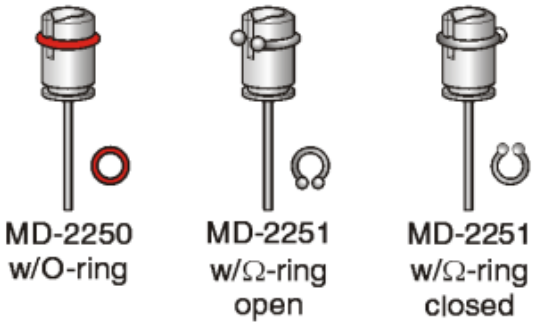
Intracerebral Guides for Brain "BR" Probes

Introduction

The role of the intracerebral guide is to target an implant site and support a microdialysis probe during in vivo sampling experiments in the brain. A guide is commonly used for studies in awake, freely moving animals. Although the microdialysis probe could also be cemented directly to the skull, the use of a guide allows more time for the animal to recover from surgical trauma.

Specifications

- Head diameter 6.4mm
- Shaft- Diameter 725 µm, exposed length 10mm
- Two options for "locking" the guide and probe. MD-2250 uses and O-ring, and MD-2251 uses an Omega Ring lock.



- MD-2250 guide is MRI compatible when used without the "dummy" stylet.
- Custom BR Guides available upon request
- All materials are biocompatible.

Benefits

The use of an intracerebral guide offers several benefits:

- The guide is positioned just above the tissue that will be sampled by the probe and thus "targets" the site.
- The guide itself does not penetrate the tissue that is eventually sampled by the probe membrane.
- There is less acute damage to the brain when the probe is inserted. The tissue recovers faster and the microdialysis experiment can begin sooner.
- Once the probe is in place, the guide/probe pair will be completely non-metallic (MD-2250 guides only). This permits magnetic imaging (NMR) during microdialysis.
- The probe is better protected from the animal.

Usage

When a BR Microdialysis Probe is placed inside a guide, only the dialysis membrane extends beyond the end of the guide cannula. When the intracerebral guide is implanted, it is placed just above the tissue that will eventually be sampled by microdialysis. After the animal recovers from the surgery (typically 3-5 days, but this is determined by your institution's animal care and use committee), the probe can be implanted into the fresh target tissue. This approach reduces the damage to the brain target, which will only be punctured with the small diameter probe membrane.

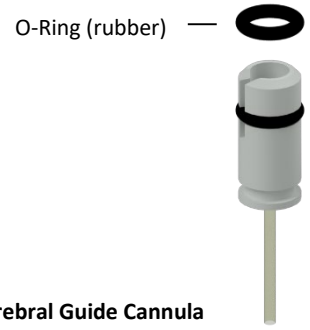
In many cases, a recovery of targeted analytes will achieve steady state within a few hours.

In studies which involve comparisons of blood-brain levels, longer healing times are required to allow the micro vasculature to heal reducing contamination from tiny, ruptured blood vessels.

Options

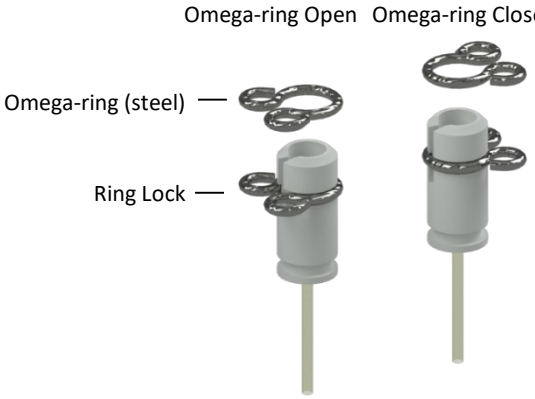
There are two locking mechanisms available for BR style guides:

- The locking mechanism in MD-2250 guides is an O-ring which secures a small notch in the probe head. The O-ring has sufficient tension to hold the probe in place, but also releases it when the probe head is pulled sufficiently.



O-Ring Intracerebral Guide Cannula (MD-2250)

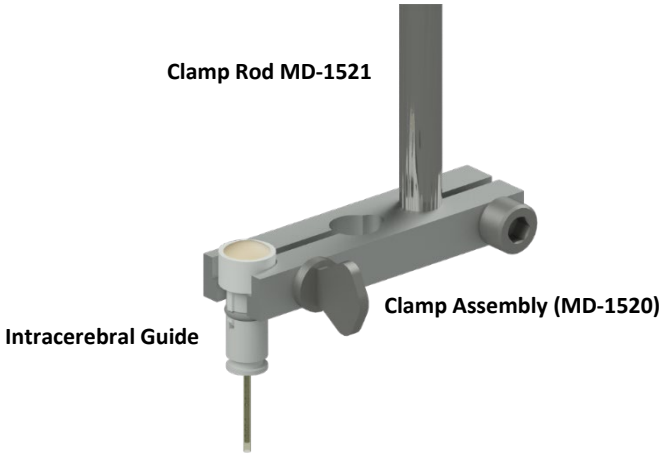
- An alternate guide with a steel lock (MD-2251) is available for users with active animals. The lock is shaped like an Omega (Ω) and rotates to lock or unlock the same notch in the probe head.



Omega-Ring Intracerebral Guide Cannula (MD-2251)

Procedure 1: Implantation of the Intracerebral Guide Cannula

Implantation of intracerebral guides requires two special accessories: the MD-1520 Clamp Assembly and MD-1521 Clamp Rod. The clamp rod has a diameter of 7.9mm (5/16") and will fit on most stereotaxic devices.



1. Attach the clamp assembly to the clamp rod. Tighten the screws to secure the clamp.

2. Mount the clamp and rod on the stereotaxic frame.

3. When placing the guide in the clamp, the guide's keyway should point toward the clamp's open U end, as shown in the previous illustration. Importantly, this insures that the probe sits squarely in the clamp, with the underside of the head resting flatly on the shelf of the clamp.

4. Tighten the front screw to secure the guide in place. This must be very secure and may need to be tightened with a tool to make sure that the guide is completely stable.

5. Anesthetize and prepare the animal for surgery and position the animal on the stereotaxic frame.

6. Expose the skull. Control excessive bleeding. Drill small holes for bone screws and insert at least two screws (MF-5182) on adjacent bone plates.

7. Position the guide above the exposed skull, using the coordinate system defined by your stereotaxic atlas. Lower it unit it is just above the bone.

8. Mark the planned entry point into the skull and then raise the guide to make room for the drill.

9. Use a sharp trephine drill bit to cut a clean circle of bone from the skull. Lift the bone circle carefully to expose the dura.

10. Carefully pierce the dura by pricking it with a sharp syringe needle, being careful not to jab it into the brain tissue below.

11. Lower the guide to the depth determined by the stereotaxic coordinates of the targeted tissue.

12. Dispense cement around the bone anchor screws and into the groove at the base of the guide cannula. While the cement begins to thicken inside the dispenser, keep dispensing it to form a mound around the base of the guide and screws.

13. Allow cement to harden according the manufacturer's specifications. Do not move to the next step until the cement is fully cured.

14. Once completely cured, release the front screw so that the guide is released from the probe clamp. Use your stereotaxic manipulator to move the clamp off of the guide.

15. If needed, add suture to fully close the incision.

16. During surgical recovery following guide implantation, the animal should be individually housed in containment without wire grids or descending protrusions upon which the animal might strike or catch the guide.

    To insert a microdialysis probe, first remove the stylet. For MD-2250 guides, pull gently on the stylet head in an upward motion. For MD-2251 guides, first rotate the Omega ring until the stylet is unlocked (if oriented as described above, to unlock turn the open end of the ring toward the animal's snout).

    Carefully insert the prepared BR probe by gently aiming the probe into the guide's lumen until it snaps in place (MD-2250) or bottoms out.

    For MD-2251 guides, after the probe has bottomed out, rotate the Omega ring to lock.

Procedure 2: Placing of the Microdialysis Probe in the Guide Cannula

1. When ready to conduct the microdialysis experiment, prepare the rat with a collar (MF-5371), a harness, or a jacket so that the animal can be connected to the tether/caging system.

2. First, remove the "dummy" stylet from the guide.

    a. For the MD-2250 O-ring guide, gently tug on the stylet to release it from the guide.

    b. For the MD-2251 Omega ring guide, twist the Omega lock to align the open end with the notch in the guide. Once aligned, the stylet will release from the guide.

3. Take a probe that has been flushed and connected to inlet and outlet tubing. Gently but firmly hold the head of the animal so that it cannot move. Slide the probe in place without forcing it. The membrane at the tip of the probe is extremely delicate. Once the probe has lined up with the shaft of the guide, gentle pressure can be applied to align the locking mechanism.

4. Connect the animal to the system with a tether.

5. Secure the tubing lines to the tether (if necessary), making sure that there is enough slack to allow the animal to move freely, but not so much that it can be pulled or chewed.

6. After insertion, it is a good idea to run blank samples, or gravimetric analysis. This will ensure that your system is working prior to dosing and sampling.

7. After the probe has been inserted, be sure to allow sufficient time for the tissue to equilibrate. This acclimation period can vary depending on the analyte of interest, but is typically just a few hours.

Other Applications

The probe and guide may also be used for other techniques. For example, a probe without a membrane can be used for injections. Ask for BASi part number MD-2262 or MD 2264.

Warranty

Brain “BR” Probes and Intracerebral Guides are NOT for use in humans. These products are designed solely for preclinical research and are viable for single use. BASi warrants its products against manufacturer defects. BASi is liable only to the extent of replacement of defective items for claims registered within 90 days of the shipping date.

BASi will not be liable for any personal injury, property damage, or consequential damages of any kind whatsoever arising from the use of the guide. This warranty does not cover damage to membranes or cannulas through improper preparation, inappropriate connections or faulty handling by the user. The foregoing warranty is in lieu of all other warranties expressed or implied but not limited to the implied warranties of merchantability and fitness for a particular purpose.

Ordering Information

Brain "BR" Microdialysis Probes

MD-2200	Brain Microdialysis Probe, 2mm Membrane, 6/pkg
MD-2204	Brain Microdialysis Probe, 4mm Membrane, 6/pkg

Brain "BR" Intracerebral Guide Cannulae

MD-2250	Standard Intracerebral Guide Cannula and Stylet with O-Ring, 6/pkg
MD-2251	Locking Intracerebral Guide Cannula and Stylet with Omega Ring, 6/pkg

Accessories

MD-1520	Clamp for Brain BR Probes
MD-1521	Clamp Rod
MD-1522	Calibration Station
MD-1300	Dental Acrylic
MD-2400	Sterile Artificial Cerebrospinal Fluid (aCSF)
MF-5182	Screw Anchors, 100/pkg
MF-5362	Drill Bits for Screw Anchors, 5/pkg
MF-5176	Trephine Bone Drill Bits, 3/pkg
MF-5371	Rat Collars, 100/pkg
MD-1404	Stand-Alone Raturn™ Movement Responsive Caging System for Rat