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The Science of Mind Reading

Microdialysis technology opens a window to the brain

By Sarah Goforth

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Microdialysis enables researchers to probe the mind's molecular architecture to determine how complex behaviors and memories are produced and how bodily functions are regulated.¹ As a method to monitor chemicals in the body's extracellular fluids, microdialysis has been most widely used in the neurosciences, and thanks to advances in technology, is getting easier. Applications include studies of behavior and metabolism, the molecular basis of learning and memory, chemical patterns of addiction, and pharmacokinetics.

Experimental studies of brain chemistry were initially limited to postmortem tissue until *in vivo* neurochemical detection methods were developed in the middle of the 20th century that helped relate neurochemistry directly to behavior. Methods such as the cortical cup and push-pull perfusion allowed scientists to sample chemicals directly in the brain; however, these techniques were limited by a lack of location sensitivity, and damage caused to the brain tissue investigated, respectively.

Conceptually, microdialysis mimics a capillary blood vessel; users perfuse a thin dialysis tube that is stereotactically inserted into the tissue. Microdialysis occurs by diffusion through a membrane that is integrated into a probe and perfused by an isotonic solution at a constant rate. Diffusion across the membrane is bidirectional, so the same probe can be used to either deliver or to sample chemicals in the target tissue. The narrow cylindrical design of microdialysis probes permits deep placement into the brain (unlike a cortical cup), and the confinement of dialysate fluid by the membrane leads to a well-defined introduction to the tissue (unlike push-pull cannulae).

Scientists originally used brain microdialysis (also called intracerebral dialysis, intracranial dialysis, or transtriatal dialysis) to measure the release of rat striatal dopamine.² Because microdialysis probes cause little damage to the blood-brain barrier, the technique is particularly useful in the study of drug penetration and pharmacokinetics. The recovered dialysate can be analyzed by high-performance liquid chromatography (HPLC), mass spectrometry, radioimmunoassays, or capillary electrophoresis, to examine the endogenous and xenobiotic contents of the

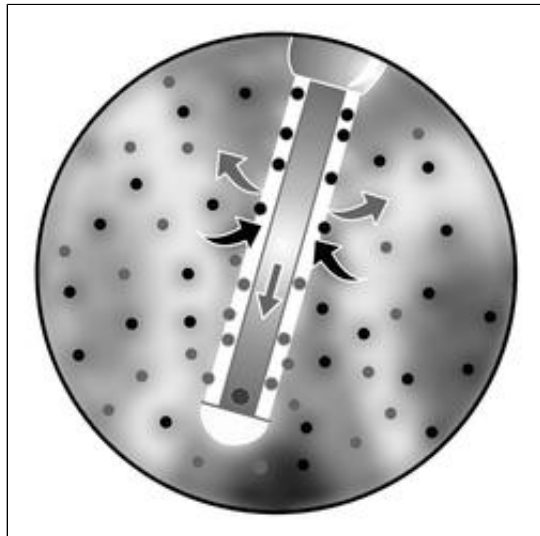


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extracellular space. Specifically, HPLC provides the high selectivity and sensitivity necessary to detect very low levels of endogenous monoamine in microdialysis perfusates.^{3,4}

Basic microdialysis systems include a syringe pump, syringe, tubing and connectors, a microdialysis probe, and a collection vial or fraction collector. The systems can be purchased for about \$1,000. Since the inception of microdialysis, a number of developments in the capabilities of these components has improved the ease, sensitivity, and range of microdialysis applications.

Courtesy of CMA Microdialysis



Picking the Brain

The central component of any microdialysis experiment is the probe itself. Designs vary depending on purpose, and many scientists design and create their own probes. Investigators most often use probes that possess a good recovery to match the sensitivity of the chosen analytical assay, that have a small diameter to ensure limited damage, and are designed for optimal implantation into the chosen tissue. When first introduced, the microdialysis probe consisted of a hollow tube of dialysis membrane inserted transversely through the brain. Many scientists now use a loop probe (a looped dialysis membrane), which reduces the extent of surgically induced injury because it is implanted vertically into the brain via a single hole in the skull. Another option is the concentric probe, which consists of a single piece of dialysis tubing blocked off at one end; these probes are more difficult to make but have a smaller overall diameter and are therefore widely used.

microdialysis samples chemicals via diffusion.

Many microdialysis studies have characterized the effects of drugs on the rat brain. With the recent boom in mouse genetics, however, there is a growing need for in vivo techniques to study drug-related effects on smaller animals. Thanks to the introduction of smaller probes to the market, it is now possible to monitor extracellular levels of multiple neurotransmitters with simultaneous measurement of behavior in the mouse.⁵

The properties of probe membranes allow users to tailor the properties of the resulting dialysate. The molecular weight cutoff defines the size of molecules that can pass through the membrane—anything smaller than the cutoff value will freely pass through the membrane pores. CMA Microdialysis of Solna, Sweden, offers a range of ready-made probes. The most basic of these, the CMA/10 series, are polycarbonate membranes 1-4 mm long, with a 20 kDa cutoff. The CMA/12 is a reusable CNS-optimized series (also with a 20 kDa cutoff) intended for chronic implantation. CMA probes with both large (100 kDa) and small (6 kDa) molecular weight cutoffs are also available for a variety of applications. In addition to its standard-design probes, CMA provides custom probes, allowing the researcher to specify the probe type, shaft material and length, membrane material and length, and cutoff size.

Bioanalytical Systems (BAS) Inc. of West Lafayette, Ind., provides microdialysis users with a comprehensive set of probe options. BAS's BR probes use a polyacrylonitrile membrane with a molecular weight cutoff of 30 kDa that minimizes tissue damage with a 340 µm outer diameter (OD). These probes are intended for use in rodents, but BAS can custom-design BR probes for larger animals with longer cannulae and membranes. BAS's IBR model microdialysis probe is suitable for injecting high molecular weight compounds such as dyes, proteins, DNA, RNA, and carbohydrates, and allows the delivery of fluid directly to the tissue being dialyzed by way of a third cannula that exits from the tip of the probe.

SciPro Inc., of North Tonawanda, N.Y., also offers a variety of probes tailored for use in microdialysis experiments. The standard probe is the Mab 6, with a 14 or 20 mm stainless steel shaft, PES membrane with lengths of 1-4 mm, a 0.6 mm OD, and 15 kDa cutoff. SciPro's Mab 2 series is identical to the Mab 6 series, except with a 35 kDa cutoff. Both probes are autoclavable and offered with both reusable and disposable guide cannulae. For studies with very small animals, the 0.2 mm OD MabCu (6 kDa cutoff) and Mab 5 (100 kDa cutoff) probes are available. Reusable and disposable guide cannulae are available for these probes as well. SciPro also custom-designs any of these types of probes with user-specified shaft and membrane lengths.

To ensure proper placement of the microdialysis probe, a stereotaxic instrument is necessary; such an instrument also makes it possible to examine the probes for air bubbles and leaks.⁶ The Lab Standard^(tm) Stereotaxic 51600, from Stoelting Co. of Wood Dale, Ill., offers a number of advantages over other U-Frame stereotaxic instruments. The Lab Standard has custom, triple-start lead screws that allow for fast positioning, and a universal joint that allows the investigator to angle the probe in either the lateral-medial or anterior-posterior planes. The 51603 Dual Manipulator Lab Standard has three-dimensional manipulator arms on both sides, and the second arm can be reversed as to handedness. Both models come with standard probe holders and can be adapted for various rodents. Also offered by Stoelting is the Stellar^(tm) Rat Stereotaxic Instrument. The Stellar's unique design allows investigators to vary the angle of approach without calculations. This is accomplished by allowing the implant carrier to be rotated left-right or anterior-posterior around the circumference of a sphere and by moving the animal, rather than the implant carrier, until the desired target area in the brain is at the center of the sphere.



Instech's 375/D/22QM Microdialysis Swivel

Go With the Flow

The flow rate used for microdialysis varies widely, from roughly 0.2 to 10 $\mu\text{l}/\text{min}$. Lower flow rates result in relatively improved recovery because the sample has a longer time to reach equilibrium with the tissue.⁷ Infusion pumps are used to accurately deliver very slow, constant flow rates to ensure that changes in the dialysate content reflect changes in release by the brain and not variable diffusion gradients resulting from occasional changes in flow rates through the probe itself. There are a variety of constant-flow syringe pumps on the market.

Stoelting offers an assortment of microprocessor- controlled syringe pumps that can be fitted with a variety of syringe models and sizes. The pumps are preprogrammed with a library of compatible syringes, so users need not measure their syringes by hand. Additionally, a volume dispense mode stops the pump after a preset volume is infused or withdrawn, preventing risk of over- injection. When the volume dispense mode is used, the pump's LCD displays the amount infused. Stoelting's Series 100 pumps are an economical option for users working with very small volumes. The 53101 Microdialysis Pump holds two syringes, in microliter sizes only, and operates at very low speeds with a smooth flow. Stoelting's model 53310 stereotaxic injector can inject nanoliter volumes at precise delivery rates; this microprocessor-controlled syringe pump mounts directly on the manipulator arm of the stereotaxic frame for stable and remote control of microdialysis.

For investigators looking for an alternative to traditional syringe pump design, BAS's Baby Bee^(tm) Syringe Drives may be the answer. The pumps on these drives are small and versatile; a variety of controllers can be used for the same syringe drive. Each Baby Bee holds one syringe, or up to three syringes when used with a bracket accessory. BAS also offers a line of controllers that work in combination with the Baby Bee Syringe Drives as a fully functional syringe pump. The Worker Bee^(tm) Controller offers a flexible control rate, ranging from 0.1 $\mu\text{l}/\text{min}$ to 100 $\mu\text{l}/\text{min}$ when used with a 1 ml gastight syringe. The Bee Hive^(tm) provides control for four Baby Bees at variable and independent flow rates.



World Precision Instrument's SP101i

Curbing Complexity

As microdialysis technology becomes more widely used for a larger variety of purposes, and as more companies enter the market with microdialysis products, experiments are

involving more and more infusion pumps, syringes, lines, and variable flow rates. These

Syringe Pump

options provide scientists with unprecedented flexibility, tempered by increasing complexity. CMA's solution is its Computer Aided Design (CAD) software, which makes it possible to plan, execute, and report microdialysis experiments at the user's computer. The CAD program controls the CMA/102 Microdialysis Pump and the CMA/142 Microfraction Collector; users can program concentration gradients, pulses, injections, and fraction times. A remote control option allows investigators to monitor and control experimental variables without disturbing the animal being tested.

According to **Robert Pelletier**, U.S. general manager for CMA, "CAD enables users to generate dose-response profiles for an unlimited number of drug concentrations, as opposed to a liquid switch that generally allows for two or three levels. Our pump and program allow the user to plan their experiments the way they wish to rather than the way existing instruments allow them to."

An important advantage of microdialysis is that it can be used to examine neurochemistry in awake, freely moving animals. In these types of studies, specially designed swivels prevent twisting and crimping of the fluid lines to and from the microdialysis probe, allowing rotational behavior to occur. Experiments that study neurotransmitter changes during ongoing drug administration may require the use of a dual-channel swivel such as the one included with the CMA/120 system for freely moving animals. This system, designed for unattended experiments in small animals, includes a balance arm with dual-channel swivel and secures tubing away from the animal, preventing twisting of the lines.

Instech Laboratories Inc. of Plymouth Meeting, Pa., recently introduced a new version of its dual channel microdialysis swivel. The new swivel has quartz lining on both channels for low dead volume and protection of neurotransmitters, and very low rotational friction, allowing it to be used with both mice and rats. In 1999, Instech introduced its Swivelless Swivel^(tm), a system designed for complex experiments involving multiple electrical or fluid lines. In this system, all lines are continuous from the stationary base to the moving animal; as the animal moves, the system senses this movement and reacts so that the tether remains untangled without rotating the animal's cage. The Swivelless Swivel allows for up to thirty microdialysis lines to be run continuously in one animal.

Another product intended to aid researchers grappling with multiple-line systems is the patented BAS Ratur^(tm) system. Ratur replaces the conventional liquid swivel with an optical switch, allowing multiple tubing lines to be threaded through a hollow shaft in the switch mechanism to syringe pumps or fraction collectors without line breaks. The interactive caging system signals the animal's containment bowl to rotate in the opposite direction as the animal, allowing the animal to move freely while preventing lines from twisting and crimping. According to **Candice Kissinger**, product manager for BAS, the Ratur system was designed to prevent accidental mixing of the inlet and outlet lines that can affect flow rates and constituents.

Neuroscientists have made great strides in the study of neurochemicals in recent years with the help of microdialysis technology. Its applications throughout the life sciences make it a tool for celebrating the molecular machinery of the synapse, the brain, and the body itself.

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