Time-Resolved Microdialysis for In Vivo Neurochemical Measurements and Other Applications

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Key Words

capillary electrophoresis, liquid chromatography, enzyme assays, amino acids, dopamine, neuropeptides

Abstract

Monitoring changes in chemical concentrations over time in complex environments is typically performed using sensors and spectroscopic techniques. Another approach is to couple sampling methods, such as microdialysis, with chromatographic, electrophoretic, or enzymatic assays. Recent advances of such coupling have enabled improvements in temporal resolution, multianalyte capability, and automation. In a sampling and analysis method, the temporal resolution is set by the mass sensitivity of the analytical method, analysis time, and zone dispersion during sampling. Coupling methods with high speed and mass sensitivity to microdialysis sampling help to reduce some of these contributions to yield methods with temporal resolution of seconds. These advances have been primarily used in monitoring neurotransmitters in vivo. This review covers the problems associated with chemical monitoring in the brain, recent advances in using microdialysis for time-resolved in vivo measurements, sample applications, and other potential applications of the technology such as determining reaction kinetics and process monitoring.

1. INTRODUCTION

Temporal resolution: the frequency by which data are collected and analyzed

Extracellular space: the fluid compartment that surrounds and bathes neurons and glial cells in the brain

Understanding normal and abnormal functioning of the brain unquestionably represents one of the greatest challenges to scientists. Behavioral control, cognition, and emotions are ultimately encoded by neurotransmission, or chemical communication between neurons, which involves the movement of ions across membranes and chemicals across the synapse between two neurons (**Figure 1**). During neurotransmission, chemicals (neurotransmitters) released from a neuron diffuse across the synaptic gap to interact with receptors found on neighboring neurons. These interactions can either promote or inhibit action potential generation in the receiving neuron, thus affecting its chemical release (for a review of neurotransmission, see Reference 1). In addition to the acute effects on action potential generation, released chemicals can alter other cellular functions such as gene expression, growth, connectivity, metabolism, and responsiveness to further stimuli (2, 3). Neurotransmitter release from other parts of neurons (such as the dendrites and cell soma) and glial cells is also important in signaling (1).

We have gained many insights into neurotransmission from ex vivo experiments on brain tissue slices and cultured cells, but ultimate understanding of the complex interconnections of the brain requires the study of living, intact subjects. Performing in vivo measurements makes it possible to discern the regulation of neurotransmission in the presence of complete neuronal circuits (e.g., inputs from different brain regions) and cellular milieu created by the activity of all cells in a brain region. Furthermore, in vivo measurements allow the correlation of neurochemistry to behavior and emotional states.

Among the factors to consider when developing and evaluating in vivo methods are chemical heterogeneity, spatial resolution, and temporal resolution. The brain extracellular space comprises a complex chemical soup that includes neurotransmitters, growth factors, and metabolites. Neurotransmitters run the gamut from small gaseous molecules (e.g., nitrous oxide), to small organic molecules (e.g., glutamate), to oligopeptides (e.g., neurotensin) with concentrations from 1 pM to 1 mM. This chemical variety places great demands on the selectivity, dynamic range, sensitivity, and versatility of the analytical methods used for in vivo chemical measurement. The structural heterogeneity of the brain places significant demands on the spatial resolution of a chemical measurement. Distinct brain regions <0.5 mm^3 can be critical for controlling a particular behavior, for example. To accurately study neurotransmission, the established method must ensure measurements are taken solely from the region of interest. Finally, temporal resolution is critically important for in vivo brain chemical measurements. Individual neurons release neurotransmitters by exocytosis, and the neurotransmitters are rapidly eliminated from the synapse by reuptake or enzymatic degradations. Fluctuations in neurotransmitter levels can occur on the millisecond time scale by this process, although sustained changes in neuronal firing or reuptake (such as what may occur during a behavioral activation) can result in longer-lived changes in neurotransmitter levels. Cellular responses to neurotransmission and the effects of pharmacological agents also occur over a longer time scale (see **Figure 2**). Thus, the temporal resolution that can be achieved in measuring neurochemicals

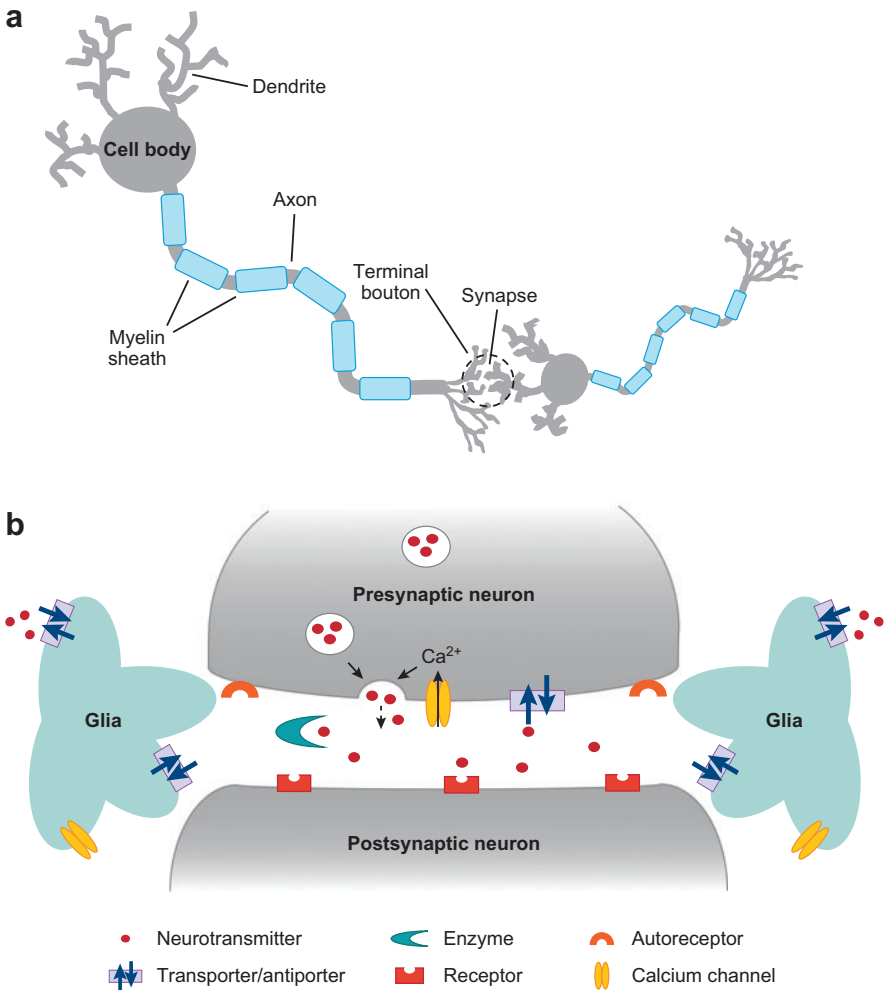

Figure 1

Neuroanatomy and neurotransmission. (*a*) Depiction of basic neuronal structure and synaptic features. Neurons consist of dendrites, cell bodies, axons, and terminal boutons. Dendrites collect chemical input from neighboring neurons, which influences the membrane potential of the neuron. Once the threshold membrane potential is reached, an action potential is generated and travels from the cell body down the axon toward the terminal boutons. Myelin ensheathment of neurons increases the speed of action potential propagation. Once the action potential reaches the presynaptic terminal, it triggers the opening of voltage-sensitive Ca^{2+} channels. The influx of calcium causes synaptic vesicles to migrate toward the synaptic cleft and release their cargo into the synapse. (*b*) Depiction of neurotransmission at synapses. Neurotransmitters diffuse across the synapse to interact with and bind to receptors on the postsynaptic neuron. These receptors can be on dendrites, cell bodies, or on another axon. If a neurotransmitter binds to an autoreceptor on the presynaptic neuron, a negative feedback loop is initiated to inhibit further release. Signaling is terminated via enzyme degradation or reuptake into the presynaptic terminal by transporters. Other supportive neuronal cells such as glia can also release and take up neurotransmitters (e.g., glutamate).

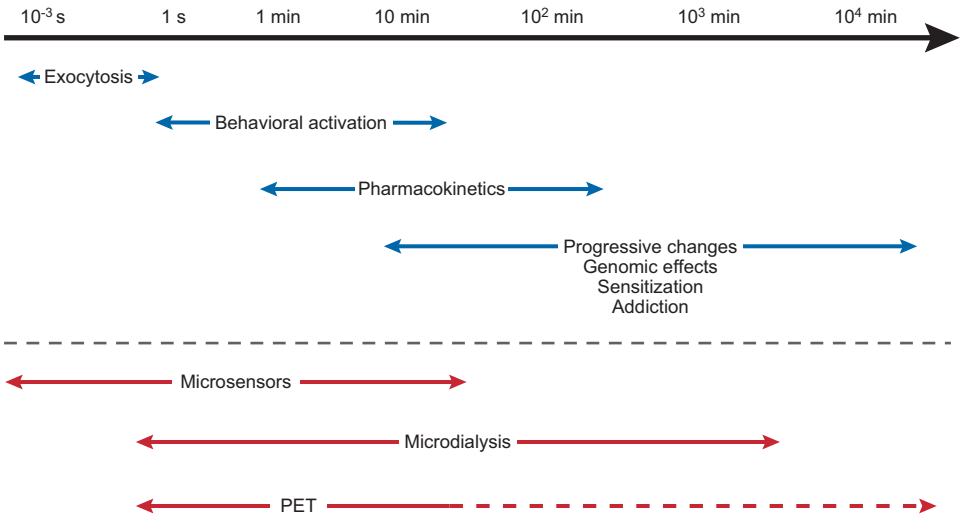

Figure 2

Temporal resolution of neurochemical events and analytical methods. The time scale of neurochemical events or their effects ranges from less than 1 s to days. The temporal resolutions of relevant in vivo chemical measurement methods are listed below the dashed line. Because it is noninvasive, positron emission tomography (PET) allows multiple measurements over many days in the same subject so that longer-term changes can be detected.

limits the types of questions that a particular method can address. In this review, we cover recent technological advances in the use of microdialysis sampling coupled with chemical measurement techniques to measure rapid chemical fluctuations.

2. IN VIVO DETECTION OF NEUROTRANSMITTERS

Researchers have developed a variety of techniques to measure neurotransmission in vivo. Prominent among these methods are positron emission tomography (PET), microsensors, and microdialysis sampling (for reviews, see References 4, 5, 6). **Figure 2** illustrates the temporal resolution in these methods.

Besides temporal resolution, other factors are also important when comparing these methods. PET and microsensors offer the advantages of noninvasive imaging and high spatial resolution, respectively. PET is expensive and is presently limited to just a few neurotransmitter systems. In addition, spatial resolution is limited when working on small laboratory animals such as rats and mice. Microsensors have excellent spatial and temporal resolution. The weaknesses of microsensors include an unproven ability to determine basal concentrations, long-term changes, and multiple analytes.

PET: positron emission tomography

Microdialysis can provide complementary information to microsensors and PET. As a general sampling method, it can be used for most transmitters, offering unsurpassed versatility and multianalyte capability when coupled with sensitive and selective

analytical techniques such as high-performance liquid chromatography (HPLC). It provides basal concentrations as well as dynamic changes. The spatial resolution is worse than sensors because the probes are generally larger. The temporal resolution has typically been in the 10–30-min range; however, recent work has pushed this to the seconds range (see **Table 1**). These properties, along with its ease of use, have made microdialysis the workhorse for in vivo neurochemical measurements, with over 11,000 published studies using this method. In addition, the recent advances in temporal resolution have potentially opened the possibility for many new applications.

HPLC: high-performance liquid chromatography

Concentration detection limit: the minimum concentration that can be detected at known confidence

Mass detection limit: the minimum mass that can be detected at known confidence

2.1. Microdialysis Overview

Microdialysis is but one of several sampling methods used in the brain. Methods that preceded microdialysis include the cortical cup (7, 8) and push-pull perfusion (9). These other methods have become less popular owing to limited spatial resolution and tissue damage associated with the collection, respectively. Microdialysis emerged as a modification of push-pull perfusion probes (**Figure 3**). In this approach, a membrane is placed around the outside of the push-pull arms to serve as a barrier between the perfusion fluid (normally pumped at 0.3 to 2 $\mu L\ min^{-1}$) and the surrounding tissue, thereby decreasing the tissue damage induced by perfusion (10–12). As fluid is perfused through the interior lumen of the dialysis probe, it washes out the material that diffused into the probe, thus creating a concentration gradient from the outside of the probe to the inside. Therefore, microdialysis samples chemicals through diffusional gradients rather than physically removing fluid from the extracellular space. Molecules with molecular weight below the cutoff of the membrane are sampled.

When considering microdialysis for temporally resolved measurements, analyte recovery by the probe is an important issue. Recovery can be defined as absolute or relative. Absolute recovery refers to the mass of analyte collected over a period, whereas relative recovery refers to the concentration of analyte in the dialysate divided by the concentration in the sampled media. Perfusion flow rate strongly controls recovery such that increasing flow rates through the dialysis probe increase the absolute recovery while decreasing the relative recovery. Absolute recovery increases because higher flow rates create a steep concentration gradient between the probe and the extracellular fluid, thus enhancing the flux of molecules to the probe. Relative recovery decreases as the flow rate is increased because there is less time for equilibrium to be reached between the solution flowing through the probe and the extracellular space. Therefore, one must choose a flow rate that allows both adequate relative recovery to meet the instrument's concentration detection limit and adequate absolute recovery to meet the instrument's mass detection limit. Besides flow rate, other factors also affect recovery. Recovery increases with increasing molecular-weight cutoff of the membrane (up to a limit), temperature, and active membrane length. In general, higher recovery (especially absolute recovery) aids in achieving high temporal resolution, as discussed below.

Table 1 High–temporal resolution dialysis methods

Analyte[a]	Experiment type	Method[b]	Temporal resolution(s)	Off-line	On-line	Reference(s)
Glu	Method development	CE-LIF	1	x		64
Glu	Method development	CE-LIF	6	x		63
D- and L-Asp	Method development	CE-LIF	3		x	95
Glu	Ascorbate effects on electrically stimulated release	CE-LIF	3		x	96
Neuroactive amines and AAs	Method development	cLC-EC	10	x		32
Glu, Asp, and DA	Apomorphine, PDC, NMDA, and nomifensine effects	CE-LIF	10	x		97
Glu and Asp	Electrical stimulation	CE-LIF	12		x	69
Neuroactive amines and AAs	Alcohol and estrogen effects	CE-LIF	15		x	98–100
Neuroactive amines and AAs	Behavioral	CE-LIF	15		x	87, 101, 102
Neuroactive amines and AAs	Method development	CE-LIF	20		x	67, 74
NA, DA, Glu, Asp, and GABA	Method development	CE-LIF	20	x		62
Neuroactive amines and AAs	Method development	Microfluidic CE-LIF	30		x	73
Neuroactive amines and AAs	Method development	MEKC-LIF	30		x	70
Glu	Behavioral	CE-LIF	30	x		103
Glu and GABA	Behavioral	CE-LIF	30	x		59
Gabapentin	Pharmacokinetics	CE-LIF	30	x		104
Glu	Haloperidol effects on flash-evoked release	CE-LIF	30	x		105
Arg, Glu, and Asp	Pain response to formalin test	CE-LIF	30	x		106
NA, DA, Glu, and Asp	Method development	CE-LIF	30	x		60
Glu	Electrical stimulation	CE-LIF	30	x		107
Glucose and lactate	Neuronal metabolism	Enzyme assay–EC	30		x	79, 83, 108
GABA, Glu, and Asp	Pain response in human spinal cord	CE-LIF	60	x		109
Glu, GABA, Arg, and Asp	Pain response in rat spinal cord	CE-LIF	60	x		110
Glu	Behavioral	CE-LIF	60	x		58
Glu and Asp	NMDA effects	CE-LIF	60	x		111

(*Continued*)

Table 1 (*Continued*)

Analyte[a]	Experiment type	Method[b]	Temporal resolution(s)	Off-line	On-line	Reference(s)
Isoproterenel	Pharmacokinetics	CE-EC	60	x		112
Lactate	Glutamate uptake effects	Enzyme assay–EC	60		x	113
Glucose	Method development	Enzyme assay–EC	60		x	114
Glutamate	Hypoxia response	Enzyme assay–FLU	60		x	115
Glucose	Behavioral	Enzyme assay–FLU	60		x	85
Lactate	Behavioral	Enzyme assay–FLU	60		x	116
Ethanol	Pharmacokinetics	GC	60	x		117
5-HT and 5-HIAA	Method development	cLC-EC	60–120	x		118
Acetaminophen and caffeine	Pharmacokinetics	HPLC-UV	60		x	119
Glu and Asp	Method development	HPLC-FLU	60	x		120
Glu and GABA	Hypoxia response	HPLC-EC	60	x		21
5-HT	Hypoxia response	HPLC-EC	90	x		21
DA	Behavior	HPLC-EC	60	x		30
DA	Cocaine effects	HPLC-EC	60	x		31
Adenosine	Hypoxia response	HPLC-UV	90	x		21
DA	Method development	MEKC-LIF	90		x	71
SR 4233 and SR 4317	Pharmacokinetics	MEKC-LIF	90		x	121
DA, NA, Glu, and Asp	Behavioral	CE-LIF	120	x		57
Glu and Asp	Circadian rhythm effects	CE-LIF	120	x		122
PEA, Glu, and Asp	Method development	CE-LIF	120	x		72
NA and Glu	Method development	CE-LIF	120	x		61
Glucose	Method development	Enzyme assay–EC	120		x	123
DA	Behavioral	HPLC-EC	120	x		124, 125
DA and 5-HT	Behavioral	HPLC-EC	120	x		22

[a]Amino acids are represented by their three-letter code. 5-HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid; AA, amino acid; DA, dopamine; NA, noradrenaline; PEA, phosphoethanolamine.

[b]CE: capillary electrophoresis; cLC, capillary liquid chromatography; EC, electrochemical detection; FLU, fluorescence detection; GC, gas chromatography;
HPLC, high-performance liquid chromatography; LIF, laser-induced fluorescence; MEKC, micellar electrokinetic chromatography;
UV, ultraviolet-visible absorbance detection.

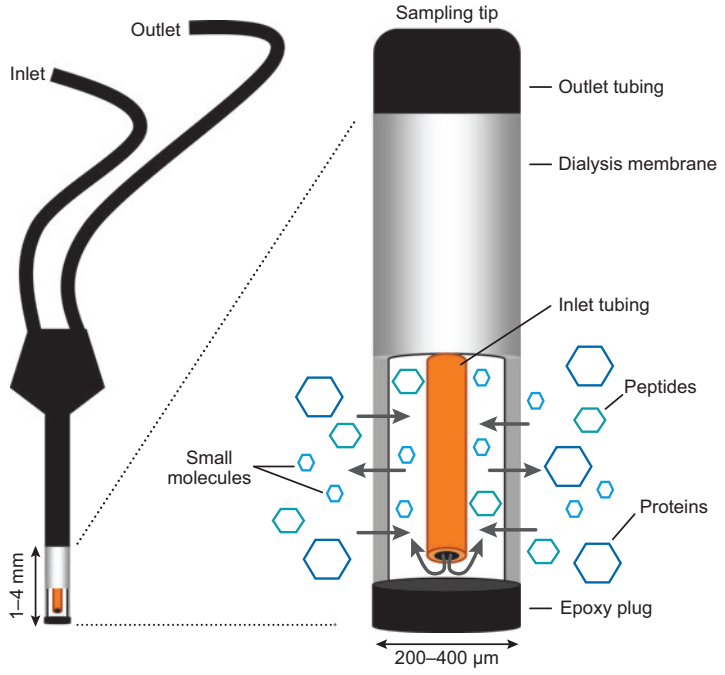

Figure 3

A depiction of a concentric microdialysis probe along with a magnified image of the sampling tip. To perform microdialysis sampling, one pushes a perfusion buffer into the inlet that mimics the ionic content of the extracellular space. When this buffer flows past the active area of the membrane, this creates a concentration gradient between the tissue and the lumen of the probe. Analytes that have a mass below the molecular-weight cutoff of the membrane diffuse into the probe and can be collected for further analysis. Dialysate exits the probe through the outlet to be collected off-line into a vial or to be sent directly toward the instrument's detector. Local pharmacological manipulations can also be performed simultaneously by adding the agent to the perfusion buffer.

2.2. Temporal Resolution of Microdialysis

The ability of microdialysis measurements to monitor fast changes (second-to-minute time scale) is greatly affected by the mass detection limits of the analytical method used for assaying dialysate. To increase temporal resolution, one collects smaller fractions, and the moles of analyte per sample decrease (**Figure 4**). For example, if a sampled compound has a concentration of 1 μM, and a flow rate of 1 μL min^{-1} is used, then 10-min temporal resolution requires the detection of 10.0 pmol of analyte in 10-μL samples, whereas 10-s temporal resolution requires the detection of 0.2 pmol of analyte in 167-nL samples. Thus, high temporal resolution requires high mass sensitivity and the analysis of small volumes. Increasing absolute recovery, especially by increasing flow rate, may help improve temporal resolution; however, this comes at the cost of more dilute samples (due to lower relative recovery).

Mass sensitivity: the smallest increment of mass that can be measured

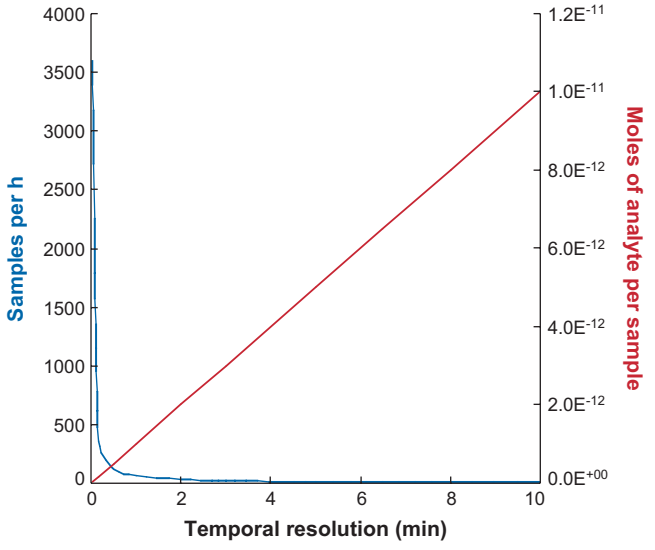

Figure 4

Effects of temporal resolution on the mass of analyte/sample and the number of samples generated. A plot of the number of samples generated and moles per sample as a function of temporal resolution for a microdialysis measurement. This plot assumes 1.0 μM of analyte in the dialysate stream, and the calculations were done using a dialysis flow rate of 1.0 μL min^{-1}. As temporal resolution increases, moles available to detect decrease and the number of samples dramatically increases.

In addition to mass detection limits, one needs to consider the method's throughput because raising the temporal resolution also rapidly increases the number of samples that need to be analyzed (**Figure 4**). For methods with 5-min temporal resolution, 12 samples are collected per hour. An increase in temporal resolution to 10 s makes the sample load 360 per hour! These two problems, diminishing analyte mass and increasing sample load, create substantial demands on the analytical system when attempting to improve the temporal resolution of microdialysis methods.

As discussed below, the use of high-throughput methods with excellent mass sensitivity has pushed the temporal resolution of microdialysis methods into the realm of seconds. With the advent of these methods, other factors can begin to limit the temporal resolution of the method. For example, adsorption to the membrane or other fluidic components can cause slow dynamics. A fundamental limit is the dispersion of a concentration plug owing to diffusion and parabolic flow (i.e., Taylor dispersion) as it is transported from the sampling probe to the analytical system or fraction collector. Previous studies have indicated that this type of broadening would limit temporal resolution to approximately 16 s if the flow rate is 1 μL min^{-1} and 85 s if the flow rate is 0.2 μL min^{-1} (13). (These limits of temporal resolution were achieved with short distances between the probe and instrument. For freely moving animals, such short distances are often not feasible, so for practical purposes, the limits of temporal resolution may be higher.) Because lower flow rates give worse temporal resolution,

a trade-off in relative recovery and temporal resolution is necessary. Although we can envision a variety of modifications to reduce this limit of temporal resolution, most work to date has focused on improving mass detection limits because this is usually the limiting factor.

On-line: a continuous method in which microdialysis sampling is coupled directly to the analytical system

Off-line: a method in which microdialysis sampling is a separate step from analysis

cLC: capillary liquid chromatography

Assays of dialysate samples may be performed either on-line or off-line. Off-line methods have the advantage of higher overall throughput because multiple sampling experiments can be performed simultaneously. This is realized because sampling is a separate step from detection. Off-line analysis also allows flexibility in assays. For example, assays that are slow, perhaps because of a long derivatization step or slow separation, may be used with off-line fraction collection. In addition, the problem of dynamic range can be reduced by assaying the same sample multiple times under different gain settings. The main difficulty of off-line analysis is sample handling because hundreds of submicroliter samples can be easily generated in a day.

On-line methods offer the advantage of minimal manual sample handling because the dialysate is continuously pumped toward the detector. In addition, with an on-line method, it is possible to monitor the output during the experiment to ensure proper sample collection. Such on-line monitoring can be useful to catch difficulties as they arise during an experiment. On-line monitoring may also be useful for providing rapid feedback in certain situations such as chemical monitoring during surgery. On-line analysis, however, places stringent requirements on the speed of the analytical method. Thus, assay time can limit the temporal resolution rather than the mass detection limit or Taylor dispersion.

3. LIQUID CHROMATOGRAPHY METHODS FOR ANALYZING DIALYSATE

Liquid chromatography methods are the most popular methods to analyze dialysate. These methods are used not only for measuring the release of neurotransmitters, but also for studying pharmacodynamics and metabolic changes. Microdialysis works well for coupling to HPLC because the dialysate is protein free, which limits biofouling and makes further purification unnecessary prior to analysis. In addition, for pharmacokinetic studies, microdialysis only samples the unbound drug, which is a measurement of the therapeutically active component of the drug. Although conventional-sized HPLC columns remain the most popular approach for analysis, advances in column miniaturization such as microbore HPLC and capillary liquid chromatography (cLC) have allowed temporal resolution to be improved from 5–30 min to 10–180 s by virtue of the improved mass sensitivity associated with these small columns.

3.1. High-Performance Liquid Chromatography Methods

The mass limit of detection (LOD) of HPLC dictates that samples are usually collected in 5–30-min fractions, thus limiting temporal resolution (14–20). The mass sensitivity strongly depends on the detector used, with fluorescence and

electrochemical detectors offering substantial improvements over ultraviolet absorbance when applicable. In some cases, high-sensitivity detection can allow temporal resolutions of 1–2 min. Glutamate, GABA, serotonin, adenosine (21), and dopamine (22) have been monitored at this temporal resolution, for example.

One advance that increased mass sensitivity was the development of microbore HPLC columns. Whereas traditional HPLC columns have inner diameters of 4–5 mm, microbore columns have inner diameters of 0.3–1.0 mm. With smaller column diameters, a given mass of material is diluted less before reaching the detector, resulting in improved detection limits. Studies have shown improved temporal resolution when switching from an HPLC column to a microbore column (23, 24). Most microbore LC methods offer temporal resolutions of 3–5 min (25–29); however, some microbore LC assays have been developed for 1–2-min monitoring of dopamine (30, 31).

GABA: γ-aminobutyric acid

MS: mass spectrometry

3.2. Capillary Liquid Chromatography

Within the past few years, cLC has emerged as a potential method for assaying dialysate samples. cLC columns have inner diameters of 25–150 μm and usually are prepared in the laboratory using them, although commercial systems are starting to become available. The greatly reduced column bore magnifies the effect on mass sensitivity such that attomole detection limits are possible with cLC columns. The use of cLC also improves compatibility with mass spectrometry (MS) and reduces mobile phase consumption.

Investigators have applied cLC with electrochemical detection to determine neuroactive amines and amino acids in dialysate. This method had LODs of 20–80 amol and as a result could achieve 10-s temporal resolution with off-line fraction collection (32). This method resolved 16 amino acids including glutamate, aspartate, and GABA from a 200-nL sample (diluted to 2 μL). Presently, the lack of automation and long analysis time have prevented this method from gaining popularity.

Most of the work using cLC for dialysate analysis has utilized reversed-phase LC columns; however, more exotic stationary phases have also been reported. One interesting method utilized an antiadenosine aptamer as a stationary phase to selectively preconcentrate and retain this purine in dialysate samples. The resulting method allowed 5-min temporal resolution for monitoring this neurotransmitter (33). In principle, by altering the aptamer, one could then apply this approach to a variety of neurotransmitters or compounds.

3.3. Capillary Liquid Chromatography–Mass Spectrometry

The low flow rates of cLC columns make them particularly amendable to coupling with mass spectrometry, allowing mass detection limits in the low attomole range. Such low mass detection limits are attainable because the background signal can be greatly reduced through selective monitoring of a specific mass-to-charge ratio. In addition, if tandem MS is available, then the sensitivity can be increased further because the chemical noise decreases faster than the signal with higher stages of MS

(34). We have used cLC-MS to achieve 2.4-min temporal resolution for acetylcholine measurements in dialysate (35). Notably, this method did not require the use of acetylcholinesterase inhibitors to artificially increase the acetylcholine level in dialysate to achieve this resolution. In principle, the resolution could be 30 s. By comparison, other LC-MS methods achieved 20-min temporal resolution for acetylcholine (36).

The high sensitivity possible with cLC-MS is especially useful for measuring neuropeptide release in vivo. Neuropeptides have picomolar basal concentrations in the brain. As a result, in dialysis samples, just a few attomoles of neuropeptide may be available. This makes studying neuropeptides an analytical challenge. Radioimmunoassays have been the main method for measuring neuropeptides in dialysate (for a review, see Reference 37). However, with detection limits of ~100 amol, one could only use these methods for ~30-min temporal resolution and measure only one neuropeptide at once. Advances in cLC and LC with electrochemical detection have allowed some neuropeptides to be detected in dialysate (38–40); however, the low concentration makes such detection difficult. Furthermore, the method is limited to peptides that either are naturally electroactive or can react to form an electroactive product. In contrast, cLC-MS is adaptable to detecting multiple peptides in one assay with sequence specificity and, in principle, is applicable to all neuropeptides without derivatization.

Caprioli's group (41, 42) first used capillary LC-MS to detect endogenous neuropeptides. This method improved the sensitivity of traditional electrospray ionization sources by creating a microelectrospray source that accommodated nanoliter flow rates (which increases the ionization efficiency of the sample). Their source consisted of a 50-μm silica capillary containing a silica frit and a 2-cm bed of reversed-phase particles. Dialysate samples were preconcentrated and desalted on this bed before being injected into the mass spectrometer. The investigators reported a detection limit for neurotensin of less than 30 amol. Moreover, miniaturization and changes in operation have further improved this method. In the cLC-MS of neuropeptides, a practical difficulty is the time required to preconcentrate microliter samples onto columns that have total volumes of just a few nanoliters. A method for met- and leu-enkephalin utilized an automated two-pressure LC system to reduce analysis time (43). One pump had a high flow rate to hasten sample loading and column rinsing, and the other pump had a low flow rate to perform the separation. Because both pumps were pressure equalized, there was no time wasted waiting for pump pressure to stabilize when switching between loading and separation. In addition, smaller column diameter and emitter dimensions, along with a lower electrospray flow rate, helped to increase separation efficiency and mass sensitivity compared with previous methods. The mass sensitivity of this method was such that a temporal resolution of 3.3 min was possible; however, as this was an on-line method, temporal resolution was limited by the analysis time, which included time required to load the sample, separate analytes, and then re-equilibrate the column (30 min). The sampling interval was cut to approximately 16.5 min by changing the gradient and other parameters (34). cLC-MS has also been utilized to study other neuropeptides, including angiotensin (44) and neurotensin (42).

3.4. Current Status of Capillary Liquid Chromatography for Microdialysis and Future Directions

Although cLC offers higher mass sensitivity and therefore higher temporal resolution than HPLC, several drawbacks limit the applicability of this method. The stability of capillary columns is far less than commercial HPLC columns because their smaller size makes it easier to clog or break them. Typically a new capillary column is required for each set of dialysate samples collected. The cLC methods also require specialized LC equipment to load the sample onto the column and perform the analysis. The continued commercialization of this technology, especially with MS interfaces, will likely make it more feasible for dialysate analysis in the future.

CE: capillary electrophoresis

LIF: laser-induced fluorescence

The enhanced temporal resolution for microdialysis monitoring by cLC is undermined by the relatively long separation times, which can create a bottleneck in routinely performing high–temporal resolution methods, either off-line or on-line. Recent advances, however, may alleviate this problem. For example, the development of ultra-high-pressure LC (for review, see Reference 45) has made faster separations possible. The use of elevated temperatures along with more stable stationary phases can also improve the separation speed (46, 47). Although these innovations have not been applied to microdialysis samples, they likely could be used to make high–temporal resolution monitoring more routine.

4. CAPILLARY ELECTROPHORESIS METHODS FOR ANALYZING DIALYSATE

Capillary electrophoresis (CE) methods are also popular methods for coupling to dialysis. CE is well suited for achieving high temporal resolution because it has both high mass sensitivity and the potential for high throughput. Mass detection limits by CE with laser-induced fluorescence (LIF) detection can be in the yoctomole range (48), suggesting the potential for subsecond temporal resolution. Such impressive detection limits are generally achieved only with LIF, but electrochemical detection can achieve attomole LODs, and ultraviolet detection can achieve femtomole LODs. The throughput of CE can enable rapid analysis, off-line or on-line, of the many samples collected by a microdialysis experiment. High throughput can be achieved through rapid separation. As recently reviewed (49), separation speed in CE can be in the seconds range compared with the minutes typically required for equivalent separations by HPLC. CE is also amenable to parallel operation (50), an innovation that is an outgrowth of the Human Genome Project, which, in principle, could improve the throughput of the off-line analysis of dialysate; however, this has not been implemented. Besides the high-throughput capability, CE is also amenable to automation, which further facilitates its use for dialysate analysis. For these reasons, CE has been highly useful for temporally resolved microdialysis measurements.

4.1. Detection and Derivatization Methods

With CE methods, LIF detection is the most popular detection method for dialysate. Some work couples CE to electrochemical detection (51–53), but this method is not

as popular because of the lack of commercial availability. For good chromophores, or compounds present at sufficiently high concentration, ultraviolet absorbance detection has also been used (54).

Although LIF provides impressive detection limits, it can only detect fluorescent compounds. Because native fluorescence of most neurotransmitters is inconsequential, a derivatization step is needed. As many transmitters contain primary amines, amine-reactive reagents have been used extensively. The relatively rapid reaction kinetics of orthophthaldialdehyde/β-mercaptoethanol (10–30 s) and naphthalene-2,3-dicarboxaldehyde/cyanide (180 s) make them good derivatization agents for on-line CE methods. These methods are also advantageous because they are fluorogenic, meaning that they have low fluorescence until they react. Fluorescent tags such as fluorescein isothiocyanate offer high quantum yields, but their reaction kinetics are extremely slow, and their native fluorescence tends to create many background peaks. The long reaction time prevents these tags from being used for on-line measurements. Fluorescent derivatization agents are not limited to being amine-reactive agents. Thiol-reactive agents such as methanolic monobromobimane are available and have been used in microdialysis/CE-LIF methods (55).

4.2. Off-Line Capillary Electrophoresis Methods for Studying Neurotransmission

The first methods developed to couple microdialysis to CE were off-line methods. Early work involved collecting dialysate into a vial, adding derivatization solution, and then injecting the derivatized sample onto the capillary (56–59). Although these approaches used CE, they required up to 7 μL of sample for injection (even though only a few nanoliters are injected) and therefore did not take advantage of the potential for high temporal resolution. The main advantage over HPLC in these instances was the analysis time.

Off-line methods have achieved temporal resolutions of 1 to 30 s by using various strategies for derivatizing and manipulating smaller samples. In continuous-flow derivatization, the dialysate stream is mixed on-line with reagents and then collected into small fractions for reaction and storage. The on-line addition of reagents is highly reproducible for small-volume fractions and creates a larger volume, at the expense of some dilution, which facilitates off-line injection and sample manipulation. Strategies for continuous-flow derivatization have evolved over time and are summarized in **Figure 5**.

On-line mixing chambers were originally a series of three small pieces of polyethylene tubing glued between pieces of capillary and connected to the outlet of the microdialysis probe (60, 61). Syringes containing internal standards and fluorescent derivatization agents were then connected to these reaction chambers via capillary tubing. As dialysate flowed from the probe toward the collection vial, the sample was continuously derivatized. This work took advantage of the off-line collection and dilution to utilize a discontinuous buffer system and achieve excellent concentration detection limits.

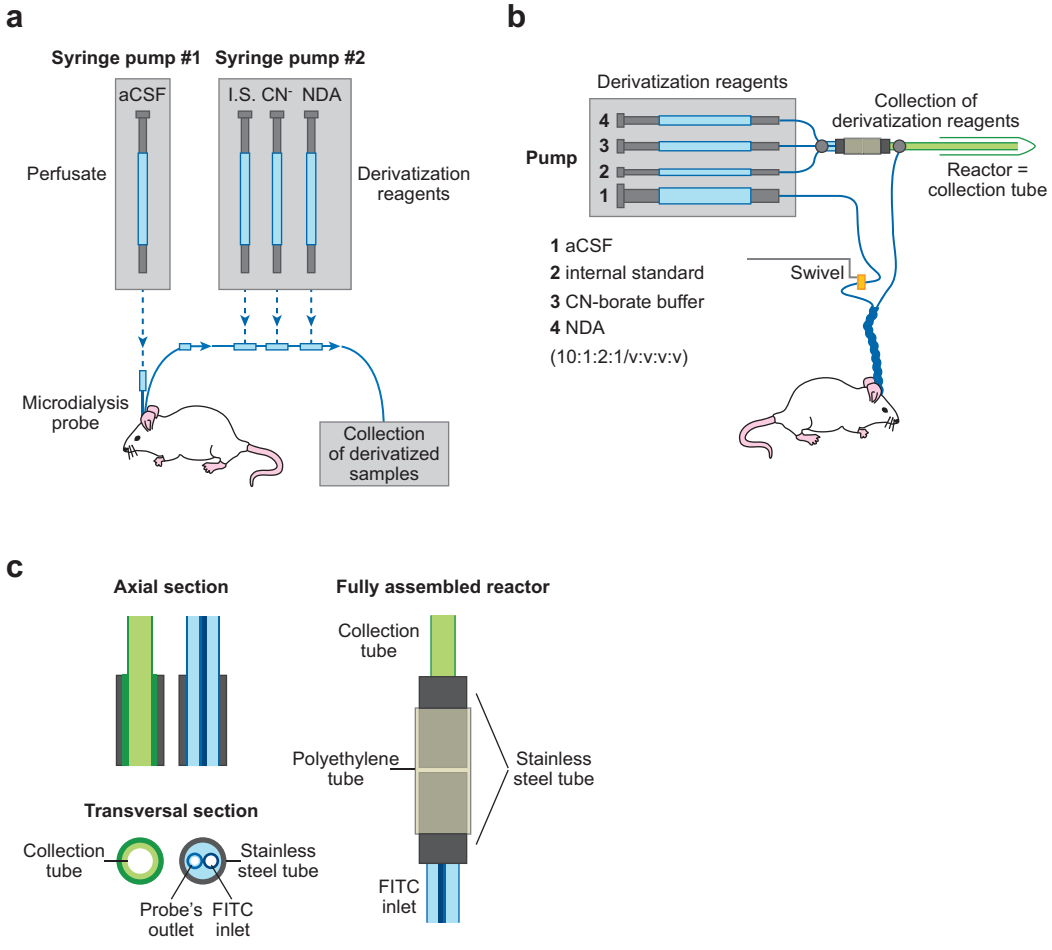

Figure 5

Evolution of the continuous-flow derivatization reactor. (*a*) The first continuous-flow derivatization agent reactor was composed of a series of three polyethylene tubes connected to the outlet of the microdialysis probe. The capillaries from the syringes were inserted into the polyethylene tubing. The derivatization reaction (naphthalene-2,3-dicarboxaldehyde/cyanide) occurs within the polyethylene tubing. **Figure 5*a*** reprinted from Reference 61, used with permission from Elsevier. (*b*) In the second-generation reactor, the dialysate is derivatized inside the collection tube rather than inside the polyethylene tubing. With this new reactor, the outlet of the probe is glued to the side of a larger bore capillary that delivers the fluorescent derivatization agents to the vial. **Figure 5*b*** reprinted from Reference 62, used with permission from Elsevier. Abbreviations: aCSF, artificial cerebral spinal fluid; CN, cyanide; I.S., internal standard; NDA, naphthalene-2,3-dicarboxaldehyde. (*c*) The Hernandez group reduced the volume of the reactor to 26 nL. The reactor is a small gap between two pieces of stainless steel tubing. In one side of the reactor, the dialysate and the fluorescent derivatization agent (FITC) are introduced through separate capillaries. On the other side of the reactor is a collection capillary. At the end of the experiment, the collection capillary is cut into 4-mm pieces representing 1-s dialysis samples. The derivatized dialysate is then transferred to a vial via centrifugation. **Figure 5*c*** reprinted from Reference 64, used with permission from Elsevier.

To further improve temporal resolution, researchers decreased the dead volume of the reaction chamber. In this modified method, dialysate collected from a freely moving animal is derivatized in the collection vial instead of inside the polyethylene tubing (**Figure 5*b***) (62). The outlet capillary from the microdialysis probe is glued to the outside of a larger bore capillary that contains the fluorescent derivatization agents as well as the internal standards. Samples were collected for 20 s corresponding to 940 nL of total volume.

The Hernandez group (63) modified the concept of continuous-flow derivatization to include three separate chambers. Their precolumn reactor resembled a three-barreled micropipette with one barrel connected to the outlet of the probe, the second barrel connected to a syringe filled with buffer, and the third barrel connected to a syringe of fluorescent derivatization solution. The solutions were mixed at the tip of the reactor, at which a capillary then delivered the samples into a collection vial for 6 s per fraction. This system used fluorescein isothiocyanate for derivatization requiring a 16-h reaction time. After this time, samples were diluted and injected onto a CE instrument.

A modification of this precolumn reactor allowed an increase in the temporal resolution to 1 s. (This temporal resolution was based on the mass sensitivity and fraction size achieved and discounts the Taylor dispersion discussed above.) The modified system reduced the volume of the reactor to 26 nL (64). In addition, samples were collected continuously into a capillary instead of as individual fractions (see **Figure 5*c***). After sample collection, the capillary was cut into short pieces (4 mm), each containing a sample fraction corresponding to 1 s of dialysate collection time. The sample was transferred to a collection vial via centrifugation. After 18 h of reaction time with fluorescein isothiocyanate, the sample was diluted and then injected onto a CE instrument.

4.3. On-Line Capillary Electrophoresis Methods for Studying Dialysate Samples

A significant research effort has also been devoted to developing on-line coupling of microdialysis and CE. In this approach, sample collection, derivatization, and separation all occur continuously and automatically, resulting in no manual sample handling. For this approach to be successful, on-line derivatization has to be rapid; therefore, reagents such as fluorescein isothiocyanate are not acceptable. Furthermore, separation time has to be short.

The Lunte group was the first to utilize on-line sample derivatization (using naphthalene-2,3-dicarboxaldehyde/cyanide) and detection. They used this approach to measure glutamate and aspartate with 70-s temporal resolution (65). This method uses two mixing crosses, one as a premixer for derivatization agents and the other as reactor that mixes the dialysate with the derivatization agents. The derivatized sample then travels to a microinjection valve, which alternatively sends the sample or running buffer to the injection interface.

The Kennedy group has developed a system in which dialysate is derivatized on-line and then periodically injected onto a CE system with a flow-gate

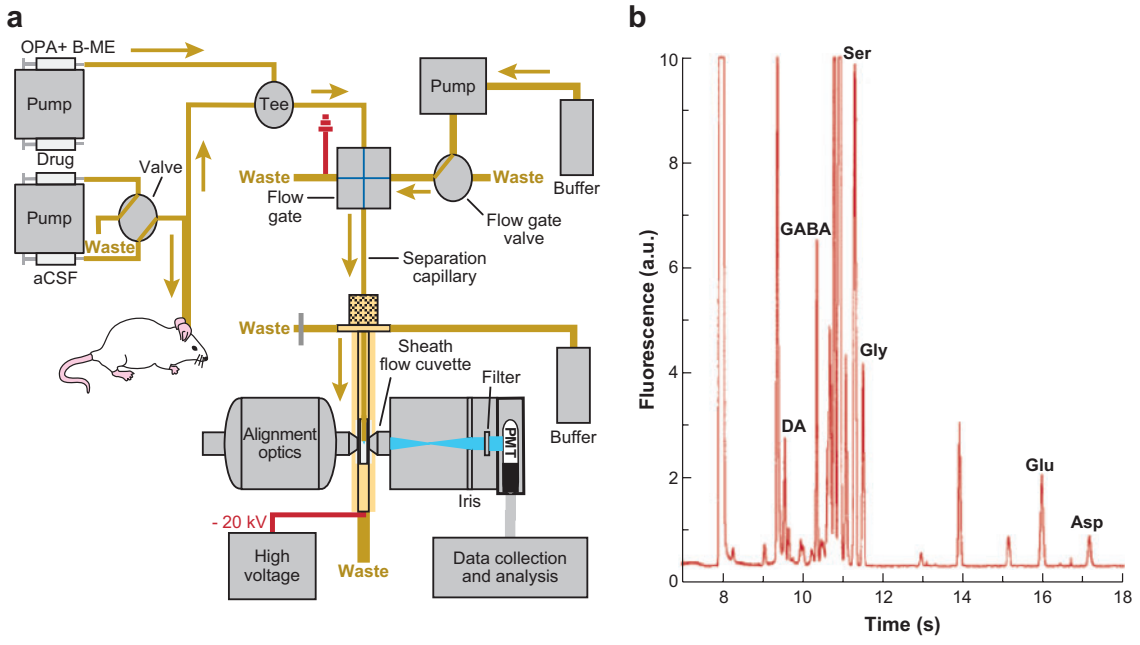

Figure 6

On-line capillary electrophoresis–laser-induced fluorescence instrument with flow gating. (*a*) Block diagram of a complete on-line capillary electrophoresis instrument. The outlet of the microdialysis probe is connected to a T-shaped junction, at which dialysate is mixed with the fluorescent derivatization agents. The derivatized dialysate is then electrokinetically injected onto a separation capillary using a flow-gate interface. The separation capillary (10 μm inner diameter) is threaded inside of a sheath flow cuvette, allowing for off-column laser-induced fluorescence detection. The fluorescent signal is collected by a photomultiplier tube (PMT), and LabView software is used to collect and analyze the data. Abbreviations: aCSF, artificial cerebral spinal fluid; B-ME, β-mercaptoethanol; OPA, orthophthaldialdehyde. (*b*) A representative in vivo electropherogram collected during a potassium stimulation. Five neuroactive amines (glutamate, aspartate, GABA, taurine, and glutamine) are separated in under 20 s. **Figure 6*b*** reprinted with permission from Reference 67. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Abbreviations: DA, dopamine; Ser, serine; Gly, glycine; Glu, glutamate; Asp, aspartate.

interface developed by the Jorgenson lab (66). The use of the flow-gate interface plus narrow-bore capillaries (10 μm) has enabled the obtainment of separations of 3–100 s. **Figure 6** presents an overview of the instrument. In this method, the dialysate sample and fluorescent derivatization agents are mixed at a T-shaped junction or in coaxial capillaries. The sample is allowed to react inside of a larger bore reaction capillary, which enters one side of the flow gate. By incorporating a flow-gate interface (for a description of operation, see Reference 67), both the temporal resolution and separation efficiencies were significantly improved over previous interfaces. High efficiencies were possible even at low dialysis flow rates (75 or 155 μL min^{-1}) (68). At such low flow rates, nearly 100%

relative recovery was achieved, allowing quantitative monitoring with good temporal resolution.

This method has evolved over time. Shorter columns and high voltages allowed 3-s temporal resolution for glutamate and aspartate analysis (69). Improvements in detector sensitivity and injection protocols allowed extremely high efficiencies (300,000 to 500,000 theoretical plates) and the resolution of five neuroactive amino acids in approximately 20 s (67). Alterations in buffer conditions (such as use of micellar electrokinetic chromatography, different derivatization reagents, and different detectors) have allowed for the detection of different compounds. Of particular note is the resolution of 17 amino acids in 30 s (70), the resolution of dopamine with 62 other amines (71), the detection of glutathione and cysteine using methanolic-monobromobimane derivatization (55), and the detection of ascorbate and lactate in dialysate (54).

The on-line methods that have been developed utilize custom-built instrumentation. This instrumentation creates a barrier to the wide use of this approach. Investigators have worked to make these on-line methods more accessible to laboratories that do not specialize in CE instrumentation. Off-line continuous-flow derivatization coupled on-line to a commercial CE instrument has been developed to meet this end (72). Instead of three separate reaction chambers, this method uses only one piece of polyethylene tubing to serve as the reaction/mixing chamber. Making this method fully on-line, the derivatized sample is sent directly to the sample loop (630 nL) of an automatic injection valve instead of a vial. An injection interface was used to electrokinetically inject the sample onto the separation capillary to simultaneously measure glutamate, aspartate, and phosphoethanolamine with 2-min temporal resolution. This is the first method reported to couple microdialysis on-line to a commercial instrument for routine sample analysis with minimal sample handling. A flow-gate system has also been assembled using almost all commercial components, which facilitates its use (70).

Another approach that may allow more widespread use of on-line electrophoretic analysis is the use of microfluidic chips. Both glass and polydimethylsulfoxane chips have been coupled to microdialysis sampling for in vivo (73–77) and in vitro experiments (78). In principle, using these chips should be fairly straightforward as all the fluidic connections can be premade. A National Institutes of Health–funded National Resource Center, the Center for Neural Communication Technology (**http://www.cnct.engin.umich.edu/**), is presently investigating the feasibility of providing such chips for user laboratories.

5. ENZYME ASSAY METHODS FOR ANALYZING DIALYSATE SAMPLES

In addition to separations-based methods, enzyme assays have also been frequently used for coupling to microdialysis. Such methods offer dedicated analysis to a small number of analytes and can be rapid and sensitive enough for high–temporal resolution measurement. In general, enzyme assays can be miniaturized with little

sensitivity loss. As a result, their mass detection limits can be extremely high. Also, it is straightforward to automate such assays as it generally only requires continuous-flow mixing.

NADP: nicotinamide adenine dinucleotide phosphate

Researchers have developed several enzyme assay methods to study glucose and lactate metabolism in the brain to understand stroke and other neuronal injuries. The sampling frequency ranges from 15 s to 2 min, depending on the analyte and the method. One common approach involves the formation of a bed of particles that contain immobilized enzymes (79–82). For the assays, horseradish peroxidase and an oxidase such as glucose oxidase are immobilized on the particles. Dialysate is injected onto the packed bed along with the electrochemical mediator ferrocene monocarboxylic acid. The oxidase enzyme creates hydrogen peroxide, which is then used by the horseradish peroxidase to reduce the ferrocene mediator to ferricinium, which is measured by a downstream glassy electrode. This change in current as the electrode reduces the ferricinium ion back to ferrocene is proportional to the amount of analyte in the sample. Modifications to this method allow multiple assays to be performed at once through the use of an intricate injection valve that can alternatively send dialysate to one specific enzyme assay (83).

Rather than immobilizing the enzymes on particles to create a packed bed, Rhemrev-Boom et al. (84) have immobilized enzymes in poly(1,3-phenylenediamine) to create a permselective membrane. This immobilization creates a low-volume (20-nL) biosensor. The fabrication of this biosensor involves inserting two platinum wires (one auxiliary and one working electrode) and an Ag/AgCl reference electrode into a piece of Tygon tubing. After washing steps, a solution of poly(1,3 phenylenediamine) plus the oxidoreductase enzyme is pushed into the Tygon tubing. After electrochemical polymerization, the permselective membrane is created. The oxidoreductase enzyme forms hydrogen peroxide when the analyte of interest flows through the biosensor, which is measured by the platinum working electrode. This biosensor can be connected to the outlet of the dialysis probe to allow real-time measurement. The authors chose poly(1,3-phenylenediamine) as the immobilization agent because it does not hinder the sensor from reducing the hydrogen peroxide.

Fluorescence enzyme assays have also been developed to selectively measure the compound of interest. In these methods, dialysate is mixed with a variety of compounds to produce NADPH, which is naturally fluorescent (85). For glucose these compounds include ATP, hexokinase, glucose-6-phosphate dehydrogenase, Mg, and NADP. When these compounds react, two reactions take place: Hexokinase converts glucose to glucose-6-phosphate, which is then used in the second enzyme reaction with glucose-6-phosphate dehydrogenase to reduce NADP to NADPH. The production of NADPH is then proportional to the glucose present. This method also can detect lactate in the so-called lactography method (86). Unfortunately, with this method, glucose levels below basal levels (25 μM) are not detectable owing to either reaction efficiency or absorption. However, this method makes up for this in its simplicity and low cost compared with its electrochemical counterparts. In principle, this approach would be amenable to almost any enzyme reaction that uses NADP as a cofactor.

6. APPLICATIONS OF HIGH–TEMPORAL RESOLUTION MICRODIALYSIS

The evolution of analytical methods with sufficient sensitivity and throughput to allow microdialysis methods good temporal resolution has resulted in new opportunities for studying brain neurochemistry that are just starting to be exploited. **Table 1** summarizes some of the analytes, methods, and applications of methods with temporal resolution better than 2 min. We discuss some illustrative examples below.

An important target of microdialysis measurements has been dopamine, a transmitter known to be involved in reward, reinforcement, and addiction behavior. The development of microbore HPLC methods has improved the temporal resolution of dopamine and its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) from 10–20 min to 1 min, enabling the correlation of rapidly changing behaviors with dopamine release and metabolism (30). In one example, dopamine and DOPAC were monitored at 1-min temporal resolution as rats self-administered cocaine (30). Dopamine levels stayed above basal levels throughout the self-administration, and animals administered another dose of cocaine to maintain these elevated levels. The duration of this elevated dopamine response was proportional to the dose of cocaine administered, with higher doses having longer latencies between shots. These results are consistent with the hypothesis that dropping dopamine levels (and not dopamine depletion) trigger subsequent cocaine-seeking behavior. A subsequent finding in this experiment was that DOPAC levels were lower during the cocaine administration than prior to drug administration, suggesting that dopamine metabolism is reduced.

The even higher temporal resolution possible with microdialysis coupled on-line to CE-LIF detection has enabled several experiments correlating neurochemistry with a variety of behaviors (see **Table 1**). One experiment examined changes in behavior and neurochemical release at 15-s intervals during the presentation of predator odor known to evoke strong behavioral activation in rats (87). One subset of animals (high responders) immediately started to dig and burrow upon presentation of the odor and showed a large biphasic response in glutamate and GABA release that lasted just 180 s but was slightly delayed from the onset of behavior. Another subset of animals (low responders) showed no behavioral response. These animals also had no neurochemical response to the odor. Indeed, an extremely high correlation between the amount of amino acid release and behavioral activation was observed. Thus, individual differences in behavior and neurochemical release were detectable, suggesting a relationship between chemical release and behavior. Because the glutamate release occurred after the behavior started, it was hypothesized that the release resulted from a response to a stress hormone. Such observations were made possible by the temporal resolution.

Interestingly, previous experiments had performed similar tests while monitoring glutamate at 10-min intervals by microdialysis (88). In those experiments, the maximal increase was 150%, maintained for 30 min after odor presentation. By contrast, at high temporal resolution, the peak increase was 500%—slightly delayed compared to odor presentation (and behavior)—biphasic, and lasted for just 3 min. When the data for the 15-s temporal resolution measurements were binned into 10-min fractions,

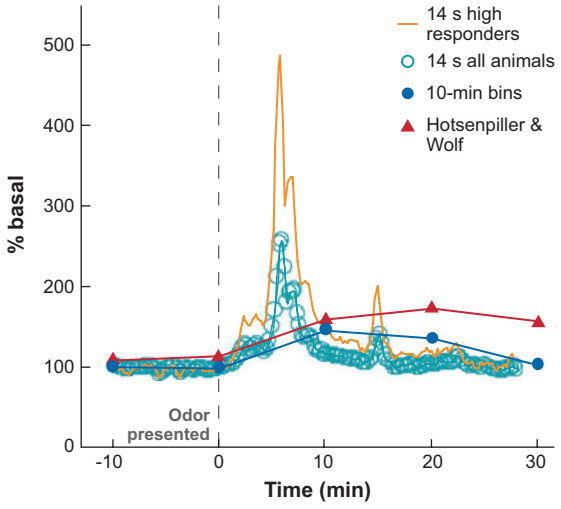

Figure 7

Capillary electrophoresis–laser-induced fluorescence method for measuring transient amino acid changes in response to predator odor. Dialysate measurements are presented in 14-s and 10-min bins to show how glutamate levels change when the predator odor (2,5-dihydro-2,4,5-trimethylthiazoline, from fox) is present. Microdialysis was performed in the nucleus accumbens. The dashed line represents the time at which the odor was presented to the rat. Rats with a high behavioral response to the odor showed an increase in glutamate that lasted only 3 min and peaked at 500%. This response is reduced if the data from both the high and low responders are grouped. Averaging the data into 10-min bins gives a response similar to that seen when using high-performance liquid chromatography to analyze fractions collected over 10-min intervals [data replotted with permission from Hotsenpiller & Wolf (88)]. Decreasing the temporal resolution from 20 s to 10 min dilutes the magnitude of the glutamate change and masks the transient biphasic nature of the response. Figure reprinted from Reference 87, used with permission from Wiley-Blackwell.

they closely matched the previous results (see **Figure 7**), proving that the differences observed resulted from temporal resolution and not the experiment. These differences dramatically highlight the significance of improved temporal resolution.

Continuous-flow derivatization with off-line CE-LIF has also been used for behavioral studies. One study measured GABA and glutamate release in the lateral hypothalamus during feeding behaviors (59). This experiment collected 500-nL dialysate samples corresponding to 30-s bins. Rats were deprived of food for 16 h before the microdialysis experiments began. Samples were taken premeal, during the meal, and after the meal. Glutamate levels spiked once the animal began to eat and then declined, whereas GABA levels slowly rose as the animal reached satiation (**Figure 8**). These findings suggest that glutamate release in the lateral hypothalamus may drive an animal to eat, whereas GABA release in this brain region opposes the drive to eat.

Off-line CE-LIF has also been used to measure amino acid and catecholamine release across the sleep-wake cycle in rats (57). Microdialysis probes were implanted in both the medial prefrontal cortex and the nucleus accumbens, and dialysate was collected off-line for 2 min. Each sample was then sorted as to whether it was collected

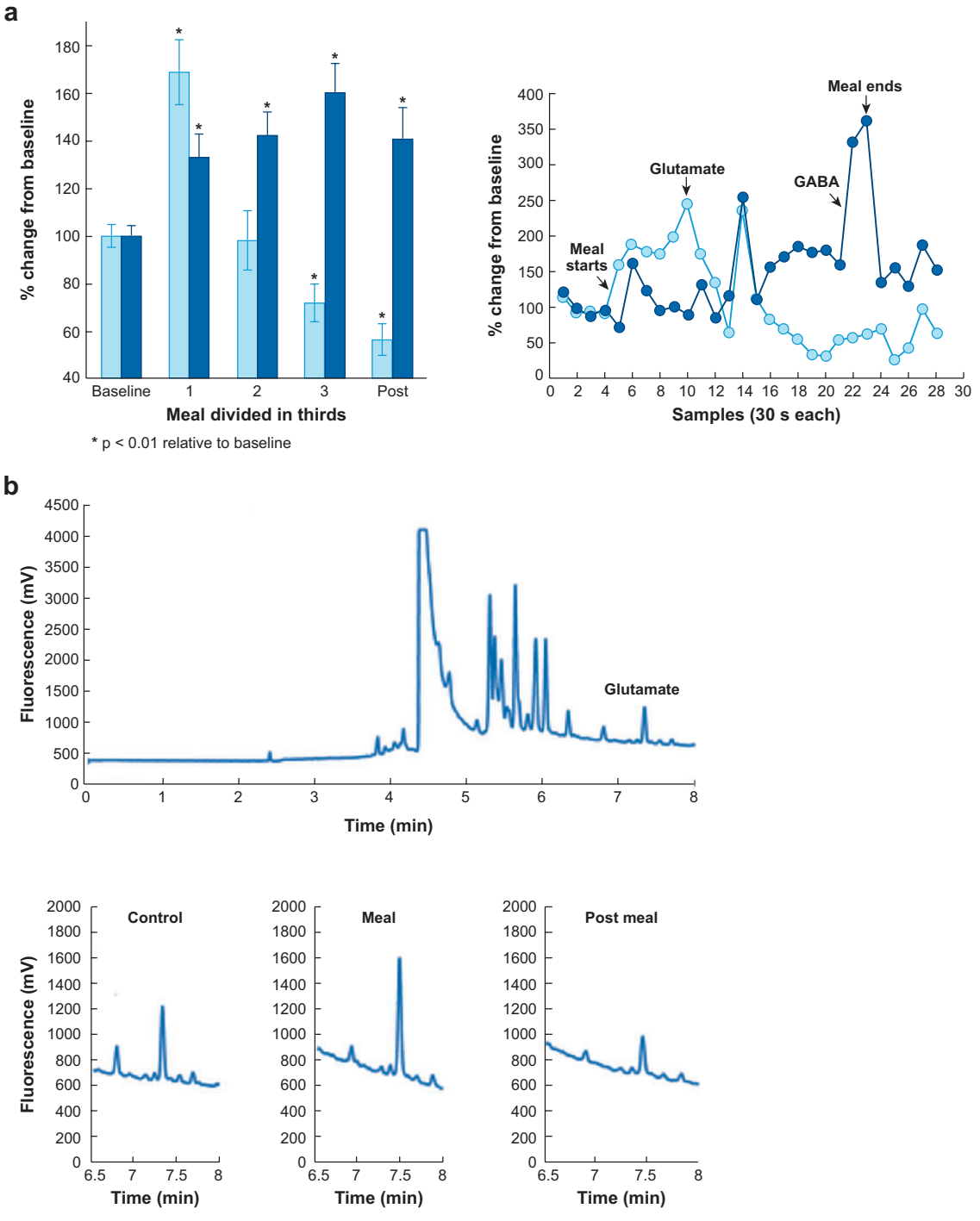

Figure 8

(*Continued*)

during waking, slow-wave sleep, or REM sleep according to electrophysiological readings. Two different separation conditions were used on each sample, one optimized for amino acids and the other for amines. The excitatory amino acids glutamate and aspartate only changed in the nucleus accumbens, in which both amino acids decreased as the animal went into slow-wave sleep and REM sleep. This change was mirrored by norepinephrine in both structures. However, dopamine release was higher in both structures during waking and REM sleep compared with slow-wave sleep. These changes in dopamine levels were hypothesized to be a result of the active cognitive processes.

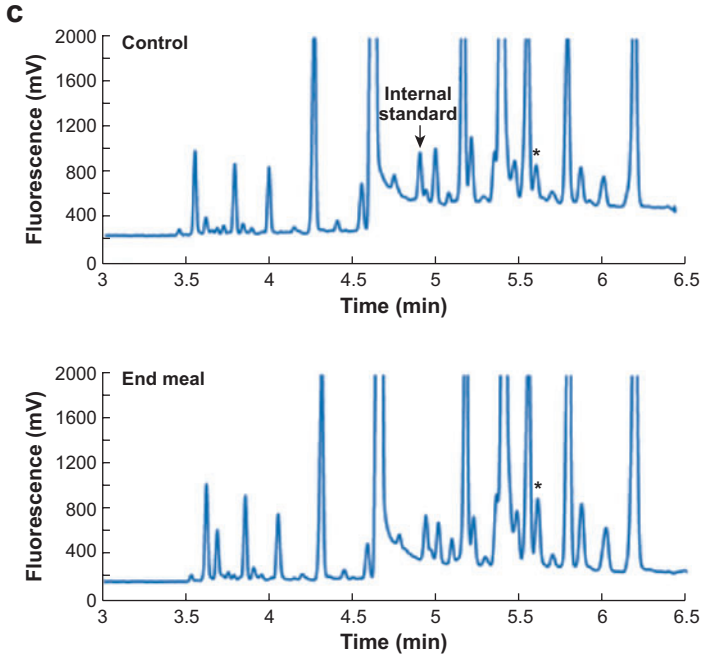

Figure 8

Off-line capillary electrophoresis–laser-induced fluorescence (CE-LIF) method for measuring changes in GABA and glutamate during eating. (*a*) Both the average data (*left panel*) and representative trace from a single animal (*right panel*) demonstrate how extracellular GABA (*dark blue*) and glutamate (*light blue*) levels change throughout a meal. Samples were collected every 30 s as the animal ate and were analyzed using an off-line CE-LIF method. Microdialysis was performed in the lateral hypothalamus. Both panels show that glutamate levels rose for the first third of the meal and then decreased, whereas GABA levels rose at the end of the meal ($^*p < 0.01$ relative to baseline). (*b*) A representative in vivo electropherogram identifying the glutamate peak. Each dialysate sample had a separation time of 10 min. Electropherograms showing changes in the glutamate peak height across the meal are depicted as well. (*c*) Sample electropherograms showing the change in GABA levels across the meal. The GABA peak is marked with an asterisk. Figure adapted with permission from Reference 59. Copyright 2003 by the American Psychological Association. The use of APA information does not imply endorsement by APA.

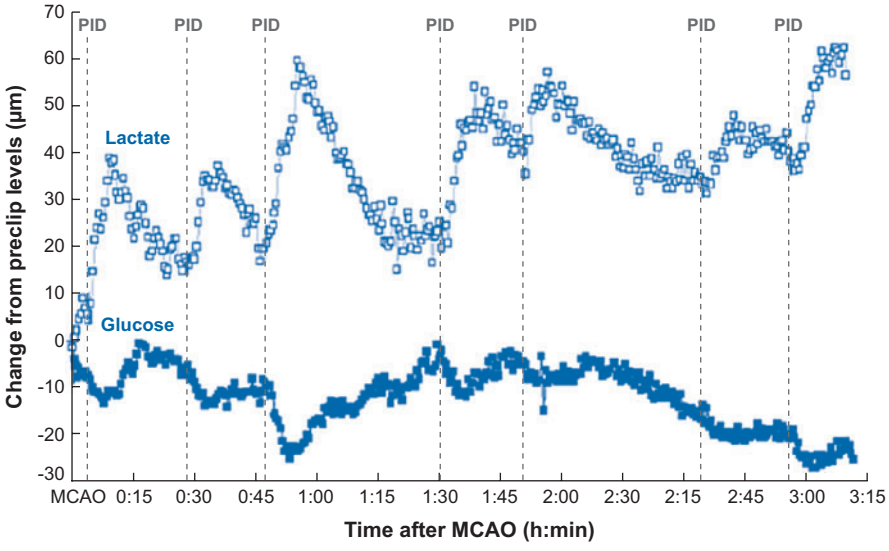

Figure 9

Microdialysis coupled to a dual enzyme assay for lactate and glucose. The occurrence of peri-infarct depolarizations (PIDs) was compared to both glucose and lactate levels after occlusion of the medial cerebral artery. Using a custom-made valve, dialysate was sent every 30 s to either a glucose or lactate assay. The occurrence of PIDs correlates well with an increase in lactate concentration and a decrease in glucose concentration. Figure reprinted with permission from Macmillan Publishers Ltd: *J. Cereb. Blood Flow Metab.* (Reference 79), copyright 2005. Abbreviation: MCAO, middle cerebral artery occlusion.

Enzyme bioassays can measure both lactate and glucose with 15–30-s temporal resolution (79). This method is applicable for clinical measurements because fast metabolite measurements can give insight into the physical state of a patient. In this experiment, the researchers occluded the middle cerebral artery in anesthetized cats and monitored the resulting changes in glucose and lactate every 30 s. When blood flow is stopped, spontaneous depolarizations occur in the surrounding tissue. These depolarizations may cause the size of the infarct to increase. Interestingly, the researchers were able to correlate changes in both glucose and lactate to the occurrence of these depolarizations. When the depolarization took place, lactate increased, while glucose decreased (**Figure 9**). These characteristic changes in glucose and lactate could then be used in the clinical setting to diagnose these spontaneous depolarizations.

As these examples show, high–temporal resolution measurements have been used in a wide variety of applications, including stress responses, learning, feeding, sleep, addiction, and stroke pathophysiology. These initial studies, however, represent a tiny fraction of all microdialysis work, suggesting that we are just starting to see the impact of high–temporal resolution dialysis methods. Especially promising is the ability to correlate behaviors, which are typically rated on 10–30-s intervals, with the

neurochemical changes occurring on the same time scale. Other examples, such as rapid pharmacological responses and short-lived processes, may also be studied. Ultimately, it may be possible to also improve the spatial resolution of such measurements. New sampling methods such as low-flow push-pull perfusion (89) and direct sampling (90) have made smaller brain regions accessible to monitoring; however, at present the temporal resolution of such measurements is limited by the flow dynamics of these systems. That is, they require exceedingly low flow rates, which results in high levels of Taylor dispersion during sampling.

Although this review focuses on using microdialysis to perform in vivo central-nervous-system measurements, this technique is applicable to other in vivo environments, in vitro systems, and even industrial processes. Microdialysis has also been used in in vitro experiments specifically investigating enzyme kinetics (91–93) and drug metabolism (94). Enzyme kinetics methods traditionally use spectrophotometry to monitor the appearance and disappearance of products and reactants. The reaction is initiated by adding the substrate, the sample is incubated, and the reaction is quenched by removing the protein or adding an enzyme inhibitor. This procedure can be arduous because, to make accurate time points, all the enzyme must be removed or the reaction will continue. Moreover, some of the analytes of interest could be lost in the separation step when the protein is precipitated out. Microdialysis can circumvent this separation process because it samples only the smaller reaction products and reactants. The membrane keeps the enzymes out, thus quenching the reaction. The sample is protein free, so it can then be injected onto an HPLC column to be separated and analyzed. With this chromatography step, one can separate analytes with similar absorbance spectra that could not be separated using spectrophotometric methods. The continuous sampling provided by microdialysis also makes these kinetic measurements more automatic because the reaction does not need to be manually quenched. These microdialysis enzyme methods have been coupled to both LC and MS with temporal resolutions of 1–10 min. The temporal resolution could possibly be improved by utilizing some of the methods outlined above.

SUMMARY POINTS

1. Microdialysis is a versatile sampling method that can be coupled on-line or off-line to a variety of methods such as LC, CE, MS, and enzyme bioassays for monitoring in vivo neurochemical events.
2. Temporal resolution is important in neurochemical studies because neurotransmitter dynamics cover a wide range, from milliseconds to days.
3. The challenge in creating high–temporal resolution dialysis methods is that as the temporal resolution increases, the mass of analyte available in the sample decreases, and the number of samples to be analyzed increases.

4. Achieving temporal resolution as high as 10 s in microdialysis is possible, but requires methods with attomolar detection limits and high throughput.
5. The temporal resolution possible with microdialysis is ultimately limited by the broadening of concentration plugs owing to flow and diffusion, as they are transported to the analytical system or fraction collector.
6. Capillary-based separation methods (cLC and CE) and enzymatic assays have high mass sensitivities, which facilitate the creation of high–temporal resolution dialysis methods.
7. Recent improvements in temporal resolution have enabled new experiments correlating neurochemical changes with rapidly changing behavior and detecting transient neurochemical events.
8. Microdialysis sampling can also be useful for in vitro experiments such as enzyme kinetics assays to make methods more simple and automated.

FUTURE ISSUES

1. Is it possible to create instruments and methods that allow high temporal resolution that are easy to use and affordable for most neuroscience laboratories?
2. Will advances in commercial HPLC equipment and the development of ultrahigh-pressure LC instrumentation improve the separation time enough to have temporal resolutions comparable with CE methods?
3. Can band broadening associated with sampling be reduced or eliminated to achieve temporal resolution better than 1 s by sampling methods?
4. Most assay development has focused on amino acids and catecholamines. What other molecules should be targeted, and can assays be developed for them?
5. Is there a way to reduce the tissue response to microdialysis probes so that measurements can be taken over several days to weeks?
6. Will improvements in sampling methods such as low-flow push-pull perfusion create high temporal methods for analyzing brain regions that are too small for dialysis probes?
7. What new neuroscience questions will be addressed by the high–temporal resolution methods currently available?
8. Will the new technology make microdialysis of sufficient interest for widespread use in clinical and surgical applications?

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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