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Optimizing microdialysis for deep brain stimulation

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1. ABSTRACT

Cerebral microdialysis is a chemical detection method capable of identifying and simultaneously sampling a wide range of substances in the micromilieu of the monitoring probe. The interstitial space of biological tissues and fluids is sampled through a thin fenestrated dialysis catheter inserted into the brain. The technique has been reported in patients with Parkinson's disease. However, the procedure is not widely used by neurosurgeons, possibly owing to unclear indications and poor effective benefits, mostly secondary to significant pitfalls. In spite of the feasibility of microdialysis in humans, many factors can affect the quality of the process. Possible pitfalls include improperly designed probe, probe insertion effects, ineffective perfusion rate, issues to optimize stabilization period, and insufficient volume sample. This article reviews those key technical features necessary for performing microdialysis in humans during deep brain stimulation for Parkinson's Disease.

2. INTRODUCTION

The basic principles of chemical transmission at the synaptic level and associated changes in functional output have been established since the mid-1930s (1-3). Early efforts to study cerebral chemical transmission through the perfusion of the extracellular space carried out via three main approaches: Ventricular perfusion or sampling, cortical cup perfusion, and push-pull perfusion (4). The ventricular perfusion method consists of the insertion of a cannula into the lateral ventricles, providing little information about chemical transmission (4). The cortical cup technique consists of the placement of a cylinder filled with physiological fluid that is replaced at regular intervals over exposed regions of the cerebral cortex. Limitations of this technique are the risk of contamination of the perfusion solution and the size of the evaluated brain region (5). Finally, the push-pull perfusion system uses two concentric cannulas. Perfusion fluid is "pushed" into a particular brain region and "pulled" out so that perfusate is collected (6).

During the 1960s, neurotransmitter and their metabolities were measured from post-mortem homogenized brain samples (7-9). Experiments using brain slices were the first to monitor dynamic changes in neurotransmitter release and examine receptor and transporter binding kinetics (10, 11). In the 1970s, voltammetry was employed to measure electroactive neurotransmitters, such as dopamine, noradrenaline and serotonin (4, 10). Over the years, several probes have been built so that semipermeable dialysis membranes could act as an "artificial blood vessel" to sample the extracellular space (12, 13). Ungerstedt and colleagues

further refined the technique so that microdialysis could be also used to measure purines, amino acids, neuropeptides and metabolic markers(14-17).

Deep brain stimulation (DBS) is an established surgical procedure to treat motor symptoms in patients with Parkinson's disease (PD). Thought its precise mechanism of action is unknown, current hypotheses include the local inhibition of neuronal population and the excitation of fibers at a distance from the electrodes(21). Real-time local monitoring of neurotransmitters during DBS surgery may provide a new tool for helping elucidate additional mechanisms as well as the kinetics of deep brain stimulation(22).

As dopamine (DA) depletion is a major mechanism in PD, different techniques have been proposed to detect this neurotransmitter in the parkinsonian brain. Traditionally two main approaches have been developed in vivo electrochemical techniques and in vivo microdialysis.

Electrochemical techniques include continuous amperometry (with carbon fiber electrodes and potentiostat), differential normal pulse voltammetry (DNPV), differential pulse amperometry (DPA) and fast scan cyclic voltammetry (FSCV). As electrochemical techniques measure oxidized molecules in the solution, the technique involves the generation of a constant potential difference between a carbon electrode (working electrode) and an Ag/AgCl electrode that acts as a reference electrode. Molecules coming in contact with the electrode surface are oxidized. Oxidation current is measurable since each DA molecule transfers two electrons to the surface of the carbon electrode. However, the total oxidation current may also include inputs from other oxidizable substances, making continuous amperometry a non-selective technique with low selectivity for DA. FSCV is much more selective compared to continuous amperometry but with a slower temporal response. The average sampling rate used with FSCV is 100 ms. compared to the 20μs used to monitor amperometric events. On the other hand, DNPV and an improved technique known as DPA employ electrochemically treated carbon fiber electrodes with a sampling rate of 500 ms. Intraoperative microdialysis is a sensitive method for detecting intraoperative changes in cerebral metabolism. It has proven to be useful for monitoring basal extracellular DA levels with time resolution at the level of one to several minutes.

This article reviews key technical features necessary for performing microdialysis in humans during stereotactic surgery for Parkinson Disease.

3. CEREBRAL MICRODIALYSIS

Microdialysis enables the sampling and collection of substances from the interstitial space(23), which plays an important role in the propagation of neurochemical substrates among neurons, glia and neurovascular elements(24-26). The method is based on the dialysis principles and involves the diffusion of water and small molecules through semi-permeable membranes (27, 28). Traditionally, microdialysis consists of the insertion of a thin fenestrated catheter into brain parenchyma either through a burr-hole drilled into the cranium or a bolt fixed in the skull(28). A pump is used to infuse artificial cerebrospinal fluid through the microdialysis catheter, which equilibrates with the surrounding interstitial space(28). The tip of the catheter has a semipermeable membrane that permits the free diffusion of molecules between the interstium and perfusate(29). Owing to the size of the membrane pores, large molecules and enzymes are not transferred from the extracellular matrix to the dialisate(24). Refined microdialysis methods have been employed for protein biomarker sampling (30-31). As no fluid aside from that perfused is driven into the probe, microdialysis is considered to be a valuable tool in patients with small blood volumes, such as children or neonates(30).

The underlying process for driving perfusion is a result of physico-chemical properties of the membrane, the physical characteristics of the analyte (molecular weight, hydrophobicity, and tertiary structure), and composition of the perfusate (concentration gradient, and osmotic pressure)(4,27). The collected dialysate is typically analyzed by high-pressure liquid chromatography (HPLC) with electrochemical or fluorometric detection, which allows multiple molecules to be identified with high sensitivity (femtomolar range) and specificity(31). One of the most common applications of microdialysis in the clinic is the measurement of neurotransmitter concentrations(24, 32).

As microdialysis permits the study of neurochemical features, it has been clinically used in patients with traumatic brain injury, Parkinson's disease, subarachnoid hemorrhage and epilepsy(33-37). In pharmacological studies, this technique has also been used to collect drugs or endogenous compounds in their cerebral site of action(23). Finally, microdialysis may help to monitor physiological processes, e.g., during traumatic brain injury (38).

There are several differences between microdialysis as used in traumatic brain injury (TBI) and deep brain stimulation. In patients with TBI the probe is often inserted in the cortex whereas during DBS it is implanted in deep subcortical structures (e.g., globus pallidus and subthalamic nucleus). In addition, the size and volume of the region for implanting the probes in TBI are quite large as compared to those in patients with DBS. In fact, to increase precision during DBS surgery, probes are often implanted under stereotactic guidance(39). To account for some of these aspects, we have made a few changes in our system. First, the length

of the probe and the dialysate membrane were adjusted to fit the stereotactic apparatus and guarantee a stable trajectory towards the targeted nuclei. DBS probes are implanted through guide tubes, inserted into the same holes used for electrophysiological recordings. In this context, our setup offers the possibility of simultaneously evaluating electrophysiology and microdialysis signals. Due to the physical properties of the sampled molecules, the perfusion rate during DBS is relatively faster than that of traditional microdialysis. A major limitation during DBS surgery is the constraint of obtaining an adequate equilibration phase after the insertion of the probes (i.e., compared to the monitoring of TBI patients in the intensive care unit where longer equilibration periods are often possible). Finally, microdialysis in the context of TBI focuses on recovery of biochemical markers such as glucose, lactate, lactate-pyruvate ratio, glutamate and glycerol. During DBS, neurotransmitters, such as dopamine, serotonin, GABA, are also analyzed.

Though the importance of the information provided by microdialysis cannot be underestimated, many factors may affect the quality of the process itself. The following paragraphs will address some of these aspects and examine how the procedure may be optimized specifically for stereotactic procedures, such as deep brain stimulation.

4. PROBE DESIGN

The probe is a major element in the microdialysis setup(24). Most are based on the original design described by Delgado et al. (1972). Different categories and variants include the linear probe, the loop probe, the side-by-side probe and the concentric probe(22,41). The linear probe has a linear and thick shape and is employed in peripheral tissue (e.g., monitoring bile return to the duodenum)(42). It is not employed for brain measurements due to an increased risk of damaging the parenchyma. The loop microdialysis probe has an enforced membrane/tubing joint and is also prone to damage the brain parenchyma. It is more commonly used in the subcutaneous tissue and peritoneal cavity as well as during in vitro studies (i.e., tissue homogenates, cell suspensions, plasma, and biological fluids). The loop probe is thicker and has longer membrane length than the linear probe(40). Finally, the side-by-side probe differs with respect to size and inflow/outflow tube composition. Two pieces of fused silica tubing are threaded through a connector that holds together a short piece of silastic tubing attached to an inlet tubing and a guide cannula of the appropriate length(40). The concentric shaped probe is the most commonly used in experimental brain research because it can reach deep cerebral tissue with a low risk of brain damage(23,24,41).

To reduce or eliminate toxicity and the risk of trauma, dialysis probes are made from high-quality biocompatible materials(24). The dimensions of the concentric cannula are defined by the target area(23). Each probe has a tip membrane that acts as a filter against large molecules present in the extracellular fluid (e.g., proteins) (28,41). Such membrane is made of cuprophane (regenerated cellulose), polycarbonate, p20 minolyamide, polysulfone, polyacrylonitrile AN69, or polyarylethersulfone(44). Membranes can also differ in pore structure and specific molecular weight cut-off, with pore sizes varying from 6-100 kDa(24). The pore cut-off is defined as the average in which 80-90% of molecules with the nominal size are retained by the membrane. Membranes should allow the diffusion of molecules across the concentration gradients with minimal transport of fluid(44). Mathematical models have been developed for understanding the diffusion coefficient of lipophilic, hydrophilic substance and analytes (45). Human microdialysis studies have not addressed whether different materials may affect results. For dopamine measurements, we have chosen to use cuprophane in clinical studies(46) based on the promising results obtained in our preclinical experiments(47, 48).

The length of the membrane is another key point that may vary according to the sampled brain region(28). Kehr (2007) emphasizes the importance of a small exposed surface. Overall, human studies have been conducted with membranes that are 2-3 mm long(31, 46) To improve stability, the very far tip of the membrane is usually covered with glue and not meant to be perfused(28, 33). For DBS targeting structures, such as the subthalamic nucleus and globus pallidus, membranes that are 2-3 mm long are ideal due to size of these nuclei.

An important variation from standard clinical microdialysis is that during stereotactic lesioning and DBS, probe length and the diameter have to fit the stereotactic apparatus. Figure 1 depicts our 450mm length concentric shaped microdialysis probe with inlet and outlet tubing in one extremity and a 3mm membrane in the other. Both are required for human DBS.

5. INSERTION

To reach brain targets with precision, catheters manufacturedfor human microdialysis may be implanted with the use of stereotactic techniques. This is a accomplished with guide tubes to ensure a stable trajectory(46). During DBS surgery, the site of catheter placement may be confirmed with fluoroscopy. Probes have also been implanted in gliomas under CT, MRI or three-dimensional ultrasonography guidance(49, 50). On the other hand, during standard clinical microdialysis, a suture is used to hold the catheter in order to make measurements in the same location throughout the procedure. As illustrated in Figure 2, our microdialysis setup includes macroelectrode and microdialysis

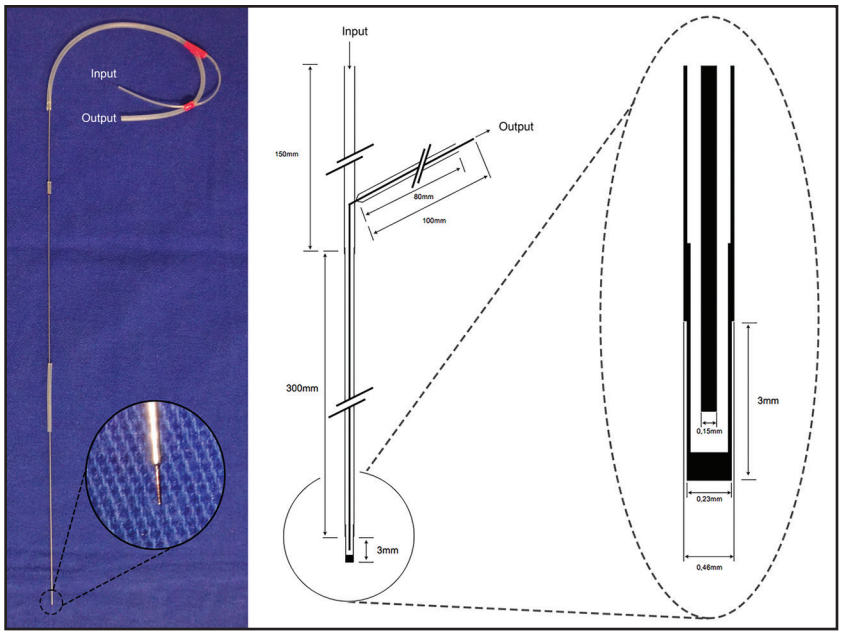

Figure 1. A. Actual photograph of the microdialysis probe that we are using during surgery and schematic microdialysis probe illustrating its components. Inlet and outlet ducts for perfusion liquid and dialysate are shown. A semi-permeable membrane is glued between the tip of the inner steel cannula and the outer steel shaft. The perfusion fluid enters the membrane space through the inner cannula and flows into the shaft to the outlet. On the right side, a magnified view of the membrane tip. It is usually covered with glue and not suitable for perfusion.

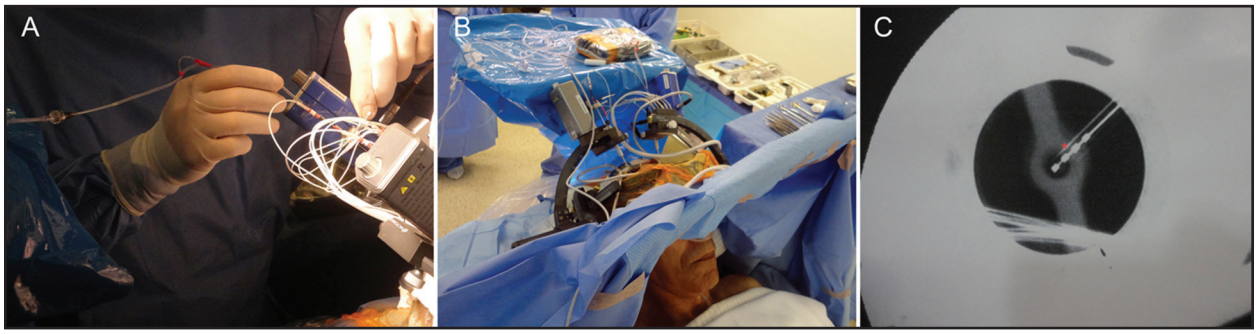

Figure 2. A. Insertion of our microdialysis probe through the same holes used for electrophysiological mapping. B. Stereotactic frame (Micromar, Brazil) allowing simultaneous bilateral microelectrode recording and intraoperative macrostimulation. On the pictured microelectrode drive (Inomed Germany) and intraoperative microdialysis catheter collecting neurotransmitters. C. X-ray image showing the tip of the microdialysis probe at the center of the frame and the microdialysis catheter on the side.

probes (Figure 2A) that are inserted side by side in the brain aside from the vials used to collect the samples (Figure 2B). Fluoroscopy has been employed to confirm the target and the alignment of the system (Figure 2C). Deep brain stimulation offers the possibility of implanting electrodes and the microdialysis probe in the target sites including globus pallidus or the subthalamic nucleus, as shown in Figure 3.

To date, the issue of minimizing tissue damage during implantation of probes has not been properly addressed. Insertion often leads to a local inflammatory reaction with subsequent edema and disturbance in microcirculation. These changes, may result in direct or indirect tissue injury, which can compromise the accuracy of measurements(51-53). Matrix metalloproteinases are activated and released during probe insertion, leading to neutrophil infiltration and subsequent tissue remodeling.

Another aspect that needs to be discussed is the integrity of the blood brain barrier(54-58). Studies using 14C-AIB autoradiography (a compound that does not cross the BBB under normal conditions) have shown that this is maintained with no changes affecting transport characteristics of hydrophilic and lipophilic drugs(59, 60). However, studies using Cr51-EDTA transport have shown a significant effect of probe insertion on blood brain barrier permeability(56). Ultimately, it seems that

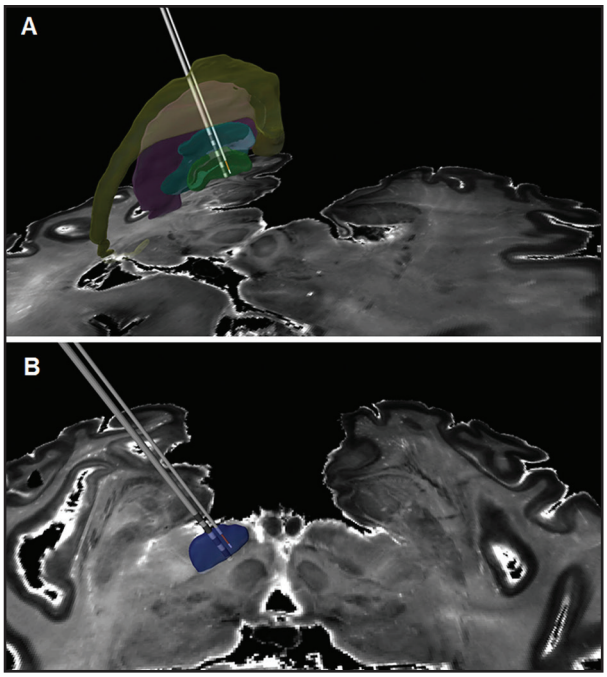

Figure 3. This Figure shows an illustrative view of the position of the microdialysis probe (orange tip) and its relationship with the deep brain stimulation (DBS) electrode (3387, Medtronic) into the globus pallidus internal part (GPi) target (in green, upper Fig.) and the subthalamic nucleus (STN) target (dark blue, bottom Fig.). Globus pallidus external part (GPe) is illustrated in light blue, putamen in pink and caudate in yellow. An horizontal histological slice is shown in the background. The 3D structures were segmented from the histological sections of the São Paulo-Würzburg Atlas of the Human Brain.

the function of the blood brain barrier is disturbed in response to insertion of microdialysis probes, but this critically depends on how close the BBB is from the tip of the probe and for how long probes remain implanted(61). In the vicinity of the probe, there is a decrease in blood flow, a reduction in oxygen uptake, and an abnormal release of neurotransmitters, which persist during the first hours after implantation(62).

After an initial equilibration phase, there is a period of stabilization during which dialysate samples can be continuously collected for a number of hours(28). A question that remains unanswered is the minimum equilibration period for human microdialysis. When patients are in the ward or intensive care unit, longer equilibration is often possible. When patients are in the operating room, however, time is often restricted and microdialysis needs to be performed at relatively short intervals. Specifically, the latter scenario is the collection of samples in patients with Parkinson´s disease treated surgically. The first study in this patient population showed consistent and reproducible results with initial high levels of neurotransmitters reaching a stable baseline 10-20 minutes after the insertion of the probes(15). However, longer equilibration periods have been described in other reports(36, 46, 63). Inasmuch as short equilibration periods are not ideal, our experience has been that in the case of DA, the main perturbation in levels of this neurotransmitter occurred during the first 10 minutes of collection (Figure 4). Bearing in mind that patients are awake during functional neurosurgical cases and, that microdialysis with 30 minutes of equilibration may prolong the surgery by over 1h, it is often difficult to justify the use of longer intervals in the operating room.

6. PERFUSION RATE

During dialysis, the perfusate is infused through the catheter using a microinfusion pump operating at a slow rate. Solutions commonly used are Ringer´s lactate or artificial cerebrospinal fluid(24, 41). These are chosen due to their similarity with the extracellular fluid in regards to ionic concentration, osmotic value and pH(41). During perfusion, the semi-permeability of the membrane allows a sufficient mass transfer without the direct contact of the perfusate with the surrounding tissue(41). Molecules can move in both directions of the membrane until a dynamic equilibrium is established(24). Transmembrane diffusion is driven by the physico-chemical characteristics of the membrane (i.e. molecular weight cut off, polarity, membrane surface) and physical properties of the molecules (i.e. concentration gradient, molecular weight of the analyte, polarity, tertiary structure)(24, 41). Transmembrane diffusion is maintained by the continuous replenishment of the molecules from more distant areas of the extracellular space(24). Probe recovery is a parameter of efficiency that designates the amount of molecules moved and retained in the dialysate relative to the external concentration(24). The recovery depends on flow rate, membrane design (i.e. probe length and diameter, physico-chemical characteristics of the substance), and diffusion through the membrane and brain tissue (24, 41).

Concentration recovery is flow dependent and has to be established so that optimal recovery of neurotransmitters and pharmacologically active substance may be ascertained(60). From animal studies, typical flow rates are between 0.5.-10 μl/min(28, 41, 64). In human microdialysis, a perfusion flow of 0.3. μl/min seems to be adequate so that the concentration of glucose, lactate, pyruvate and glutamate in the dialysate recovered is of approximately 70% of that in the interstitial fluid(65). For sampling neurotransmitters during DBS (e.g. GABA, dopamine, glutamate and cGMP) flow rate may be relatively faster, in the order of 2 or 5 μl/min(36, 46, 63, 66, 67). The faster perfusion rate in clinical studies as compared to preclinical experiments may be related to differences in the concentration gradient of the external medium (e.g., tissue), which will partially depend on the smaller proportion of traumatic implantation site relative to the human brain volume (much larger in humans). In theory, ultra-slow and slow rates (< 0.2. microl/min) permit extensive diffusion and

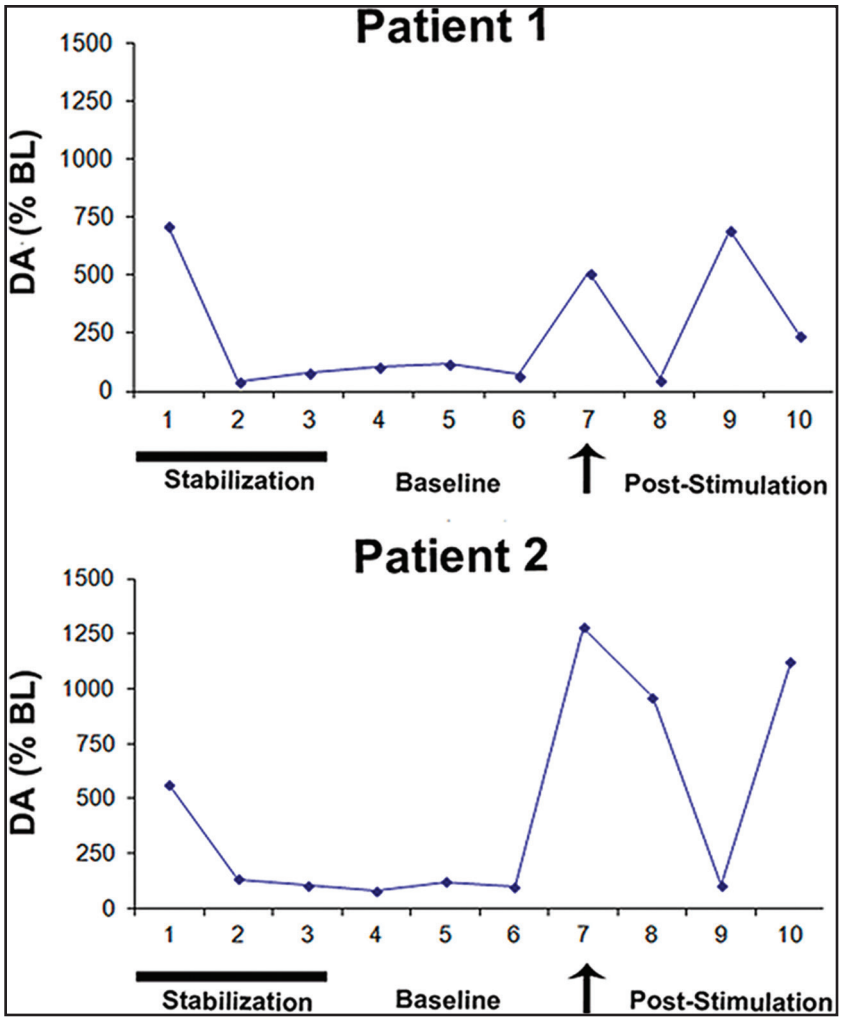

Figure 4. Example of microdialysis data collected in two patients with Parkinson's disease in our center. Dopamine levels (% of baseline) were recorded every 10 minutes during the stabilization phase (samples 1-3), at baseline (samples 4-6), during high frequency stimulation (arrows) of the globus pallidus, and after stimulation was discontinued (post-stimulation samples 8-10). Note the striking increase in DA release recorded during stimulation as well as the relatively stable recordings obtained after the first 10 minutes of stabilization(42). (Supplemental Material)

allow concentrations to reach a steady state of maximum of recovery (e.g. over 90%). This method, however, is not suitable for monitoring fast changes in the extracellular content and operates with fairly large sample volumes(68). In practice, low flow rates are responsible for 5–20% in vivo recovery, depending on the analytes(68).

Another fundamental aspect is the time resolution, which is dependent on the detection limit of the assay and the neurotransmitter recovery from the extracellular fluid(28). During deep brain stimulation, samples are collected every 10-15 min(31, 35, 46, 63, 67). The temporal resolution required will influence the choice of analytical technique(69). Flow rates that result in small and concentrated samples (10-50 μl) may be easily processed in analyses systems(28). These are continuously collected into microvials to be stored at −80 °C and analyzed as soon as possible(24). Results are generally displayed as trend curves(34). Because many factors can affect in vivo recovery, microdialysis is employed for within- and between-subject comparisons of relative changes in the extracellular concentration of the analyte that are expressed as a percentage of average basal levels(24).

7. SAMPLE ANALYSIS

One purpose of microdialysis is the quantification of small and middle-sized molecules that can diffuse throughout the membrane. This depends on the sensitivity of the chosen analytical techniques(24). Due to the clean properties of our dialysate, which is a high filtered (protein-free), low volume, aqueous solution of polar analytes, the method of choice is often liquid chromatography(69). In order to improve sensitivity and work with nanoliter sample volumes, high-pressure liquid chromatography (HPLC) with small internal

diameter columns and capillary-zone electrophoresis is routinely employed(70). The most important aspect for a successful microdialysis is the selectivity and sensitivity of the applied analytical method(69).

Analyses include neurotransmitters, amino acids, neuromodulators, such as certain peptides, drugs and their metabolites(69). Reliable measurements of extracellular levels of glutamate and GABA with microdialysis are possible, though the neuronal origin of these neurotransmitters in dialysate samples continues to be debated(71, 72). As the probe has no access to the synaptic space(73), extrasynaptic transmission may explain detectable levels of glutamate and GABA in dialysate samples(74, 75). These amino acid neurotransmitters (i.e., GABA, glutamate, aspartate) can be quantified using liquid chromatography and fluorescence or electrochemical detection after a derivatization process(28). In addition to liquid chromatography, capillary electrophoresis may be employed for the analysis of amino acid levels. This technique requires a small sample volume such as that obtained after derivatization. Derivatization is a process in which functional groups of reacting compounds are changed while the chemical structure of the compound remains the same so that they become more easily detectable for analysis. This has been currently performed for determining serotonin, norepinephrine and dopamine in microdialysis samples(76). Coupled with laser-induced fluorescence detection, capillary electrophoresis may yield high-temporal resolution(69).

Dopamine, noradrenaline, serotonin, melatonin and related metabolites can be analyzed with HPLC. This is also true for most neurotransmitters that have extracellular levels comprising a small percentage of their true extracellular concentration(69). Microdialysis of acetylcholine can be performed using a post-column enzyme-reactor. First, acetylcholine is converted into choline by acetylcholinesterase. Choline oxidase metabolizes choline generating hydrogen peroxide, which can be detected by a platinum electrode(28). Immunoassays for neuropeptides are highly sensitive(77, 78). If compounds are present in large concentrations, chromatographic separation is not necessary and the direct analysis by online biosensors can be used to record levels of molecules such as lactate and glucose(79). These compounds are clinically used for assessing the bioenergetics state of the brain and can be monitored in patients with traumatic brain injury and subarachnoid hemorrhage(25, 80). Overall, an increase in lactate and a decrease in glucose indicate a state of bioenergetics crisis in which anaerobic metabolism is active(41).

Technical improvements, such as the introduction of capillary columns and capillary electrophoresis as well as new electrochemical cell shapes, have been able to reduce sample size and improve time resolution(24, 28). However, as these variables decrease, aspects such the concentration of the analyte in the extracellular space and probe recovery need to be rethought so that sampling can occur at intervals of seconds to minutes(69). Major advances have been made in analytical chemistry in order to evaluate most small and middle-sized molecules with microdialysis.

8. CONCLUSIONS

Microdialysis is a versatile and practical method to study dynamic changes in the extracellular space of defined brain structures. During DBS, brain microdialysis may be performed with optimized concentric shaped probes with 2-3 mm long membrane and an adjusted low flow rates of perfusion. To ensure a stable trajectory, the insertion is often guided using stereotactic techniques with equilibration phase as long as possible, according to the patient condition (intensive care unit or operating room). The choice of the targets and the goal molecules (neurotransmitters, amino acids or neuromodulator) are based on the main characteristic of the brain disorder. The purpose is to evaluate biochemical findings of particular relevance for early detection of secondary damage and the evaluation of therapeutic interventions during neurosurgical procedures and in the intensive care unit. In the future, we hope that controversial issues such as those related to equipment, equilibration period, sampling and analysis may be resolved. With accumulation of experience, we expect to gain further insight into the meaning of changes detected in different brain disorders so that the real value of this technique during surgical procedures may be better ascertained.

9. ACKNOWLEDGMENTS AND DISCLOSURES

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Abbreviations: BBB: blood brain barrier; CT: computed tomography; DA: dopamine; DNPV: differential normal pulse voltammetry; DPA: differential pulse amperometry; dPPN: peduncularpontine nucleus dorsal part; EDTA: Ethylenediaminetetraacetic acid; FSCV: fast scan cyclic voltammetry; GABA: Gamma amino butyric acid; GPe: globus pallidus external part; GPi: globus pallidus internal part; HPLC: high-pressure liquid chromatography; MRI: magnetic resonance imaging; PD: Parkinson disease; SNc: substantia nigra compacta; SNr: substantia nigra reticulate; STN: subthalamic nucleus; VL: ventral lateral nucleus of thalamus; VLPPN: peduncularpontine nucleus ventrolateral part

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