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Use and Future Prospects of *In Vivo* Microdialysis for Epilepsy Studies

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Abstract

Epilepsy is a common neurological disease characterized by recurrent unpredictable seizures. For the last 30 years, microdialysis sampling has been used to measure changes in excitatory and inhibitory neurotransmitter concentrations before, during, and after seizures. These advances have fostered breakthroughs in epilepsy research by identifying neurochemical changes associated with seizures and correlating them to electrophysiological data. Recent advances in methodology may be useful in further delineating the chemical underpinnings of seizures. A new model of ictogenesis has been developed that allows greater control over the timing of seizures that are similar to spontaneous seizures. This model will facilitate making chemical measurements before and during a seizure. Recent advancements in microdialysis sampling, including the use of segmented flow, “fast” liquid chromatography (LC), and capillary electrophoresis with laser induced fluorescence (CE-LIF) have significantly improved temporal resolution to better than 1-min, which could be used to measure transient, spontaneous neurochemical changes associated with seizures. Microfabricated sampling probes that are markedly smaller than conventional probes and allow for a much greater spatial resolution have been developed. They may allow the targeting of specific brain regions important to epilepsy studies. Coupling microdialysis sampling to optogenetics and light-stimulated release of neurotransmitters may also prove useful for studying epileptic seizures.

Keywords

Microdialysis; LC-MS; CE-LIF; temporal resolution; epilepsy; seizures

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A. Brain microdialysis in Epilepsy

Many brain diseases involve abnormal concentrations of select molecules in the brain extracellular space. As such, *in vivo* measurements can be invaluable in determining the underlying causes of such diseases and how treatments modulate these causes. Microdialysis has been used for such studies of many brain disorders including Alzheimer's disease, Parkinson's disease, Huntington's disease, traumatic brain injury, stroke, schizophrenia, and depression. Our experience in epilepsy research has shown the power of this method to uncover fundamental aspects of this disease and here we review insights gained by this approach. We then discuss emerging improvements in technology that may prove useful in further study of epilepsy.

Epilepsy as an imbalanced neurotransmission between glutamatergic and GABAergic systems

Epilepsy is a chronic neurological disorder affecting over fifty million people worldwide.¹ Specifically Temporal Lobe Epilepsy (TLE) is the most common form of partial epilepsy, characterized by recurrent unpredictable seizures that often originate in the limbic system. Despite the availability of pharmacological treatment options, one third of patients do not respond to them.^{2, 3} In this sense, drug resistance is likely multifactorial in nature, and an active area of research for future therapeutics strategies.⁴ Therefore, a better understanding of the neurochemistry underlying epilepsy would be beneficial in the search for novel therapeutics.

Microdialysis has been used to measure neurochemical changes specifically before, during, and after seizures are triggered with a focus on glutamatergic, GABAergic, monoaminergic, cholinergic, and histaminergic systems. Clinical and experimental brain microdialysis studies carried out in the last three decades support the notion that an imbalance between excitatory (mainly mediated by glutamate) and inhibitory (mainly mediated by the γ -aminobutyric acid or GABA) neurotransmission is a central factor in the pathophysiology of pharmacoresistant TLE.^{5–12}

Glutamate and GABA extracellular levels as biomarkers associated with drug-resistant TLE

Glutamate is considered the major excitatory neurotransmitter in the mammalian central nervous system (CNS), and due to its important role in synaptic plasticity and activation of hippocampal neuronal pathways, its extracellular concentration has been measured under both normal and pathological conditions.^{13, 14} In refractory TLE, several clinical and experimental brain microdialysis studies have reported dysregulated glutamate levels, which may be due to an altered expression of glutamate-metabolizing enzymes favoring glutamate production^{15, 16} or to an altered glial expression of glutamate uptake transporters, leading to an overstimulation of the glutamate receptors.^{5, 17–19} In patients with TLE, the increased extracellular glutamate levels in the brain are associated with excitotoxicity,^{20, 21} increased hippocampal cellular excitability,²² higher seizure frequency, decreased hippocampal volume, and decreased neuronal cell count.^{10, 11}

Conversely, GABA is the main inhibitory neurotransmitter in the mammalian brain, and regulates neuronal excitability. Tissues from human patients and chronically epileptic rats have demonstrated some specific alterations in the GABAergic system that may lead to enhancement of excitatory signals and disruption of the neurotransmission balance. For example, inhibition can become impaired due to reduction of GABA release,²³ desensitization of post-synaptic GABA_A receptors,^{24–26} and/or loss of GABAergic neurons and interneurons.^{27–30} Unfortunately, little is known about the dynamic changes in the glutamatergic and GABAergic systems during the natural course of TLE and its progression toward refractoriness.

In humans, During and Spencer⁵ were the first to report a seizure-related increase outflow of GABA and a significant increase in glutamate extracellular concentration before and during partial seizures with secondary generalization in mesial TLE patients undergoing surgery, using bilateral intrahippocampal microdialysis and the non-epileptogenic hippocampus of each patient as control. In line with these findings, Sherwin¹⁷ and Thomas et al^{7–9} confirmed that glutamate extracellular levels are significantly augmented in the epileptogenic brain tissue under interictal and ictal conditions in epilepsy surgery patients. Moreover, the interictal extracellular glutamate concentrations in the non-epileptogenic hippocampus were considerably lower compared to those in the epileptogenic area of the evaluated patients.¹¹ In the case of GABA, only Wilson et al¹⁸ and Thomas et al⁹ supported the previously described ictal increase, whereas Pan et al²² reported a non-significantly reduced interictal GABA outflow in the epileptogenic hippocampus. Although these results indicate a strong relationship between elevated glutamate and epileptiform activity, it is evident that all these human studies lack appropriate controls and do not provide information on the dynamic changes occurring in the natural development of the disease. Interestingly, later clinical studies had considered the excessive extracellular glutamate concentrations reached between seizures and during the epileptic seizures itself as a biomarker linked to the altered expression and function of P-glycoprotein. P-glycoprotein is a transmembrane transport protein expressed in the luminal cell membrane of brain capillary endothelial cells of the blood-brain barrier whose primary function is to regulate the active uptake and expulsion of a broad range of substrates including a variety of therapeutic drugs.^{31–35} In this context, studies in human and rodent brain capillaries have revealed that glutamate can upregulate P-glycoprotein via an *N*-methyl-D-aspartic acid (NMDA) receptor/cyclooxygenase-2 signaling pathway.^{36–39} These findings might have general implications for other neurologic disorders with excessive glutamate release.

Several animal studies support the human brain microdialysis results, especially those where hippocampal glutamate levels increase after microperfusion of chemoconvulsants such as pilocarpine, picrotoxin, and 3,5-dihydrophenylglycine into the hippocampus⁴⁰ or during chronic-phase seizures following intrahippocampal kainate injection in rats.^{18, 41} However, a very small number of studies describe interictal extracellular levels of glutamate and GABA in models of epilepsy. In fact, the results obtained from these models have been more or less inconclusive to support alterations in extracellular levels of glutamate and GABA in the epileptic brain.^{18, 42–44} The lack of uniformity could be attributed in part to the fact that the epileptic animals used in those experiments were not classified as to whether they were responsive or non-responsive to antiepileptic drugs. To date, the study conducted by Luna-

Munguia et al¹² is the only report focused on characterizing the interictal and ictal extracellular levels of glutamate and GABA at the epileptic hippocampus of phenytoin-non-responsive kindled rats. This experimental model offers unique approaches to the biological basis of refractoriness, particularly because pathological alterations in such rats can be directly compared with those of rats that respond to phenytoin.⁴⁵ The results obtained by Luna-Munguia et al¹² conclude that alterations of glutamate and GABA release in the epileptic focus of non-responsive animals resemble those found in hippocampus of human patients with refractory TLE.

The monoaminergic system

In addition to the previously-described imbalance between glutamatergic and GABAergic neurotransmission, other neurotransmitter systems are known to be involved in epileptogenesis. Clinckers et al⁴⁶ reported that increased extracellular glutamate levels alone are not enough to induce seizures when administered intrahippocampally in concentrations found after limbic seizures in the pilocarpine rat model. Therefore, an extra stimulus is required for inducing seizures. Monoamines such as dopamine and serotonin are good candidates. Different types of dopamine and serotonin receptors are located on the neocortical and hippocampal glutamatergic or GABAergic nerve terminals, where they can cause a significant shift in the balance towards excitation in these networks.^{47, 48} However, previous genetic and classical pharmacological studies have described the complex neuromodulatory responses of both monoamines. In the case of dopamine, the activation of D1-like receptors usually exerts pro-epileptogenic effects, whereas D2-like receptor stimulation can block the seizure induction.^{48, 49} On the other hand, serotonin and seizure inhibition have been linked for almost six decades and through the years several studies have reported that selective serotonin reuptake inhibitors can be used as antiepileptic drugs, depletion of brain serotonin can lower seizure threshold, and the lack of 5-HT_{1A} receptors can increase lethality after kainic acid-induced seizures.^{47, 50–52}

In line with these studies, brain microdialysis experiments describing the proconvulsant and anticonvulsant effects of dopamine and serotonin based on their hippocampal extracellular levels find similar complexity. Clinckers et al⁵³ reported a complete protection against pilocarpine-induced seizures when enhanced preictal dopamine levels or serotonin levels were established (for example, with 2 nM dopamine or serotonin perfusion). Similar effects on seizure severity and hippocampal monoamine levels were obtained by intrahippocampal perfusion of selective dopamine or serotonin reuptake blockers.⁵⁴ With protective levels of either dopamine or serotonin, no significant pilocarpine-induced increases in extracellular hippocampal glutamate were observed. However, high preictal concentrations of dopamine or serotonin (10 nM) aggravated pilocarpine-induced seizures compared with control conditions and were, therefore, defined as proconvulsant concentrations. In these cases, extracellular glutamate levels were increased before, during, and after pilocarpine administration.

The cholinergic system

Although acetylcholine is the second most prevalent excitatory neurotransmitter in the brain, few microdialysis studies have focused on its extracellular levels in epilepsy. Recently,

Hillert et al⁵⁵ used the lithium-pilocarpine model to induce *status epilepticus* in young adult rats and monitor acetylcholine levels in the hippocampus both during seizures and after their termination by diazepam or ketamine. Similar to the previously report by Joep et al,⁵⁶ Hillert's brain microdialysis study confirms that acetylcholine accumulates in tissue, and describes for the first time its active release in hippocampus and striatum as long as seizures prevail. Moreover, they report that ketamine, a NMDA receptor blocker, rapidly lowers the acetylcholine release and achieves a faster seizure termination when compared to diazepam. Thus, it can be concluded that increased acetylcholine release during *status epilepticus* is correlated with epileptic activity.

The histaminergic system

The histaminergic system has also been associated with the complicated pathogenesis of epilepsy. For several years, histamine has been considered an anticonvulsive neurotransmitter as its low levels were associated with convulsions and seizures.^{57, 58} However, recent microdialysis experimental evidence shows a seizure-induced increase of histamine release, which may lead to neurotoxicity, blood-brain barrier breakdown, and brain edema.^{59, 60} The variable results between studies could be due to the inhibitory or excitatory effects of the histaminergic system, depending on the type of epilepsy and the evaluated brain region.^{61, 62} Further studies, based on the histamine H₃ receptors (H₃R) ability to modulate the release of a wide spectrum of molecules such as glutamate, GABA, dopamine, serotonin, and acetylcholine in a pathway-dependent manner,⁶³ should determine whether H₃R antagonists bear therapeutic value in epilepsy.

B. Methods development and future outlook in using microdialysis to study epilepsy

Emerging advances in epilepsy models and microdialysis methods may be expected to help elucidate causes and therapies. Here we review recent uses of new models and measurements techniques that are relevant to epilepsy.

1. A Model for Ictogenesis

Decades of research have improved our understanding of some of the physiological changes that lead to epilepsy. However, we still have a very limited understanding of the basic mechanisms of ictogenesis, the process by which an individual seizure begins. Without this understanding, epilepsy studies rely upon waiting for seizures to occur randomly, which represents an uncontrolled process. Recently a novel method of controlling ictogenesis in an *in vivo* model of TLE in rats has been developed.⁶⁴ This model is capable of increasing the risk of seizures in TLE, providing experimental control of seizure threshold via endogenous pathways that mirror normal brain activity. In this model, it is possible to increase the risk of seizures by exciting a seizure focus (the hippocampus) via physiological pathways (afferent synaptic activity from the *nucleus reuniens*). As shown in Figure 1, the seizure hazard rate (seizures per hour) is significantly increased upon the injection of KCl in the *nucleus reuniens* of the epileptic animals as opposed to PBS.⁶⁴ Combining this method with microdialysis measurements will enable exploration of the basic mechanisms of ictogenesis by searching for chemical biomarkers associated with that risk in a controlled fashion.

Future experiments are expected to be multimodal recordings, combining EEG with multiple chemical measurements.

2. Improved Temporal Resolution Microdialysis

Microdialysis has traditionally only sampled brain extracellular space at 5–10 min intervals. The limiting step for temporal resolution is usually the sensitivity of the analytical method used, i.e. enough sample must be collected to have enough material to analyze. Use of methods that allow high mass sensitivity, such as CE-LIF, can allow better temporal resolution.

The potential utility of higher temporal resolution has been revealed in a study that used CE-LIF to monitor neurochemical changes underlying 3-MPA induced seizures.^{65, 66} The changes in glutamate and GABA were measured at “fast” (60 s) and “slow” (5 min) time scales using CE-LIF.⁶⁶ Figure 2 shows the amino acid/neurotransmitter data using fast 1 min microdialysis sampling.⁶⁶ A unique trend was observed where biphasic activity was now shown with glutamate. Within the 5–25 min timeframe, glutamate markedly spiked and again at 40–65 min. There was also a sustained increase in glutamate during the final 30 min of the experiment. The 60 s sampling showed that 3-MPA caused glutamate changes that were bimodal, which was not apparent with the “slow” 5 min sampling timescale. For the first time, a relatively high temporal resolution had been achieved with *in vivo* microdialysis sampling during *status epilepticus*, which could provide novel insight into the unpredictable and spontaneous seizures caused by epilepsy.

These results show that improved temporal resolution can reveal better detail on the chemical changes underlying seizures. Presumably other studies, such as changes leading up to spontaneous seizures or even higher resolution changes (second by second) could reveal more about the patterns of neurochemistry. Recent advances in technology may allow such measurements.

Fast LC—Because natural spontaneous seizures occur randomly, a method that offers continuous high resolution methods (as opposed to collecting fractions for a limited amount of time) would be useful. Recently some groups have reported the development of advanced HPLC methods coupled to microdialysis that allow up to 1 min temporal resolution for certain monoamines with continuous, on-line operation.^{67–70} These methods involve careful optimization of the HPLC flow paths and high sensitivity electrochemical or photoelectrochemical detectors. The novel methods have been used to monitor dopamine in the rat striatum during the delivery of potassium and nomifensine in live freely behaving animals.⁶⁸ Having achieved a 1 min-temporal resolution, it was possible to determine the pharmacokinetics of locally delivering nomifensine and also the use of dexamethasone to increase basal levels of dopamine.⁶⁸

An important feature of these methods is that they allow continuous operation so it is possible to detect unpredicted and spontaneous chemical transients. For example, a recording from SERT+/+ mice revealed large, spontaneous surges in serotonin levels during the first half of the dark phase (Figure 4).⁷⁰ The spontaneous spiking in serotonin levels associated with the light-dark cycle by continuous sampling of dialysate at 3 min intervals

for 20 h and throughout the dark phase. This “fast microdialysis technique” was also used to measure serotonin transporter (SERT) and sex-mediated differences in stimulated serotonin. The novel development of fast microdialysis, when combined with potassium stimulation, showed that differences in extracellular serotonin levels that were associated with normal hormonal cycles and pharmacologic vs. genetic loss of the function of the serotonin transporter.

3. Droplets and Fast CE—Other technology is emerging to allow temporal resolution of a few seconds.^{71–74} Such high temporal resolution could be useful for better defining the chemical dynamics that drive seizures. If sufficient sensitivity is available, a technical challenge of collecting samples at a few seconds intervals, corresponding to nanoliter volumes if the microdialysis perfusion rate is 1 $\mu\text{L}/\text{min}$, must be met. One way to collect such small samples is by segmented flow wherein sample is interspersed with an immiscible fluid to create an array of small samples.⁷¹

Droplet-based techniques have significantly increased the temporal resolution of microdialysis. Applications of the concept have been shown where a tee channel on a microfabricated PDMS chip with an immiscible carrier phase (perfluorodecalin/PFD) was used to segment dialysate into nanoliter plugs.⁷⁵ The temporal resolution of 15 s was measured by the fluorescence of individual sample plugs as opposed to a temporal resolution of 25 to 160 s without the sample plugs.

The segmented flow microdialysis approach was also utilized to measure glucose in anesthetized rats illustrating its potential utility for *in vivo* monitoring with high temporal resolution. The technique was further developed where a microdialysis probe was integrated with a PDMS chip that merged dialysate and fluorescent reagent that segmented the flow into plugs.⁷⁴ The plugs then flowed to a glass stream where they were analyzed via electrophoresis with fluorescence detection. The 35 s temporal resolution was independent of the distance between analysis and sampling. The system allowed for the measurements of six amino acids and neurochemical dynamics upon infusing glutamate into the striatum of anesthetized rats.

Further pushing the boundaries of temporal resolution, electrospray ionization mass spectrometry (ESI-MS) was coupled with droplet microdialysis for *in vivo* acetylcholine monitoring for a 5 s temporal resolution.⁷³ Furthermore, 2 s temporal resolution was achieved when 2 nL microdialysate fractions were collected in plugs for off-line *in vivo* chemical monitoring.⁷⁶ This method was then applied for the collection and analysis of nanoliter microdialysis samples from awake animals *in vivo*.⁷² Droplet-based sampling techniques would be ideal to increase the temporal resolution of microdialysis sampling to the sub-min timescale to measure neurochemical fluxes during random, spontaneous seizures.

3. Miniaturized Sampling Probes by Microfabrication

To make neurochemical measurements with microdialysis during epileptic seizures, specific brain regions must be monitored such as the hippocampus and nucleus reuniens. Conventional microdialysis probes are too large to solely target these brain regions. The

large size also causes tissue damage that might also confound results in some cases. Recent efforts to miniaturize the probes have been made. However, there is a requirement to obtain chemical information from small volumes of the central nervous system (CNS) without sacrificing neurochemical recovery. Such information can be obtained by push-pull perfusion sampling. This method utilizes 10–50 nL/min flow rates to minimize tissue damage with up to 80% *in vitro* recoveries.⁷⁷ The spatial resolution of the probes was illustrated as they were able to measure chemical gradients across small brain nuclei.⁷⁸ It has also been shown that such probes generate lower tissue damage than conventional microdialysis probes and had minimal tissue damage.⁷⁹ The utility of such probes for behavioral studies was shown in a study the recorded glutamate surges in the rat lateral hypothalamus during feeding.⁸⁰ Ultimately it may be possible to couple the small probes with high temporal resolution monitoring.^{81,82}

Microfabrication of push-pull perfusion probes in silicon has led to further miniaturization, which could be applicable to epilepsy studies.⁸³ The probes contained two 20 μ M channels with ports where the fluid (buffer/dialysate) is either injected or collected as shown in Figure 3. The utility of these low-flow push-pull sampling probes was illustrated by sampling the brain of live anesthetized rats. Sampling occurred at 50 nL/min in the striatum of live rats and 17 neurotransmitters and their metabolites were detected from the push-pull perfusate using liquid chromatography-mass spectrometry (LC-MS). It has also been possible to embed a membrane into a silicon probe so that microdialysis sampling is possible from a similar sized probe.⁸⁴ These microfabricated microdialysis probes were found to perform comparably to conventional microdialysis probes in response to dynamic *in vivo* chemical changes in response to amphetamine administration to an anesthetized animal with 14 neurochemicals being detected consistently in 20 min fractions. The probes have a 79% smaller cross-sectional areas than conventional microdialysis probes, which allows for their use in smaller animal models and brain regions. The use of these microfabricated probes for high spatial resolution measurements targeting small brain regions such as the hypothalamus and nucleus reuniens would be ideal for studying epilepsy by measuring neurochemical changes caused by seizures with microdialysis sampling.

3. Incorporating Optogenetics with Microdialysis Sampling and Applications for Epilepsy

Optogenetics is another technology that will have use in epilepsy studies. Optogenetics⁸⁵ uses light to control neurons that have been genetically modified to express light sensitive ion channels, which may be used to study models of epilepsy. In epilepsy, optogenetics has become an important tool to mute overactive neurons, while also stimulating other neurons to controllably start seizures.⁸⁶ A recent study reported the arrest of spontaneous seizures using a real-time, closed-loop, response system and *in vivo* optogenetics in a mouse model of temporal lobe epilepsy.⁸⁷ Either optogenetic inhibition of excitatory principal cells, or activation of a subpopulation of GABAergic cells in hippocampal neurons halts seizures rapidly upon light application. Furthermore, photostimulation of glutamatergic neurons triggers seizure-like activity in the presence of an intact GABAergic transmission.⁸⁸ This could allow for a controllable method for both inducing and alleviating epileptic seizures.

The integration of optogenetics with microdialysis sampling, however, has been rather limited in scope as opposed to integration with electrophysiology and behavioral changes observed during light stimulation.⁸⁹ Some challenges to optogenetic neurochemical monitoring include the expertise in neurochemical techniques, the availability of analytical methods enabling high sampling rates, and implementing controls to determine the effects of laser light or heat on non-targeted endogenous ion-channels or biochemicals.⁸⁹ These advances will enable a more thorough understanding of underlying neural circuits and their effect on behavior such as in epilepsy. A few examples of integrating microdialysis and optogenetics include tuning arousal of the locus coeruleus, which has caused reversible behavioral arrests that suggests that it is finely tuned to regulate organismal arousal.⁹⁰ Figure 5 shows the novel combined optogenetic microdialysis probe used for these experiments.⁹¹ The use of a combined microdialysis-optogenetics probe would be ideal for neurochemical monitoring during optogenetic neuronal stimulation for epilepsy studies to measure neurochemical changes specifically during, before, and after controlled epileptic seizures. This would allow for the correlation of neurochemical biomarkers to epileptic seizures and complex behavioral states.

Conclusions

This review has summarized the use of microdialysis for the *in vivo* neurochemical monitoring of several neurotransmitters before and during epileptic seizures. A technical limitation of studying epilepsy is that seizures arise unpredictably so it is difficult to monitor the spontaneous chemical changes before and during the seizure. It is complicated to do these studies because there is no clear way to control ictogenesis. The new ictogenesis model and use of optogenetics to control excitability of neurons are two ways to alleviate this problem. Coupling these models with microdialysis may prove fruitful. Similarly, continuous, on-line HPLC methods may allow detection of neurochemical activity associated with a spontaneous seizure. Finally, improvements in smaller probes and better temporal resolution may allow more refined examination of neurochemistry *in vivo* during seizures.

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Figure 1.

Time-dependent seizure hazard rates. The KCl solution injection significantly increases the seizure hazard rate immediately after the first injection was administered (time 0), more than doubling the risk of seizures during the experiment. In contrast, PBS injections had no effect on seizure risk. Dotted lines indicate the average risk across all animals before and during the injections. Figure is reproduced with permission from reference.⁶⁴

Figure 2.

Glutamate and GABA data from 60 s microdialysis sampling. Percent change in glutamate and GABA upon the administration of 3-MPA over the time course of the experiment ($n = 3$ rats). The box marks the time (20–52 min) during which a steady-state concentration of 3-MPA was achieved in each sampled region. The arrow marks the time at which the infusion was stopped. Figure is reproduced with permission.⁶⁶

Figure 3.

Layout of probe with scanning electron micrographs of different sections. Drawing at right shows the probe layout. Dark lines in interior are buried channels with 20 μm diameter. (a) Port where connection is made to capillaries. Drawing shows close view, and micrograph shows cross section from a probe that was broken to show the buried channel. (Probes had three ports. Two were in use for these designs; the third is available for a possible third fluidic arm but was not used in this work). (b) Micrograph of channel after backside etching to reveal the channels. Slight defect is due to partial unfinished backside etching. (c) Close up drawing and micrograph of the sampling orifice. Figure is reproduced with permission from reference.⁸³

Figure 4.

Reduced SERT expression produces different patterns of spontaneous oscillation in extracellular serotonin levels during the dark phase. Overlay of typical examples from mice. Spontaneous serotonin surges during the dark phase were large in SERT^{+/+} mice (solid circles, approximately 1500 times the basal level), while SERT^{-/-} mice (open circles) did not show surges but rather serotonin fluctuated around basal levels. Spontaneous serotonin surges during the dark phase in SERT^{+/-} mice (half-filled circles) fell between those of the SERT^{+/+} and SERT^{-/-} mice. There was usually only one large serotonin surge in SERT^{+/+} mice during ZT13–18, while multiple intermediate-sized serotonin surges occurred in SERT^{+/-} mice throughout the dark phase, with more changes tending to cluster during ZT13–18. Typical examples of circadian serotonin surges (black symbols) measured using 3 min sampling. Figure is reproduced with permission from reference.⁷⁰

Figure 5.

Optogenetic-microdialysis probe. A. Light dispersion on a 6 μm diamond-coated film from typical optical fiber used for optogenetic stimulation (albeit one-half the diameter). B. Light dispersion of the same type of fiber with a conical sculpted tip. C. Light dispersion of the fully assembled optogenetic-microdialysis probe. D. Magnification of the probe tip, showing the conical sculpted optical fiber and the dialysis inlet tips surrounded by dialysis membrane and epoxy seal at the bottom. Figure is reproduced with permission from reference⁹¹.