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Technical Note

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Localized Drug Application and Sub-Second Voltammetric Dopamine Release Measurements in a Brain Slice Perfusion Device

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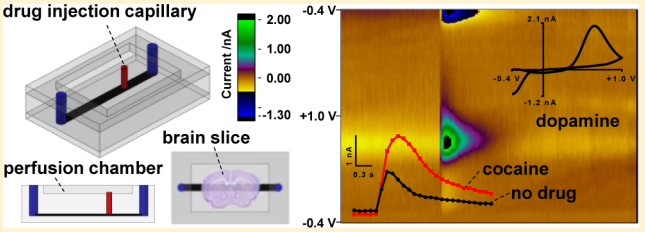
Supporting Information

ABSTRACT: The use of fast scan cyclic voltammetry (FSCV) to measure the release and uptake of dopamine (DA) as well as other biogenic molecules in viable brain tissue slices has gained popularity over the last 2 decades. Brain slices have the advantage of maintaining the functional three-dimensional architecture of the neuronal network while also allowing researchers to obtain multiple sets of measurements from a single animal. In this work, we describe a simple, easy-to-fabricate perfusion device designed to focally deliver pharmacological agents to brain slices. The device incorporates a microfluidic channel that runs under the perfusion bath and a microcapillary that supplies fluid from this channel up to the slice. We measured electrically evoked DA release in brain slices before and after the administration of two dopaminergic stimulants, cocaine and GBR-12909. Measurements were collected at two locations, one directly over and the other 500 μm away from the capillary opening. Using this approach, the controlled delivery of drugs to a confined region of the brain slice and the application of this chamber to FSCV measurements, were demonstrated. Moreover, the consumption of drugs was reduced to tens of microliters, which is thousands of times less than traditional perfusion methods. We expect that this simply fabricated device will be useful in providing spatially resolved delivery of drugs with minimum consumption for voltammetric and electrophysiological studies of a variety of biological tissues both in vitro and ex vivo.



Fast-scan cyclic voltammetry (FSCV) at carbon-fiber microelectrodes[1−3] is commonly used for the subsecond measurement of dopamine (DA) as well as other electroactive neurotransmitters and neuromodulators in a variety of model biological systems, including cultured cells,[4−6] anesthetized and awake rodents,[7−12] and viable brain tissue slices.[5,13−20] Of these systems, brain slices have the advantage of allowing investigators to obtain measurements from multiple preparations, taken from a single animal, while also maintaining the native three-dimensional architecture of the neuronal tissue. Usually, the viability of these brain slices is maintained by bathing the slices in continuously flowing oxygenated artificial cerebrospinal fluid (aCSF) in a perfusion chamber. Although specific conditions may differ, typical flow rates required to maintain slice viability are on the order of 1−2 mL min^{-1}.[21]

Oftentimes, it is necessary to introduce pharmacological agents in order to study specific mechanisms that govern neurotransmitter release and uptake. For example, cocaine, which competitively inhibits DA uptake[22] and causes the DA reserve pool to be mobilized,[23] has frequently been introduced to slices by switching a three-way valve from a supply reservoir containing aCSF solution without drug to a reservoir containing aCSF with drug. Although this method has the advantage of simplicity, it also may have complications. First, drugs applied in this way must be dissolved in the aCSF perfusate, which is continuously oxygenated. These conditions may limit the ability of researchers to use compounds that are sensitive to oxygen-rich environments. Additionally, since dispersion and diffusion exist in the flow, the exact amount of time for the drug to reach sufficient concentrations to act on the slice remains unknown, and a few minutes are needed for the bath to reach a steady state with a uniform drug concentration. The entire slice is then treated continuously in the bath of drug, leading to a lack of both spatial and temporal resolution when applying drugs. Moreover, with the high flow rates required to maintain slice viability, hundreds of milliliters of pharmacological solution could be consumed in a single slice experiment, presenting a problem when only small quantities of drug are available to carry out selected experiments. Thus, certain experiments that make use of expensive drugs or custom-synthesized compounds may be rendered cost- or time-prohibitive. Therefore, developing a simple perfusion system that would accommodate low flow rates of solution delivered at spatially defined locations would be useful in such cases.

Delivery through microfluidic channels[24,25] has the potential to provide good spatial resolution when applied to a perfusion chamber. The inherent properties of using a low flow system for this purpose have the advantage of decreasing fluid consumption from hundreds of milliliters to just a few

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microliters. Additionally, one small area of the slice could be exposed to pharmacological agents, while another area that is analyzed in the same experiment remains drug naive. For the conduct of FSCV experiments, such a system would optimally apply compound from the bottom of the slice, compared to placing microfluidic probes[26,27] or pipettes[28] above the slice, where the chemicals could be diluted by the massive aCSF perfusion flow. Moreover, delivering drugs from the bottom is preferable also because it will not interfere with electrodes or block the microscopic view when using an upright microscope. Such a strategy has been realized previously by attaching an add-on bottom[29] to a conventional perfusion chamber or by fabricating the micropost array,[30] injection and suction ports,[31] or stencil and microporous membrane,[32] in multilayer microfluidic devices to physically support and administer the brain slice on the top from bottom microchannels. The construction of all these unique devices, however, requires additional, sophisticated photolithographic procedures. Moreover, the delivery of drugs by these methods has been demonstrated in only a short-period (less than a minute),[29,31,32] or in a larger rectangular area across the entire slice immersed in a laminar flow.[30] Technically and ideally, the techniques developed by Tang et al.[31] and Scott et al.[32] were able to deliver drugs to a slice for a relatively long time, but they substantially relied on the stability of two competing pump flows and the pure diffusion of chemicals. The latter would lead to large variations in the temporal resolution, ranging from seconds to minutes, based on the different diffusion coefficients of drugs. Also, to our knowledge, the use of these devices with FSCV has not been published in the literature.

Another useful drug delivery approach is iontophoresis,[33] a method that relies on the application of an electrical current to eject charged chemical species from a micropipet barrel. Iontophoresis has previously been combined with FSCV measurements by using a multibarrel probe integrated with a carbon-fiber microelectrode and fluid delivery barrels.[34] This technique is simple in that it does not involve any micromachining technologies. Moreover, iontophoresis has the advantage of minimized tissue disruption compared to pressure ejection.[35] However, this technique requires specialized electronic equipment to deliver current to the barrels in order to eject chemicals. Corrections, applied by interrogating the flow profile of electroosmotic flow (EOF) markers, are required for accurate ejections of given amounts of drugs.[34,36] In addition, by introducing an external electric field to drive the flow, hydrogen peroxide, formed by the electrolysis of water, may interfere with the voltammetric detection of DA.[37]

Herein, we introduce a simple method to make a through hole in a three-layer microfluidic device to pump chemicals from a bottom channel to a brain slice in the top perfusion chamber. Our collective approach differs from other reported microfluidic delivery chambers in several key respects. First, since the through-hole is made of replaceable fused silica capillary, various diameters necessary to provide desired spatiotemporal characteristics of agent delivery can be used. Moreover, the use of a syringe pump to deliver the microfluidic flow eliminates the need for extra electrical sources to charge and drive the EOF, and long periods of continuous pumping can be accommodated. Finally, the open-face design allows an unobstructed microscopic view and facilitates the easy manipulation of multiple electrodes to obtain FSCV measurements of DA release and uptake. For proof of concept, we tested our device by comparing DA release before and after the administration of GBR-12909 and cocaine. These drugs were successfully administered through the injection capillary, as indicated by the change in DA release and uptake characteristics. Moreover, the ability of this method to apply pharmacological agents at spatially resolved points within brain slices was demonstrated.

EXPERIMENTAL SECTION

Chemicals and Animal Procedures. The procedures are described in the Supporting Information. Cocaine was purchased from Sigma-Aldrich through a permit granted by the United States Drug Enforcement Administration (DEA).

Brain Slice Preparation. Coronal brain slices, 300 μm thick and having approximate dimensions of 0.8 cm (dorsal to ventral) and 0.5 cm (lateral), were harvested from C57BL/6 mice (Charles River, Wilmington, MA) using published procedures,[18] which are described in the Supporting Information. A slice was immobilized on top of the injection capillary by a harp slice anchor (Warner Instruments, LLC, Hamden, CT) and maintained in the perfusion chamber (Figure 1D) with continuously oxygenated aCSF at a flow rate of 2 mL min^{-1}. Slices were equilibrated for at least 60 min in the chamber prior to obtaining electrochemical recordings.

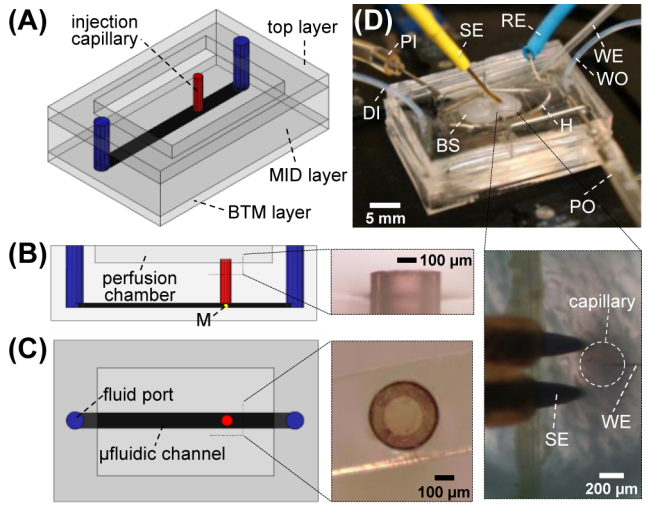

Figure 1. Design and setup of the perfusion device for the detection of DA release in a brain slice with FSCV. Schematic drawing of (A) isometric, (B) side, and (C) top views of the device. The blow-up images of parts B and C show the same injection capillary connected to the bottom microfluidic channel and top perfusion chamber. (D) Image of the experimental setup, and a close view of the injection capillary under the brain slice and electrodes on top of the capillary. The position of the capillary under the slice is indicated by the white dashed circle. Abbreviations: BS, brain slice; H, harp; WE, working electrode; SE, stimulating electrodes; RE, reference electrode; PI, perfusion inlet; PO, perfusion outlet; DI, drug inlet; WO, waste outlet.

FSCV in Brain Slices. Previously described procedures were used to fabricate 7 μm diameter carbon-fiber microelectrodes (Goodfellow Cambridge Ltd., Huntingdon, U.K.).[20] The following waveform was applied to the electrode every 100 ms: −0.4 V increasing to +1.0 V then decreasing to −0.4 V (versus Ag/AgCl reference electrode) at a constant scan rate of 300 V s^{-1}. Color plots, in which cyclic voltammograms were unfolded, stacked, and color-coded for current, were generated for each recording. Data were sampled on each color plot at the

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potential that provided the greatest DA oxidation current (~0.6 V). The carbon-fiber microelectrode was positioned 120 μm below the surface of the dorsolateral striatum, between two stimulation electrodes (Plastics One, Roanoke, VA) separated by a distance of ~400 μm. A single, biphasic electrical stimulus pulse, with a total duration of 4 ms and current of 350 μA, was used to evoke the release of DA. Before applying drugs, electrically evoked DA release was measured every 5 min until the magnitude of DA release was constant between measurements. During drug treatment, stimulated DA release was also measured every 5 min. After use, carbon-fiber electrodes were calibrated by injection of a 2 μM standard solution in a flow cell. Three of these measurements were averaged and used as a calibration factor to calculate the concentration of DA released.

Fabrication of Perfusion Device. The tissue perfusion device, with a microdelivery capillary, was fabricated following standard photolithography procedures.[38] The device consists of three polydimethylsiloxane (PDMS) layers, which were made by pouring the mixtures of degassed PDMS and curing agent with varying mass ratio (mr) onto the molds with subsequent baking at 70 °C for 1 h. The top layer (~3 mm thick, mr = 20:1) has an opened rectangular chamber (0.9 cm wide and 1.8 cm long) for the continuous perfusion of aCSF. The bottom layer (~1 mm thick, mr = 20:1) was embossed with a channel, having cross-sectional dimensions of 500 μm wide by 160 μm deep, for introduction of pharmacological agents to brain slices. The middle layer (~5 mm thick, mr = 5:1) contains a through hole to connect the channel at the bottom and the brain slice on top. A rough hole was made first by punching the PDMS layer through with a 0.35 mm i.d. puncher (Harris Micro-Punch, Whatman, Maidstone, U.K.). To produce a smooth inner surface of the hole, a short fused silica capillary (360 μm o.d., 200 μm i.d., Molex, Lisle, IL) was inserted into the rough hole. One end of the capillary was leveled with the bottom of the middle layer (i.e., the roof of the bottom layer); the other end was 100–150 μm taller than the top surface of the middle layer to support the brain slice in the top chamber (seeing Figure 1B). Both ends of the capillary were ground and polished on a micropipet beveler (BV-10, Sutter Instrument Co., Novato, CA) to make them flat before inserting into the device. The partially cured top and middle layers were bonded together first after another 30 min baking at 70 °C and then access holes for the channel were punched. Under a stereo microscope, the two-layer was aligned and permanently bonded to the bottom layer and formed the final microcapillary perfusion device (Figure 1D). The perfusion device could be reused by cleaning thoroughly with DI water and isopropanol after each experiment. All the data collected in this work were obtained using the same device.

RESULTS AND DISCUSSION

Perfusion Device Design and Flow Evaluation. In order to administer pharmacological agents in a temporally- and spatially defined manner to brain slices through the embedded capillary, it is critical to know how long it takes the solution to travel from the point where the drug was loaded to the opening of the injection capillary. Here, we used a short air plug flowing ahead of the drug to locate its front end in the microfluidic device. To prevent all undesired fluids (e.g., aCSF and air) from entering the injection capillary, we designed the device with a lower flow resistance from M (as indicated in Figure 1B) to the waste outlet (WO) than M to the outlet of the injection capillary. Therefore, the fluid supplied prior to drug injection will preferably flow to the WO. The hydrodynamic resistances of these two flow paths were calculated in the Supporting Information.

To visually demonstrate the ability to introduce solutions containing chemicals and pharmacologically active compounds into this device, red and blue dyes were sequentially injected into the device. The injection process is illustrated in Figure 2A–D (top view) as well as in Figure S1 (side view) and Movie

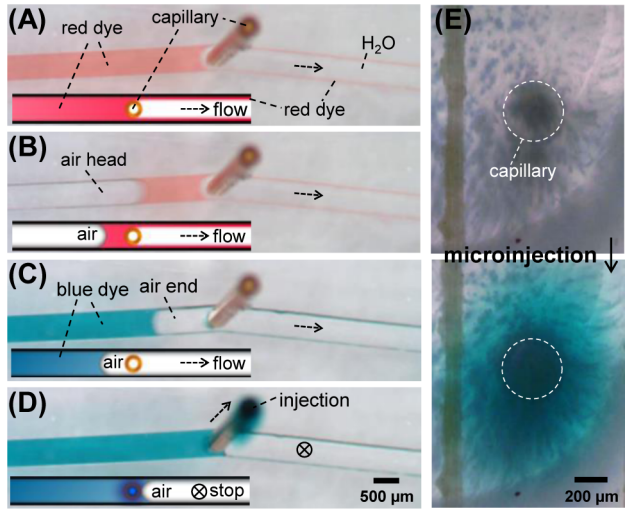

Figure 2. Demonstration of microfluidic drug injection into a brain slice using blue dye to represent the drug and red dye to represent aCSF. (A–D) Snapshots of a representative injection without the brain slice. The inlets show the schematic illustration of the injection process. (A) The leading red dye merges with H_2O and forms a laminar flow. (B) The front of air spacer is approaching the capillary. (C) The end of the air spacer is passing by the injection capillary and carrying blue dye to the injection spot. (D) Blue dye is injected from the bottom channel to the top chamber through the embedded capillary after the air passed by the capillary and the microfluidic channel was blocked. (E) Representative microinjection of blue dye to a brain slice resting on top of the capillary. The white dashed circles indicate the locations of the injection capillary under the slice. The pump flow rates are 5 μL min^{-1}.

S1 in the Supporting Information. At the beginning of the experiment, water was perfused through the top chamber at a constant flow rate of 2 mL min^{-1}. Next, all of the flow paths and connecting tubes were filled with red dye. Prior to injecting blue dye, an air plug (<1 cm in length) was introduced into the tube in order to separate the red and blue dyes and to index the position of blue dye flowing through the device. We also lowered WO and left the waste vial 12 cm below the perfusion chamber to increase the resistance from M to the capillary outlet and decrease the resistance from M to WO. Thus, after we started the pump (CMA 402, CMA Microdialysis, Kista, Sweden), the red dye would merge at point M with the water flow induced by the difference of liquid (water) levels between perfusion chamber and waste vial and lead the air plug continuing to flow in the bottom channel, with lower resistance, directly to the waste vial (Figure 2A–C). As the air plug passed M, we immediately blocked the WO by applying a clamp at WO downstream and the following blue dye was therefore being pumped to the top chamber in about a second through the embedded capillary, which was the only outlet remaining for the fluid inside the device to escape, as shown in Figure 2D and Movie S1 in the Supporting Information. We defined the

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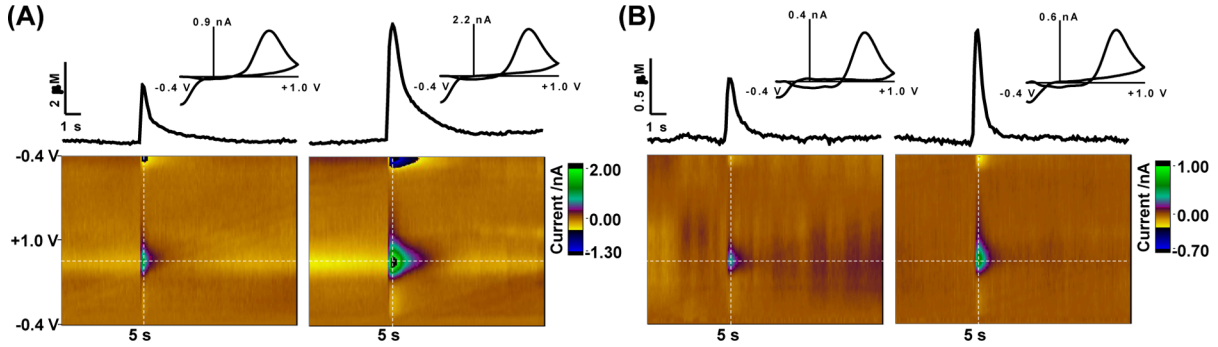

Figure 3. Comparison of responses of electrically evoked DA before and after drug administration. Dopamine release was measured at a single point directly above the microcapillary opening. Representative color plots (bottom) shown with current–time plots (top) sampled at the horizontal white dashed lines on the color plots and cyclic voltammograms (insets) sampled at the vertical white dashed lines on the color plots. Each data set is 15 s in duration. (A) Evoked DA release at 5 min before (left) and 5 min after (right) the administration of 5 μM cocaine. (B) Evoked DA release at 5 min before (left) and 45 min (right) after the administration of 1 μM GBR-12909. Single electrical stimulus pulses were applied at 5 s. Drugs were constantly delivered to the slices at a flow rate of 0.5 μL min^{-1}.

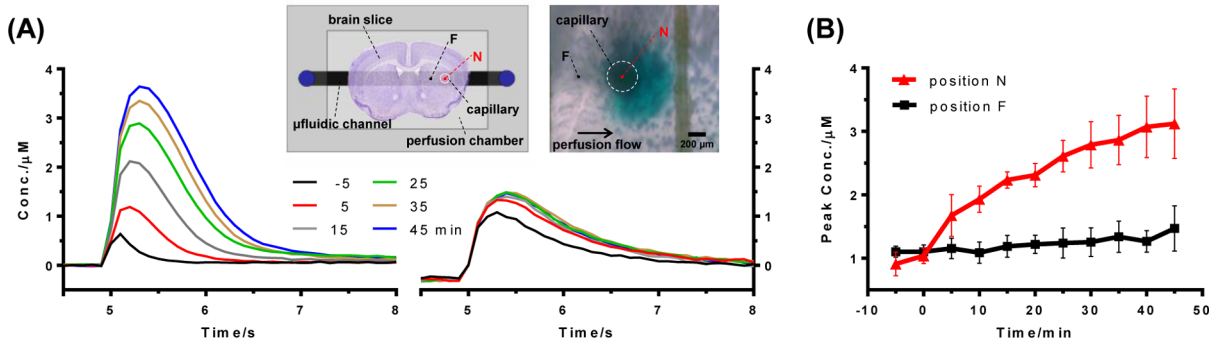

Figure 4. Spatially resolved drug delivery by microcapillary injection. (A) Two representative plots showing the changes in DA release over the course of the administration time: left plot, detected at position N (right on top of the injection capillary); right plot, detected at position F (500 μm away from the capillary). These two distinct spots on brain slices are indicated in the top insets. The snapshot showing blue dye injected to the slice at 0.5 μL min^{-1} with continuous aCSF flowing in perfusion chamber at 2 mL min^{-1}. The white dashed circles indicate the location of the injection capillary underneath the slice. (B) Comparison of peak DA concentrations evoked at spots N (red triangle) and F (black square) with continuous treatment of 5 μM cocaine feeding at 0.5 μL min^{-1}. There is a significant difference in response between these two locations ($p < 0.001$, two-way ANOVA, $F_{1,92} = 115$, $n = 5$).

moment when the blue dye was about to be injected as time zero, i.e., the moment we blocked the WO and the air plug was immobilized. Images in Figure 2E show blue dye diffusing in a brain slice 5 s after injection.

Delivery of DA Uptake Inhibitors through an Embedded Capillary. Following the aforementioned flow evaluations, we tested our device on freshly harvested, functioning brain tissue slices. FSCV was used to measure DA release and uptake in the slice in response to electrical stimulation. DA release measurements were also carried out before and after exposure to cocaine and GBR-12909 (Figure 3). Our initial results have demonstrated that DA release occurs normally in brain slices even after 6 h in the perfusion chamber; therefore, our device has allowed the slices to remain properly oxygenated and viable.

To supply either cocaine or GBR-12909 to the slice, the drug was pumped through the interior channel, thereby allowing drug to flow through the injection capillary at the bottom of a slice after the WO was blocked. The two drugs were separately applied to freshly harvested brain slices while measuring stimulated DA release every 5 min. Upon exposure to cocaine, an almost immediate increase in extracellular DA concentration was noted. As shown in Figure 3A, a 1.86 ± 0.27-fold ($n = 5$) increase of DA concentration was observed after only a 5 min treatment with 5 μM cocaine. Two factors likely contribute to the dramatic increase in DA levels measured after electrical stimulation: (1) inhibition of DA clearance by competitive inhibition of the dopamine transporter[22] and (2) mobilization of the DA reserve pool.[23] GBR-12909 selectively inhibits DA uptake and binds with greater affinity to the dopamine transporter than cocaine.[39] Similar to the results found upon cocaine administration, the application of GBR-12909 through the capillary resulted in a dramatic increase of DA concentration following electrical stimulation, shown in Figure 3B (1.93 ± 0.09-fold, $n = 2$). Importantly, the flow rate through the channel used for these experiments was 0.5 μL min^{-1}, compared to the perfusion flow rate of 2 mL min^{-1}. Thus, our device is able to effectively deliver compounds in an efficient manner, using far less quantities compared to traditional bath perfusion.

Spatially Resolved Drug Delivery. When using the traditional bath application to treat brain slices with pharmacological agents, a substantial amount of drug is required and the entire slice is exposed to the drug. The latter issue presents a problem when the goal is to study drug-exposed and drug-naive areas of the same slice. The inner diameter of the injection capillary is small (200 μm); therefore, it is possible in this case that the drug would affect only a

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limited area of the slice around the injection spot. In order to investigate this phenomenon, we monitored evoked DA release from a location directly above the injection capillary (position N) and a location 500 μm upstream of the capillary (position F). As we observed in the inset image of Figure 4A, right after the blue dye was injected from the capillary at a slow rate (0.5 μL min^{-1}), it was immediately pushed downstream by the aCSF flow (2 mL min^{-1}). Thus, we anticipated the drugs delivered to the slices through the tiny opening of the capillary would only affect a limited area around position N and not the tissue at F upstream. These two locations were independently tested on 10 brain slices, 5 slices for each position. Cocaine (5 μM) was continuously delivered to the slices through the injection capillary at 0.5 μL min^{-1}. As shown in Figure 4B, at position N, the extracellular DA concentrations gradually increased over time under continuous exposure to the drug through the capillary, while at position F, the DA levels remained almost constant. Thus, this microfluidic injection method is able to focally deliver pharmacological agents to a confined region of brain slice without influencing the neighboring area. The attenuated response to cocaine is no doubt a result of the negligible amount of cocaine at point F resulting from diffusion from the injection site as well as the drug being carried away downstream.[40] Of significance, only ~22.5 μL of pharmacological solution was consumed in a 45 min experiment. Using the traditional perfusion method, 45–90 mL of solution would be required; therefore, the capillary application method represents a 2 000–4 000-fold reduction in chemical consumption.

CONCLUSIONS

In this paper, a microfluidic capillary injection method for the pharmacological treatment of a viable brain slice was demonstrated. FSCV was used to measure electrically stimulated DA release before and after drug administration at two adjacent locations on separated slices. This method of application provided enough spatial resolution to measure DA release in regions of the slice exposed and not exposed to drug. Although the limits of spatial resolution were not explored, we speculate that a higher resolution could be attained if a thinner injection capillary (e.g., 10–50 μm i.d.) was incorporated in this device. By using current microfluidic injection techniques, only microliters of drug were consumed in a 45 min experiment, which is a three-order-of-magnitude reduction in volume consumption compared to the traditional perfusion method. This device is easily reusable and allows the analyses of multiple slices in multiple different assays carried out over weeks or months. Moreover, this device was intended for use in measuring electrically evoked DA release in brain slices by FSCV, but the open-face design is amenable to the application of other experiments, such as electrophysiology and imaging. Additionally, it is possible to incorporate multiple injection capillaries in order to monitor multiple regions on single slices simultaneously or to investigate drug interactions. This methodology could also easily be expanded to analyze other biological tissues, further broadening the applicability of this device for lab-on-a-chip research.[41−43]

ASSOCIATED CONTENT

Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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