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Minimizing tissue damage in electroosmotic sampling

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Abstract

Electroosmotic sampling is a potentially powerful method for pulling extracellular fluid into a fused-silica capillary in contact with the surface of tissue. An electric field is created in tissue by passing current through an electrolyte-filled capillary and then through the tissue. The resulting field acts on the counter ions to the surface charges in the extracellular space to create electroosmotic fluid flow within the extracellular space of a tissue. Part of the development of this approach is to define conditions under which electroosmotic sampling minimizes damage to the tissue, in this case organotypic hippocampal slice cultures (OHSCs). We have assessed tissue damage by measuring fluorescence resulting from exposing sampled tissue to propidium iodide solution 16 – 24 h after sampling. Sampling has been carried out with a variety of capillary diameters, capillary tip-tissue distance, and applied voltages. Tissue damage is negligible when the power (current $\times$ potential drop) created in the tissue is less than 120 μ W. In practical terms, smaller capillary IDs, lower voltages, and greater tissue to capillary distances lead to lower power.

Driven by the need to understand the chemistry of life processes better, the analysis of the extracellular space of brain tissue has emerged as an invaluable window into the mechanisms of signal transduction and processing of neuroactive substances in the brain. Methods such as push-pull perfusion¹, microdialysis², and direct sampling³ have previously been developed to sample and control the environment in the extracellular space *in vitro* (cultures and slices) and *in vivo*. Each method has been significantly improved over its first introduction and major advances in the detection of neurotransmitters have occurred over the past decade⁴. Of particular note is the reduction in the damaging nature of early push-pull methods. The push-pull method was altered for compatibility with lower flow rates (10–50 nL/min) in order to minimize damage stemming from the higher flow rates and large fluid volume introduction in to the tissue⁵. This new improved method has since been applied to routine *in vivo* sampling^{6–8}. Analysis of the low-volume samples has been improved by coupling it to online microfluidic devices⁹ and creating selective membranes for the removal of proteins from biological samples in the interest of enhancing peptide collection and detection by mass spectrometry¹⁰. A further advantage of methods such as microdialysis and push-pull perfusion is the versatility and potential for introduction of pharmaceuticals into the sampling area via the inlet portion of the apparatus. The chemical microenvironment of the sampling site can be carefully controlled while monitoring the brain's chemical and metabolic response to the stimuli.

A valuable *in vitro* brain preparation is the organotypic hippocampal slice culture (OHSC), developed in 1991 by Stoppini¹¹. This culture involves dissecting the hippocampus of an

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Supporting Information Available

Cell death when sampling at different capillary-to-tissue distances, different capillary inner diameters, and all data presented as a function of current. There is additionally a table with calculated voltage at the surface of the OHSC under each sampling condition.

immature rat pup, sectioning perpendicular to the septotemporal axis, and culturing the slices atop a porous membrane over medium. *In vitro*, these tissues can be maintained from a week up to 1–2 months, developing quite similarly to the intact tissue in the living animal and maintaining most hippocampal structural integrity and neuronal organization^{12,13}. The well-organized laminar structure of the neurons in the hippocampus allows for easy visual discernment of the different structural areas. This organization combined with the architectural similarities to the hippocampus *in vivo* make the OHSC an attractive model for exploring ill-understood neurochemical mechanisms.

Due to the size of the OHSC (only 100–200 μm thick), not all of the *in vivo* approaches to sampling the extracellular space are practical. The most common approach to analyzing the extracellular fluid for neurotransmitters in an OHSC is to simply submerge the tissue in an oxygenated buffer or medium, apply the stimulus (i.e. remove the oxygen to simulate stroke-like conditions), then analyze the buffer/medium. This method, while simple, lacks spatial resolution, relies on slow diffusion to the buffer solution, does not have good time resolution, and poses analytical challenges because of the dilution of typically low-concentration analytes. Tackling the issue of collection efficiency, Roy, et al. developed a microsampling device, similar to a microdialysis probe, but using suction to increase recovery of high molecular weight peptides¹⁴. To overcome the limitations of methods designed for *in vivo* applications, Bradberry, et al. developed a modified microdialysis method to be used on acute tissue slices¹⁵. However, much like traditional *in vivo* microdialysis, spatial resolution still suffers as well as recovery rates, especially for higher molecular weight molecules like peptides. Addressing the shortcomings of currently employed methods for sampling the extracellular space of living tissue, we have developed an alternative method that is well suited to the OHSC model. This method improves current spatial resolution limitations, and has the potential to better the temporal resolution of microdialysis. It additionally carries the same advantage of push-pull perfusion and microdialysis allowing introduction of exogenous compounds into the sampling area. While we envision wider application, here we focus on application to the OHSC

We now know that the extracellular space of OHSCs has a ζ -potential and thus supports an electrically induced bulk fluid flow referred to as electroosmotic (EO) flow¹⁶. The ζ -potential arises from fixed charges on the cell surfaces and the (immobile) extracellular matrix. The counterions to the fixed charges are free to move under the influence of an externally applied electric field, but also have a thermodynamic tendency to remain near the surface. As a result of the interaction of the externally applied electric field with the mobile counterions to the fixed surface charges, there is a bulk fluid movement called electroosmotic flow. The velocity of the flow is directly proportional to the ζ -potential and the externally applied field. It is therefore possible to imagine withdrawing a sample of extracellular fluid from tissue electrokinetically. As this approach will be applied for sampling of living tissue, understanding and minimizing tissue damage is imperative. Thus, we have assessed tissue damage arising from the local electric field created in the OHSC under a variety of conditions relevant to the collection of the extracellular fluid of the tissue. We have found conditions under which little to no tissue damage (defined below) occurs at the sampling site from the sampling procedure. Additionally, when there is damage at the sampled site, no damage is found elsewhere in the OHSC. A companion paper describes the sampling itself and application to ectopeptidase activity determination in more detail¹⁷.

EXPERIMENTAL SECTION

Results have been acquired over about a two-year period. During that time, there were some difficulties with the tissue culture, which led to changes in protocols for the preparation and incubation of the OHSC. The results using both protocols are the same. We will refer to one

protocol as "A", and the other as "B". In the description below, parenthetical statements give details for the two protocols. The elements of the two protocols have been used routinely in tissue culturing procedures by others in the field. We currently use protocol "B".

Solution and Reagents

Medium for protocol A is 50 % basal medium eagle (BME), 25 % Earle's balanced salt solution (EBSS), 23 % heat inactivated horse serum, 25 U/mL penicillin-streptomycin (PEST), 1 mM L-glutamine, 41.6 mM D-(+)-glucose, all from Sigma-Aldrich (St. Louis, MO, USA). Medium solution for protocol B is 50 % opti-MEM, 25 % horse serum, 25 % Hank's balance salt solution with phenol red (all from Gibco, a subsidiary of Invitrogen, Carlsbad, CA, USA), supplemented with 1 % D-(+)-glucose (Sigma), and filtered through a Nalgene filter (0.45 μ m was utilized until a 0.1 μ m size, designed to combat mycoplasma contamination, became available, Fisher Scientific, Waltham, MA). The buffer used during tissue preparation (GBSS) was Gey's balanced salt solution (Sigma) supplemented with 0.5 % D-(+)-Glucose and 2.7 mM MgSO₄. In protocol B, this solution was filtered as above.

Two biological buffers were used over the time period of the experiments. One was prepared with the following composition in purified Millipore water (Synthesis A10) : NaCl (134 mM), KCl (5.40 mM), MgSO₄ (1.20 mM), NaH₂PO₄ (1.38 mM), CaCl₂ (2.65 mM), 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES 5.00 mM, all from Sigma) and β -D-glucose (10.0 mM, from MP Biomedicals, LLC; Solon, OH, USA). The pH of the buffer solution was adjusted to 7.40 with NaOH solution then vacuum filtered through a 0.45 μ m PTFE membrane and frozen until the day of use. For convenience, later experiments used Hank's balance salt solution (no phenol red), purchased from Gibco. Both had identical conductances (1.57 S/m). Propidium iodide (PI) solutions were prepared by dissolving solid PI in GBSS at a final concentration of 0.35 mM and freezing until use.

Culturing Procedure

The hippocampal region of a Sprague-Dawley rat pup (A: 7 days post-natal (p7); B: p9) was dissected and chopped perpendicular to the septotemporal axis using a tissue chopper (McIlwain, model TC752. A: 400 μ m; B: 350 μ m). Two tissue cultures were placed onto each transparent porous (0.4 μ m) PTFE insert membrane surface (Millipore, Bedford, MA, USA), and cultured in a 6-well plate (Sarstedt, Newton, NC, USA) over 1.2 mL of serum-containing medium. In Protocol B, during the pre-experimental culturing period (days *in vitro* (DIV) 0–5), the medium was supplemented with 2 % filtered B-27 (Gibco). The OHSCs were incubated in 5 % CO₂ and 95 % air (A: 37 °C B: 36.5°C) for 5–9 days before experimental use. The medium was exchanged every 2–3 days during incubation.

Characterization of Cultures

Stoppini initially stated the thickness of the OHSC to be roughly 150 μ m after two to three weeks in culture¹¹. We verified the OHSC thickness for days 6–8 in culture by measuring the position of a metal probe when it is in contact with the top surface of the tissue, and comparing that to the position of the same probe when it is in contact with the insert membrane nearby. Positions were determined by noting the readings on an electronic micromanipulator (MP-285, with ROE-200 controller and MPC-200, Sutter, Novato, CA). Contact of the conducting probe with the tissue/insert membrane surface was determined by using an ohmmeter (Fluke Corporation, Everett, WA) connected to the conducting probe held by the micromanipulator and a second probe in the medium. Each thickness measurement resulted from four insert membrane readings and four OHSC readings.

To assess culture viability prior to performing experiments, PI was added to culture medium 16–24 hours prior to the desired sampling time. For each experimental cycle, a pair of

cultures on one insert was intentionally damaged irreversibly by treating them with methanol. A few milliliters of methanol were pipetted onto the insert membrane, submerging the tissues in the methanol solution. After at least 5 minutes of exposure, any excess methanol was then aspirated away. Prior to fluorescence microscopy, the medium was replaced with warmed (37°C) GBSS. The OHSCs were screened for pre-existing cell death and structural integrity using an inverted fluorescence microscope (IX-71 with U-MGIW2 cube from Olympus, Melville, NY) with image acquisition software (Simple PCI). The exposure time used for assessing PI fluorescence in experimental OHSCs was set to the auto-exposure time for the methanol-treated OHSC. Any OHSC showing extensive cell death was noted and not used for sampling. GBSS was then replaced with fresh medium and the tissues were returned to the incubator until sampling.

Sampling Procedure

The sampling time was 5 minutes. Initial experiments demonstrated that voltages greater than 1600 V were quite damaging. Voltages applied ranged from 1000–1600 V. Capillary inner diameters ranged from 75 μm to 280 μm and capillary-to-tissue distances ranged from 0 μm (surface sampling) to 100 μm .

One 6-well plate was removed at a time from the incubator for experiments. Electroosmotic flow was induced in viable OHSCs before the plate was returned to the incubator (OHSCs remained outside the incubator for approximately 1.5 hours). Figure 1 shows the apparatus used to induce electroosmosis. Fused-silica capillaries (Polymicro Technologies, L.L.C., Phoenix, AZ), 30 cm long, of various inner diameter (ID) were cut with a Shortix capillary cutter (Scientific Instrument Services, Ringoes, NJ, USA) with diamond blade to ensure a clean, straight cut to the end. The capillary was filled with biological buffer and ends were submerged in biological buffer-filled dishes. The electrodes were 0.3 mm diameter platinum wire and were held in place contacting the buffer solution through slits cut in the sides of the plastic dishes. The voltage source was a high voltage power supply, model PS350 from Stanford Research Systems (Sunnyvale, CA, USA). A manual manipulator held the capillary in place in the dish absent of tissue while the electronic micromanipulator held the end of the capillary above the tissue surface.

Positioning of the capillary occurred in three separate ways. Method 1 relied on an established tissue thickness, measured to be $148 \pm 8 \mu\text{m}$ at 6–8 days in culture. In this method, a dry, empty capillary was slowly lowered perpendicularly to the surface of the insert membrane near the tissue edge. Movement of the capillary was carefully determined visually through a Stereomaster Zoom microscope (Fisher). Once the capillary tip contacted the surface of the insert membrane, the position was noted and the tip was raised 200 μm . The capillary was then moved laterally to the desired area of the tissue. This position was noted, the capillary raised and filled with an biological buffer solution with syringe such that a droplet of buffer formed on the end of the capillary but did not fall off. The capillary was then returned to the previously saved position, which was approximately 50 μm above the tissue. Contact between capillary and tissue occurred via the droplet of buffer, which spread out upon contact with the tissue surface. Sampling then commenced by switching on the voltage. Protocol A relied on positioning via method 1.

Method 2 is a refinement of Method 1 to remove variability from the natural variation in OHSC thickness. In Method 2, the dry capillary was slowly lowered directly to the surface of the OHSC (not the insert membrane), until it made contact. The capillary was then raised the desired distance (CTD) or kept at this location if the desired CTD is '0 μm '. Similar to method 1, this position was saved, the capillary raised and filled with biological buffer leaving a droplet to hang off the end, then returned to the sampling site. Sampling then commenced. Protocol B relied on positioning via method 2.

While these methods ensure accuracy and precision of the CTD and thus the electric field in the tissue, they proved impractical for routine sampling. Method 3 arose out of Method 2 and is identical in all but one aspect – the capillary starts out filled with biological buffer solution. It was identically lowered to the surface until contact was made then raised a particular distance (CTD). Experiments investigating feasibility of sampling with parameters denoted as ‘safe’ relied positioning via Method 3, as did the sampling carried out in the companion paper¹⁷.

To apply potential across the tissue, a specified voltage was applied across the Pt electrodes (Fig. 1); current was monitored during this time. Following each 5-minute sampling period, the capillary was raised, buffer replaced with fresh solution, and then repositioned on the next tissue to be sampled. We did not experience difficulties with capillary clogging. Once both tissues on an insert were sampled, the insert was returned to its original position in the 6-well plate. At least 2 tissues (one insert membrane) were reserved for negative controls and were not exposed to any electric field. An additional 2 tissues were reserved for positive controls wherein a few milliliters of methanol were added to the top of each tissue to ensure 100% cell death.

Once the electric field was applied to all desired OHSCs, medium under each insert membrane was replaced with fresh PI-containing medium (7 μ M) and incubated 16–24 hours overnight. The next day, the medium was removed and replaced with warmed GBSS. All OHSCs were imaged using the IX-71 inverted fluorescence microscope with an exposure time determined by the auto-exposure time of the methanol treated cultures. To analyze the cell death quantitatively, five equal-sized regions of interest (ROI) were drawn in the area where the capillary had been positioned (no cell death arising from sampling is incurred elsewhere in the OHSC.) The average fluorescence intensity of the PI (‘mean red’) was measured in these areas. Figure S-1 shows a representative image with ROIs indicated. Mean red values of the corresponding area of the methanol-treated control is the “mean red dead control”. Mean red of the live cultures is the “mean red live control”. A ‘% death’ value was then calculated using Equation 1. Each value in the results section is reported as an average of all % death values for a particular voltage applied or power induced in the tissue.

$$\% \text{ Death} = \frac{\text{mean red experimental culture} - \text{mean red live control}}{\text{mean red dead control} - \text{mean red live control}} \times 100 \quad (1)$$

RESULTS AND DISCUSSION

In any sampling procedure applied to tissue, there is a tradeoff between conditions supporting simple, accurate and rapid analysis of the sample, and conditions that perturb the tissue minimally. For example, acquiring more sample at higher flow rates in push-pull perfusion leads to fewer difficulties in sample handling and analysis, but more damage. In electroosmotic sampling, we anticipate that the same sort of considerations will lead to optimum conditions. Higher electric fields in the tissue created by increasing the applied voltage or current, or positioning the capillary closer to the tissue surface create a higher flow rate and larger sample volumes in a given time. We anticipate that higher electric fields will at the same time induce more damage in the sampled tissue. Additionally, larger capillary inner diameters afford larger sample volume but also induce a larger current through the tissue. As a result of these considerations, we examined a range of capillary diameters and applied voltages that encompassed what we believe to be suitable for sampling¹⁷. Our goal was to find conditions that caused minimal damage (<10% cell death) in the tissue culture.

A companion manuscript demonstrates the determination of membrane-bound peptidase activity (ecto-peptidase) in OHSC by drawing the peptide Leu-enkephalin through the tissue electroosmotically and determining the rate of its hydrolysis¹⁷. It was found that, when using a 30 cm-long sampling capillary, the applied voltage should be greater than about 900 V in order to pull the peptide from the biological below the OHSC through the tissue in the sampling time of five minutes. Lower voltages can be used, but in the five minute sampling period, only the extracellular fluid in the OHSC would be drawn into the sampling capillary. In those experiments, a 150 μm inside diameter capillary was used to acquire a convenient volume of sample. Therefore, in our investigations of the effect of sampling conditions on the tissue, we used capillaries with inside diameters both smaller and larger than 150 μm , voltages greater than or equal to 1000 V, and a sampling time of five minutes. For sampling from other tissues types, or with other objectives, these conditions may not apply.

Tissue Thickness Measurements

We measured OHSC thicknesses for days 6–8 in culture using protocol B culture medium beginning with two different initial tissue chopper settings: 400 μm and 500 μm . The mean value for both sets of OHSCs is $198 \pm 4 \mu\text{m}$ ($n=61$ OHSCs). There was a small dependence of the thickness on the day in culture, and no dependence on the tissue chopper setting. We also measured the OHSC thicknesses for the same days in culture using protocol A culture medium, beginning with 500 μm tissue chopper setting. The mean thickness for these tissues was $148 \pm 8 \mu\text{m}$ ($n = 30$ OHSCs), thus thinner than those cultured with protocol B culture medium. Since the tissue chopper setting did not influence OHSCs cultured with protocol B medium, we have assumed that the OHSC thickness from protocol A, with an initial tissue chopper setting of 400 μm , is also $148 \pm 8 \mu\text{m}$.

Numerical Simulation

Using numerical solutions of the Laplace equation (COMSOL Multiphysics v3.5a), we calculated the potential distribution outside of the capillary. In each sampling experiment, there is a layer of buffer ranging anywhere from 0 to 100 μm thick, depending on the specified CTD. The brain has a porosity of about 0.2 μm ¹⁸, thus the average conductance is lower and the field higher in the tissue than in the buffer. Figure 2 shows an example of the voltage drop along the cylindrical capillary's axis of symmetry outside of a 30 cm long, 200 μm ID capillary operating at 1400 V, with the tip placed 50 μm from the tissue surface. Because the capillary is quite long, a small fraction of the total voltage is dropped outside of the capillary, and an even smaller fraction is dropped within the tissue. The field within the tissue decreases with distance away from the tip because the current is diverging. However, the field does not decay to zero within the tissue. Therefore, although the resulting velocities may be small, there will be electroosmotic flow throughout the depth of the tissue, at least along the z-axis.

Tissue Damage

Our goal is to determine conditions under which sampling results in minimal cell death at the sampling site. We will refer to conditions as “safe” when the propidium iodide fluorescence is less than 10% of the positive control after correction for the negative control (Eq. 1). We recognize that this is an arbitrary criterion, and that some experiments may be uninfluenced by more damage while some experiments may be perturbed by the electric field even if there is no resulting cell death 24 h after the experiment.

We have focused our efforts, after initial experiments, on conditions that are safe (as described above) and that will yield useful samples. Thus, the number of repeat experiments under a particular set of conditions is small when it is clear that either the current is too low to produce adequate sampling velocity or that substantial cell death results. Sampling

parameters investigated include the region of the hippocampus sampled, the CTD, and the inner diameter of the capillary. Capillary-to-tissue distances of 0, 50, 75, and 100 μm were investigated at applied voltages ranging from 1000 to 1600V, using a 200 μm ID capillary (see Figure S-2, for plot of % death vs. voltage for different CTDs). In all cases, n equals the number of OHSCs. Shorter distances between the capillary tip and tissue surface tend to cause more cell death. At all voltages tested, sampling is safe (cell death $\leq \sim 10\%$) for both 75 and 100 μm CTDs; at the 50 μm distance, an applied voltage of 1400 V or less is safe. Figure 3 shows results from two sets of data. The left panel shows data from protocol A (180 μm ID, 50 μm CTD). Cell death is minimal (mean % death = 3.7 %, standard deviation of the data = 7.0 %, $n = 89$) in both areas of the hippocampus when ≤ 1400 V is applied. For higher voltages, the CA1 becomes more vulnerable than the CA3 (for $V \leq 1400$, $p > 0.21$; for $V \geq 1500$, $p < 0.0198$). The right panel shows data obtained from sampling (200 μm ID, 100 μm CTD) with protocol 'B'. Cell death is minimal (mean % death = 1.0 %, standard deviation of the data = 2.7 %, $n = 69$) in both areas of the hippocampus when ≤ 1300 V are applied. For larger voltages, the CA1 becomes more vulnerable than the CA3 (for $V \leq 1300$, $p > 0.08$; for $V \geq 1400$ V, $p < 0.007$). We note that the variability of the data in panel A (protocol A) is greater than that in panel B (protocol B). This we believe to be the result of the better control of CTD. Both sets of data illustrate the same general trend: lower voltages are safer for sampling and the CA1 tends to be more vulnerable to cell death than the CA3. Fig. 3 also shows representative images of control and sampled tissue. The visual impression of the PI fluorescence reflects the quantitative data shown in the accompanying graphs. Damage occurs at the site of sampling, not elsewhere in the tissue.

The effect of different capillary inner diameters was also investigated (see Figure S-3, for a plot of % death vs. voltage for different sized capillaries). Diameters explored include 150, 180, 200, and 280 μm ; all sampling was at a 50 μm CTD. Larger inner diameters (280 μm) cause the most cell death, where a smaller capillary (150 μm) produces very little cell death at all tested voltages.

In the interest of predicting sampling parameters outside the realm that was tested here, it is desirable to find a single variable that describes the cell death induced during electroosmotic sampling of the OHSC. When comparing cell death incurred by the electric field, for example, at 1500 V, five statistically different % death values exist for capillary inner diameters (75, 150, 180, 200, and 280 μm) investigated at that voltage. Thus it is clear that electric field/applied voltage cannot serve alone to explain cell death induced by electroosmotic sampling. Current did not have predictive value either (see Figure S-4 for a plot of % death vs. current). We thought that power might have predictive value. We have estimated the power as the product of the calculated voltage at the top surface of the tissue on the cylindrical axis and the experimental current. Power seems to lead to a general overall trend illustrated in Figure 4. The data in Fig. 4 span a variety of total voltages, capillary diameters, CTDs, and tissue from both protocols. When the power dissipated in the tissue is 120 μW or less, cell death in the tissue is less than 10% of the positive control.

The measurements described above used a dry capillary to make accurate CTD measurements (capillary positioning methods 1 and 2). Additionally, the capillary-to-tissue distances were quite large. In the interest of efficient and reproducible sampling, it is more convenient to begin with a filled capillary (despite the added variability in CTD measurement) and sample at a shorter distance to avoid dilution of collected sample (positioning method 3). Based on the established power trend, sampling parameters were chosen such that minimal cell death would occur while keeping the CTD short. A 150 μm ID capillary was positioned using method 3 with a CTD of 15 μm . The power model shows that sampling under these conditions at 1400 and 1500 V should be safe with powers of 85 and 99 μW . The % death values obtained experimentally were 7.9 ± 2.2 % (SEM) and $5.6 \pm$

4.7 % respectively. At a CTD of 0 μm and 1400 V applied (positioning still by method 3), the predicted power is somewhat greater than 120 μW , and indeed there was significantly more than 10 % cell death in this case. These values are highlighted in Fig. 4. Thus, with the help of the power model, conditions appropriate for minimizing cell death in a particular sampling experiment were established here with the possibility of extending for additional parameters with additional experiments. With a range of parameters deemed 'safe', the feasibility of sampling was tackled under these conditions. The specific details of these experiments are outlined in the companion paper.

Sampling at 0 μm CTD

In Fig. 4A, it is clear that sampling at CTD = 0 μm when establishing the position with a dry capillary is quite damaging. We believe that other cell death-inducing factors arise when the dry capillary is placed directly on the surface. After the capillary has been placed on the surface and sampling has occurred, there is visible physical damage to the tissue, which may contribute to cell death. The damage could arise for several reasons. There is a mismatch in ζ -potential between the capillary and the tissue. The capillary's ζ -potential is significantly more negative than the tissue. Due to this mismatch, a small portion of the induced flow is pressure driven. The pressure will tend to compress the tissue that touches the capillary. By raising the capillary only micrometers, damage from pressure is avoided.

Based on these experiments, we can state conditions conducive to minimizing cell death during electroosmotic sampling in organotypic hippocampal slice cultures. At lower power (<120 μW), cell death is minimal in the CA3. While power has emerged as the best descriptor of the cell death incurred from electroosmotic sampling, power is not directly controlled by experimental means. Since voltage is directly controlled in all experiments, it is more useful to dictate the conditions for minimizing cell death in terms of this variable. A low CTD is desirable for sample collection. A larger capillary ID is desirable for larger sample volumes. A variety of sampling scenarios that minimize cell damage rise from these desired conditions – these conditions are shown in Table 1.

Conclusion

We have established a voltage/current/power threshold in the CA1 and CA3 regions in an OHSC. This information can be used for predicting safe experimental conditions for future electroosmotic sampling designs, which will unavoidably subject living tissue to similar electric fields. The decision about what is 'safe' depends on the experiment. For experiments such as measuring the chemical properties of the extracellular space (e.g., the determination of ectoenzyme activity, binding of antibodies to extracellular epitopes, chemical reactions with elements of the extracellular matrix, etc.), the fact that a cell is dead the following day is probably not important. On the other hand, for investigating neurotransmitter release from excitable cells, cell death 24 h later may not be the critical issue. Rather, it may be the influence of the electric field on the transmembrane potential during the sampling.

The electroosmotic sampling approach in its current form does not seem well suited to true *in vivo* sampling. However, it is certainly applicable to acute and cultured tissues and cell cultures. While applicability to other tissues remains to be established, it is very likely that all tissues have a negative ζ -potential, and therefore this sampling technique would be useful for acquiring samples from them. The key finding is that electrokinetic flow in brain tissue can be used to sample the extracellular space, and that cell death can be immeasurably small. It is true that the applied voltage may influence the transmembrane potential, and thus influence the result of a sampling experiment. Investigations into these more subtle effects are underway.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Apparatus for electroosmotic sampling. A 30 cm long fused-silica capillary held in place by two micromanipulators connects a buffer filled reservoir with the surface of an OHSC cultured on a PTFE Biopore membrane (0.45 μm pore size). Two platinum electrodes connect each buffer reservoir to a voltage source. When voltage is switched on with the indicated polarity, electroosmotic flow in the OHSC and the capillary will pull fluid from the tissue into the capillary.

Figure 2.

Voltage drop between the tip of a sampling capillary and the sampled tissue at a variety of applied voltages; CTD = 50 μm ; the discontinuity in the graph at 50 μm is a result of a change in conductivity between buffer solution and the sampled tissue; sampling capillary = 200 μm ID

Figure 3.

Cell death resulting from electroosmotic sampling of the CA3 and CA1 regions of an OHSC (A) Data obtained from sampling (180 μm ID, 50 μm CTD) via protocol 'A'. (B) Data obtained from sampling (200 μm ID, 100 μm CTD) via protocol 'B'; Legend: solid black square - cell death in CA3 from sampling at CA3, cross - cell death in CA1 from sampling at CA1 (C) Representative images of control and sampled tissue: (1) Live control. (2) Methanol-treated (100% dead) control. (3) and (4) 1300 V applied to CA3 and CA1, respectively, little to no cell death incurred. (5) and (6) 1400 V applied to CA3 and CA1 respectively; no cell death to CA3 but CA1 appears damaged. (7) and (8) 1700 V applied to CA3 and CA1 respectively; significant cell death to both areas, but CA1 cell death is more widespread. Overall, the CA1 is more susceptible to damage arising from the applied electric field than the CA3.

Figure 4.

Cell death in CA3 from electroosmotic sampling of the CA3 using capillaries of various diameters at various CTDs, as a function of power. Power was calculated by multiplying the voltage at the surface of the culture, found using the modeling program, Comsol (see Table S-1) and experimentally observed current. Panel A illustrates results from 11 separate geometries, with 7–8 voltages for each geometry, while panel B enlarges the area from 0 – 400 μW for better resolution of the threshold area. In panel A, the data for 0 μm CTD clearly do not fit the trend of the remainder of the data. In panel B, data for 0 μm CTD and 15 μm CTD (distances established with a filled capillary) are indicated. Legend: solid black squares - 75 μm ID, 50 μm CTD; solid red circle - 150 μm ID, 50 μm CTD; solid blue

triangle - 180 μm ID, 50 μm CTD; upside down solid pink triangle - 200 μm ID, 100 μm CTD; solid green diamond - 200 μm ID, 75 μm CTD; solid 5-pointed star - 200 μm ID, 50 μm CTD; open 5-pointed star - 250 μm ID, 100 μm CTD; asterisk 8-pointed star - 280 μm ID, 50 μm CTD; solid red upside down triangle - 200 μm ID, 0 μm CTD; a cross - 150 μm ID, 0 μm CTD; solid black diamond - 150 μm ID, 15 μm CTD

Table 1

Summary table of sampling conditions that cause less than 10% death.

Sampling Area	Capillary-to-tissue distance (μm)	Capillary inner diameter (μm)	Safe Sampling Range (V) ¹
CA3	15	150	≤1500
CA3	50	75	≤1900 ²
CA3	50	150	≤2000 ²
CA3	50	180	<1400
CA1	50	180	≤1400
CA3	50	200	≤1300
CA3	50	280	≤900
CA3	75	200	≤1600
CA3	100	200	≤1600 ²
CA1	100	200	≤1300
CA3	100	250	≤1000

¹ Ranges valid for electroosmotic sampling of an OHSC 6–9 DIV using a 30 cm long fused-silica capillary and set up as shown in Fig. 1.

² Voltages above value this were not tested. Further experiments are required to find a ceiling voltage.