SUPPLEMENTARY INFORMATION

Development of μ-low-flow-push-pull perfusion probes for *ex vivo* sampling from mouse hippocampal tissue slices

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Figure S1. Schematic of probe pulling device.

The process of creating µLFPS probes with a pulling device. While first efforts utilized a commercial laser-based micropipette puller, it was discovered that damaging soot was being deposited onto the mirrors in the system likely from the combustion of the polyimide coating. As a result, an in-house, flame-based puller was developed (Figure S1). Pre-pulled probes were pushed through the alumina rods and clamped into place on the pulling device. These adjustable rods are fire resistant and allow for a finely adjusted area of heating. It was determined that a heated capillary length of 3.7 ± 0.4 mm produced symmetrical tips with desired o.d. Weighted metal blocks ranging from 200 to 550 g were used and the pulling force was fine-tuned with 17 - 21 g metal washers that could be added. The mass used in our system was 225 g. Once a butane flame heats the fused silica glass for several seconds, the glass reaches its softening temperature and begins to pull, the weight will drop creating a fine tip as the final result. These conditions were highly reproducible for creating the µ-LFPS probe tips.

A particular challenge that needed to be addressed with pulling these concentric fused-silica probes is the complication from the polyimide coating of the inner capillary. Asymmetric probe tips were produced due to the need to burn the polyimide coating on the interior capillary before softening. Even when a probe was pulled with reasonable tip shape, there was often tip blockage due to partially combusted polyimide flaking off from the interior capillary and blocking the probe tip. To prevent these problems, the polyimide coating of the inner capillary would be burned away from the section of the capillary to be pulled prior to probe construction.

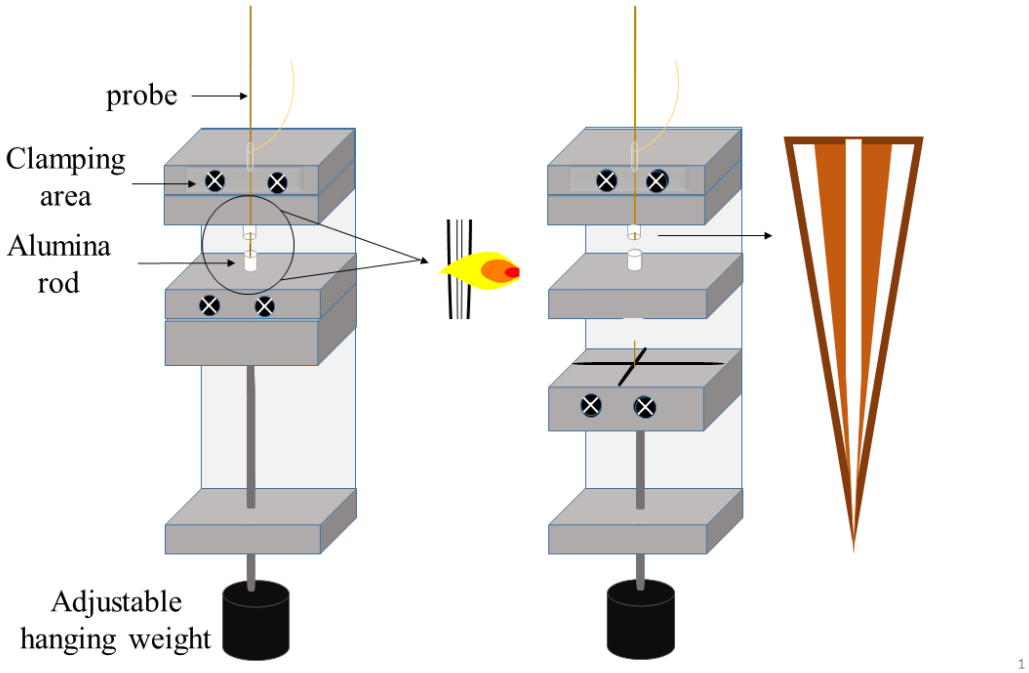

Figure S1. Schematic of pulling process. Cannula alumina rods are adjustable to control the area of applied heat to the probe. Once both fused silica capillaries reach their softening temperature, the capillaries are pulled to a fine tip as the weight drops. The weights are adjustable allowing for different probe tip lengths and shapes.

Figure S2. Representative images of initial µ-LFPS probes taken with SEM.

Micrographs of various probe designs. Initial pulling experiments were performed with 170/100 µm o.d./i.d. (infusion) and 90/20 µm o.d./i.d. (withdrawal) capillary. Show in Figure S2A and S2B are representative scanning electron micrographs (SEM) that display a withdrawal line opening that is estimated to be less than 1 micron wide. While this demonstrates an ability to construct sub-micron probes, this size was found to be highly subject to blockage and was not used in further testing. Often probes were pulled closed as seen in Figure S2C. In these instances, the probe tip could be opened by cutting with a razor blade or a microdissection scissor tool for a more polished probe tip.

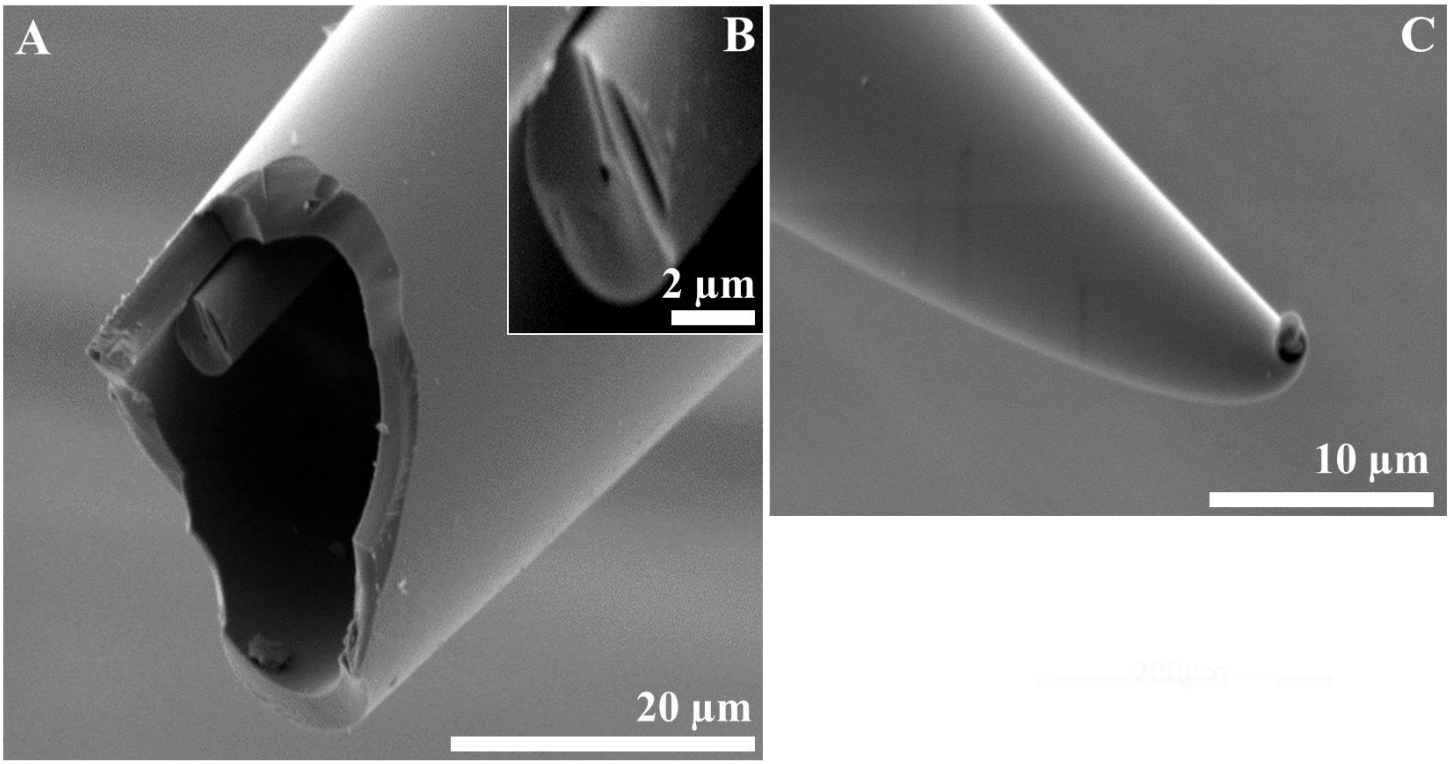

Figure S2. Representative images of µ-LFPS probes taken with SEM. Each probe is referenced by the original inner diameter (i.d.) of the withdrawing line pre-pull. (A,B) 20 µm i.d. probe. (B) Magnified image of the 20 µm i.d. withdrawing line to show the sub-micron i.d. of the withdrawing line once pulled. (C) 50 µm i.d probe that was pulled so quickly it fused together on the tip. It will need to be cut to be patent.

Figure S3. Representative calibration curves of withdrawing line probe calibration.

Measuring pressure differentials vs flow rate of withdrawing lines of different probe designs. Probe designs were calibrated with respect to withdrawing line inner diameter in order to determine the working range of flow rates and applied pressure vacuums for withdrawing rates. Each probe design required slightly different pressures in order to achieve a constant flow rate. For example, the probe design with 150/20 µm o.d,/i.d. required higher pressure differentials since the inner capillary i.d. was much smaller than the other designs with 50 or 75 µm i.d.

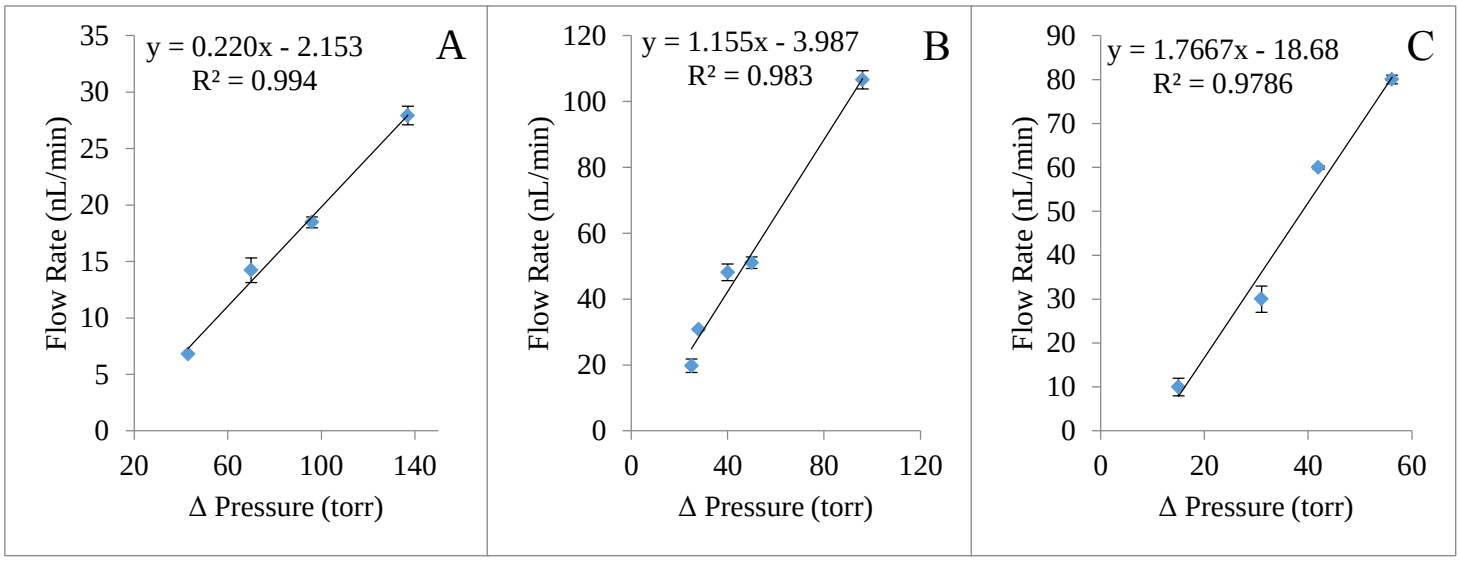

Figure S3. A. withdrawing line calibration for 150/20 µm o.d./i.d. B withdrawing line calibration for 150/50 µm o.d./i.d. C. withdrawing line calibration for 150/75 µm o.d./i.d. The slope for each withdrawing line calibration increases as the i.d. of each withdrawing line increases.

Figure S4. Determining the back pressure in relation to withdrawing line inner diameter.

The effect of backpressure on multiple probe designs. Backpressures were found by subtracting theoretical pressures from experimentally required pressures needed for a given withdrawal flow rate. These backpressures were plotted against the measured flow rates. The contribution of backpressure is larger with smaller inner diameter withdrawing lines and with higher flow rates. These changes would be expected based upon the Poiseuille equation. More variation is found with the smallest i.d. of 20 µm. This variation is likely due to 20 µm i.d. probes having the most variation in probe tip o.d. corresponding to withdrawal line i.d. variation.

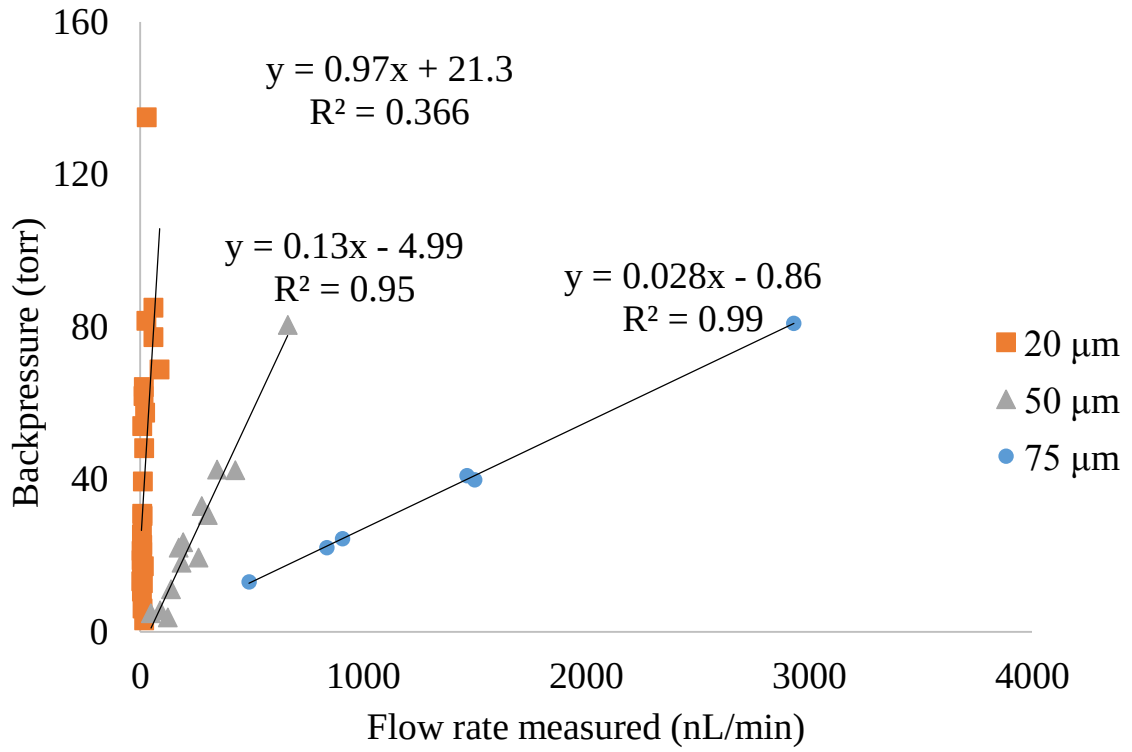

Figure S4. Determining the backpressure (torr) with different withdrawing capillary i.d. vs. measured flow rate (nL/min). Backpressure was calculated for three probe withdrawing line i.d.s. The absolute pressures are relatively small at <100 torr. The 20 µm i.d. varied the most which might be attributed to the large variation in probe tip o.d.

Figure S5. Average primary amine concentrations split into beginning and ending sampling time regions.

Measuring primary amines over time for loss of extracellular content to bath superfusion. Measuring primary amine content over time has several advantages. Being able to determine the effect of loss of extracellular content to the superfusion bath is important to understand what mechanisms are occurring in tissue slices as not much is known about the chemical content in the extracellular space. In order to understand if there is significant extracellular composition concentration loss occurring later in tissue sampling, averages were taken from 0-3 hours and 3-6 hours. The data was statistically similar to results from Figure 6.

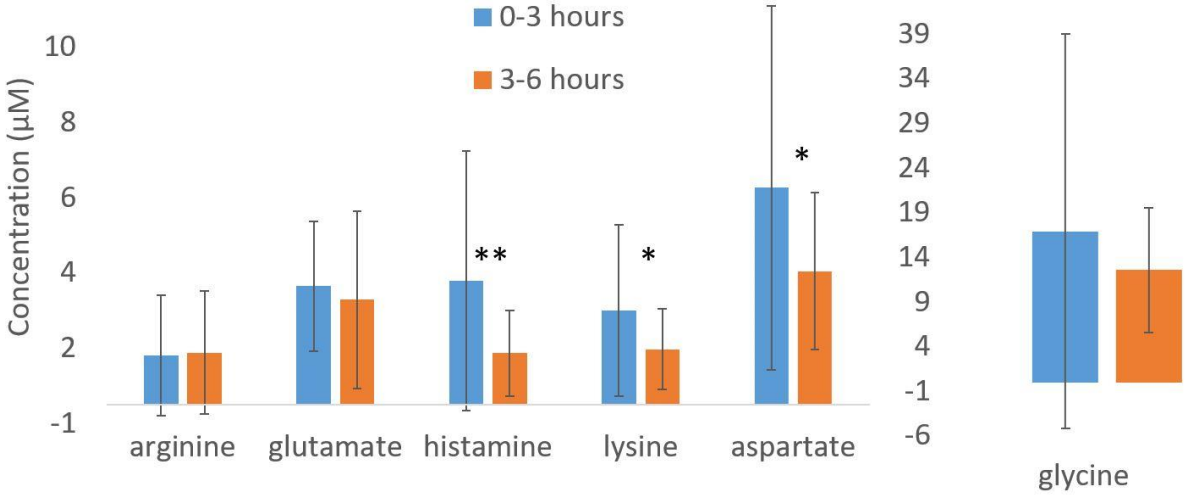

Figure S5. Average of amino acids over time ranges 0-3 hours (n=45) and 3-6 hours (n=18). This figure complements Figure 5. Despite averaging different time periods, 0-3 and 3-6 hrs for this figure, no additional primary amines were found to have significant differences over the sampling time. Significant difference via student *t*-test between primary amine levels for brain perfusate samples between the earlier or later time period of sampling *(P<.05) or **(P<.01).