Enhancing Continuous Online Microdialysis using Dexamethasone: Measurement of Dynamic Neurometabolic Changes during Spreading Depolarization

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Abstract. Clinical microdialysis is well-established for bedside monitoring of brain glucose levels in human patients with acute severe traumatic brain injuries. Spreading depolarization, a mechanism for secondary brain injury that leads to poor patient outcomes, causes dynamic falls in brain glucose levels. Nevertheless, clinical microdialysis is not yet a standard of care in medical practice. One issue that remains to be addressed is the penetration injury inevitably caused by the insertion of a microdialysis probe into brain tissue. This injury induces ischemia and gliosis in the vicinity of the probe. Once the probe is surrounded and engulfed in a glial barrier the responsiveness of the probe to changes in the brain tissue is dampened. Emerging evidence suggests that retrodialysis of dexamethasone may be an effective mitigation strategy. Herein, we show that the presence of dexamethasone in the perfusion fluid enhances the performance of microdialysis for the detection of K^+ and glucose transients in the rat cortex associated with experimentally induced spreading depolarization 2 hrs, 5 days, and 10 days after probe insertion. In the case of microdialysis recordings performed 10 days after insertion, dexamethasone was removed from the perfusion medium 5 days after probe insertion. Thus, the enhancement by dexamethasone outlasts its retrodialysis. Immunohistochemical analyses show that dexamethasone retrodialysis for 5 days is highly effective at preventing ischemia and gliosis in the vicinity of microdialysis probes implanted for 10 days.

Keywords. Microdialysis, dexamethasone, biosensor, microfluidic, spreading depolarization

The high incidence of traumatic brain injury (TBI) is a significant health crisis and it has been described recently as a 'silent epidemic'.[1,2] In the United States alone, 1.7 million brain injuries per year lead to 275,000 hospitalizations and 52,000 deaths.[3] At the heart of this problem is secondary brain injury, which can occur over the many days a TBI patient spends in the intensive care unit. Secondary injury occurs when tissue in the ischemic penumbra of the primary injury is subject to secondary insults, such as rises in intracranial pressure, transient ischemia, seizures, and spreading depolarizations. Neuromonitoring after severe TBI detects episodes of spreading depolarization[4-9] (SD) in approximately 60% of patients.[10-13] SD is a pathological mechanism for secondary injury that drives expansion of the primary brain lesion into the penumbra.[9-15] Incidence of SD is significantly correlated with poor patient outcomes, including death, vegetative state, and severe disability.[7-9] SD disrupts the concentration gradients of ions and molecules between intra- and extracellular spaces, and repolarization after SD requires vast amounts of energy.[7-9] Clusters of SD impose particularly severe energy demands on the injured brain.[15-18]

Clinical microdialysis enables monitoring of the local metabolic status of brain tissue in patients with TBI.[18-24] Boutelle and coworkers developed a rapid sampling microdialysis (rsMD) system that monitors SD-associated glucose transients with 30-s temporal resolution.[19] By the combination of rsMD with an online ion selective electrode (ISE), simultaneous rapid monitoring of glucose and K^+ is now possible.[21, 24-25]

Microdialysis is well-established for real time intracranial chemical monitoring.[26-27] Nevertheless, inserting the probe into brain tissue causes a penetration injury.[28-35] In response, the host tissue engulfs the probe in a glial barrier that, once formed, impairs further microdialysis sampling.[32,36-41] This is a limitation for clinical microdialysis, as TBI patients often remain under

intensive care for 10 days or more. Emerging evidence suggests that retrodialysis of dexamethasone (DEX), a glucocorticoid anti-inflammatory steroid, may be a simple yet effective strategy for mitigating the probe penetration injury. Recent work on the microdialysis of dopamine in the rat striatum shows that DEX reduces ischemia, suppresses glial activation, protects neurons and neuronal terminals, and reinstates normal dopamine activity in the tissues surrounding microdialysis probes.[32-36]

Here, we report that DEX also offers substantial benefits to the microdialysis of SD-associated glucose and K^+ transients in the rat cortex. We inserted microdialysis probes into the cortex of rats and monitored glucose and K^+ 2 hrs, 5 days, or 10 days later. Retrodialysis of DEX improved the detection of SD-associated transients at all three time points. In our 10-day studies, DEX retrodialysis was performed only during the first 5 days, confirming that continuous DEX delivery for the entire 10-day time window is not required. Finally, histochemical analyses show that 5 days of DEX retrodialysis prevented ischemia and gliosis near probes implanted for 10 days. Our findings confirm that DEX enhances the performance of microdialysis for monitoring glucose and K^+ in the rat cortex for at least 10 days after probe insertion.

RESULTS AND DISCUSSION

Experimental Design. Microdialysis probes were inserted into the rat cortex and used to monitor K^+ and glucose in response to SD induced by needle pricks 2 hrs, 5 days, or 10 days later. Groups of different animals were used for each time point. For observations 2 hrs after insertion, the animals were anesthetized with isoflurane and remained anesthetized for the duration of the experiment. For observations at 5 and 10 days, the animals were anesthetized for probe insertion, allowed to recover from anesthesia, and housed in a Raturn system until being re-anesthetized for the measurements. Once inserted, all probes were perfused continuously, with

only occasional brief interruptions to reload the syringe used to deliver the perfusion fluid. The perfusion fluid was either artificial cerebrospinal fluid (aCSF) or aCSF with DEX. For observations 10 days after probe insertion, the perfusion fluid was switched from DEX to aCSF on day 5.

Glucose and K^+ were monitored with rsMD and an on-line ISE, as previously described (Figure 1).[25] *In vitro* probe recovery was 64.0 ± 6.2 % and 10.1 ± 2.6 % for K^+ and glucose, respectively. The higher recovery of K^+ can be explained by its relatively small size in comparison to glucose. Probe recovery was constant when probes were stored in aCSF for 10 days (Table S1). Concentrations reported herein have not been corrected for probe recovery.

Probe Insertion and Representative Observations. Figure 2 shows an example of a complete recording of K^+ and glucose from an acute experiment in a single rat. The vertical black lines mark the times at which needle pricks were performed. Each needle prick induced a clear K^+ spike and a clear glucose dip, hallmarks of SD. The glucose dip is indicative of the energy consumed as the tissue repolarizes after SD.

A prominent K^+ spike also occurred just after the microdialysis probe was inserted into the cortex (Figure 2). A few moments later, the glucose signal, which had been increasing towards an initial basal level, also dipped. This initial K^+ spike and glucose dip, which indicate that probe insertion induces SD, were observed in all animals regardless of whether or not the perfusion fluid contained DEX (Figure 3). The insertion SD supports prior conclusions that probe insertion causes a penetration injury.[28-41]

Two additional examples of complete recordings of K^+ and glucose from single animals are provided in the Supplementary Information document. Figure S1 is an example with needle pricks that produced neither a K^+ spike nor a glucose dip. Of the 104 needle pricks performed

during this work, only 11 (~11%) produced neither a K^+ spike nor a glucose dip. We assume that the needle prick either did not induce SD or that SD did not reach the location of the microdialysis probe. When this occurred, extra pricks were performed so that three SD responses were recorded from each animal.

Figure S2 shows a unique adverse event: in this one animal, the needle prick induced a long-lasting elevation in K^+ and a long-lasting decline in glucose. The rat died about 1 hr after the needle prick, by which time neither K^+ nor glucose had returned to basal levels. This seems characteristic of an extreme variant of SD called spreading ischemia, although without direct measurement of blood flow this cannot be confirmed.[42] Anecdotally, this event illustrates the potential of intracranial microdialysis to provide an alarm signal in near real time of a severe, in this case lethal, metabolic crisis. It remains to be seen, of course, whether such an alarm signal would be valuable in a clinical setting.

SD-associated Transients 2 hours after Probe Insertion. Three needle pricks were induced at 30-min intervals beginning 2 hrs after insertion of microdialysis probes into the rat cortex. DEX enhanced the SD responses compared to those recorded with aCSF (Figure 4 a,b). DEX significantly increased the maximum amplitudes (Figure 4 c,d) and the area-under-the-curves (Figure S3) of the K^+ and glucose transients.

SD-associated Transients 5 days after Probe Insertion. Probes were inserted and perfused with aCSF or DEX for 5 days. Then, rats were re-anesthetized 1 hr before the first needle prick. If a needle prick produced neither a K^+ spike nor a glucose dip, an extra needle prick was performed, as explained above. In some cases, however, the needle prick produced a K^+ spike but no detectable glucose dip from the basal glucose level (Figure S4). These needle

pricks were not repeated: instead, the glucose dip was rated as non-detectable. All needle pricks that induced a K^+ spike were included in the data analysis explained below.

When the microdialysis probes were perfused with aCSF, the K^+ spikes were of low amplitude and the glucose dips were essentially non-detectable (Figure 5a). Fifteen K^+ spikes were recorded (5 animals, 3 responses each), only 4 of which were accompanied by a quantifiable glucose dip, defined as a dip larger than 3 times the baseline noise of glucose (Figure S4 explains how the detection limit for the glucose dip was determined). Previously we showed that microdialysis probes are completely engulfed in a glial barrier 5 days after insertion,[32,36] thus we attribute these non-detectable glucose dips to the presence of the glial barrier.

When the microdialysis probes were perfused with DEX, both K^+ spikes and glucose dips were clearly observed after needle pricks (Figure 5b). Amplitudes of the K^+ spikes in the presence of DEX were significantly larger than in the absence of DEX (Figure 5c: comparisons of the glucose dips were not possible because the glucose dips recorded without DEX were not quantifiable). With DEX, 80% of the K^+ spikes were accompanied by a quantifiable glucose dip 5 days after probe insertion (12 of 15, Figure 5d).

SD-associated Transients 10 days after Probe Insertion. Microdialysis probes were inserted into the rat cortex and responses to needle pricks were recorded 10 days later. DEX retrodialysis was performed only during the first 5 days. On the fifth day the perfusion fluid was switched from DEX to aCSF, which was perfused through the probes for the remainder of the experiments. There were two principal reasons for adopting this protocol. First, DEX is an exogenous agent. In most instances, whether working in animals or patients, it would likely be preferable to use a minimum sufficient amount of such an exogenous agent. Second, prior work

on neuroprosthetic devices appears to suggest that continuous delivery of DEX might not be necessary for long-term benefits.[43-45]

Needle pricks performed 10 days after probe insertion induced clear K^+ spikes and corresponding glucose dips (Figure 6): 87% of the fifteen K^+ spikes were accompanied by a quantifiable glucose dip (Table 1). Figure 6 confirms that the benefits of DEX retrodialysis for monitoring K^+ and glucose in the context of SD outlast the DEX retrodialysis itself. Figure 6 is our first report of DEX-enhanced microdialysis at 10 days after probe insertion. Previously, we showed that 5 days of DEX retrodialysis reinstated normal dopamine neurochemical activity near the probe.[36] Here we extend that work not only to 10 days after probe insertion, a clinically relevant time window, but also to include the enhancement of monitoring K^+ and glucose transients in the context of SD.

Quantitative Comparisons. In the presence of DEX, the amplitudes of the K^+ spikes were significantly larger at 2 hrs compared to 5 and 10 days (Figure 7a). In contrast, there were no significant differences between the amplitudes of the glucose dips at the three time points (Figure 7b), possibly due to the variation in the amplitude of the glucose responses. In the presence of DEX, the fraction of K^+ spikes accompanied by quantifiable glucose dips was relatively constant across the three time points (Table 1). In the absence of DEX, glucose dips were essentially non-detectable 5 days after probe insertion (Table 1).

Prior to the start of the needle pricks the average basal glucose concentration was 374 ± 36 μM. There were no significant differences between the basal levels of the 5 groups analyzed in this study (one-way ANOVA). This confirms that none of the probes failed during this study, including those perfused for 5 days without DEX.

Immunohistochemistry. We used fluorescence microscopy to examine thin sagittal sections of brain tissue containing the tracks of probes implanted for 10 days. Non-implanted control tissues from the contralateral hemisphere were used for comparison. When probes were perfused with DEX for 5 days after probe insertion, immunohistochemistry showed no evidence of ischemia (lack of nanobeads) or gliosis (GFAP) 10 days after probe insertion (Figure 8). When the percent of fluorescent pixels in images of probe tracks were compared to images of non-implanted control tissue, there were no significant differences between either the blood flow or gliosis images (Figure 8f: during the analysis the probe tracks themselves were excluded from the regions of interest). Figure 8 extends our previous reports that DEX prevents ischemia and gliosis.[32-33]

Figure 8 is our first report that DEX prevents ischemia and gliosis near probes inserted into the rat cortex, that the benefits of DEX last for 10 days after insertion, and that the benefits of DEX retrodialysis long outlast the DEX retrodialysis itself.

CONCLUSIONS

Our findings confirm that DEX retrodialysis significantly enhances the monitoring of K^+ and glucose responses associated with SD for at least 10 days after probe insertion. The probe triggers an insertion SD, supporting prior conclusions that insertion causes a penetration injury. To our knowledge, there is no indication in the existing literature that the penetration injury has any negative consequences for the subject. Quite the contrary, as microdialysis is well tolerated by animals and human patients alike. The issue at hand in this work, however, is the impact of the response to the penetration injury on the probe's ability to provide a near real-time report of chemical events occurring in the brain. Previous studies from the rat striatum have established that probe insertion triggers ischemia in the vicinity of the probe and that a glial barrier

eventually surrounds and engulfs the probe, thereby hindering long-term sampling.[28-36] Adding DEX to the perfusion fluid reduces ischemia and suppresses gliosis. Consistent with these prior reports, the present study confirms that DEX retrodialysis enhances the performance of microdialysis monitoring also in the rat cortex. In the absence of DEX, glucose responses became essentially non-detectable 5 days after probe insertion. In contrast, in the presence of DEX, SD-associated K^+ and glucose transients were consistently observed 2 hrs, 5 days, and 10 days after probe insertion. This work is our first extension of DEX enhanced microdialysis to K^+ and glucose, to the rat cortex, and to 10 days after probe insertion.

We hypothesize that the combination of rsMD with DEX-enhanced microdialysis has the potential to impact clinical microdialysis in important ways. Traditional clinical microdialysis has been previously performed in TBI patients for 10 days with a microdialysis sampling time of 1 hr and without DEX or any other strategy to mitigate the consequences of the probe insertion.[22-23] A correlation was found between dialysate glucose levels during the first 50 hrs of microdialysis and patient outcome.[22] However, no such correlation was found 2-10 days after probe insertion. This latter observation is perplexing, because ECoG detects SD in TBI patients well beyond 50 hrs.[11,14,17-18] Although it remains to be seen, we are hopeful that the combination of rsMD, online ISEs, and DEX-enhanced microdialysis will permit the real time monitoring of SD-associated K^+ and glucose responses in TBI patients over the entire duration of the clinically relevant 10-day time window, eventually aiding in patient care. Our long term goal is to establish microdialysis as a standard of care in medical practice.

METHODS

All procedures involving animals were approved by the University of Pittsburgh Institutional Animal Care and Use Committee.

Reagents and Solutions. All solutions were prepared with ultrapure water (Nanopure; Barnstead, Dubuque, IA). Artificial cerebrospinal fluid (aCSF) contained 142 mM NaCl, 1.2 mM $CaCl_2$, 2.7 mM KCl, 1.0 mM $MgCl_2$, and 2.0 mM NaH_2PO_4, adjusted to a pH 7.4. Dexamethasone sodium phosphate (APP Fresenius Kabi USA, LLC, Lake Zurich IL) was diluted in aCSF. The microdialysis perfusion fluids were filtered with Nalgene sterile filter units (Fisher, Pittsburgh, PA; PES 0.2 μm pores). Glucose oxidase (GOx, 100-200 units/mg) and horseradish peroxidase (HRP, ≥ 250 units/mg) were obtained from Sigma Aldrich. The ferrocene solution contained 1.5 mM ferrocenecarboxylic acid, 1 mM EDTA, 150 mM sodium chloride and 100 mM sodium citrate and was filtered before use with 0.1 μm and 0.02 μm pore size filters.

Microdialysis Probes, Surgical Procedures, and Experiment Protocol. Concentric style microdialysis probes were built in-house with hollow fiber membranes (13 kD MWCO, Specta/Por RC, Spectrum, Ranco Dominguez, CA), 4 mm in length and 280 μm in outer diameter. Fused silica capillaries (75 μm I.D., 150 μm O.D., Polymicro Technologies, Phoenix, AZ) were used for the inlet and outlet lines. Prior to use, probes were soaked in 70% ethanol and then flushed and immersed in aCSF (or aCSF with DEX) for several hrs prior to implantation into the brain. Prior to insertion, the probe inlet was connected to a 1 mL gas tight syringe driven by a microliter syringe pump (Harvard Apparatus, Holliston, MA) at a perfusion rate of 1.67 μL/min. The probe outlet (50 cm long) was connected to the K^+ ISE detector.

Rats (male, Sprague-Dawley, 250-350g, Charles River, Raleigh, NC) were anesthetized with isoflurane (5% induction, 2.5% maintenance), placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA) and adjusted to flat skull[46] for probe insertion. Aseptic technique was used throughout. The microdialysis probe was lowered at a 51° angle into the cortex using the coordinates 4.2 mm posterior to bregma, 1.5 mm lateral to midline, and 4 mm

below dura: the entire active portion of the probe came to rest in the cortex. For the 2-hr study, the rats (n=8 per group) remained under anesthesia for the duration of the experiment and responses to needle pricks were monitored beginning 2 hrs after probe insertion. To perform the needle pricks, a second hole was drilled through the skull ipsilateral to the probe, just anterior to bregma. The surface of the cortex was manually pricked with the tip of an 18-gauge hypodermic needle. Three pricks, 30 min apart, were performed per animal. For the 5- and 10-day experiments (n=5 per group), the probes were inserted as described above and secured with bone screws and acrylic cement. The incision was closed with sutures, anesthesia was removed, and the rats were housed in a Raturn Microdialysis Bowl (MD-1404, BASI, West Lafayette, IN) with continuous perfusion of the microdialysis probe. When used, the concentration of DEX was 10 μM for the first 24 hrs and then 2 μM for the next five days.[32,36] After 5 or 10 days the rats were re-anesthetized 1 hr prior to recording responses to needle pricks.

Potassium and Glucose Detection. The K^+ ISE electrode and poly(dimethyl)siloxane (PDMS) microfluidic system have been described previously.[24] Briefly, a miniaturized K^+ ISE was made in-house. A membrane containing 2 mg potassium ionophore, 0.2 mg potassium tetrakis(4-chlorophenyl)borate, 150.0 mg bis(2-ethylhexyl) sebacate, and 66 mg poly(vinyl chloride) (PVC) dissolved in tetrahydrofuran (reagents from Sigma-Aldrich) was cast over a segment of perfluoroalkoxyalkane tubing (PFA, IDEX Health Sciences, 360 μm O.D. and 150 μm I.D). The electrode was assembled with an internal Ag/AgCl reference and aCSF filling solution. The potential of the K^+ ISE was measured against an external Ag/AgCl reference electrode using custom electronics built in-house. The K^+ ISE and external reference electrode were inserted into a PDMS chip fabricated with soft lithography, as shown in Figure 1. The total

internal volume of the PDMS chip is approximately 700 nL. Connections to and from the PDMS chip were made with 0.15 mm and 0.13 mm ID FEP tubing, respectively.

The rapid sampling microdialysis (rsMD) system has been described previously.[19] Briefly, after the dialysate flows through the K^+ detection chip it enters a custom built sampling valve (Valco, Switzerland) with two 100 nL internal sampling loops. A standard HPLC pump (flow rate: 200 μL/min) mixed ferrocene solution with the dialysate and injected the mixture at 30 second intervals into one of two paths, both of which contain dual enzyme reactors in-line with a 3 mm disk glassy carbon electrode. The enzyme reactor contained two 6 mm-diameter membranes (0.025 μm pores, Millipore) loaded with glucose oxidase (1 mg/mL) and horseradish peroxidase (0.5 mg/mL). The reduction of ferrocenium ion was measured at 0 V with a three-electrode system with a Ag/AgCl reference electrode (UniJet, BASi, USA) and custom-built electronics.

Data Analysis. The rsMD data were analyzed with previously published algorithms[47] and converted to concentration with post-experiment calibrations. In Figures 2-6 the K^+ and glucose recordings were time-aligned to t_0 (Figure 1) to account for the travel time to and between the sensors. The K^+ spikes were used to confirm SD at the probe site: if there was no K^+ spike the needle prick was repeated (see discussion of Figure S1). A 10-min baseline prior to the expected glucose response was used to calculate a threshold for a detectable glucose signal, defined as 3x the noise of the 10 min baseline. If the glucose level dropped below the threshold in the next 15 minutes it was included as a detectable response (see Figure S4). Only detectable glucose responses were included in the data analysis and figures. A K^+ threshold was calculated in a similar manner using the signal recorded for 2 mins prior to needle pricks.

Immunohistochemistry and fluorescence microscopy. The procedures for immunohistochemistry and fluorescence microscopy are described in our previous papers.[32,48-49] Rats were deeply anesthetized with isoflurane (2.5% by volume O_2) and perfused transcardially with 200 ml 0.01 M phosphate-buffered saline (PBS), pH 7.4, followed by 160mL of 4% paraformaldehyde, and then with 50mL of a solution containing commercially available (Molecular Probes) 100-nm fluorescent beads (1:1000 dilution PBS). The entire brain was quickly removed and further fixed in 4% paraformaldehyde for 24 h at 4°C before being equilibrated in a 30% sucrose solution at 4°C for 24 hrs. The brain was then frozen in 2-methylbutane in a bath of liquid nitrogen to prevent freeze fracturing. The tissue containing the probe track was cut to 20-μm sagittal sections (n=3, 3 slides per animal). Free floating sections were rinsed in PBS, three times (10 min each), then blocked with 5% goat serum in PBS containing 0.1% Triton X-100 for 1 h at room temperature and subjected to immunohistochemical staining. The sections were incubated overnight at 4°C with the primary antibody anti-glial fibrillary acidic protein (GFAP; 1:100; #Z033401, DAKO Agilent Technologies). As a negative control, PBS was used instead of the primary antibody. Sections were then washed with PBS (5 min) and incubated in a secondary solution consisting of 5% goat serum, 0.1% Triton X-100, and antibody (1:500 goat anti-rabbit Alexa 568, Invitrogen, Carlsbad CA) for 2 hrs at room temperature. Sections were then rinsed with PBS for 10 min before being cover slipped with Fluoromount-G (Southern Biotech, Birmingham AL). Sections were imaged using fluorescent microscopy (FluoView 1000, Olympus, Inc., Tokyo, Japan) at 20x magnification.

Tissue images were processed and analyzed with the Metamorph/Fluor 7.1 software package (Universal Imaging Corporation; Molecular Devices). Quantitative analysis was based

on individual sections containing the microdialysis probe track in the ipsilateral region. Sections from the contralateral region (non-implanted control tissue) were treated in identical fashion to the ipsilateral region. The fluorescence intensities of GFAP and blood flow were determined by setting threshold values; the total number of pixels was expressed as the percent of fluorescence in the region of interest.[33] Samples were compared to a non-implanted region of the tissue slice.

Statistics. IBM Statistical Package for the Social Sciences (SPSS) 22 software was used for all statistical analysis. For all tests a $p < 0.05$ was used for statistical significance.

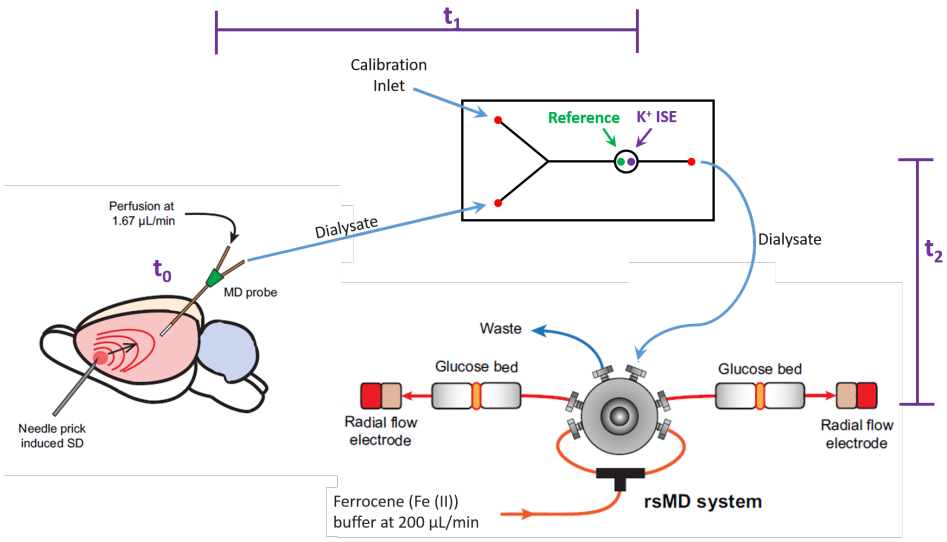


Figure 1: Experimental design. The needle prick model was used to induce SD in the cortex of anesthetized rats. The SD arrives at the microdialysis probe at t_0. Next, the sample travels through the probe outlet to the K^+ ISE detection chip with a travel time, t_1, of approximately 4 min. Finally, 7 minutes are required, t_2, for the sample to travel to the rsMD system for glucose detection.

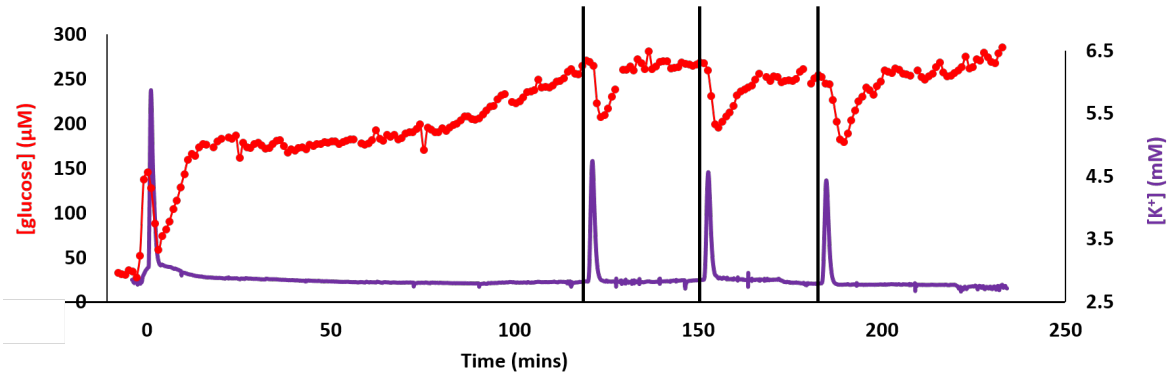


Figure 2: An example of a complete recording of K^+ (purple) and glucose (red) from an acute experiment in one rat. The microdialysis probe was inserted at time 0. Then, 2 hrs later, three needle pricks were performed 30 minutes apart (black lines mark when the needle pricks occurred). This experiment was performed in the presence of DEX.

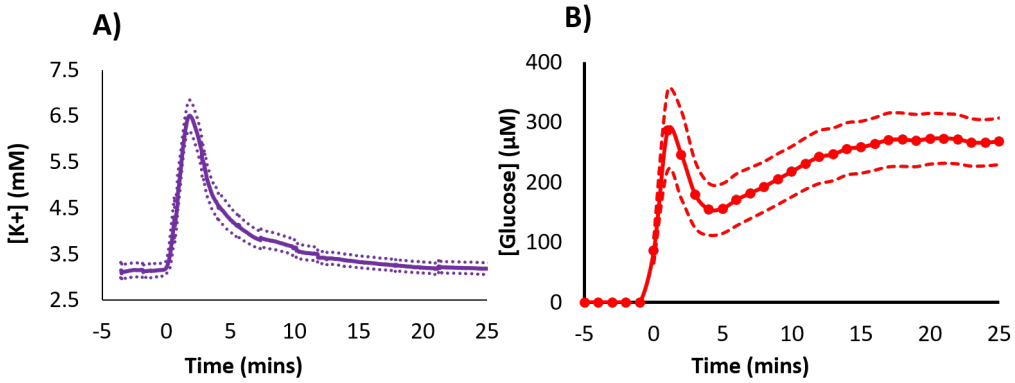


Figure 3: Probe insertion SD in the rat cortex characterized by A) an increase in K^+ (K^+ spike) and B) a decrease in glucose (glucose dip) time-aligned to the probe insertion at time zero (mean ± SEM, n=16 rats).

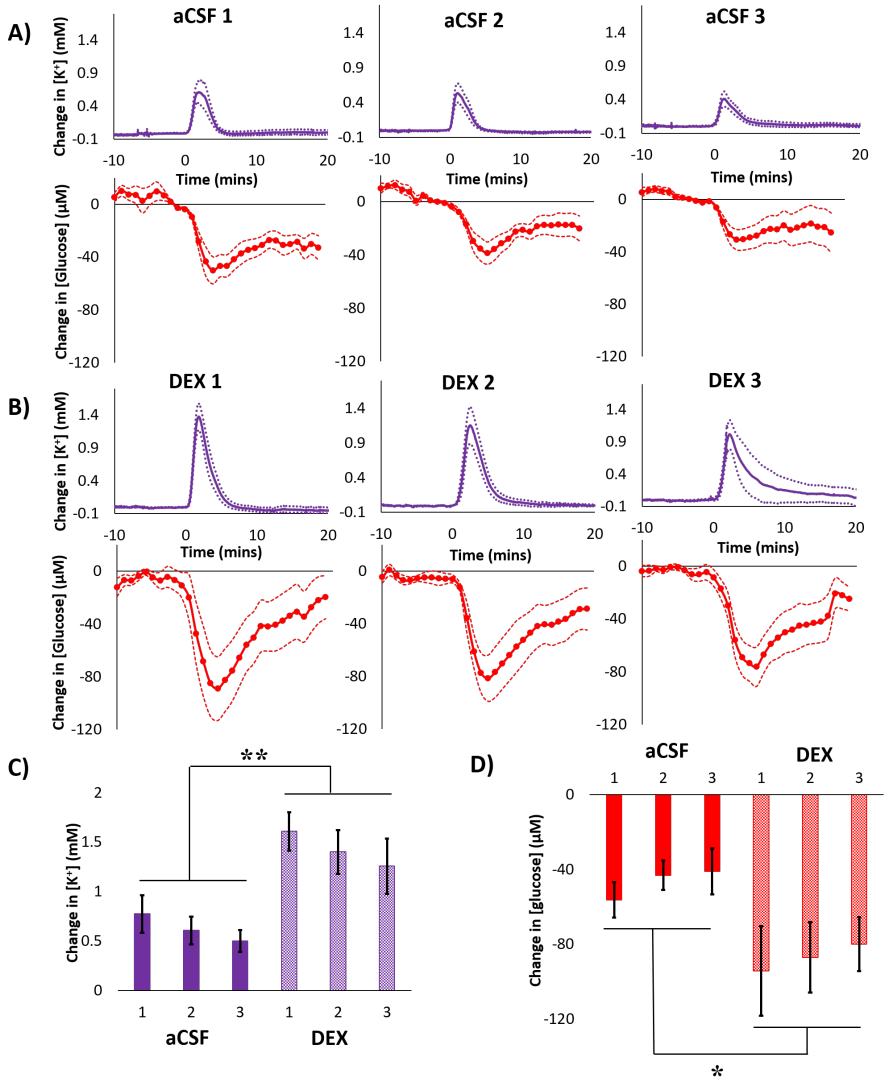


Figure 4: Cortical responses to 3 needle pricks recorded 2 hrs after probe insertion with A) aCSF or B) DEX (mean ± SEM, n = 8 rats (24 needle pricks) per group). Maximum changes in C) K^+ and D) glucose were analyzed with 2-way ANOVAs with group (aCSF, DEX) and needle prick (1,2,3; repeated measures) as the factors. The needle prick and interactions were not significant, but group was significant for both K^+ ($F(1,14) = 13.422$) and glucose ($F(1,14) = 6.253$).

$^{**}p < 0.005$, $^{*}p < 0.05$

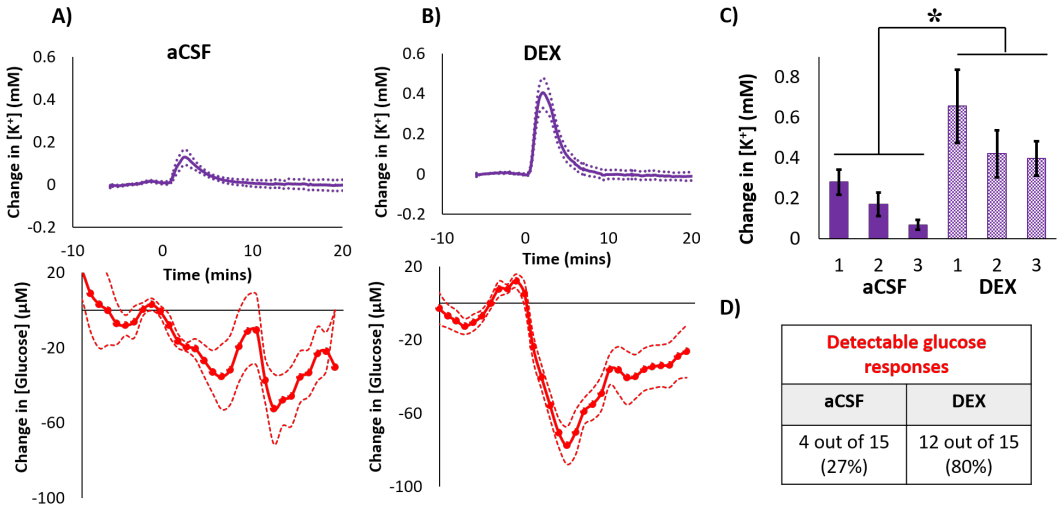


Figure 5: Average cortical response to needle pricks recorded 5 days after probe insertion with A) aCSF or B) DEX (mean ± SEM, n = 5 rats (15 needle pricks) per group). C) Changes in K^+ to the 3 needle pricks were analyzed with a 2-way ANOVA with group (aCSF, DEX) and needle prick (1,2,3; repeated measures) as the factors. Group ($F(1,8) = 5.844$, $p < 0.05$) and needle prick ($F(2,16) = 12.689$, $p < 0.001$) are significant, interaction is not significant. D) Only detectable glucose responses are represented in A and B, see Table1 and S4 for details. *$p < 0.05$.

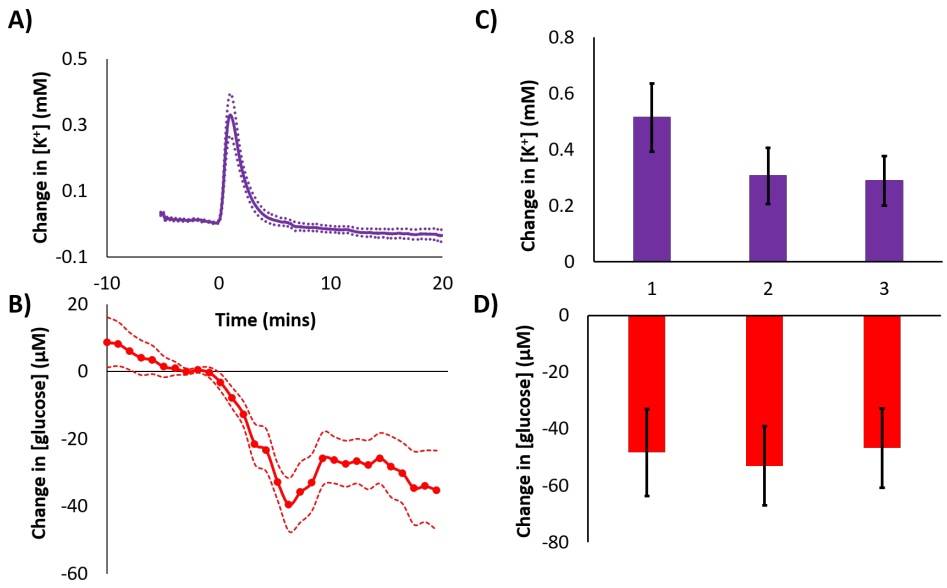


Figure 6: A) K^+ and B) glucose response (average ± SEM) to needle pricks performed 10 days after probe insertion (n=5 rats, 15 needle pricks). C and D provide the changes in K^+ and glucose, respectively, to each of the 3 needle pricks. The microdialysis probe was perfused with DEX for days 1-5 and then aCSF for days 6-10.

	Total SD events	Detectable changes in [Glucose]
2hr DEX	24	24 (100%)
2hr aCSF	24	21 (88%)
5d DEX	15	12 (80%)
5d aCSF	15	4 (27%)
10d DEX	15	13 (87%)

Table 1. The noise in a 10-min baseline prior to each needle prick was used to create a threshold for detection of the glucose dip (see Figure S4 for details). Observation of a K^+ spike confirmed SD in the vicinity of the probe. In every case, except for 5d aCSF, the majority of the K^+ spikes were accompanied by a quantifiable glucose dip: these were classified as detectable. Note that only detectable glucose dips are included in the figures and data analysis.

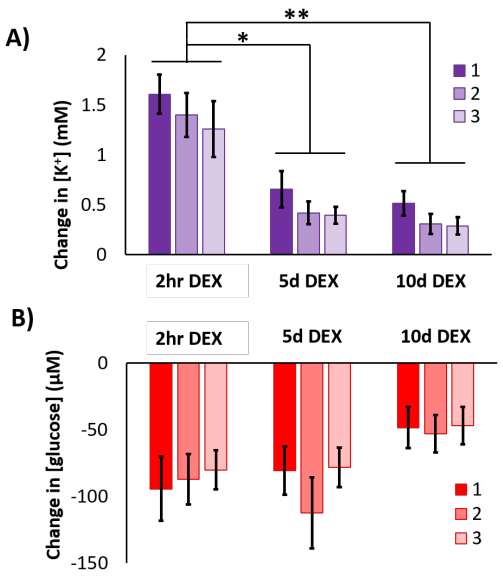


Figure 7. Summary comparison (mean ± SEM) of the amplitudes of A) K^+ spikes and B) glucose dips in response to 3 needle pricks recorded 2 hrs, 5 days, and 10 days after probe insertion in the presence of DEX. Data were analyzed with a 2-way ANOVA with time (2 hr, 5 d, 10 d) and needle prick (1, 2, 3; repeated measures) as the factors. K^+: time is significant ($F(2,15)=15.878$, $p < 0.0005$ while needle prick and the interaction are not significant. Glucose: neither factor was significant. Stars represent Games-Howell post-hoc tests, $^{**}p < 0.001$ and $^{*}p < 0.005$.

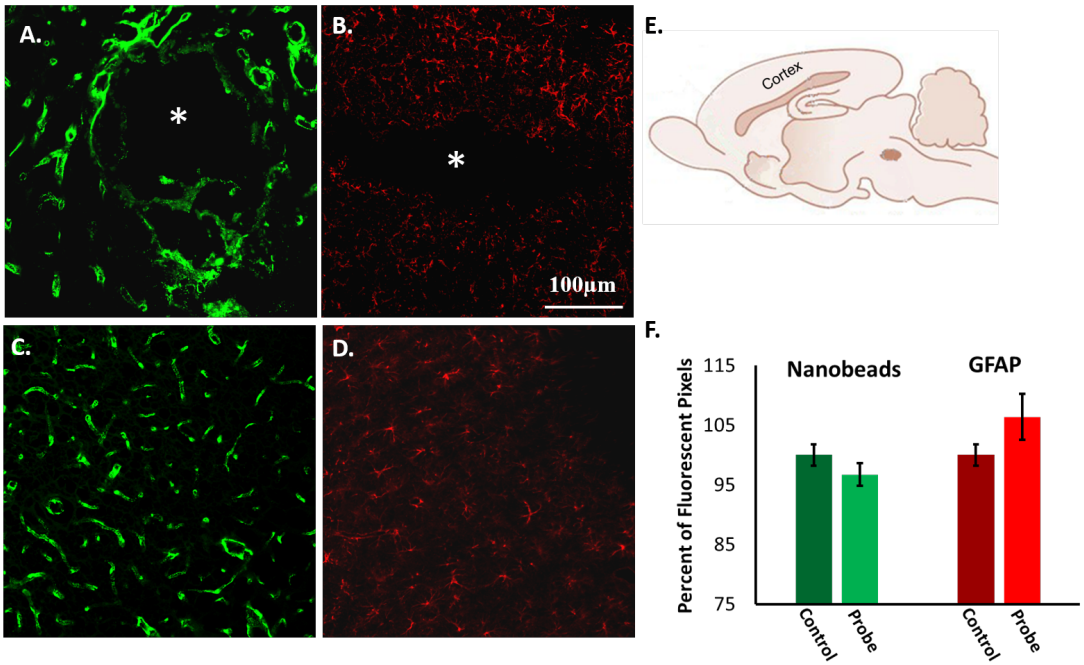
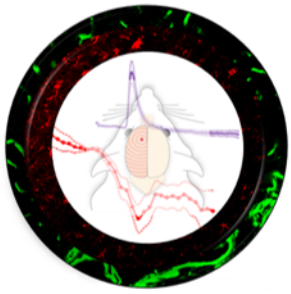


Figure 8: Representative fluorescent microscopy images of the probe tracks after 10 days. The cortex is labeled for GFAP immunoreactivity and blood flow (fluorescent nanobeads). A) Is the green nanobeads indicative of blood flow and B) represents GFAP immunoreactivity around the probe. Asterisks represent the center of the probe track. C) and D) are control cortex tissue of the contralateral hemisphere. Scale bar is 100 µm. E) is a sagittal schematic of a rat brain. F) Normalized graph comparing the blood flow and GFAP immunoreactivity in the areas of interest in both the ipsilateral and contralateral hemispheres. There is no significant difference between probe tracks and control tissue for either nanobeads or GFAP (t-tests). Results are reported as the percent of fluorescent pixels (mean ± SEM).

TOC.



ASSOCIATED CONTENT

Supporting Information

Additional information as noted in text (Supporting Figures S1-S4 and Table S1). This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

ELV, CLL, AJG, and KMN collaborated on the collection of microdialysis results. CLL set up the rsMD and K^+ detector systems in Pittsburgh and trained ELV and AJG in its use. AJG collected brain tissues, performed the immunohistochemical procedures, collected the microscope images, and quantitatively analyzed the images. All authors participated in the design of the experiments, evaluation of the results, and preparation of the manuscript.

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Conflict of Interest

The authors declare no competing financial interest.

ABBREVIATIONS

SD, Spreading Depolarization; TBI, traumatic brain injury; DEX, dexamethasone; aCSF, artificial cerebrospinal fluid; ISE, ion selective electrode; rsMD, rapid sampling microdialysis; PDMS, poly(dimethyl)siloxane; ANOVA, analysis of variance; GFAP, glial fibrillary acidic protein; PBS, phosphate-buffered saline.

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