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In Vivo Microdialysis Sampling of Cytokines from Rat Hippocampus: Comparison of Cannula Implantation Procedures

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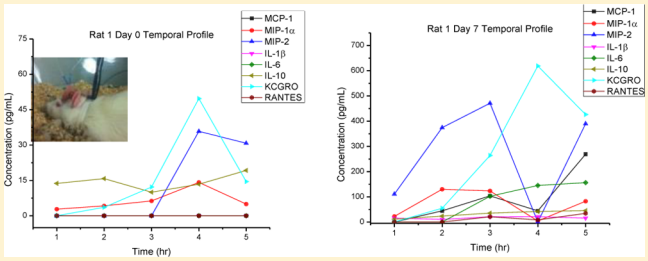
ABSTRACT: Cytokines are signaling proteins that have been of significant importance in the field of immunology, since these proteins affect different cells in the immune system. In addition to their immune system significance, these proteins have recently been referred to as a third chemical communication network within the CNS. The role that cytokines play in orchestrating the immune response within tissues after a mechanical injury leads to potential complications if the source of cytokines (i.e., trauma vs disease) is of interest. Microdialysis sampling has seen wide use in collection of many different solutes within the CNS. Yet, implantation of microdialysis guide cannulas and the probes creates tissue injury. In this study, we compared the differences in cytokine levels in dialysates from 4 mm, 100 kDa molecular weight cutoff (MWCO) polyethersulfone membrane microdialysis probes implanted in the hippocampus of male Sprague–Dawley rats. Comparisons were made between animals that were dialyzed immediately after cannula implantation (day 0), 7 days post cannula implantation (day 7), and repeatedly sampled on day 0 and day 7. Multiplexed bead-based immunoassays were used to quantify CCL2 (MCP-1), CCL3 (MIP-1α), CCL5 (RANTES), CXCL1 (KC/GRO), CXCL2 (MIP-2), IL-1β, IL-6, and IL-10 in dialysates. Differences in cytokine concentrations between the different treatment groups were observed with higher levels of inflammatory cytokines measured in day 7 cannulated animals. Only CCL3 (MIP-1α), CXCL1 (KC/GRO), CXCL2 (MIP-2), and IL-10 were measured above the assay limits of detection for a majority of the dialysates, and their concentrations were typically in the low to high (10–1000) picogram per milliliter range. The work described here lays the groundwork for additional basic research studies with microdialysis sampling of cytokines in rodent CNS.



KEYWORDS: *Chemokine, cytokine, hippocampus, rat, microdialysis sampling*

Cytokines and chemokines (*chemo*attractant cyto*kines*) are chemical signaling proteins that are commonly emitted from various cells of the immune system. Cytokines are becoming of great interest in neurobiology and a neuroimmune axis has been described.[1−7] Cytokines and their receptors have been found in the central nervous system (CNS) within astrocytes, glial cells, and neurons. Cytokines are now believed to comprise a third generation of neuromodulatory chemicals.[8] These cell-signaling proteins have been associated with many different aspects of neurological disease or injury.[9−13] For this reason, there is a significant need to unravel the cytokine network within the CNS allowing for a greater prognostic capability and ultimately treatment of various neurological disorders with an inflammatory etiology.

Different experimental approaches have been applied to elucidate the presence of cytokines in the CNS. These methods include (1) mRNA analysis through in situ hybridization or Northern blot analysis; (2) radioimmunoassay or ELISA for protein content; (3) radiolabeled cytokine studies in brain slices using autoradiography to map out receptor sites; (4) immunochemistry or immunohistochemistry; and (5) different imaging methods to map receptors.[14−16] While these different measurement methods allow the cytokine locations to be mapped, none of these methods allows for real-time in vivo cytokine collection from the extracellular fluid space (ECF), which is of great interest to numerous neuroscience researchers.

Microdialysis sampling is a diffusion-based sampling technique that has been used to sample solutes within the ECF of the CNS.[17] Microdialysis sampling works well for collection of low molecular weight, hydrophilic solutes such as the catecholamines and amino acid neurotransmitters. Microdialysis sampling has been used to collect neuropeptides.[18,19] However, sampling and quantitation for some neuropeptides such as insulin and neuropeptide Y that exist at less than nanomolar concentrations within the ECF is challenging.[20]

The success of microdialysis sampling for so many different solutes within the CNS has led researchers to want to apply the technique to collection of soluble signaling proteins such as the cytokines. Collection of cytokines with microdialysis sampling using 100 kDa or larger MWCO membranes requires strict attention to the role of fluid loss and there have been studies to minimize this problem.[21] Microdialysis sampling has been used

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Table 1. In Vitro Extraction Efficiency and Molecular Weight Information for Cytokines[a]

cytokine {cells that secrete}	mode of action	monomer MW (kDa)	active structure	EE (%)	assay LOD (pg/mL)
CCL2 (MCP-1) {M, MΦ, DC}	chemokine; P-Inf.; Monocyte attractant	13.1	monomer or homodimer	3.0 ± 0.6 (n = 8)	9.0
CCL3 (MIP-1α) {MΦ, DC, LP}	chemokine; P-Inf; recruits PMNs	8−200	monomer and may exist as higher MW aggregates	13.8 ± 4.2 (n = 8)	0.8
CCL-5 (RANTES) {M,MΦ}	chemokine; P-Inf.; recruits leukocytes	8	homodimer	10.5 ± 5.9 (n = 8)	1.3
CXCL1 (KC/GRO) {MΦ, N, E}	chemokine; P-Inf. and A-Inf.; multiple functions	7.8	monomer and homodimer	9.3 ± 3.2 (n = 8)	0.7
CXCL2 (MIP-2) {M,MΦ}	chemokine; P-Inf.; recruits PMNs	7.9	homotetramer	8.5 ± 2.4 (n = 8)	11.3
IL-1β {MΦ}	P-Inf.	17.4	monomer	2.1 ± 0.8 (n = 8)	2.8
IL-6 {MΦ, T}	P-Inf. and A-Inf.	21.7	monomer	1.4 ± 0.7 (n = 2)	30.7
IL-10 {M}	A-Inf.	18.7	homodimer	0.9 ± 0.9 (n = 4)	2.7

[a]All samples were collected using a CMA/12 probe with a 4 mm PES membrane at 1.0 μL/min with 2% BSA in the perfusion fluid. EE% is presented as mean ± SD. Abbreviations are as follows: A-Inf, anti-inflammatory; DC, dendritic cells; E, epithelial cells; LP, lymphocytes; MΦ, macrophage; M, monocytes; N, neutrophils; PMN, polymorphonuclear leukocytes; P-Inf., pro-inflammatory; T, T-cells.

for collection of cytokines within the human brain.[12,22,23] However, in human studies, the catheters used are typically 10 mm long, flow rates of 0.3 μL/min are commonly used, and the dialysate samples are frequently pooled. Most of these published reports describing microdialysis sampling in humans are focused on understanding the inflammatory cytokine signature associated with traumatic brain injury (TBI). In these cases, it is likely the inflammation from the TBI and thus the cytokine response would override the inflammatory response that implantation of the microdialysis probe causes. As a greater understanding of cytokine interactions and control of brain function become available, it will be critically important to be able to discriminate between cytokine signals associated with the disease state versus those associated with the implantation of a foreign object, the microdialysis probe.[24] This is especially important as neurosurgeons consider using microdialysis sampling to elucidate the levels of inflammation within a patient undergoing surgery for either placement of stimulating electrodes for controlling Parkinson's disease or removal of epileptic foci, both diseases in which cytokines have been implicated in part of the disease process. In such instances, the surgeon only gets one attempt to collect cytokines within the ECF. Thus, a critical understanding of which cytokines one can confidently state are due to the disease process versus the insertion of the microdialysis probe is essential for making informed clinical decisions.

During microdialysis sampling of the CNS in rodent models, there are many different procedures used in different research groups for guide cannula implantation, probe implantation and perfusions, and post implantation waiting periods before initiation of sampling. Given the known progression of the foreign body reaction that occurs via different stages,[25] it would be anticipated that cytokines collected may vary among these different implantation procedures. For this reason, we chose to study the response of eight different cytokines, IL-1β, IL-6, IL-10, KC/GRO, MCP-1/CCL2, MIP-1α/CCL3, MIP-2/CXCL2, and RANTES/CCL5, after acute microdialysis probe implantation, which more closely matches what would be seen in the clinic versus a 7 day recovery period after cannula implantation prior to probe implantation which is performed by some research groups. This set of cytokines was chosen based on their known involvement with different neurological disease states and their role in the foreign body reaction. We recognize there are many other waiting schemes used after microdialysis probe implantation. These include waiting several hours or perfusing the dialysis probe at flow rates of 0.2 or 1.0 μL/min overnight prior to the start of sampling. Quantifying cytokine levels in dialysates after these other postimplantation procedures is certainly important, but is not part of this study. The hippocampus was chosen in these preliminary studies because of its importance in different disease states such as behavior and epilepsy.

RESULTS AND DISCUSSION

Table 1 shows the in vitro extraction efficiency (EE) values using a flow rate of 1.0 μL/min for the eight different cytokines across the CMA-12 4-mm PES 100 kDa MWCO probe. The in vitro EE is simply the ratio of the dialysate concentration over the sample concentration. The mathematical description for EE has been well-described by Bungay et al., and in the numerous citations to their original paper.[26] In general, the in vitro EE for cytokines matches what would be expected for their molecular weight values with a few exceptions. IL-10 has a much larger hydrodynamic radius than the other cytokines, and we have previously observed that this cytokine is difficult to recover across the PES membranes.[27] All of the cytokines had EE% values less than 14%, illustrating the known challenges with collecting low pg/mL solutes within the ECF.

Only a few cytokines have been quantified in brain dialysates obtained from rats and include IL-1β, IL-6, and MCP-1.[28−33] Several other cytokines including RANTES and MIP-1α have been quantified from microdialysis probes placed into an in vitro astrocyte culture system that was stimulated using vasoactive intestinal protein.[34] With relatively few publications of cytokine measurements from brain dialysates combined with differences in probe placements into different brain regions, it is not possible to predict what the cytokine concentrations should be within a particular brain region after a particular treatment.

In this study, we chose to compare how cytokine levels change as a function of the cannula implantation time as well as differences in cytokine levels after repeated microdialysis measurements. There have been no standardized procedures for the waiting periods and dialysis protocols to use for measurements of any solute after implantation of microdialysis probes into the brain. There is variation with respect to guide cannula placements to the time between cannula implantation and probe implantation. Others implant the cannulas and then have an overnight washout period prior to sampling. Different

Table 2. Dialysate Cytokine Concentration Range (pg/mL) from Day 0 Cannula Implantations[a]

	rat 1	rat 2	rat 3	rat 4
CCL2 (MCP-1)	N.D.	N.D.	N.D.	N.D.
CCL3 (MIP-1α)	3−21	4−10	3−5	3−35
CCL-5 (RANTES)	N.D.	N.D.	5−9	N.D.
CXCL1 (KC/GRO)	6−70	1.5−98 (1)[b]	3−1052	21−206 (1)[b]
CXCL2 (MIP-2)	27−45 (1)[b]	27−33	26−73 (1)[b]	25−50
IL-1β	N.D.	N.D.	N.D.	N.D.
IL-6	N.D.	N.D.	N.D.	N.D.
IL-10	10−13 (1)[b]	14−20 (1)[b]	12−25 (1)[b]	11−22

[a]Samples were collected once per hour for 5 h at 1.0 μL/min. N.D. = Not detected (<LOD). [b]Indicates the number of samples out of five that had concentrations below the LOD.

Table 3. Dialysate Cytokine Concentration Range (pg/mL) from Day 7 Cannula Implantations[a]

	rat 1	rat 2	rat 3	rat 4	rat 5	rat 6	rat 7
CCL2 (MCP-1)	48−83	50−110 (2)[b]	48−103 (1)[b]	48−77 (1)[b]	23−35 (1)[b]	23−177	23−83
CCL3 (MIP-1α)	4−13 (1)[b]	13-21	29−65	17−46	4−12	12−311 (1)[b]	10−600
CCL5 (RANTES)	N.D.	7 (4)[b]	5−20 (2)[b]	8−34 (2)[b]	N.D.	7−10 (3)[b]	6−20 (2)[b]
CXCL1 (KC/GRO)	4−90	18−120	32−550	23−200	2−210 (1)[b]	2−1460	8−1885
CXCL2 (MIP-2)	40−80 (1)[b]	20−245	110−305	60−300	35−75 (1)[b]	70−2130 (1)[b]	30−1990
IL-1β	6−26 (1)[b]	12−36 (1)[b]	25−65	7−40	6−12 (1)[b]	3−17	3−11
IL-6	70 (4)[b]	130 (4)[b]	120 (4)[b]	N.D.	80 (4)[b]	125 (4)[b]	80−100 (2)[b]
IL-10	45−195	13−45	10−30	9−14	9−15	10−40	9−26

[a]Samples were collected once per hour for 5 h at 1.0 μL/min. N.D. = Not detected (<LOD). [b]Indicates the number of samples out of five that had concentrations below the LOD.

variations among these procedures also exist. There is also interest in performing repeated dialysis measurements in animals where the guide cannula will be implanted chronically and repeated dialysis sampling will occur. For these reasons, we chose to start with a comparison of cytokine levels from animals that had a cannula implanted on the day of sampling (Day 0), animals that had a guide cannula implanted for 7 days (Day 7), and repeated dialysis measures from Day 0 and Day 7. In each of these cases, we then placed the dialysis probe into the cannula and began sampling immediately.

Microdialysis sampling has historically been touted as a minimally invasive sampling procedure. Yet, in reality, the implantation of a microdialysis sampling probe into the brain as well as any tissue creates a stab wound.[24,35−37] Stab wounds to rat brain have been shown to exhibit alterations in inflammatory chemokine and cytokine gene expression.[38−40] Chemokines produced from tissue surrounding a stab wound would be expected to come from activated microglia.[41] Histological observations demonstrating altered tissue structures in the brain surrounding implanted microdialysis probes have also been reported.[42,43]

Tables 2 and 3 show the concentration range for the cytokines quantified in the dialysates obtained from animals that had sampling occur either after the cannula implantation (Day 0) or seven days post cannula implantations (Day 7). Five samples were obtained once an hour for 5 h using a flow rate of 1.0 μL/min. While some of the cytokines typically expressed an expected pattern of gradual increase in their concentrations within the sequential collection period, many others did not exhibit this trend and occasionally had large spikes in their concentrations (data not shown). The most reliable cytokine with respect to exhibiting a trend of increasing concentration after probe implantation was KC/GRO. Since it is not clear if these observed concentration spikes are true biological events or anomalies, we chose to report the range of concentrations from the dialysates collected and the number of samples out of five that had cytokine concentrations below the limits of detection (LODs) of the immunoassay.

When comparing the cytokine concentrations obtained in the dialysates between the Day 0 and Day 7 cannula implantations, it is clear there is a higher level of inflammatory cytokines in the Day 7 animals. In the Day 0 cannula implantation animals, the concentrations of CCL2, CCL5, IL-1β, and IL-6 were all below the LOD for the immunoassay kit. However, for the Day 7 animals, all of these cytokines were quantified although some were at low levels. There are also much higher concentrations for MIP-2 observed in the Day 7 vs the Day 0 animals. IL-10 concentrations also appear to be slightly elevated in Day 7 animals, but it is not clear if this increase is significant. While it is possible to pool this data to compare the animals, the time series information is then lost. This issue combined with the time series data giving different results such that samples really cannot be pooled as we have previously demonstrated for glucose by repeated measures ANOVA.[44] Additionally in many of the data sets, there are samples below the LOD suggesting the need for a far more complicated statistical analysis to characterize this preliminary data. Figures 1−8 show the concentrations of cytokines collected from four separate animals that were repeatedly dialyzed on Day 0 after the cannula implantation and then on Day 7. As with the Day 7 only samples, the repeated dialysate samples on Day 7 show cytokine concentrations that appear to be elevated compared to those observed on Day 0.

KC/GRO, CXCL1, a chemokine, is a neutrophil chemoattractant protein and has reported to be produced by neurons and endothelial cells in rat brain.[45] CXCL1 protein has been quantified from tissue samples from rat brain after a TBI.[46] In this study, CXCL1, was detected in nearly all dialysates which would be expected given the mechanical injury induced by the implantation of the microdialysis probe. This mechanical injury

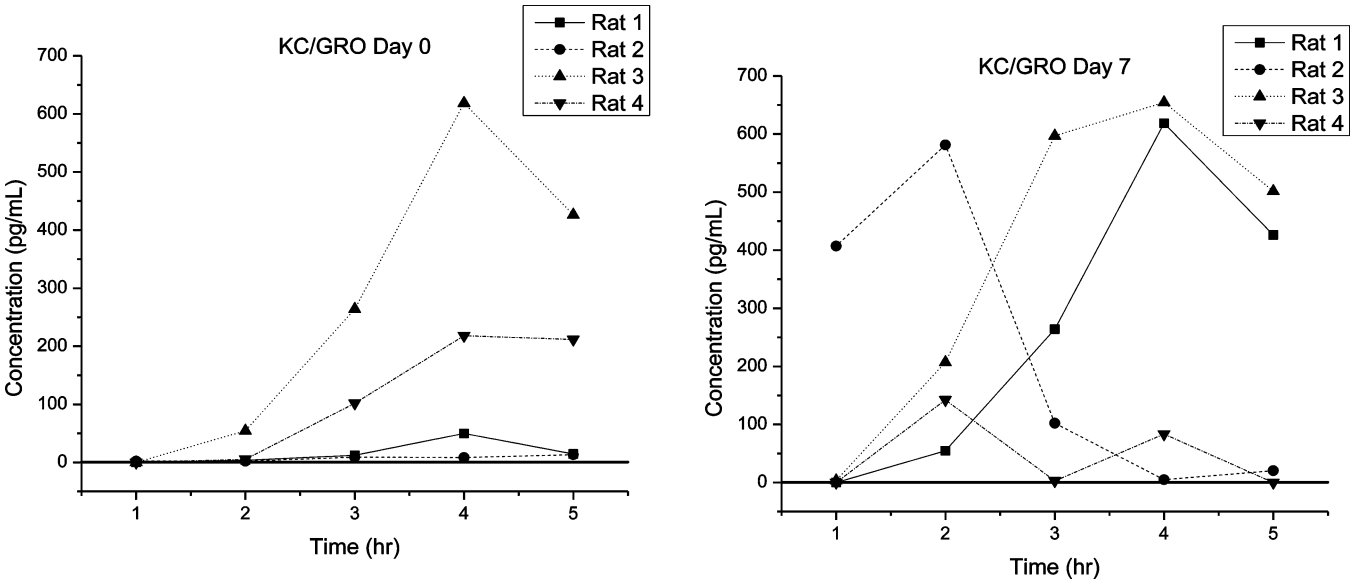

Figure 1. KC/GRO concentrations from four different rats sampled on Day 0 and Day 7 after cannula implantation. The solid line indicates the LOD for the bead-based immunoassay for KC/GRO. Any value < LOD was set to zero.

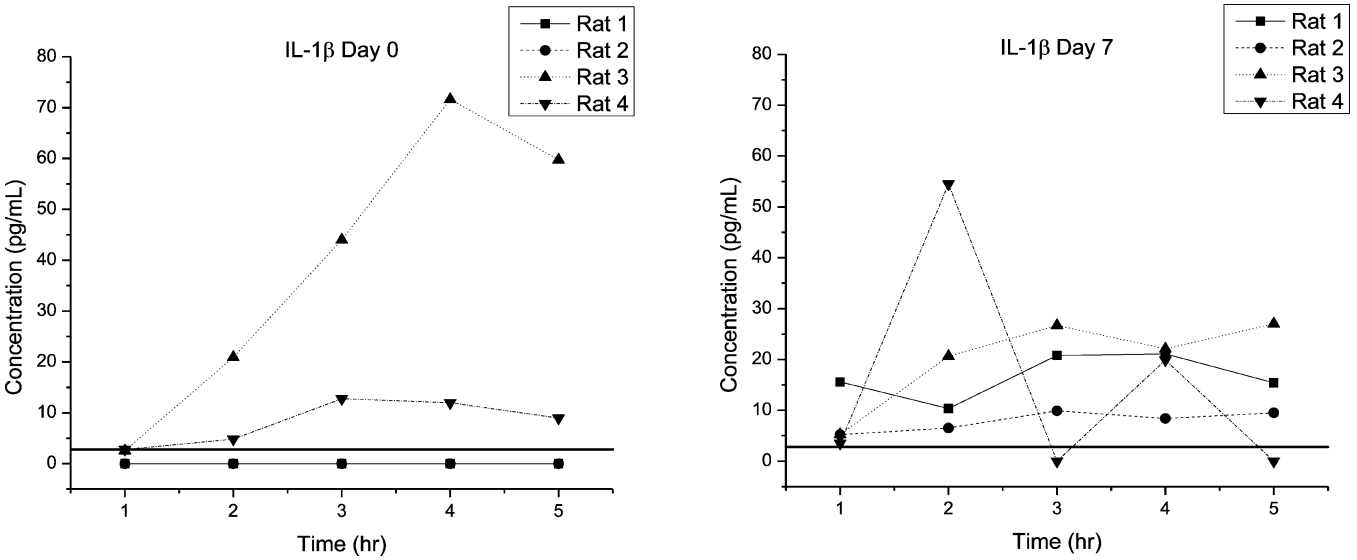

Figure 2. IL-1β concentrations from four different rats sampled on Day 0 and Day 7 after cannula implantation. The solid line indicates the LOD for the bead-based immunoassay for IL-1β. Any value < LOD was set to zero.

could cause release from the different cells that release CXCL1. Elucidating the exact cell type would likely require animals with an appropriate genetic knockout for the cell type that is desired to be isolated.

MCP-1/CCL2 is a chemokine that serves to attract monocytes. While we have previously quantified CCL2 in dialysates in a study where the dialysis probe was implanted at different stereotaxic coordinates,[33] the concentrations of MCP-1 quantified in dialysates from this work were far lower in the tens of pg/mL range versus the 100s to 1000s of pg/mL range in our previous work. There may be several contributing factors to this observation including differences between the measurement platforms and localization of CCL2 within the brain. There have been several reports describing large differences in absolute cytokine concentrations for the same samples when multiplexed kits are compared to standard ELISA measurements.[47−49] A recent large international study that compared multiple cytokine detection platforms reported the Luminex assays to have the lowest interlaboratory reproducibility.[50] CCL2 is produced in neurons and differences among brain regions for the mRNA expression have been reported with hippocampus being lower than other regions such as the hypothalamus and the pituitary.[51] The CCL2 receptors have been mapped within different brain regions and the receptor levels appear to match the gene expression levels for this protein.[52]

RANTES/CCL5 is a chemokine that like other chemokines has been found in the rodent in varying distributions within different brain regions.[8] In this work, CCL5 concentrations in the dialysate were either not detected or in low pg/mL concentrations. This is despite the reasonable in vitro EE% of 10% for this chemokine. CCL5 has been reported to be expressed during stab wounds in the rat brain although its expression was reported to be more diffuse and not from

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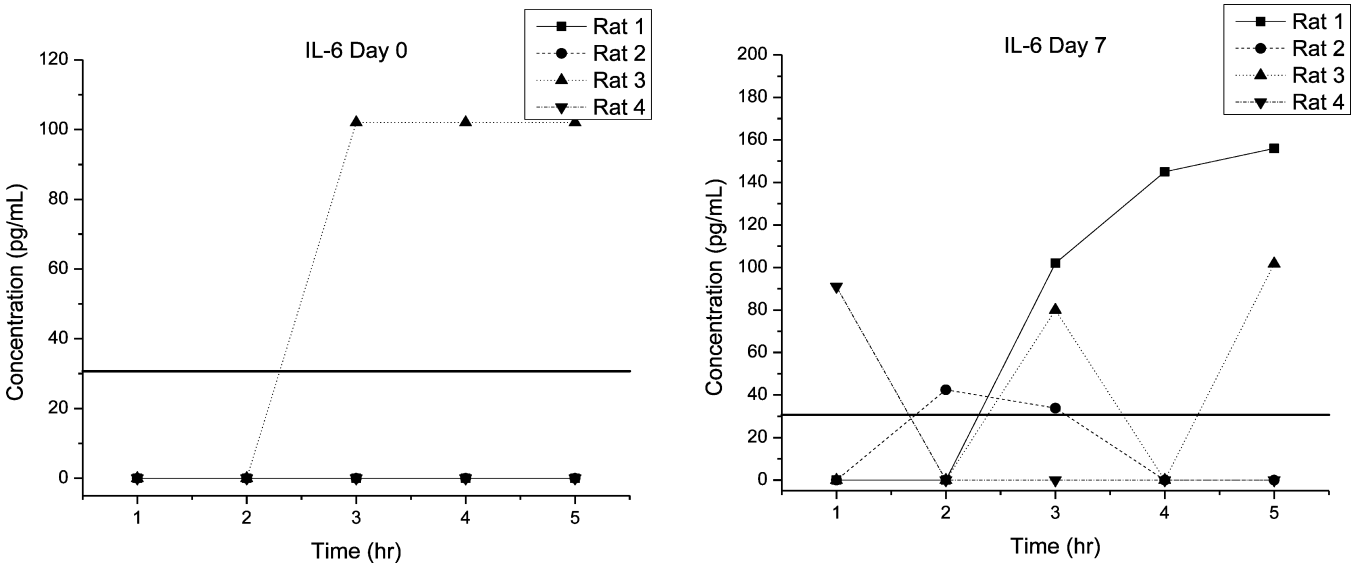

Figure 3. IL-6 concentrations from four different rats sampled on Day 0 and Day 7 after cannula implantation. The solid line indicates the LOD for the bead-based immunoassay for IL-6. Any value < LOD was set to zero.

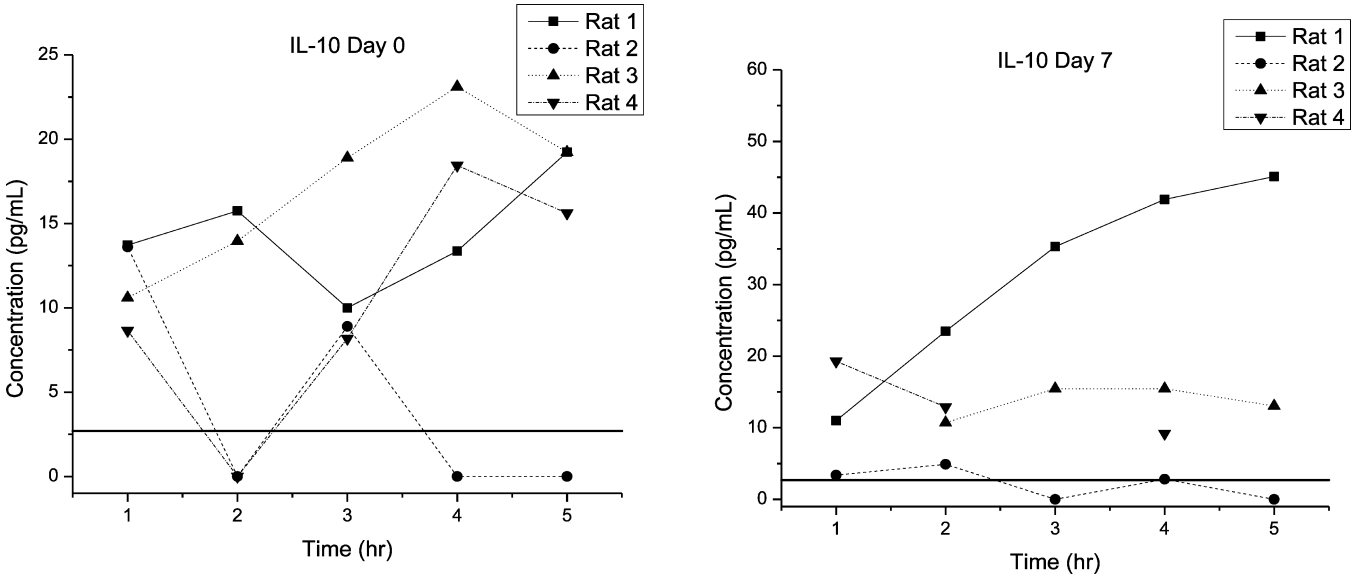

Figure 4. IL-10 concentrations from four different rats sampled on Day 0 and Day 7 after cannula implantation. The solid line indicates the LOD for the bead-based immunoassay for IL-10. Any value < LOD was set to zero.

astrocytes.[38] RANTES has also been collected from dialysis catheters that have been implanted into the human brain after TBI.[12,53]

MIP-1α/CCL3 is a chemokine that serves to recruit and activate polymorphonuclear leukocytes. In this work, CCL3 was detected in most dialysates among the different groups. This is expected as this chemokine also has been detected from stab wounds.[38]

MIP-2/CXCL2 is a chemokine that attracts both polymorphonuclear leukocytes and hematopoietic stem cells. CXCL2 was quantified in most dialysates. MIP-2 gene expression has been found to be slightly upregulated after hemorrhage in the rat.[54]

IL-10 is an anti-inflammatory cytokine. In the brain, this cytokine is produced from microglia.[55,56] While IL-10 was measured in many of the dialysates, the concentrations were in the low pg/mL range. It may not be expected to have IL-10 in dialysates right after implantation since typically IL-10 regulation would be upregulated after an inflammatory event. However, in a postnatal rodent study involving the creation of cytotoxic lesions using *N*-methyl-D-aspartate in the sensorimotor cortex, IL-10 concentrations from tissue were found to be approximately 6 pg/mg total protein of tissue at 10 h postlesion for controls versus 8 pg/mL for the lesion, which was reported as significantly higher at the $p < 0.05$ level. At 7 days post lesion, the controls had approximately 8 pg/mL and the lesions contained 9 pg/mL of IL-10. Given the small level of changes between control and chemically lesioned tissue, it may not be surprising that IL-10 concentrations remain fairly constant across the dialysate collections within the different cannula implantation procedures.

There is more literature about brain dialysis in rodents and quantitation of the cytokines IL-1β and IL-6 as compared to the other cytokines measured in this study. In most of these

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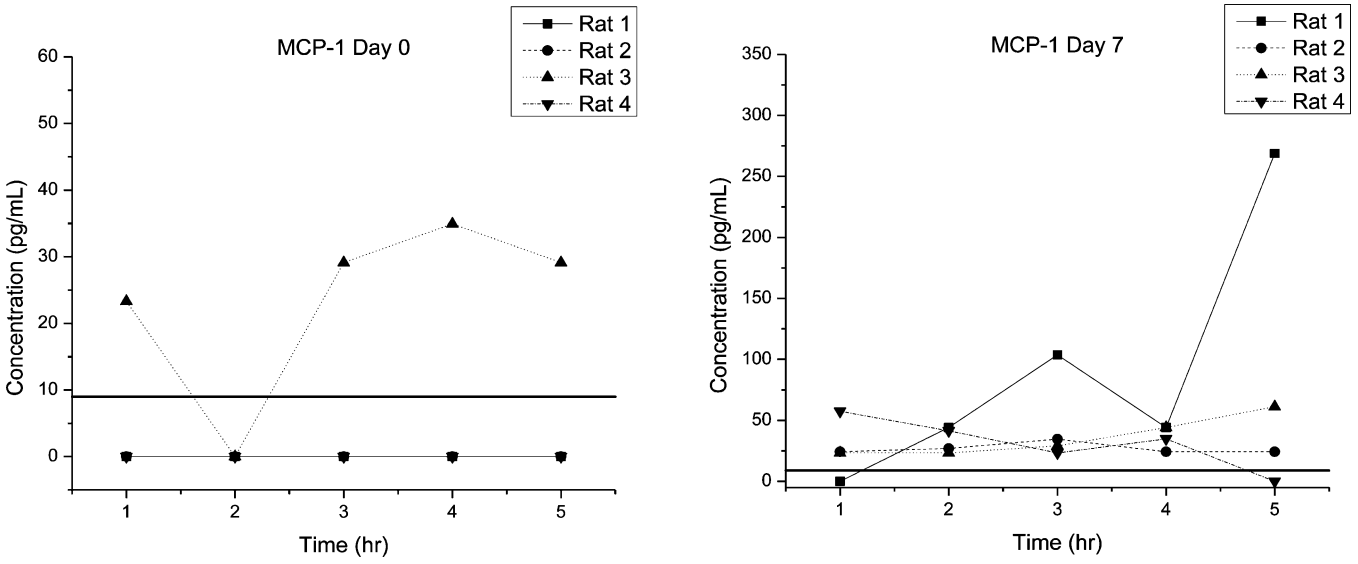

Figure 5. MCP-1 concentrations from four different rats sampled on Day 0 and Day 7 after cannula implantation. The solid line indicates the LOD for the bead-based immunoassay for MCP-1. Any value < LOD was set to zero.

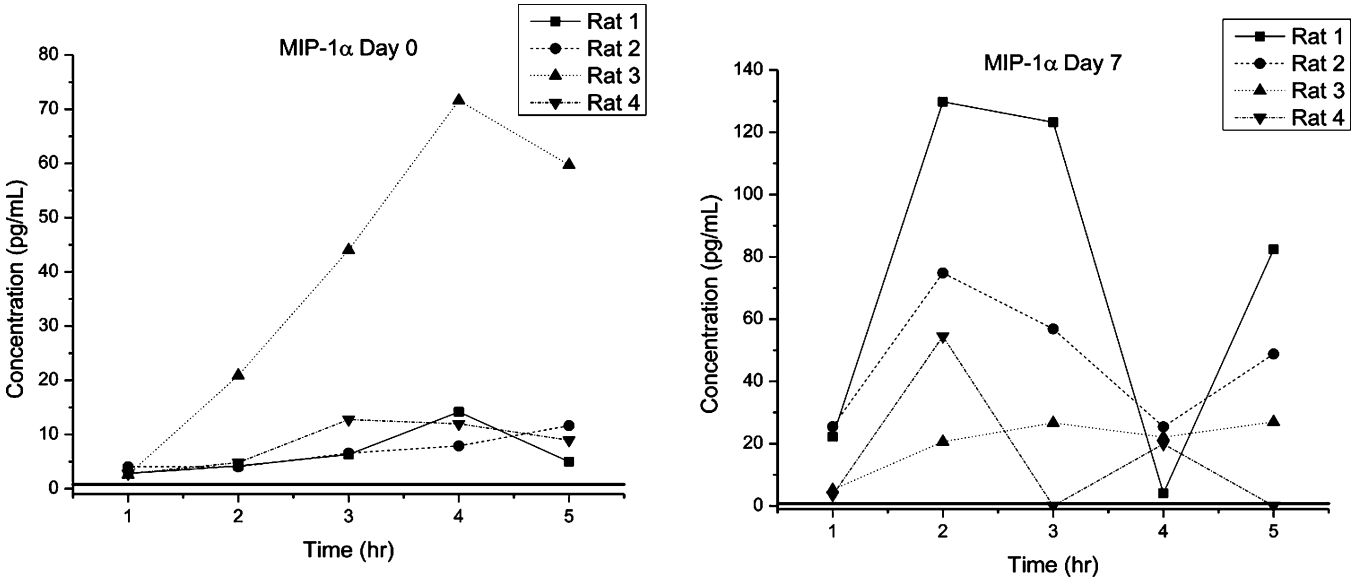

Figure 6. MIP-1α concentrations from four different rats sampled on Day 0 and Day 7 after cannula implantation. The solid line indicates the LOD for the bead-based immunoassay for MIP-1α. Any value < LOD was set to zero.

reports, these two cytokines have been measured under significant stress. Folkersma and colleagues report concentrations of IL-1β and IL-6 after TBI in the parietal lobe and reported only low pg/mL levels in dialysates from 2 mm, CMA-12 probe with a 100 kDa-MWCO and flow rates of 0.5 μL/min.[31] In our work, these two cytokines were not recovered from Day 0 implantations which was surprising. It is not clear if this observation is due to the biology or the immunoassay kit as described above for MCP-1. Additionally, the LOD for IL-6 is much higher on the new immunoassay bead kits than the previous kits or standard ELISA kits from companies such as R&D Systems. This higher assay LOD could also contribute to the reason that IL-6 was not quantified in many of the samples.

The measurement and elucidation of cytokine concentrations within ECF of the rodent brain is uncharted territory. While cytokine collections using microdialysis catheters have been performed in humans and multiplexed measurements have been made, there are challenging differences between the current state of the art with respect to human cytokine measurements vs those for use in basic research.

The majority of human brain microdialysis sampling of cytokines has been from subjects that have incurred a TBI and have subarachnoid hemorrhage.[12,22,53,57,58] Alternatively, the collections have come from other severe injuries such as a subarachnoid hemorrhage from an aneurysm.[59] Traumatic brain injury activates different inflammation pathways including disruption of the blood brain barrier allowing for the infiltration of peripheral immune cells, glial activation, and neuronal cell death which are all processes that affect cytokine production.[60,61] It is not surprising that quantifiable cytokine levels are observed in human brain after a TBI due to the high levels of inflammation that are known to exist after such an injury.

In addition to expecting a significant level of inflammation from a TBI, the membrane lengths (surface area) are longer

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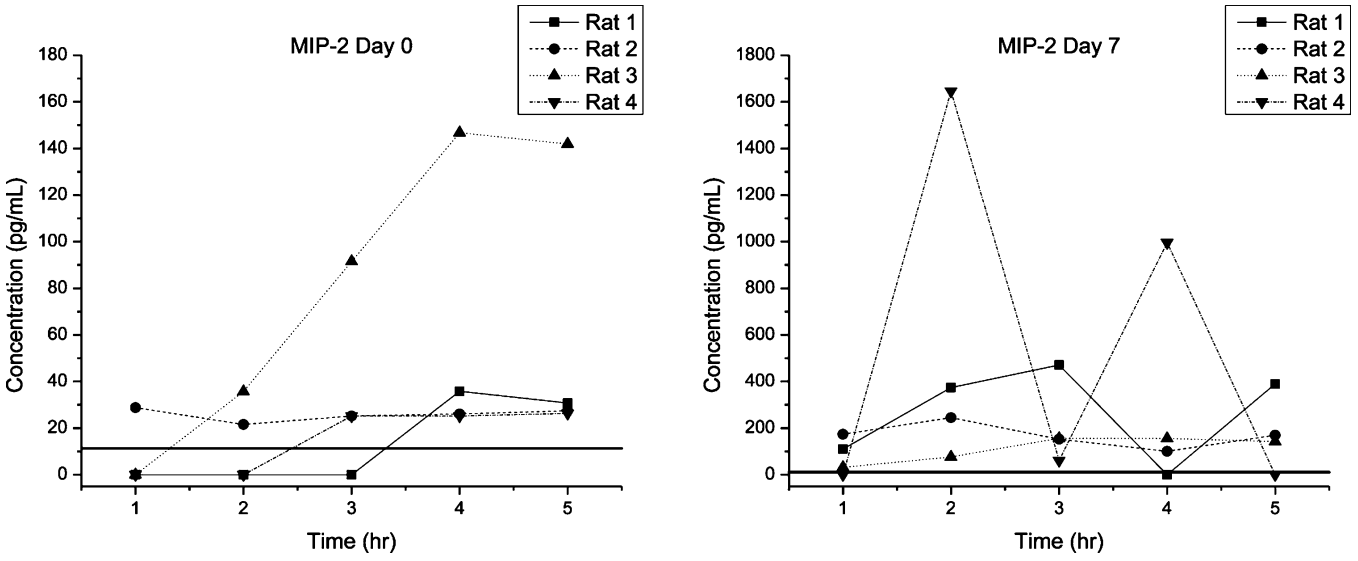

Figure 7. MIP-2 concentrations from four different rats sampled on Day 0 and Day 7 after cannula implantation. The solid line indicates the LOD for the bead-based immunoassay for MIP-2. Any value < LOD was set to zero.

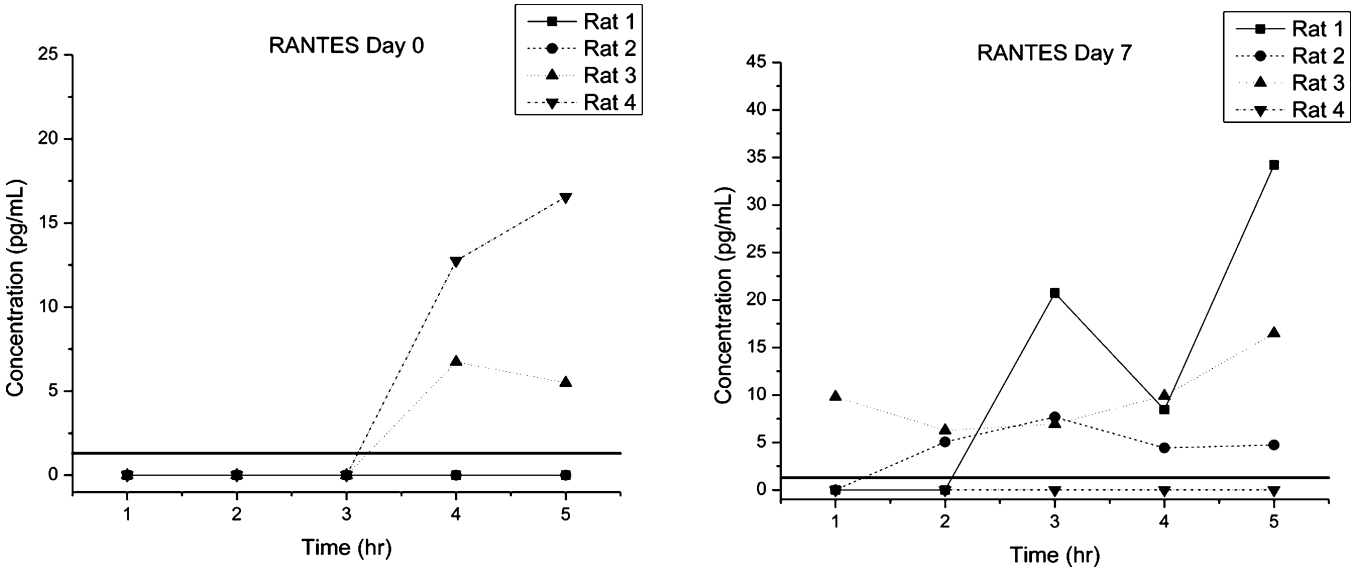

Figure 8. RANTES concentrations from four different rats sampled on Day 0 and Day 7 after cannula implantation. The solid line indicates the LOD for the bead-based immunoassay for RANTES. Any value < LOD was set to zero.

(10 mm vs 4 mm) and the flow rates commonly used for human cerebral microdialysis studies are significantly reduced (0.3 vs 1.0 μL/min) resulting in a significantly greater recovery of these proteins through the dialysis membranes.[26,27] Collections of dialysates from humans are often taken over long periods of time representing a weighted average of concentrations over many hours, rather than a specific concentration at a specific time point.[62]

CONCLUSIONS

Eight different cytokines known to be present in the rodent brain and important to wound healing have been quantified in dialysates collected from the rat hippocampus. This work provides an initial baseline for making other comparisons among collection/implantation methods as well as alterations in cytokine levels associated with different disease states. Such work has been reviewed with different neurotransmitters to elucidate the role of tissue damage from microdialysis implantations with respect to collection of classical neurotransmitters finding that dopamine, noradrenaline, serotonin and acetylcholine likely is related to functional release whereas glutamate and GABA is not so convincing.[63] The effect of the tissue damage caused by microdialysis sampling for both collections of neurotransmitters as well as cytokines will continue to be debated.[42,64−66] The animal-to-animal variability observed also provides a basis to more fully understand cytokine expression and measurement within the ECF. Unlike the known classical neurotransmitters, the expected basal concentrations for cytokines have not been summarized. Many studies have shown differences in concentrations among various brain regions for classical neurotransmitters such as glutamate.[67,68] Finally, the observed differences in cytokine concentrations between day 0 and day 7 cannulated animals keenly illustrates that microdialysis probe implantations and sampling is not necessarily as "minimally invasive" as the

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microdialysis sampling community has adopted. A foreign body reaction will occur at the site of implantation, and elucidating its role will be necessary for interpreting future studies using microdialysis sampling for measurement of cytokines.

METHODS

Chemicals. Sodium chloride, potassium chloride, calcium chloride, sodium hydroxide, and D-glucose were obtained from Sigma-Aldrich (St. Louis, MO). Magnesium chloride was purchased from Alfa Aesar. Ascorbic acid was sourced from Amreco (Selen, OH); bovine serum albumin (BSA, 100%) was purchased from Rockland Immunochemicals (Gilbertsville, PA). All solutes were dissolved in HPLC grade water (Fisher Scientific, Fair Lawn, NJ). The carprofen injectable was purchased from Butler Schein (North Dublin, OH), and carprofen/enrofloxacin tablets (2 mg/2 mg) and placebo tablets were sourced from Bioserv (Beltsville, MD)

Microdialysis Sampling Materials. CMA 12 PES 4 mm membrane 100 kDa molecular weight cut off (MWCO) probes and CMA 12 guide cannulas (Harvard Apparatus, Holliston, MA) were used for all collections. Isoflurane was purchased from Abbott Laboratories (North Chicago, IL). Self-tapping bone screws and the microdrill were obtained from Fine Science Tools (Foster City, CA). Dental cement was purchased from Stoelting Co. (Wood Dale, Illinois).

Artificial cerebral spinal fluid (aCSF) was used as the perfusion fluid for all studies and contained 145 mM NaCl, 2.8 mM KCl, 1.2 mM $CaCl_2$ 1.2 mM $MgCl_2$, 0.25 mM ascorbic acid, 5.4 mM D-glucose, pH altered to 7.2−7.4 using NaOH in HPLC grade water (Fisher Scientific, Fair Lawn, NJ). To this was added 2% (w/v) BSA, and the entire solution was sterile syringe-filtered (0.2 μ m) (GE Healthcare, Pittsburgh, PA) before use. Halt protease inhibitor (Thermo Scientific, Rockford, IL) was added to all collection vials before dialysates were collected. Bead-based immunoassays (8-plex) were obtained from Millipore (Bellerica, MA).

In Vitro Recovery. Two CMA 12 probes were placed into an Eppendorf 0.5 mL LoBind centrifuge tube containing 1000 pg/mL IL-1β, 30 000 pg/mL IL-6, 3000 pg/mL IL-10, 500 pg/mL KC/GRO, 3000 pg/mL MCP-1/CCL-2, 1000 pg/mL MIP-1α, 10 000 pg/mL MIP-2, 2000 pg/mL RANTES/CCL-5 and aCSF. The solution was incubated at 35 °C, and collections made at 1 μL/min for 60 min over a 4 h period. Some cytokine in vitro recovery experiments were repeated and the extraction efficiency data was pooled to give values of n = 8. Dialysates were analyzed immediately following collection using a MAGPIX instrument and 8-plex bead based immunoassays.

Surgical Procedures. All surgical procedures were approved by the University of Arkansas IACUC and were in compliance with the NIH Guide for the Care and Use of Laboratory Animals. Male Sprague−Dawley (275−325 g) rats (Harlan, Indianapolis, IN) were housed in an environmentally controlled facility with 12 h on/off light cycle, bedded with a hardwood chip/cellulose fiber mixture. Standard rodent diet and tap water was provided ad libitum. For 2 days prior to surgery, animals were given placebo tablets to acclimate them to the medicated carprofen/enfloxacin tablets provided for pain management.

Animals were anesthetized using isoflurane 5% at a flow rate of 0.8 L/min. After sedation, the rat was transferred to a Kopf stereotaxic unit (Tujunga, California) and anesthesia maintained through a nose cone at 2−4% isoflurane at 0.5 L/min. To maintain hydration, 2 mL of 0.9% saline solution was administered subcutaneously. Aseptic technique was maintained during the surgical procedure. Two dental screws were implanted laterally to the probe. Coordinates of +4.6 mm anterior, −5.6 mm lateral, and −3.3 mm dorsal with respect to bregma were used for guide cannula implantation. The cannula was inserted over a 7 min period. Dental cement was applied to seal the wound, and to provide stability for the cannula. After cannula implantation, an injection of carprofen (5 mg/kg) in 0.9% saline containing was administered s.c. For 2 days postsurgery, animals were dosed with carprofen/enrofloxacin tablets (2 mg/2 mg). Consumption of the tablet was assessed during daily health/weight checks. After collections were made the animal was euthanized with carbon dioxide.

Microdialysis Sampling. All collections were made in a Raturn (BASi, West Lafayette, IN) while the animal was awake and freely moving. For day 0 collections, probes were acutely implanted after surgery. For day 7 collections, animals were lightly sedated and probes implanted acutely. Using a syringe pump (BASi BEE), a 30 min flush was performed through the microdialysis probe at 1 μL/min after which collections began at 1 μL/min for 60 min for 5 h. All collection vials contained a 1× solution of halt protease inhibitor. Samples were stored on ice, and kept frozen at −80 °C until analyzed using a MAGPIX instrument and 8-plex bead based immunoassays.

Quantitation Procedures. Dialysates were quantified using 8-plex bead based immunoassays and a MAGPIX instrument. Samples were analyzed following the kit protocols except, samples were not diluted further, were allowed an initial 18 h incubation step, and resuspended in 1× wash buffer prior to analysis. The manufacturer reported LOD for these assays for each analyte is (pg/mL) KC/GRO (0.7), IL-1β (2.8), IL-6 (30.7) IL-10 (2.7), MCP-1 (9), MIP-1α (0.8), MIP-2 (11.3), and RANTES (1.3).

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Author Contributions

T.W.V. and M.R.J. have contributed equally to this work. T.W.V., M.R.J., T.M.P., and J.A.S. designed the experiments. Experiments were performed by T.W.V., M.R.J., and T.M.P. Data was analyzed and interpreted by T.M.V., M.R.J., and J.A.S. The bulk of the manuscript was written by J.A.S. with section drafts from T.W.V. and M.R.J. The manuscript was edited by T.W.V., M.R.J., T.M.P., and J.A.S.

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Notes

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ABBREVIATIONS

CCL2, chemokine (C−C motif) ligand 2, also called monocyte chemoattractant protein-1 (MCP-1); CCL3, chemokine (C−C motif) ligand 3, also called macrophage inflammatory protein-1α (MIP-1α); CCL5, chemokine (C−C motif) ligand 5, also called regulated and normal T cell expressed and secreted protein (RANTES); CXCL1, chemokine (C−X−C motif) ligand 1, also called KC/GRO; CXCL2, chemokine (C−X−C motif) ligand 2, also called macrophage inflammatory protein 2-(MIP-2); ECF, extracellular fluid space; EE, extraction efficiency; IL-1β, interleukin-1β; IL-6, interleukin-6; IL-10, interleukin-10; KC/GRO, see CXCL1; MCP-1, see CCL2; MIP-1α, see CCL3; MIP-2, see CXCL2; MWCO, molecular weight cutoff; RANTES, see CCL5; TBI, traumatic brain injury

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