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Acute, regional inflammatory response after traumatic brain injury: Implications for cellular therapy

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Abstract

While cellular therapy has shown promise in the management of traumatic brain injury (TBI), microenvironment interactions between the intracerebral milieu and therapeutic stem cells are poorly understood. We sought to characterize the acute, regional inflammatory response after TBI.

Rats underwent a controlled cortical impact (CCI) injury or sham injury, were sacrificed at 6, 12, 24, 48, and 72 hours, and intracerebral fluid (IF) was isolated from the direct injury, penumbral, ipsilateral frontal, contralateral regions. Cortical and hippocampal areas were also isolated. Regional cytokine levels were measured. PMN oxidative burst and marker expression were assessed after incubation with the IF. Immunohistochemistry identified intracerebral CD68⁺ cells (microglia/macrophages).

The pro-inflammatory cytokines IL-1 α , IL-1 β , IL-6, and TNF- α were significantly elevated after CCI in the injury and penumbral regions. Increases in the same cytokines were localized to the cortex and the hippocampus. Increased PMN expression of CD11b and L-selectin was identified after incubation with injury or penumbral area IF, without change in PMN oxidative burst. CD68⁺ cells were noted in the direct injury and penumbral areas.

The local cerebral milieu in the first 48 hrs after TBI is highly pro-inflammatory. This response is most pronounced in areas at or proximal to the direct injury. The local, acute pro-inflammatory response after TBI may serve as a therapeutic target of early cell therapy or, conversely, may create an unfavorable local milieu, limiting the efficacy of early cellular therapy.

Keywords

Traumatic brain injury; inflammation; cellular therapy; cytokine

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Introduction

Our therapeutic ability to mitigate cell damage and improve functional recovery after traumatic brain injury (TBI) is limited. Despite a significant body of research, there has been minimal success in translating potential therapies to the clinical arena. Cellular therapy is an area of investigation that has shown promise as a therapy for TBI. Pre-clinical research using adult stem cells to treat TBI has shown that these cells can mitigate motor and cognitive functional deficits in animals.¹⁻³ These findings have led to the initiation of an ongoing phase I clinical trial evaluating the safety of autologous mononuclear cell therapy for TBI in children.

Alongside early success using cell therapy to treat TBI, a range of alternative theories surrounding the optimal timing of delivery have been proposed. Most early studies chose to deliver the cells acutely (<24 hours) after TBI,^{1, 2} perhaps because most interventions are more efficacious when applied earlier or because the open blood brain barrier (BBB) was more likely to allow the cells to reach the area of injury. In addition, acutely produced chemokines may attract cells to the area of injury.⁴ Recently, administration of cells at later time points has shown efficacy⁵ and the acute inflammatory response has been shown to be detrimental to cell survival, integration, and functional improvement.^{6, 7}

The central nervous system (CNS) is capable of mounting a significant inflammatory response to an insult.^{8, 9} This (CNS) inflammatory response is mediated by cytokines, polymorphonuclear cells (PMN's), macrophages, and glial cells (particularly astrocytes and microglia). Previous work has characterized general cytokine alterations and cell migration in cerebral homogenate or cerebrospinal fluid (CSF), but the inflammatory response at specific intracerebral areas, relative to the area of injury and time after injury, is less characterized. Understanding the intracerebral inflammatory response to TBI at specific locations over time will enable targeted intracerebral cellular therapy. Thus, this study was designed to characterize the acute, regional inflammatory response in multiple areas of the brain in a rat model of TBI. Our hypothesis was that the magnitude of the inflammatory response would be most pronounced in the area of injury and in the penumbral area. We further hypothesized that TBI would elicit a local milieu characterized by an increased PMN oxidative burst, increased CD11b expression, and decreased L-selectin expression.

Materials and Methods

Controlled cortical impact injury

A controlled cortical impact (CCI) device (eCCI Model 6.3; Custom Design) was used to administer unilateral brain injury as described previously.¹⁰ Rats were anesthetized with 4% isoflurane and a 1:1 mixture of N₂O/O₂ and the head was mounted in a stereotaxic frame. The head was held in a horizontal plane, a midline incision used for exposure, and a 7–8 mm craniectomy was performed on the right cranial vault. The center of the craniectomy was placed at the midpoint between bregma and lambda, ~3 mm lateral to the midline, overlying the tempoparietal cortex. Animals received a single impact of 3.1 mm depth of deformation with an impact velocity of 6 m/s and a dwell time of 150 ms (moderate-severe injury) at an angle of 10° from the vertical plane using a 6 mm diameter impactor tip, making the impact orthogonal to the surface of the cortex. An audible baseline monitor was used to ensure that the location of the tip, relative to the surface of the brain, was consistent prior to each impact. The impact was delivered onto the parietal association cortex. Sham injury was performed by anesthetizing the animals, making the midline incision, and separating the skin, connective tissue, and aponeurosis from the cranium, before closing the incision. The body temperature was maintained at 37°C by the use of a heating pad. Previously obtained

serial arterial paO_2 and paCO_2 have shown that animals do not become hypoxic or hypercarbic during this procedure.

Intracerebral interstitial fluid and plasma isolation

Five groups of eight rats underwent a severe controlled cortical impact (CCI) injury (4 groups) or sham injury (1 group). The CCI groups were sacrificed at 6, 12, 24, 48, and 72 hours after injury and the sham group was sacrificed at 6 hours after sham injury. Their brains were extracted and four regions, relative to the injury, were isolated: 1) site of direct injury, 2) penumbral region, 3) ipsilateral frontal region, and 4) contralateral region (Figure 1). The sections were weighed to ensure each section was ~120 mg, gently minced with a pellet pestle, diluted in low glucose DMEM (GIBCO®), vortexed for 30 seconds, and centrifuged for 2 minutes at $1000\times g$. The supernatant, containing intracerebral, interstitial fluid, was collected. The blood was simultaneously collected via cardiac puncture and centrifuged for 2 minutes at $1000\times g$ to isolate the plasma.

Additionally, the cortical and hippocampal anatomic / functional regions, ipsilateral and contralateral to the area of injury, were isolated at the 6 hour timepoint from four additional rats. The intracerebral, interstitial fluid from these specific areas was isolated as described above. For areas where less than 120 mg of tissue was isolated, the same volume/weight ratio of diluent was used.

Cytokine analysis

Cytokines were detected in the intracerebral interstitial fluid and the plasma using the Bio-Plex™ cytokine assay system (Bio-Rad Laboratories, Inc., Hercules, CA). Concentrations of IL-1, IL-1, IL-2, IL-4, IL-6, IL-10, IFN-, GM-CSF, and TNF- were simultaneously evaluated using the commercially available multiplex bead-based immunoassay (Rat 9-Plex, Bio-Rad Laboratories, Inc.). The assay was performed per the manufacturer's instructions and the details have been previously published by our group and others elsewhere.^{11, 12} High standard curves (Low RP1 target value) for each soluble cytokine were used, ranging from 2 – 32,000 pg/ml. A minimum of 100 beads per cytokine region were evaluated and recorded. Values with a coefficient of variation beyond 10% were not included in final data analysis. All samples were run in duplicate.

PMN superoxide anion production

Superoxide anion production was measured by hydroethidine (HE) conversion to ethidium bromide (EB), detected by flow cytometry (BD LSR II; BD Biosciences, San Jose, CA). Polymorphonuclear cells (PMN) were isolated from healthy, human volunteers. The PMNs were suspended in a Krebs's-Ringer's Phosphate with dextrose (KRP-d)¹³ solution (100 μl) and were incubated with HE (10 μM final concentration) for 15 minutes at 37° C. The intracerebral, interstitial fluid was added to the PMN solution and allowed to incubate for 5 minutes at 37° C. The 'priming' agent, PAF (10 μM final concentration), was added to certain control and experimental wells and allowed to incubate for 5 minutes at 37° C. The intracerebral, interstitial fluid was added to the appropriate experimental wells and allowed to incubate for 5 minutes at 37° C. The NADPH-dependent activating agent, fMLP (1 μM final concentration), or the NADPH-independent activating agent, PMA (10 μM final concentration), was added and allowed to incubate for 20 minutes at 37° C. The 96-well plate was placed on ice for 5 minutes to stop the reactions and immediately run on the high throughput system (HTS) of the BD LSR II flow cytometer. The PMN population was gated on the FSC/SSC plot and the mean fluorescent intensity (MFI) was recorded. All samples were run in duplicate.

PMN CD11b and L-selectin surface marker expression

Surface marker expression was measured by conjugated antibodies binding to the PMN cell surface, detected by flow cytometry. After PMN isolation, the intracerebral, interstitial fluid was added to the PMN+KRP-d solution and allowed to incubate for 5 minutes at 37° C. The 'priming' agent, PAF (10 µM final concentration), was added to certain control and experimental wells and allowed to incubate for 5 minutes at 37° C. The intracerebral, interstitial fluid was added to the appropriate experimental wells and allowed to incubate for 5 minutes at 37° C. The NADPH-dependent activating agent, fMLP (1 µM final concentration), or the NADPH-independent activating agent, PMA (10 µM final concentration), was added and allowed to incubate for 20 minutes at 37° C. The conjugated antibodies (CD11b and L-selectin, 2 µl each antibody) were added to all wells and allowed to incubate for 30 minutes at 37° C. The 96-well plate was immediately run on the HTS of the BD LSR II flow cytometer. The PMN population was gated on the FSC/SSC plot and the MFI (mean receptor density) was recorded. All samples were run in duplicate.

Immunohistochemistry of brain tissue

At 48 hours after CCI injury, coronal brain sections were obtained for immunohistochemistry analysis. After intraperitoneal ketamine injections, the thoracic cavity was opened and room-temperature PBS was infused for 15 minutes via the left ventricle. The heart was spontaneously beating on initiation of infusion and the animals were simultaneously allowed to exsanguinate via right atrium puncture. Cold 4% paraformaldehyde (PFA) was then perfused for 15 minutes via the left ventricle. The brain was extracted and placed in 4% PFA at 4°C for 24–48 hours. The brain was embedded in 3% agarose and sectioned at 50 µM in the coronal plane using a vibrating-blade microtome (Leica Microsystems; Bannockburn, IL). The brain slices were incubated with a fluorescein isothiocyanate (FITC) conjugated anti-CD68 antibody (AbD Serotec, Kindlington, Oxford, UK), stained with 4',6-diamidino-2-phenylindole, dihydrochloride (DAPI) (Invitrogen), and placed on a slide. Sections from the area of direct injury, penumbra, and contralateral hemisphere were examined for CD68 (FITC) positive cells (macrophages/microglia).

Data and statistical analysis

Data were collected using the Bio-Plex Manager software version 4.1.1 (Bio-Rad Laboratories). Standard levels between 80 and 120% of the expected values were considered to be accurate and were used. At least 6 of 8 standards were used to establish standard curves following a 5-parameter logistic regression model (5 PL). Cytokine values are expressed as pg/ml and presented as mean ± SEM. Multiple analysis of variance (MANOVA) and a post-hoc Dunnett's test versus a control were used to determine significance at $p < 0.05$.

Results

Intracerebral cytokines after traumatic brain injury

In the *area of direct injury*, IL-1 α , IL-1 β , IL-6, and TNF- α were significantly elevated in the intracerebral interstitial fluid (Figure 2) ($p < 0.0001$ for all, MANOVA). IL-1 α was increased from baseline 25–50 fold at 6 hours ($p < 0.01$, Dunnett's test), 10–15 fold at 12 hours ($p < 0.05$, Dunnett's test), and remained elevated ~3 fold (not statistically significant) at 24 hours. IL-1 β was increased 60–80 fold from baseline at 6 hours ($p < 0.01$, Dunnett's test), 18–22 fold at 12 hours ($p < 0.05$, Dunnett's test), and remained elevated ~5 fold (not statistically significant) at 24 hours. IL-6 was increased 30–36 fold from baseline at 6 hours ($p < 0.05$, Dunnett's test), 23–30 fold at 12 hours ($p < 0.05$, Dunnett's test), 17–21 fold at 24 hours ($p < 0.05$, Dunnett's test), and remained elevated ~4 fold (not statistically significant) at

48 hours. TNF- α was increased 6–10 fold from baseline at 6 hours ($p < 0.05$, Dunnett's test) and returned to baseline by 12 hours.

In the *penumbral area*, significant increases in the same cytokines, IL-1 β , IL-1 α , IL-6, and TNF- α , were seen at 6 hours after TBI (Figure 2). The increases seen in the penumbral area were less pronounced than the increases seen in the area of direct injury. IL-1 β was elevated 10–12 fold at 6 hours ($p < 0.05$, Dunnett's test), 3–4 fold at 12 hours ($p < 0.05$, Dunnett's test), and returned to baseline at 24 hours. IL-1 α was elevated 22–26 fold at 6 hours ($p < 0.05$, Dunnett's test), 8–10 fold at 12 hours ($p < 0.05$, Dunnett's test), and remained slightly elevated (4–5 fold, not statistically significant) at 24 hours. IL-6 was increased 16–20 fold from baseline at 6 hours ($p < 0.05$, Dunnett's test), 16–26 fold at 12 hours ($p < 0.05$, Dunnett's test), 20–28 fold at 24 hours ($p < 0.05$, Dunnett's test), and remained elevated ~3 fold (not statistically significant) at 48 hours. TNF- α was increased 3–5 fold from baseline at 6 hours ($p < 0.05$, Dunnett's test) and returned to baseline by 12 hours.

In the *frontal area*, IL-6 was significantly increased at 24 hours (33–50 fold, $p < 0.05$, Dunnett's test), but not at 6 or 12 hours after TBI. No other cytokines were increased in the frontal area. No significant changes in intracerebral cytokines were identified in the *contralateral area*. Additionally, no significant changes in plasma cytokines were identified. Irrespective of intracerebral area or time after TBI, no changes in IL-2, IL-10, or IFN- γ were identified. In addition, the levels of IL-4 and GM-CSF were below detectable limits at baseline and at every subsequent measurement. All cytokines in all locations returned to near baseline levels (no statistically significant difference) by 48 hours and remained there at 72 hours.

In the *ipsilateral cortex*, IL-1 β , IL-1 α , IL-6, and TNF- α were significantly elevated in the intracerebral interstitial fluid at 6 hours after TBI (Figure 3). Significant increases were also identified in the *ipsilateral hippocampus*, although the increases identified in the hippocampus were less elevated than the cortex for IL-1 β , IL-1 α , and IL-6. For IL-1 β , IL-1 α , and IL-6, no increases were identified in the contralateral cortex or hippocampus. TNF- α , however, was noted to be elevated in the ipsilateral and contralateral cortex and hippocampus. No changes in IL-2, IL-10, or IFN- γ were identified.

CD11b marker expression

The expression of CD11b on unstimulated PMN's was significantly increased in the area of direct injury and penumbral area at 6 hours in the presence of the intracerebral interstitial fluid (Figure 4). At 12, 24, and 48 hours, CD11b expression returned to baseline. There was no increase in CD11b expression when PMN's were exposed to intracerebral interstitial fluid from the frontal or contralateral areas. PMN's that were primed and/or activated did not have increased CD11b expression.

L-selectin marker expression

The expression of L-selectin on unstimulated PMN's was significantly increased in the area of direct injury and penumbral area at 12 hours in the presence of the intracerebral interstitial fluid (Figure 4). At 6, 24, and 48 hours, L-selectin expression remained at baseline. There was no increase in L-selectin expression when PMN's were exposed to intracerebral interstitial fluid from the frontal or contralateral areas. PMN's that were primed and/or activated did not have increased L-selectin expression.

Superoxide anion production

Superoxide anion production by PMN's in the presence of the intracerebral interstitial fluid was unchanged, irrespective of the intracerebral location or time after TBI (Table 1).

Intracerebral macrophages/microglia after traumatic brain injury

Numerous CD62 positive cells (macrophages/microglia) were identified in the area of direct injury (Figure 5). The CD62 positive cells were also present in the penumbral area, but became significantly less frequent as areas further away from the area of direct injury were examined. There were very few CD62 positive cells identified in the frontal area or contralateral area (Figure 6).

Discussion

Our findings indicate that the area of direct injury and penumbral area are highly pro-inflammatory between 6 and 48 hours after CCI TBI. We found that the pro-inflammatory cytokines IL-1 α , IL-1 β , IL-6, and TNF- α were significantly elevated six hours after CCI in the direct injury and penumbral regions when compared to sham animals. IL-1 α , IL-1 β , and IL-6 remained elevated through 12, 12, and 24 hrs, respectively. IL-1 α , IL-1 β , and IL-6 were elevated in the ipsilateral cortex and hippocampus, while TNF- α was elevated in the ipsilateral and contralateral cortex and hippocampus. Increased PMN expression of CD11b was identified after incubation with early, post-traumatic injury or penumbral area intracerebral interstitial fluid. Increased PMN expression of L-selectin was identified after incubation with injury or penumbral area intracerebral interstitial fluid isolated 12 hrs after injury. Although PMN's in the presence of this pro-inflammatory intracerebral interstitial fluid increase their expression of CD11b and L-selectin, they do not seem to be stimulated enough to increase their superoxide anion production. CD68 positive cells were noted, indicating that large numbers of microglia/macrophages also migrate to these areas at the same time after TBI. This pathophysiologic response has important implications when deciding the timing and location of cellular therapy for traumatic brain injury.

Inflammation is an integral part of the pathophysiology that follows a traumatic brain injury. Nearly 3 decades ago, the previously held tenet that the nervous system was incapable of producing an inflammatory response, began to be challenged by accumulating evidence of a robust inflammatory response.⁸ The acute rise of pro-inflammatory cytokines (IL-1 α , IL-1 β , IL-6, and TNF- α) after TBI has been generally characterized.^{9, 14} TNF- α is elevated in the cerebrospinal fluid (CSF) and intracerebrally in a number of neurologic diseases.^{8, 15} IL-6 has also been shown to be acutely elevated in the CSF, intracerebrally, and systemically after TBI.^{8, 14} Increased expression of IL-1 has also been shown after mechanical injury.¹⁶ Our work supports previous findings, but identifies more specific intracerebral locations of cytokine production. It seems intuitive that the cytokines are highest at the location of injury, but previous work has shown increases in the CSF or the entire hemisphere homogenate, indicating that there may be a more general pro-inflammatory response. While the cytokine response in the direct injury and penumbral areas was significantly greater than the response in the ipsilateral frontal area or the contralateral area, we also found a significant increase in IL-6 levels in the frontal area 24 hours after injury, and the functional significance of this remains to be fully elucidated.

The source of the cytokines is an area of ongoing investigation. Astrocytes,¹⁷ mature neurons,¹⁴ peri-vascular/endothelial cells,¹⁸ microglia,¹⁹ and macrophages²⁰ are currently thought to be sources of the 'pro-inflammatory' cytokine response. The time course of genetic expression and arrival of proteins, chemokines, growth factors, cytokines, and cells that collectively constitute the intracerebral inflammatory response to trauma^{8, 9} reveals that cytokines are early mediators of the inflammatory cascade. The resultant effects of cytokine expression include general pathophysiologic actions (temperature alterations, edema), vasculature alterations, and cellular effects (neurons, glia).⁹ Cytokines induce endothelial upregulation of adhesion molecules involved in PMN migration,²¹ and PMN's have been shown in significantly increased number in the traumatically injured brain.²² We identified

upregulation of PMN adhesion molecules CD11b and L-selectin, but no increase in oxidative burst was detected. Immediately following PMN migration, macrophages are identified, although resident intracerebral macrophages (microglia) are ubiquitously distributed throughout the central nervous system. Differentiating the role of resident microglia versus invading peripheral macrophages is an ongoing area of investigation.²³ However, the immunophenotype of macrophages/microglia are identical (including expression of CD68), making it difficult to differentiate their respective roles in intercerebral inflammation.

Cytokines induce responses that make them simultaneously autodestructive and neuroprotective.^{9, 14, 24} Higher IL-6 levels have been shown to correlate with survival and improved recovery after TBI.²⁵ Other cytokines (such as TNF- α) have been shown to be cytotoxic to neural stem cells under proliferative conditions *in vitro*,²⁶ and the synergistic effects of multiple cytokines (IL-1, TNF- α , and/or IFN- γ) have been shown to result in significantly greater neurotoxicity.²⁷ On the other hand, mice lacking the genes for TNF- α and IL-6 showed slightly increased mortality after a closed head injury, suggesting that these cytokines offer some neuroprotection or neurorecovery effect.²²

In the same way that the overall affect of the acute, local 'pro-inflammatory' milieu is currently felt to be simultaneously beneficial and deleterious, the potential impact of this inflammatory response on cell therapy may also be symbiotic and/or antagonistic. The interplay between transplanted cells and the intracerebral inflammatory response to TBI is largely unknown. Irrespective of route of administration, transplanted stem and progenitor cells appear to migrate or home to areas of inflammation^{4, 28}, predominantly the area of direct injury and the penumbral area. Stem and progenitor cell populations are known to express a variety of chemokine receptors,⁴ although the optimal chemokines for cell attraction are unknown. Although we did not measure an exhaustive number of cytokines/chemokines, our results are similar to previous work that indicates early cell transplantation would be optimal for maximizing the beneficial homing effects of cytokines. On the other hand, recent work has shown that cells transplanted intracerebrally immediately following TBI survive better after a mild injury than a severe injury,⁶ suggesting that a highly pro-inflammatory, edematous milieu is suboptimal for stem cell survival. Additionally, Molcanyi and colleagues recently identified activated macrophage phagocytosis as the mediator of intracerebrally transplanted cell death after TBI.⁷ Placing the cells in the hostile, local, intracerebral environment after TBI may even exacerbate the inflammatory response, leading to rapid cell death.

In conclusion, we have further clarified the details of the acute, pro-inflammatory response to TBI. Although the interactions between the intracerebral inflammatory milieu after TBI and transplanted cells remain undercharacterized, this work contributes to the background necessary for targeted cellular therapy focused at mitigating the inflammatory response or avoiding the inflammatory response, maximizing cell survival.

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Figure 1. Intracerebral areas isolated

Four intracerebral areas were isolated, minced, centrifuged, and the supernatant was collected for analysis of cytokines and stimulation of PMN's.

Figure 2. Elevated intracerebral cytokines identified in specific areas and at specific time points relative to the TBI

The pro-inflammatory cytokines IL-1 α (A), IL-1 β (B), IL-6 (C), and TNF- α (D) were significantly elevated six hours after CCI in the injury and penumbral regions when compared to sham animals (* $p < 0.01$ for all). IL-1 α , IL-1 β , and IL-6 remained elevated through 12, 12, and 24 hrs, respectively (* $p < 0.01$ or † $p < 0.05$). In the frontal area, IL-6 was significantly increased at 24 hours (33–50 fold, $p < 0.01$, Dunnett's test), but not at 6 or 12 hours after TBI.

Figure 3. Elevated intracerebral cytokines identified at specific anatomic locations (1. cortex and 2. hippocampus) at 6 hours after TBI

The pro-inflammatory cytokines IL-1 (A), IL-1 (B), IL-6 (C), and TNF- (D) were significantly elevated six hours after CCI in the ipsilateral cortex and hippocampus when compared to sham animals (* $p < 0.05$ for all). TNF- was also elevated in the contralateral cortex and hippocampus. Note – brain section taken from Paxinos and Watson, 2005.

Figure 4. Increased PMN expression of CD11b and L-selectin in the presence of intracerebral interstitial fluid after TBI

The expression of CD11b (A) and Lselectin (B) on unstimulated PMN's was significantly increased in the area of direct injury and penumbral area at 6 and 12 hours, respectively, in the presence of the intracerebral interstitial fluid.

Figure 5. Macrophages/microglia identified in the area of injury

Brain tissue sections were isolated after TBI, stained with DAPI (blue) to identify nuclei, and incubated in FITC-conjugated anti-CD68 antibodies (green) to identify macrophages/microglia. Numerous CD68 positive cells were identified in the area of injury, a moderate number in the penumbral area, and the number of cells decreased rapidly in areas further away from the injury. A complete coronal section, after nissl staining, is shown for perspective.

Figure 6. Macrophages/microglia were not identified in the contralateral hemisphere
Brain tissue sections were isolated after TBI, stained with DAPI (blue) to identify nuclei, and incubated in FITC-conjugated anti-CD68 antibodies (green) to identify macrophages/microglia. The contralateral hemisphere contained few CD68 positive cells.

Table 1
Superoxide anion production by PMN's in the presence of intracerebral interstitial fluid after TBI

No statistically significant changes in superoxide anion production were noted. All data is shown as mean±SEM. (Note – For the electronically published version, not to be included in paper printed version)

				Time after CCI					
		Control	Sham	6 hours	12 hours	24 hours	48 hours		
INJURY									
Unstimulated		187±10	109±18	82±5	121±31	126±18	129±22		
PAF		258±14	164±12	133±8	139±10	180±11	162±12		
PAF+fmLP		442±18	151±23	97±11	170±32	226±19	187±43		
PAF+PMA		4306±358	1811±407	691±155	3242±1048	2471±642	2149±817		
				Time after CCI					
PENUMBRA									
Unstimulated		Control	Sham	6 hours	12 hours	24 hours	48 hours		
PAF		187±10	105±17	84±4	151±36	124±21	112±12		
PAF+fmLP		258±14	136±6	146±4	137±6	168±12	146±13		
PAF+PMA		442±18	140±24	103±7	219±46	171±18	141±31		
		4306±358	1133±192	359±46	2638±960	1662±425	1353±461		
				Time after CCI					
FRONTAL									
Unstimulated		Control	Sham	6 hours	12 hours	24 hours	48 hours		
PAF		187±10	105±19	65±5	114±17	105±11	120±14		
PAF+fmLP		258±14	142±10	116±3	171±7	162±17	142±8		
PAF+PMA		442±18	132±20	78±6	195±20	142±16	117±12		
		4306±358	826±176	284±46	1740±480	1095±323	672±204		
				Time after CCI					
CONTRALATERAL									
Unstimulated		Control	Sham	6 hours	12 hours	24 hours	48 hours		
PAF		187±10	120±16	78±8	117±10	129±27	110±13		
PAF+fmLP		258±14	154±4	134±9	170±9	148±11	140±7		
PAF+PMA		442±18	140±12	96±12	179±8	163±37	143±24		
		4306±358	1730±226	342±81	3142±675	2090±983	1587±443		