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Characterization of the temporo-spatial effects of chronic bilateral intrahippocampal cannulae on interleukin-1 β

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Abstract

The implantation of a foreign object in the brain produces an acute neuroinflammatory state in which glia (astrocytes and microglia) may remain chronically activated in response to the inert foreign object. Activated glia can exhibit a sensitized pro-inflammatory response to immunogenic stimuli. This may be relevant to intracranial cannula implantation, which is commonly used to administer substances directly into the brain. If intracranial cannulation activates glia, a subsequent neuroinflammatory stimulus might induce a potentiated pro-inflammatory response, thereby introducing a potential experimental confound. We tested the temporal and spatial responses of interleukin-1 β (IL-1 β) to an acute immune challenge produced by lipopolysaccharide (LPS) in animals with chronic bilateral intrahippocampal cannulae implants (stainless steel). Cannulation increased the gene expression of the microglia activation antigens MHC II and CD11b, but not the astrocyte antigen GFAP. Moreover, this activation was temporally and spatially dependent. In addition, IL-1 β mRNA, but not IL-1 β protein, was significantly elevated in cannulated animals. Administration of LPS, however, significantly potentiated the brain IL-1 β response in cannulated animals, but not in stab wounded or naïve animals. This IL-1 β response was also temporo-spatially dependent. Thus, the pro-inflammatory sequelae of intracranial cannulation should be considered when designing studies of neuroinflammatory processes.

Keywords

cannula; interleukin-1; glia; lipopolysaccharide; GFAP; MHCII; CD11b; method

1. Introduction

For the past 40 years, the use of cannula implants has been very well accepted. Injecting agents directly into the brain via a cannula is a useful approach to manipulate the function of a specific brain region without altering whole brain function. One of the earliest studies that validated this method, reported that animals can be ready for experimental use as early as seven days after cannulation (Hilliard et al., 1968). This assumption was based on the motor activity and other behaviors of the cannulated animals compared to that of naïve animals. It has been implicitly assumed that the presence of a cannula does not induce an effect by itself. However, Hilliard et. al., (1968) noted inflammation of the lateral ventricle, the site of cannula placement.

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At the time, it was not clear what induced the inflammation. However, it is now known that the implantation of a foreign object in the brain induces an inflammatory immune response, mediated primarily by glia (astrocytes and microglia) (Schultz and Willey, 1976; Stensaas and Stensaas, 1976; Turner et al., 1999; Szarowski et al., 2003). An acute immune response (< one week) occurs, wherein the glial reaction is towards the mechanical trauma produced by the foreign object. The spread of glial activation to distant tissues is directly proportional to the size of the foreign object (Turner et al., 1999). Subsequently, a chronic immune response ensues characterized by marked glial mobilization towards the site of injury, where glia form an interwoven network to isolate the foreign object from healthy tissue (i.e. a glial scar). At 2 weeks, there is a marked alignment of glia along the foreign object and glial immunoreactivity is still detected at tissues distant from the injury site. By four weeks, this distant glial activation dissipates. By six weeks, only those glia that are strongly adhered to the foreign object maintain a level of activation and do so as long as the foreign object remains in situ (Turner et al., 1999). This temporal and spatial glial response is of critical importance because the durations mentioned (1, 2 and 4 weeks) are durations that researchers usually choose to allow animals to recover after implanting cannula(e).

Since cannulation is a frequently used method, it is important to determine whether intracranial cannulation can sensitize a central nervous system immune response to an immunogenic stimulus. The effects of cannulae implanted in the dorsal hippocampus were examined on pro-inflammatory responses, specifically interleukin-1 β (IL-1 β) to a peripheral immune challenge produced by LPS.

2. Materials and Methods

Animals

Adult viral-free Sprague-Dawley male rats (350–400 gm) (Harlan Labs, Madison, WI) were used for all experiments. They were housed in pairs on a 12/12 light/dark cycle (lights on at 0700 hr; room temperature 23 $\pm$ 3 $^{\circ}$ C) with food and water ad libitum. All animals were allowed to acclimate for one week. Experimentation began at 0700 hr and lasted between 4–5 hr. All procedures were in accordance with protocols approved by the University of Colorado Institutional Animal Care and Use Committee.

2.2. Intrahippocampal Cannulae Implantation

Chronic indwelling stainless steel guide cannulae were implanted or used to induce a stab wound in the dorsal hippocampus using Watson and Paxinos rat brain atlas coordinates (AP: –3.5 mm; ML: +2.4 mm; DV: –3.0 mm, relative to Bregma) (Paxinos and Watson, 1986) (see Figure 1A,B). Cannulae were secured using dental acrylic and a dummy injector (4 mm in length) was placed to maintain patency. Rats were allowed to recover for 1, 2, or 4 wk.

2.3. Lipopolysaccharide (LPS) preparation and administration

LPS (*Escherichia Coli*, serotype 0111: B4 Sigma Lot No. 072k4096) or equal volume 0.9 % endotoxin-free saline (Abbott Laboratories, North Chicago, IL) was used. The dose used was 25 μ g/kg. Lyophilized LPS (25 mg) was reconstituted with 0.9% sterile saline (10 ml) to derive a concentration of 2.5 mg / ml and then LPS was aliquoted in 100 μ l vials and stored at –20 $^{\circ}$ C until used. At the time of experimentation, one 100 μ l aliquot was thawed and added to 9.9 ml of 0.9% sterile saline yielding a concentration of 25 μ g/ml. LPS for all experiments was administered intraperitoneally (i.p.).

2.4. Tissue collection

Animals were anesthetized using sodium pentobarbital (kg/ml). Prior to perfusion, cardiac blood was collected. Animals were transcardially perfused with 250 ml 0.9% chilled saline. Immediately after perfusions the brains were extracted and several brain regions were dissected. The tissues dissected were hippocampus (site of cannula placement), posterior cortex (peri-cannula tissue) and frontal cortex (tissue at a distant from injury site) (see Figure 1C). The tissues were immediately flash frozen in liquid nitrogen and stored at -80°C until assayed. To obtain both protein and mRNA measures from the same animal, left hemisphere of the hippocampus (and frontal cortex) was used for protein analyses and the right hemisphere for mRNA analyses.

2.5. Tissue Preparation

To prevent tissue degradation, a buffer cocktail was prepared composed of three parts: 1) extraction buffer (190ml Iscove's media, 10 ml fetal calf serum), 2) 20 mM phenylmethylsulfonyl fluoride (PMSF, Boehringer Mannheim, .174 gm PMSF and 50 ml isopropanol), and 3) enzyme cocktail (13.1 gm amino-n-caproic acid (Sigma), 3.722 gm ethylene-diamine-tetra-acetic acid (EDTA)(Mallinckrodt), and .783 gm benzamidine (Sigma), in a total volume of 100 ml distilled water). The buffer cocktail was immediately added to the tissue-containing vial after retrieving from -80°C . The tissues samples were sonicated (ultrasonic cell disruptor: Heat system, Inc, model #MS-50) for 10 sec at a setting of 10 to liberate cell contents. The sonicated samples were centrifuged at 4°C , 14,000 rpm for 10. The supernatants were extracted and stored 4°C until assayed.

2.6. Tissue Assay

A rat IL-1 β enzyme linked immunosorbent assay (ELISA) kits (R&D systems, cat #RLB00) was used to quantify the concentration of IL-1 β in the supernatant. All procedures were performed at room temperature ($23 \pm 3^{\circ}\text{C}$). The microplate contained wells that are pre-coated with rat IL-1 β monoclonal antibody. The dilution follows a 1:1 ratio (50 μl of sample, standard, & control and 50 μl of assay diluent; 100 μl total volume). Absorbance was measured using a ultra microplate reader (ELx808; Biotex Instruments, INC Vermont, USA) at a wavelength of 450 nm and wavelength correction was set at 570 nm to correct for optical imperfections in the plate as suggested by the manufacturer. KC Junior software (Biotex Instruments) loaded into a PC (EV500) was used to obtain the microplate reader sample values. The sample values were read off the standard curve by generating a regression line employing Microsoft Excel spreadsheet software.

2.7. Coomassie Brilliant Blue G-250 protein assay

In order to compensate for variability in tissue sample size, total protein concentration of tissue was determined by a Coomassie Brilliant Blue (CBB) G-250 protein assay (Bradford, 1976). The CBB G-250 protein reagent was composed of 100 mg CBB G-250 (Bio Rad: Sigma, St. Louis, MO), 50 ml of 95% ethanol, and 100 ml 85% o-phosphoric acid. One liter of distilled water was added and the solution was filtered. Dilutions were as follows: hippocampus 1:30; frontal cortex 1:40; posterior cortex 1:40. After the dilutions, samples (10 μl) were pipetted into 96-well flat-bottom Immulon-4 plates (Dynex Tech Inc; Chantilly, VA). CBB G-250 reagent (250 μl) was added to each well and incubated for 10 min. Standards were derived from bovine serum albumin (Sigma; St. Louis, MO) ranging from 5.0–35.0 μg protein. The plate was incubated for 10 min at room temperature ($23 \pm 3^{\circ}\text{C}$). After incubation the plate was read employing an ultra microplate reader 595 nm (a ultra microplate reader (ELx808; and KC Junior software Biotex Instruments, Vermont) Sample total proteins were calculated from the standard curve by generating a regression line employing Microsoft excel spreadsheet

software. Lastly, a ratio was taken between IL-1 β (pg/ml) and total tissue protein (pg/100 μ l) to derive the amount of IL-1 β (pg/100 μ g total protein)

2.8. RNA isolation and enrichment

Total RNA was isolated from tissue based on the method of Chomczynski (Chomczynski and Sacchi, 1987). Tissue was rapidly homogenized in 1 ml TRIzol reagent (Invitrogen, Carlsbad, CA). After incubation at room temperature for 5 min, homogenate was centrifuged (12,000 x g) for 15 min at 4° C to remove excess lipids. Chloroform (200 μ l) was added to supernatant, vortexed, and centrifuged (12,000 x g) for 15 min at 4° C to achieve phase separation of nucleic acids. Isopropyl alcohol (500 μ l) was added to the aqueous phase to precipitate nucleic acids. Samples were vortexed (1 min) and incubated at room temperature for 10 min followed by centrifugation (12,000 x g) for 10 min at 4° C. Nucleic acid precipitate was washed twice in 75% ethanol (1 ml) and centrifuged (7,500 x g) for 5 min at 4° C. UV spectrophotometric analysis of nucleic acid was performed at 260 nm to determine concentration. The 260:280 absorbance ratio was utilized to assess nucleic acid purity. Mean 260:280 absorbance ratio ($2.004 \pm .008$ SE) indicated a high degree of nucleic acid purity across all experimental samples. Samples were Dnase-treated (DNA-free kit, Ambion, Austin, TX) to remove contaminating DNA from total nucleic acid and requantitated prior to cDNA synthesis.

2.9. Real time Reverse transcription-polymerase chain reaction (Q RT-PCR)

cDNA synthesis—Total RNA was reverse transcribed into cDNA using the SuperScript II First Strand Synthesis System for RT-PCR (Invitrogen). RNA (2 μ g) was incubated for 5 min at 65° C in a total reaction volume of 12 μ l containing random hexamer primers (5 ng/ μ l) and dNTPs (1 mM). Samples were chilled on ice for at least 1 min. A cDNA synthesis buffer (7 μ l) as described by the manufacturer was added to the reaction and incubated at 42° C for 2 min. Reverse transcriptase (1 μ l; 200 units SuperScript II) was added to the reaction and incubated at 25° C for 10 min followed by 42° C for 50 min. Reaction was terminated by heating to 70° C for 15 min. cDNA was diluted 2-fold in nuclease-free water and stored at -20° C.

Primer specifications—cDNA sequences were obtained from Genbank at the National Center for Biotechnology Information (NCBI; www.ncbi.nlm.nih.gov). Primer sequences (Table 1) were designed using the Qiagen Oligo Analysis & Plotting Tool (oligos.qiagen.com/oligos/toolkit.php) and tested for sequence specificity using the Basic Local Alignment Search Tool (Altschul et al., 1997) at NCBI. Primers were obtained from Prologo (Boulder, CO). Primer specificity was verified by melt curve analysis (see *Quantitative real time PCR*).

Quantitative real time PCR—PCR amplification of cDNA was performed using the Quantitect SYBR Green PCR Kit (Qiagen, Valencia, CA). cDNA (1 μ l) was added to a reaction master mix (25 μ l) containing 2.5 mM MgCl₂, HotStar Taq DNA polymerase, SYBR Green I, dNTPs, fluorescein (10 nM) and gene specific primers (500 nM each of forward and reverse primer). For each experimental sample, triplicate reactions were conducted in 96-well plates (BioRad, Hercules, CA). PCR cycling conditions consisted of a hot-start activation of HotStar Taq DNA polymerase (94° C, 15 min) and 40 cycles of denaturation (95° C, 15 s), annealing (55° – 58° C, 30 s), and extension (72° C, 30 s). A melt curve analysis was conducted to assess uniformity of product formation, primer dimer formation, and amplification of non-specific products. PCR product was denatured (95° C, 1 min) and annealed (55° C, 1 min) prior to melt curve analysis, which consisted of incrementally increasing reaction temperature (0.5° C/10 s) from 55° C to 95° C. The negative first derivative of the melt curve (fluorescence vs temperature) plotted against temperature will yield a single peak (T_m of product) if primers are specific to the gene of interest.

Real time detection and relative quantitation of PCR product—Formation of PCR product was monitored in real time using the MyiQ Single-Color Real-Time PCR Detection System (BioRad). Fluorescence of SYBR Green I was captured at 72° C. Threshold for detection of PCR product above background was set at 10x the standard deviation of mean background fluorescence for all reactions. Background fluorescence was determined from cycle 1 to 5 cycles prior to exponential amplification of product and subtracted from raw fluorescence of each reaction/cycle. Threshold for detection of PCR product fell within the log-linear phase of amplification for each reaction. Threshold cycle (C_T ; number of cycles to reach threshold of detection) was determined for each reaction.

Relative quantitation of gene expression—Relative gene expression was determined using the $2^{-\Delta\Delta C_T}$ method (Livak and Schmittgen, 2001; Pfaffl, 2001). Mean C_T of triplicate measures was computed for each sample. Sample mean C_T of glyceraldehyde 3-phosphate dehydrogenase (GAPDH; internal control) was subtracted from the sample mean C_T of the respective gene of interest (ΔC_T). The sample with the absolute highest mean ΔC_T was selected as a calibrator and subtracted from the mean ΔC_T of each experimental sample ($\Delta\Delta C_T$). $2^{-\Delta\Delta C_T}$ yields fold change in gene expression of the gene of interest normalized to the internal control gene expression and relative to the calibrator sample.

Experimental Design

Animals were randomly assigned to a 3 (Cannulated, Stab wound, or Naïve) $\times$ 2 (Endotoxin or .9% sterile saline) $\times$ 3 (Hippocampus, Frontal Cortex, and Parietal Cortex) group design. Three timepoints were chosen; one, two, and four weeks.

Data Analyses

IL-1 β protein measures for each timepoint was analyzed using a three-way (Surgery $\times$ Endotoxin $\times$ Brain Region) ANOVA. Fisher's posthoc tests were performed where appropriate. Furthermore, sera IL-1 β levels were determined by a two-way ANOVA. The mRNA measures were analyzed using a one-way ANOVA.

3. Results

The temporo-spatial effects of LPS on interleukin-1 β in animals implanted with bilateral intrahippocampal cannulae

3.1 One WEEK Post-Surgical Treatment: Effects of LPS—Intracranial cannulation potentiated the IL-1 β response to peripheral LPS in hippocampus posterior cortex, and frontal cortex (Fig. 2). A 3-way ANOVA failed to show a three-way interaction between Surgery $\times$ Endotoxin $\times$ Brain Region ($p > .05$). However, there was a significant two-way interaction between Surgery $\times$ Endotoxin [$F(2, 155) = 12.07$; $p < .02$]. Post hoc analyses are described below for each brain region.

In hippocampal tissue, there were significant differences in IL-1 β levels between the LPS treated groups; Cannulated + LPS ($n=10$) and Stab Wound + LPS ($n=10$) ($p = .01$) and Cannulated + LPS and Cannulated + Naïve ($n=9$) ($p = .03$) (Fig. 2A). No differences in IL-1 β levels were found between the Stab Wound + LPS and Naïve + LPS groups ($p > .05$). Moreover, vehicle treated groups (Cannulated + Vehicle ($n=10$), Stab Wound + Vehicle ($n=10$) and Naïve + Vehicle ($n=9$)) do not differ from each other ($p > .05$ for each comparison).

In posterior cortex, peri-cannula tissue, IL-1 β levels were significantly different between the LPS treated groups; Cannulated + LPS ($n=9$) and Stab Wound + LPS ($n=10$) ($p = .02$) and the Cannulated + LPS as well as Naïve + LPS ($n=9$) ($p = .04$). No differences were found between the Stab Wound + LPS and Naïve + LPS groups ($p > .05$). Moreover, the Vehicle treated groups

(Cannulated + Vehicle (n=10), Stab Wound + Vehicle (n=10) and Naïve + Vehicle (n=9) did not differ from each other ($p > .05$ for each comparison).

In frontal cortex, IL-1 β levels were significantly different in the LPS treated groups; Cannulated + LPS (n=10) and Stab wound + LPS (n=10) ($p = .03$) as well as Cannulated + LPS and Naïve + LPS (n=9) ($p = .04$) (Fig. 2A). There were no differences between the Stab wound + LPS and Naïve + LPS groups ($p > .05$). Furthermore, vehicle treated groups (Cannulated + Vehicle (n=10), Stab Wound + Vehicle (n=10) and Naïve + Vehicle (n=9)) did not differ from each other ($p > .05$ for each comparison).

3.2 Two WEEKS Post-Surgical Treatment: Effects of LPS on IL-1 β protein—The IL-1 β response to LPS was potentiated in cannulated animals in hippocampus, posterior cortex, and frontal cortex (Fig. 2B). A 3-way ANOVA failed to show a three-way interaction between Surgery $\times$ Endotoxin $\times$ Brain Region ($p > .05$). However, there was a significant two-way interaction between Surgery $\times$ Endotoxin [$F(2, 87) = 12.23$; $p < .0001$] Post hoc analyses are described below for each brain region.

In hippocampal tissue, there were significant differences in IL-1 β levels between the LPS treated groups; Cannulated + LPS (n=5) and Stab Wound + LPS (n=6) ($p = .01$) and Cannulated + LPS and Cannulated + Naïve (n=6) ($p = .03$) (Fig. 2B). No differences in IL-1 β levels were found between the Stab Wound + LPS and Naïve + LPS groups ($p > .05$). Moreover, vehicle treated groups (Cannulated + Vehicle (n=6), Stab Wound + Vehicle (n=6) and Naïve + Vehicle (n=6)) do not differ from each other ($p > .05$ for each comparison).

In posterior cortex, peri-cannula tissue, IL-1 β levels were significantly different between the LPS treated groups; Cannulated + LPS (n=5) and Stab Wound + LPS (n=6) ($p = .02$) and the Cannulated + LPS as well as Naïve + LPS (n=6) ($p = .04$). No differences were found between the Stab Wound + LPS and Naïve + LPS groups ($p > .05$). Moreover, the Vehicle treated groups (Cannulated + Vehicle (n=6), Stab Wound + Vehicle (n=6) and Naïve + Vehicle (n=6)) did not differ from each other ($p > .05$ for each comparison).

In frontal cortex, IL-1 β levels were significantly different in the LPS treated groups; Cannulated + LPS (n=5) and Stab wound + LPS (n=6) ($p = .03$) as well as Cannulated + LPS and Naïve + LPS (n=6) ($p = .04$) (Fig. 2B). There were no differences between the Stab wound + LPS and Naïve + LPS groups ($p > .05$). Furthermore, vehicle treated groups (Cannulated + Vehicle (n=6), Stab Wound + Vehicle (n=6) and Naïve + Vehicle (n=6)) did not differ from each other ($p > .05$ for each comparison).

Additionally, there was an interaction between Brain Region and Endotoxin [$F(2, 87) = 5.16$; $p < .01$] (Fig. 3). The post hoc test revealed that LPS-induced IL-1 β was significantly higher in posterior cortex (n=17–18/grp) compared to hippocampal tissue (n=17–18/grp) ($p = .0004$), but not frontal cortex (n=17–18/grp) ($p > .05$). No significant differences were found between hippocampal tissue and frontal cortex ($p > .05$).

3.3 Four WEEKS Post-Surgical Treatment: Effects of Endotoxin on IL-1 β protein—By four weeks, again a three-way interaction was not found (Fig. 2C). However, there was a two-way interaction between Brain region and Endotoxin [$F(2, 87) = 12.59$; $p < .0001$] (Fig. 3). Post hoc test revealed that IL-1 β response to LPS was significantly higher in posterior cortex (n=17–18/grp) compared to hippocampal tissue (n=17–18/grp) ($p < .0001$) and frontal cortex (n=17–18/grp) ($p < .0001$). There were no significant differences between hippocampal tissue and frontal cortex ($p > .05$).

Sera: A two-way (Surgery $\times$ Endotoxin) ANOVA was performed for each timepoint (one, two, and four week(s)) (Fig 4A–C). *At one week*, the IL-1 β response to LPS between animals with (n=10) and those without cannulae (n=9) were not significantly different (Surgery $\times$ Endotoxin $F(1, 52) = 1.31$; $p > .05$) (Fig. 4A). There was a main effect of Endotoxin (n=29/treatment) [$F(1, 155) = 25.23$; $p < .0001$] but not Surgery ($p > .05$). *At two weeks*, there was a main effect of Endotoxin [$F(1, 87) = 56$; $p < .0001$; n=17/treatment], but not Surgery ($p > .05$). Also, no interaction between Surgery and Endotoxin was evident ($p > .05$). (Fig 4B). *At four weeks*, consistent with the one and two week timepoints, Endotoxin increased peripheral IL-1 β [$F(1, 87) = 47.71$; $p < .0001$; n=18, LPS grp; n=17, Vehicle grp] but not Surgery ($p > .05$) or Surgery and Endotoxin ($p > .05$) (fig. 4C).

3.4 One Week Post-Surgical Treatment: Effects of Surgery Treatment on hippocampal IL-1 β , MHCII, CD11b, Iba-1, and GFAP mRNA—One week after surgery, hippocampal IL-1 β mRNA, unlike IL-1 β protein, was elevated [$F(2, 24) = 7.16$, $p < .01$] (Fig. 5A). Significant differences in IL-1 β mRNA levels were found between the cannulated animals (n=10) compared to stab wound (n=9) ($p = .003$) and naïve (n=8) ($p = .004$) animals. MHCII mRNA levels were also elevated [$F(2, 24) = 3.57$, $p < .04$] (Fig. 5B). Post hoc tests revealed significant differences between the cannulated group (n=10) compared to the naïve (n=8) ($p < .003$) but not stab wound (n=9) ($p = .12$) group. CD11b mRNA levels were also elevated [$F(2, 24) = 3.22$, $p < .04$] (Fig. 5C). Post hoc test revealed significant differences between the cannulated group (n=10) compared to the stab wound (n=9) ($p < .03$) and naïve (n=8) ($p < .04$) group. No group significant differences were found for Iba-1 ($p = .18$) or GFAP ($p = .10$) mRNAs (Fig. 5D,E). Furthermore, comparisons between stab wound and naïve did not differ from each other for each dependent measure ($p > .05$ for each comparison).

4. Discussion

The infusion of pharmacological agents directly into the brain is a routinely used method. In order to infuse these agents into the brain it is necessary to first implant cannulae. It has often been implicitly assumed that the presence of cannulae does not contribute to the outcome. We have demonstrated that intracranial cannulation potentiates a neuroinflammatory response, specifically IL-1 β , to a peripheral immune challenge.

Factors that may contribute to the IL-1 β potentiation

The insertion of a cannula induces a significant trauma to the brain in which the blood brain barrier (BBB) is compromised. In the rat, it takes approximately 10 days for the BBB to heal (Baldwin et al., 1996), and during that time, peripheral immune cells (monocytes, granulocytes, and lymphocytes) can infiltrate the brain parenchyma (Dinarello, 1988; Soares et al., 1995; Dinarello, 1996; Tonelli and Postolache, 2005). Disturbance of the BBB also leads to the activation of BBB resident cells (pericytes and perivascular macrophages) (Angelov et al., 1998) and glial cells. The initial signaling that follows the trauma, however, is primarily through activation of microglia with the release of IL-1 β (Vincent et al., 1996).

Peripheral immune cells that infiltrate the brain, as well as those cells that reside at the BBB, have the capacity to release IL-1 β . Peripheral immune cells and BBB resident cells can also express antigen-presenting markers as well as complement receptors, such as MHCII and CD11b, respectively. Thus, several factors may contribute to the potentiated IL-1 β response that occurs in the presence of an inert cannula. These factors may include, but are not limited to, proliferation of microglia or other monocytes, sensitized (or primed) microglia and / or other monocytes and increased number of proinflammatory mediators due to infiltrating peripheral immune cells. First, microglia and other monocytes proliferate locally in response to a central nervous system (CNS) disturbance such as a cortical stab wound (Giordana et al.,

1994). Second, glia and other monocytes can exist in an activated “primed” state, without producing proinflammatory mediators (Perry et al., 2003). Cells in this primed state over express proinflammatory products if further stimulated. Third, infiltrating immune cells may contribute to the potentiated hippocampal IL-1 β response (Soares et al., 1995; Ling et al., 2006) and also may explain the consistently higher IL-1 β levels in posterior cortex compared to the other brain regions.

An open wound in the brain parenchyma can potentially allow circulating LPS, IL-1 β , and pathogens to gain access to the CNS. This would allow for these proinflammatory stimuli to directly produce neuroinflammatory responses in the CNS. (Soares et al., 1995; Raivich et al., 1998; Fawcett and Asher, 1999; Raivich et al., 1999). Taken together, the exaggerated hippocampal IL-1 β response may be the product of a sensitized as well as proliferating microglial and that of other monocytic cells in addition to an increase of immune cells infiltrating the brain.

Spread of neuroinflammation to frontal cortex

Frontal cortex IL-1 β increases produced by LPS were exaggerated at one and two weeks, but not four weeks after cannula implantation in the hippocampus. These findings are consistent with others that have reported glial activation in tissues at a distance from the injury site after a cortical stab wound (Moumdjian et al., 1991) or implanting a foreign object (Szarowski et al., 2003). To date, evidence has not been provided that indicates how this occurs. A possible explanation, however, is that the spread of the increased IL-1 β response is due to direct contact between glia (and neurons) via gap junctions. It is well known that neurons and astrocytes can form intra- and intercellular gap junctions. More recent research has shown that microglia can also express gap junctions (Eugenin et al., 2001). Subsequent administration of LPS may thus induce the transmission of signals through gap junctions and that, in turn, may lead to an exaggerated LPS-induced IL-1 β response in distant regions such as frontal cortex. It is also possible that autocrine and paracrine functions of IL-1 β result in a signal that is propagated from the injury site to uninjured tissue.

Hippocampal IL-1 β mRNA but not IL-1 β protein increases in cannulated animals

An interesting finding was that IL-1 β mRNA was increased in cannulated animals one week after surgery, but IL-1 β protein levels from the same group of animals were not increased. As previously described, the role of IL-1 β in the recovery process after a CNS disturbance involves initiating glial scarring, inducing growth factors, and recruiting cells (Basu et al., 2004). However, overproduction of IL-1 β can turn a recovery process into a pathological outcome. For example, IL-1 β can induce the release of inflammatory mediators through autocrine and paracrine actions on glial cells, in turn, glia can contribute to the inflammation through their release of mediators such as prostaglandins (Basu et al., 2004) and/or reactive oxygen species (ROS) (Van der Goes et al., 2001). The degree and duration of IL-1 β release can be the difference between IL-1 β mediating recovery or instigating pathology. Perhaps after a brain injury such as that produced by inserting a cannula, IL-1 β protein levels are kept in check to allow recovery to take place and prevent further damage.

In conclusion, it is clear from the present data that implantation of cannulae activates recovery and protective processes that ensue an open wound brain injury. This is important because cannulation is frequently used as a means to carry out studies that involve neuroinflammation in brain injury. Thus, the effects of intracranial cannulation should be considered when designing studies that will stimulate neuroinflammatory processes.

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Figure 1. Illustrations include the cannula used for these studies and the brain regions assessed for analyses of IL-1 β . (A) Plastic One stainless-steel cannula (3mm $\times$ .46 mm diameter) were used to test the effects of cannulation on altering IL-1 β levels. (B) Site of cannulation placement, dorsal hippocampus (CA1 region). (C) Brain regions dissected, parietal cortex (per-cannula tissue, $\sim 2\text{mm}^2$), hippocampus (left and right hemispheres), and frontal cortex ($\sim 3\text{mm}$ each hemisphere).

Figure 2.

The spatial effects of bilateral intrahippocampal cannulation on hippocampal, frontal cortex, and parietal cortex IL-1 β response to LPS at *one, two, and four weeks* after surgery. Cannulae were either implanted bilaterally into the dorsal hippocampus (stripped bars), or used to induce a stab wound injury (black bars) in the rat. Non-manipulated animals (Naïve) (white bars) served as controls. At one, two, or four week(s) following the surgical procedure, animals were injected i.p. with either LPS (25 μ g/kg) or equivolume vehicle. Two hr after LPS (or vehicle), hippocampus, frontal cortex, and parietal cortex were dissected to determine IL-1 β protein levels. (A) At one and two weeks after surgical treatment, hippocampal, parietal, and frontal IL-1 β levels were exacerbated in cannulated animals after LPS treatment (A, B). At four weeks, cannulation no longer had a significant effect on IL-1 β response to LPS (C). Data are represented as mean and sem. *Significant difference set at $p < .05$.

Figure 3.

The interaction between Brain Region and LPS is depicted here to show differences between the IL-1 β response to LPS between hippocampus (Δ), parietal cortex ($\square$), and frontal cortex ($\circ$) at one, two, and four weeks after surgery. Data are represented as mean and sem.

*Significant difference set at $p < .05$.

Figure 4.

Sera IL-1 β response to LPS *one, two, and four weeks* after surgery. To determine the peripheral IL-1 β response to LPS in animals implanted with cannulae intrahippocampally (bilaterally) (stripped bars), that received a stab wound (black bars), or that were non-manipulated (Naïve) (white bars). At one, two and four weeks, LPS (i.p., 25 μ g/kg) or equivolume vehicle was administered. Two hr after LPS (or vehicle) treatment, cardiac blood was collected to measure sera IL-1 β levels. Data are represented as mean and sem. *Significant difference set at $p < .05$.

Figure 5. Effects of cannulation on hippocampal IL-1 β , MHCII, CD11b, Iba-1, and GFAP mRNA. To determine if glial markers and IL-1 β were altered at the mRNA level, hippocampal tissues were processed for mRNA analyses from the cannulated, stab wound and naïve group one week after surgical procedure. mRNA was assessed using real-time PCR. Data are represented as mean and sem. *Significant difference set at $p < .05$.

Table 1

PCR primer specifications

GENE	Cell Type Specificity	Function	Primer Sequence (5' → 3')	Genbank Acc. #
IL-1β	Glia, Perivascular macrophage	Proinflammatory cytokine	F-GAAGTCAAGAGACCAAAAGTGG R-TGAAGTCAAACTATGTCCCG	M98820
MHCII	Microglia, Perivascular macrophage	Antigen presentation	F-AGAGACCATCTGGAGACTTG R-CACTCTGGGGTGTGTTGGA	AJ554216
CD11b	Microglia, Perivascular macrophage	Complement Receptor	F-CTGGGAGATGTGAATGGAG R-ACGTGCTGCTGGCTACTGATG	AF268593
Iba-1	Microglia, Perivascular macrophage	Cell motility and Phagocytosis	F-GAATGATGCTGGGCAAGAGA R-CAGTTGGCTTCTGGTGTC	D82069
GFAP	Astrocytes	Cytoskeleton Protein	F-AGGACAATCTCACACAGG R-GACTCAACCTTCTCTCTCCA	AF028784