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Interleukin-10/Ceftriaxone prevents *E. coli*-induced delays in sensorimotor task learning and spatial memory in neonatal and adult Sprague–Dawley rats

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Abstract

Intrauterine infection during pregnancy is associated with early activation of the fetal immune system and poor neurodevelopmental outcomes. Immune activation can lead to alterations in sensorimotor skills, changes in learning and memory and neural plasticity. Both interleukin-10 (IL-10) and Ceftriaxone have been shown to decrease immune system activation and increase memory capacity, respectively. Using a rodent model of intrauterine infection, we examined sensorimotor development in pups, learning and memory, via the Morris water maze, and long-term potentiation in adult rats. Pregnant rats at gestational day 17 were inoculated with 1×10^5 colony forming units of *Escherichia coli* (*E. coli*) or saline. Animals in the treatment group received IL-10/Ceftriaxone for 3 days following *E. coli* administration. Intrauterine infection delayed surface righting, negative geotaxis, startle response and eye opening. Treatment with IL-10/Ceftriaxone reduced the delay in these tests. Intrauterine infection impaired performance in the probe trial in the Morris water maze (saline 25.13 ± 1.01 ; *E. coli* 20.75 ± 1.01 ; *E. coli* + IL-10/Ceftriaxone 20.2 ± 1.62) and reduced the induction of long-term potentiation (saline 141.5 ± 4.3 ; *E. coli* 128.7 ± 3.9 ; *E. coli* + IL-10/Ceftriaxone 140.0 ± 10). In summary, the results of this study indicate that *E. coli* induced intrauterine infection delays sensorimotor and learning and memory, while IL-10/Ceftriaxone rescues some of these behaviors. These delays were also accompanied by an increase in interleukin-1 β levels, which indicates immune activation. IL-10/Ceftriaxone prevents these delays as well as decreases *E. coli*-induced interleukin-1 β activation and may offer a window of time in which suitable treatment could be administered.

Keywords

Ceftriaxone; Interleukin-1 β ; Interleukin-10; Long-term Potentiation; Intrauterine Infection; Morris water maze

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Conflict of interest

The authors declare that they have no competing financial interests.

1. Introduction

Recent evidence indicates that events causing fetal inflammatory response syndrome during gestation can have adverse consequences on the developing fetus [40]. Intrauterine infection (IUI) resulting from bacterial and viral sources is associated with an increased risk of white matter damage [21,20,29,41], and neurobehavioral disorders [5,9,28,47]. IUI leads to a series of changes which induce both maternal and fetal inflammatory cytokines [13,19] facilitating changes in brain formation [6,50,69,71] resulting in possible neurobehavioral changes.

Interleukin-1 β (IL-1 β) is a well characterized inflammatory cytokine which is elevated after immune activation or brain injury and is correlated with neurodegenerative disorders, ischemia and neuronal damage [13,68]. IL-1 β is also increased in the brain after prenatal or postnatal endotoxin injection [11,12,43,64,71].

We have established a rodent model of IUI that demonstrates white matter damage in rat pups [52,56]. Ceftriaxone, a third generation cephalosporin, is routinely used to treat bacterial infections during pregnancy and has been shown to offer neuroprotective effects in rodent models [46,57,67]. Interleukin-10 (IL-10) and other anti-inflammatory cytokines such as interleukin-4 have also been shown to prevent neurological damage and decrease inflammatory cytokines which are activated through bacterial infections [3,24,36,56]. Our previous studies indicate that IL-10 and Ceftriaxone when combined together as a treatment, suppresses white matter damage in this model [55]. The objective of this study was to utilize this animal model to determine the effects of perinatal IL-1 β activation on rodent sensorimotor skills as well as any long-term effects on learning and memory or hippocampal synaptic plasticity in adult rodents.

2. Methods

2.1. Experimental animals

All experiments were carried out with the approval of the Institutional Animal Care and Use Committee of the University of MS Medical Center which follows the NIH Guidelines for the Use and Care of Laboratory Animals. Twenty-four timed-pregnant Sprague–Dawley rats (Harlan Sprague–Dawley, Indianapolis, IN, USA) were obtained at gestational day 13. Food and water were available *ad libitum* and the colony was maintained in a 12:12-h light/dark schedule.

2.2. Experiment 1

2.2.1. Surgical procedures and treatments—On GD15 twelve timed-pregnant dams, between 270 ± 5 g, were randomly assigned to either saline ($n = 4$), *E. coli* (ATCC #25922, Manassas, VA, USA) ($n = 4$) or *E. coli* plus recombinant IL-10 (Biosource International, Carlsbad, CA, USA) and Rocephin[®] (Ceftriaxone) ($n = 4$) group. Without altering dam weight, IL-10 was administered intravenously via femoral vein catheters implanted on GD15 under general anesthesia with 5% isoflurane. On GD17 animals were anesthetized and inoculated with saline or 1×10^5 colony forming units (CFU) of *E. coli* at the bifurcation of the uterine horns. At the time of saline or *E. coli* injection none of the dams had fetal reabsorptions. On GD18 animals in the treatment groups received 1 μ g/kg body weight of rat recombinant IL-10 I.V. and 20 mg/kg Ceftriaxone I.M., b.i.d. until GD20. Our previous studies have shown that this dosing and timing of IL-10/Ceftriaxone treatment decreases astrogliosis, ventriculomegaly and white matter lesioning [52,56]. Administration of IL-10 alone after *E. coli* injection decreases fetal weight and increases pup morbidity, while Ceftriaxone alone after *E. coli* injection does not significantly affect fetal weight or

pup morbidity (Supplementary data 1). Treatment with Ceftriaxone alone after *E. coli* injection has also been shown to prolong delivery time compared to animals receiving saline injections [2]. Based on this data the combination of IL-10/Ceftriaxone was utilized. All animals were observed daily and allowed to deliver without any additional experimental manipulation. After delivery, pups were weighed and remained with dams for the length of the study. Throughout the study rat pups were separated from dams for a period not exceeding 1 h a day until weaning. At postnatal day 8 all female pups were culled, so that only male pups remained in the study.

2.2.2. IL-1 β analysis—One hallmark of immune system activation is increased IL-1 β . IL-1 β receptors are the most dense in the hippocampus [45], where cytokine damage during development affects memory [4]. Pilot studies from our lab indicates that when *E. coli* is administered to pregnant Sprague–Dawley dams on GD17 there are no statistically significant differences in protein levels of tumor necrosis factor- α or interleukin-6 in placentas or fetal brains after 48 h of *E. coli* administration when compared to animals who received saline injections. Protein levels of IL-1 β remained elevated in both placentas and fetal brains at least until 96 h post-*E. coli* injection, for this reason we focused our studies only on IL-1 β . On PN days 8 and 75, four rats from each group were sacrificed via decapitation and brains removed. The hippocampus was dissected out and quickly frozen with dry ice. Tissue samples were homogenized in 5 volumes of ice-cold PBS containing a cocktail of serine and cysteine protease inhibitors (Roche, Mannheim, Germany). The homogenate was centrifuged at 10,000 rpm at 4°C for 10 min, and the supernatant was stored for analysis. BCA protein assays (Pierce, Rockford, IL, USA) were performed to determine the total protein concentrations in hippocampal supernatant samples. IL-1 β ELISA (RnD Systems, Minneapolis, MN, USA) was carried out according to manufacture's instructions.

2.2.3. Early age sensorimotor analysis—Beginning at PN2, male pups were placed through a series of sensorimotor tests (saline, $n = 19$; *E. coli*, $n = 19$; *E. coli* + IL-10/Ceftriaxone, $n = 15$). Surface righting (PN2–12) was defined as the time in seconds required for a pup lying on its back to right itself on all four limbs. Pups were observed for up to 60 s. Negative Geotaxis (PN2–12) was defined as the time in seconds required for a pup placed head down on a 45° incline to turn 180° and begin to crawl up the slope. Pups were observed for up to 60 s. Cliff avoidance (PN2–12) was defined as the time in seconds required for a pup with paws and snout over the edge of a shelf to turn and begin crawling away from the edge. Pups were observed for up to 60 s. Open Circle (PN8–16) was defined as the time in seconds required for a pup to move off a circle 13 cm in diameter. Pups were observed for up to 300 s. Audio startle (PN8–16) was defined as whether or not a pup stopped voluntary movement or jumped after a loud noise was administered 10 in. above the pup.

2.3. Experiment 2

2.3.1. Surgical procedures and treatments—On GD15 twelve timed-pregnant dams, between 270 ± 5 g, were randomly assigned to either saline ($n = 4$), *E. coli* ($n = 4$) or *E. coli* plus recombinant IL-10 and Ceftriaxone ($n = 4$) group. All animals underwent the same surgical schedule as in Experiment 1. After delivery, pups were weighed and remained with dams until PN30 at which they were weaned. At postnatal day 8 all female pups were culled, so that only male pups remained in the study.

Appendix A. Supplementary data: Supplementary data associated with this article can be found, in the online version, at doi: 10.1016/j.brainresbull.2009.10.016.

2.3.2. Immunohistochemistry—At PN70 brains from eight animals in each group were fixed via intracardial perfusion with 4% paraformaldehyde prior to paraffin embedding. 20- μ m sections were cut with a microtome and every 5th section was used to detect microglia. Slides were treated with Trilogy (Cell Marque, Rocklin, CA), followed by antigen retrieval with 0.1% phenylhydrazine for 30 min. Slides were then blocked with 0.05 M Tris, 5% dry milk (BioRad, Hercules, CA), 2% goat serum and 0.2% SDS for 1 h. The slides were then incubated overnight at 4°C with 1:200 OX-42 (Chemicon, Temecula, CA) in 0.05 M Tris, 5% dry milk, and 0.05% Tween 20. After washing with PBS and 0.05% Tween 20, the sections were incubated for 1 h with 1:200 of affinity purified goat anti-mouse IgG HRP (Santa Cruz). Antibody binding was visualized with diaminobenzidine (Invitrogen) as a chromogen. Coverslips were placed over sections on the slides and the sections air-dried overnight. Sections incubated without primary antibody served as a negative control.

2.3.3. Locomotor activity—At PN60 male adult rats (saline $n = 19$, *E. coli* $n = 19$, *E. coli* + IL-10/Ceftriaxone $n = 15$), were placed individually into a monitoring system (Opto-Varimex-Minor System, Columbus Instruments, Columbus, Ohio, USA) under low lights for 30 min to test locomotion.

In the following set of studies male adult rats (PN70+) from our treatment groups were randomly divided into two study arms, Morris water maze (saline $n = 12$, *E. coli* $n = 12$, *E. coli* + IL-10/Ceftriaxone $n = 10$) or LTP (saline $n = 7$, *E. coli* $n = 7$, *E. coli* + IL-10/Ceftriaxone $n = 5$).

2.3.4. Morris water maze (MWM)—The water maze was a circular pool (2 m diameter, 35 cm deep; water, 23 ± 1 °C) filled to 31 cm with opaque (non-toxic dry tempera paint) water. Rats could escape the water by climbing onto a submerged (1 cm below water level) white Plexiglas platform (29 cm $\times$ 10 cm). The pool was divided into four quadrants, with four visual cues placed around the quadrants. All trials were taped with a digital camcorder for further analysis. Rats were subjected to the water maze for 5 consecutive days. Four training trials a day were given, in which each animal was released from a different quadrant each trial [39,62]. This was done in a pseudo-random manner and each start quadrant varied for each day. During the training trials the rats had to search for the stationary hidden platform during a 120 s time period. If a rat failed to find the platform during the training period it was placed on the platform and for 15 s. Following the fourth trial, a 60 s probe trial was given in which the platform was removed from the pool and the time the rat spent in the quadrant assigned to the platform was recorded. The platform was moved to a different quadrant each day, to test working memory rather than reference memory.

2.3.5. Long-term potentiation (LTP) studies—Immediately prior to experimentation animals were anesthetized (isoflurane) and decapitated. Brains were extracted and immersed in cold oxygenated dissection buffer. The hippocampus was extracted and sliced (400 μ m) using a tissue chopper. Slices were transferred to a recording chamber (Fine Science Tools, Foster City, CA, USA) containing preheated running artificial cerebrospinal fluid (1–2 ml/min). Slices were continuously perfused with this solution and maintained in an oxygenated, humidified atmosphere. Slices were allowed to equilibrate in the chamber for at least 2 h before starting electrophysiological recordings. For the recordings, a glass-pipette electrode filled with 0.15 M NaCl (impedance 2–3 M Ω) was placed in the stratum radiatum of the CA1 region of the hippocampus. A concentric bipolar stimulating electrode (FHC, Inc., Bowdoinham, ME) was positioned to stimulate the Schaffer collateral–commissural fibers in CA1. Extracellular field potentials were evoked by a 1–150 μ A square wave stimuli of 0.2 ms duration. After electrode placement, a full input/output curve was obtained, and stimulation intensity was set at 50% of the maximum response. A stable 20 min baseline was recorded utilizing the half-maximal stimulation intensity (1–30 V) delivered once every

minute. LTP was induced with 10–13 theta bursts (TBS), with each burst consisting of four pulses at 100 Hz, and individual bursts separated at 5–7 Hz and continuous with the same half-maximal intensity, stimulation was thereafter delivered once every minute and recordings followed for 30 min. The stimulus duration was increased to 0.4 ms during TBS. The evoked field potentials were digitized at 20 kHz and stored for later analysis. They were analyzed for a single-waveform parameter, the slope of the field excitatory postsynaptic potential (fEPSP). Changes in fEPSP after TBS were expressed and analyzed as percent change from average baseline levels [42].).

2.4. Statistical analysis

Surface righting, negative geotaxis, cliff avoidance and open circle behavioral data were analyzed by two-way repeated measures ANOVA, with postnatal age serving as the repeated measure and prenatal treatment a fixed factor. Response to audio startle and the day of eye opening was analyzed using Kruskal–Wallis, since yes or no values were recorded. Results from IL-1 β ELISA, locomotor activity and data from LTP were analyzed using a one-way ANOVA. MWM was analyzed using WATER-MAZE – Infallible Software 3.0. Time in each quadrant, number of crosses into each quadrant, latency to reach target for each training and probe trial were calculated. Data was analyzed by two-way repeated measures ANOVA. Bonferroni, corrected t tests were used for post-hoc evaluation. Differences of $p < .05$ were considered significant.

3. Results

3.1. Experiment 1

3.1.1. General newborn characteristics—All dams delivered between GD21–22. Table 1 shows that there were significant differences ($F(2,48) = 4.39, p = .026$) between pup weights, with pups in the IL-10/Ceftriaxone group weighing significantly more than pups in the *E. coli* alone group on PN2–6 and from pups in the control group on PN10–14. There were no significant differences in weights among the groups after PN18 (data not shown). It should be noted that the dose of *E. coli* used in this study does not significantly affect litter size or rate of reabsorption in Sprague–Dawley rats. Likewise administration of IL-10/Ceftriaxone does not affect litter size or rate of reabsorption.

3.1.2. IL-10/Ceftriaxone prevents *E. coli*-induced increased IL-1 β expression the hippocampus—IUI significantly increased IL-1 β expression in the hippocampus at both PN8 ($F(2,9) = 14.439, p < .05$) and PN75 ($F(2,9) = 19.264, p < .05$). IL-10/Ceftriaxone prevented the increase in IL-1 β when at PN8 (Fig. 1). However, IL-10/Ceftriaxone did not have long-term effects in preventing IUI-induced IL-1 β activation, as it was also significantly increased compared to controls at PN75. There was an age-related decrease in IL-1 β expression in the hippocampus, which was observed regardless of experimental treatment.

3.1.3. IL-10/Ceftriaxone prevents *E. coli*-induced delays in sensorimotor skills
—*Surface righting*: IUI significantly increased ($F(2,48) = 4.25, p = .017$) time taken to complete surface righting on PN2–3 (Fig. 2A) as compared to the control and IL-10/Ceftriaxone groups. IL-10/Ceftriaxone prevented the delay in surface righting and decreased the time taken to complete the task compared to the control group on PN3–5. *Negative geotaxis*: IUI with and without IL-10/Ceftriaxone significantly increased ($F(2,48) = 41.05, p = .01$) the time taken to complete this task (Fig. 2B) from PN2–6. *Cliff avoidance*: IUI did not have a significant effect on cliff avoidance, when compared to the control group (Fig. 2C). Pups in the IL-10/Ceftriaxone group were able to complete this task at a faster rate than the controls or the pups in the *E. coli* alone group on PN5–6, and 10 ($F(2,48) = 11.58, p = .$

000). *Open circle*: IUI did not have a significant effect on the amount of time spent in an open circle, when compared to the control group (Fig. 2D). Pups in the IL-10/Ceftriaxone group spent significantly less time ($F(2,48) = 5.75, p = .005$) in the open circle on PN10–11 compared to pups in the *E. coli* alone group. Pups from dams who received IL-10 alone after *E. coli* injection demonstrated marked delays in the performance of all of the above tests. Pups from dams who received Ceftriax-one alone after *E. coli* injection initially performed worst than pups in the *E. coli* injection group, but were able to surpass pups in the *E. coli* group after a few days of testing (Supplementary data 2). *Audio startle response*: IUI with or without IL-10/Ceftriaxone significantly decreased ($X^2 = 26.75 (n = 55), p = .009$) the response to an audio startle compared to controls. Pups in the control group began responding to an audio stimulus on PN9, *E. coli* alone on PN14 and *E. coli* + IL-10/Ceftriaxone on PN13. *Eye opening*: IUI significantly delayed ($X^2 = 13.64 (n = 55), p = .014$) eye opening compared to the control and the IL-10/Ceftriaxone groups. On average pups in the control group had both eyes open on PN14, *E. coli* alone on PN16 and *E. coli* plus IL-10/Ceftriaxone on PN15.

3.1.4. IL-10/Ceftriaxone increases locomotor activity—There was a significant difference between the groups in locomotor activity ($F(2,55) = 12.36, p < .05$) (data not shown). IL-10/Ceftriaxone administration significantly increased locomotor activity in the adult rat when compared to controls ($t_{29} = 5.74, p < .05$) and the *E. coli* alone group ($t_{29} = 6.33, p < .05$).

3.2. Experiment 2

3.2.1. IL-10/Ceftriaxone prevents ramified microglia morphology in adult rats

—Staining of amoeboid microglia with OX-42 was observed in all brain sections ($n = 8$) from the saline and IL-10/Ceftriaxone groups (Fig. 3A and C). Staining of Ramified microglia with OX-42 was observed in all brain sections ($n = 8$) from the *E. coli* alone group (Fig. 3B). These microglial cells were observed in white matter regions and in the internal capsule area of the hippocampus.

3.2.2. IL-10/Ceftriaxone improves *E. coli*-induced impairment in Morris water maze—No significant differences ($F(2, 33) = 2.640, p = .090$) were seen in latency to reach the target over the testing trials among groups (Fig. 4A). A significant treatment effect ($F(2, 33) = 5.14, p < .05$) (Fig. 4B) was seen between groups in the probe trial. Post-hoc analysis revealed that animals in the *E. coli* without IL-10/Ceftriaxone administration had an impaired performance in the probe trial relative to the saline group ($p < .05$).

3.2.3. IL-10/Ceftriaxone improves *E. coli*-induced reduction in LTP—After 20 min of stable baseline recording, LTP was induced by TBS. This produced an immediate and pronounced increase in the slope of the fEPSP (percentage of baseline) in the three groups (saline: 141.5 ± 4.3 ; *E. coli*: 128.7 ± 3.9 ; *E. coli* + IL-10/Ceftriaxone: 140.0 ± 10 reported as Avg $\pm$ SEM). Thirty minutes after TBS, there were no statistically significant differences in fEPSP slope between experimental groups relative to the last 10 min of baseline ($F = 1.2, p < .05$). Animals from the *E. coli* alone group showed a nonsignificant reduction in the fEPSP slope as compared to the saline group (Fig. 5A). This reduction in the LTP magnitude was not present in the *E. coli* + IL-10/Ceftriaxone animals (data not shown), suggesting that the latter treatment prevented any reduction due to *E. coli* administration.

4. Discussion

We investigated the role of host immune activation during the perinatal period through a variety of developmental sensorimotor skills and learning/memory tasks. Our data suggests that prenatal *E. coli* infection delays select sensorimotor skills as well as eye opening and audio startle. IUI significantly increased the amount of time taken to complete surface righting and negative geotaxis. IL-10/Ceftriaxone administration prevented IUI-induced delays. In addition as adults, rats in the *E. coli* alone group demonstrated impairments in spatial memory that was reversed with IL-10/Ceftriaxone treatment. Even though the response to LTP was not significantly reduced in the *E. coli* alone group, IL-10/Ceftriaxone brought all LTP levels back to baseline/control levels. These results show that intrauterine inoculation of *E. coli* does cause permanent impairment in learning skills and that treatment with IL-10/Ceftriaxone offers some protection in these behaviors. Furthermore, the behavioral changes seen here are accompanied by increases in IL-1 β levels in the hippocampus. This indicates that an immune response is present and that immune activation may be correlated with deficits in behavior. The combination of IL-10/Ceftriaxone was able to decrease levels of IL-1 β in early juvenile rats, but was less efficacious in adult rats.

The increase in body weight due to IL-10/Ceftriaxone administration was unexpected, as there were no differences in interval to delivery or litter size among the treatment groups. In our preliminary studies IL-10 administration alone after *E. coli* decreased pup weight, while Ceftriaxone administration alone did not lead to any significant differences in pup weight compared to the saline group (data not shown). Some clinical studies have shown that Ceftriaxone administration is used to improve weight gain among malnourished patients [22]. Based on the current study, the combination of IL-10/Ceftriaxone appears to improve weight gain. It should also be noted that prenatal administration of IL-10/Ceftriaxone increased overall birth weight in pups.

Previous work has shown that endotoxin exposure either prenatally or in the immediate postnatal time period leads to changes in sensorimotor skills [23,59,70], such as the ones examined in this study. Lipopolysaccharide administered at 3 time points during pregnancy results in delays in surface righting, negative geotaxis and eye opening [23,70]. While other models have seen a delay in cliff avoidance [70], we were not able to demonstrate a significant difference in this skill. It would also appear that a continual infection as seen in this model or several injections of LPS [70] is needed to see a delay in these skills. When single injections of LPS are given on GD15, there are no alterations in sensorimotor skills [53], thus suggesting a robust activation of the fetal immune system might be needed to see alterations in sensorimotor skills.

Ceftriaxone serves not only as an antibiotic, but also increases glutamate transporter activity which improves motor neuron degeneration and protects against neurologic disorders [57,58]. When Ceftriaxone is administered to mice it increases motor ability in the tail-suspension and forced swim test [48], thereby increasing overall motor ability. Likewise peripheral administration of IL-10 to adult mice also increases motor activity in the locomotor chamber [30]. It is possible that the increase in locomotor activity seen at PN60 in the IL-10/Ceftriaxone group is due to the effects of Ceftriaxone and IL-10 on motor ability.

IL-1 β has been found to impair LTP in the hippocampus [7,31,35,36,72], and to inhibit hippocampal-dependent forms of cognitive learning [34,54,60,63] as well as to affect spatial memory [27,51]. Accordingly, we examined the presence of IL-1 β in the hippocampus after IUI. The chronic increase in IL-1 β was somewhat surprising since others have found that LPS lowers IL-1 β in the brain [8,74]. One explanation for this may be the activation of

microglia, which are sensitive to endotoxins [26]. Microglia are a major source of IL-1 β in the brain [1,38] and remain activated in response to infection [15,18,65]. Ours and other models of endotoxin exposure have shown that microglia are activated after infection [52,66]. Intrauterine *E. coli* infection led to long-lasting increases in microglia activation, indicated by the presence of ramified microglia, which was prevented by IL-10/Ceftriaxone treatment in this model. While these results may explain the continual production of IL-1 β seen in the *E. coli* animals, it does not explain why animals in the IL-10/Ceftriaxone group still had elevations in IL-1 β as adults. Further studies are needed to determine the mechanisms of microglia activity in response to IL-10/Ceftriaxone.

Our findings in respect to endotoxin exposure and the MWM are consistent with reports that *E. coli* and/or IL-1 β impair spatial memory [16,27,51,61]. IUI decreased the amount of time spent in the target quadrant (Fig. 4B), while IL-10/Ceftriaxone increased the amount of time spent in the target quadrant. The majority of the studies indicating significant changes in spatial memory utilize an animal model where a direct injection of the endotoxin or IL-1 β is given into the brain, or the animals are tested shortly after peripheral endotoxin administration. This is one of the first studies to show that neonatal infection with *E. coli* can lead to long-term memory changes in adult rats. Early-life stress in rodents has been shown to impair hippocampal structure and decrease pyramidal cells in the hippocampus, and subsequently lead to impairments in learning and memory [10]. In the current study we did not examine hippocampal volume, but it is possible that prenatal *E. coli* administration in this model decreases hippocampal volume which when combined with increased hippocampal concentrations of IL-1 β leads to impairments in spatial memory, as demonstrated here.

Administration of IL-1 β or endotoxin has been shown to impair induction of LTP in the hippocampus [7,35,73]. Even though there was a reduction seen in LTP, a significant impairment was not seen due to *E. coli*. When LPS is given acutely, the response to LTP is diminished [43]. Previous studies have issued an immune challenge shortly before testing LTP [17], whereas our immune challenge was during the prenatal period. It is possible that while prenatal exposure sensitizes the immune system and affects early age behavior, it does not damage the immune system enough to have robust effects on synaptic plasticity.

Elevations in inflammatory cytokines in rodents often lead to a set of behaviors, primarily weight loss and lethargy, termed sickness behavior [37]. As a result, dams exhibiting these behaviors are often too sick to care for pups, which decreases pup survival and potentially has long-lasting changes on the neurological and behavioral development of the pups [25,33]. Work by Champagne et al. [14] has shown that the frequency of licking and general pup grooming given by dams has long-lasting effects on hippocampal plasticity, again demonstrating that maternal care in rodents has neurobehavioral consequences in pups. In this study we did not use surrogate dams or monitor mothers for sickness behavior. Although pup weight in the *E. coli* group was not significantly less than pups in the saline group, with the exception of PN2, it is possible that there were some effects of sickness behavior among the dams.

We have previously shown that IL-10/Ceftriaxone is capable of reducing astrogliosis, and increasing pre-oligodendrocytes precursors [52] as well as preventing ventricular white matter lesion formation [56]. Although the exact mechanisms of the neuroprotective action of IL-10/Ceftriaxone are not completely known, Ceftriaxone has a role in the promotion of neuronal survival and improving neurological outcome by increasing glutamate transporter expression and glutamate uptake [57,67]. IL-10, through its actions as an anti-inflammatory cytokine can decrease IL-1 β activation and protect oligodendrocytes from endotoxin [49]. Anti-inflammatory cytokines such as IL-10 and IL-4 have been shown to have a role in

learning and memory, therefore, future studies are designed to determine alterations in these cytokines due to prenatal IL-10/Ceftriaxone administration [32,43,44]. Data from this study indicates that the combination of IL-10 and Ceftriaxone offers neuroprotection from sensorimotor and learning deficits as a result of *E. coli* infection. As a result of infection fetal programming is altered, thus causing irreversible changes in the fetus. Although animals are able to recover from initial delays in learning, these data suggests that the prenatal period offers a unique window of time in which the neuroimmune system can be permanently altered. We have demonstrated that the effect of IL-10/Ceftriaxone is a potential anti-inflammatory/antibiotic therapy that may provide an opportunity for further therapeutic treatment for white matter diseases of prenatal origin.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Intrauterine *E. coli* inoculation effects on IL-1 β protein levels. *E. coli* inoculation without treatment leads to increased IL-1 β protein levels in the hippocampus ($p < .05$). Treatment with IL-10/Ceftriaxone after *E. coli* administration, decreased IL-1 β protein levels ($p < .05$). Rats were sacrificed and the hippocampus was collected at the times indicated. $N = 4-5$ per group at each time point.

Fig. 2.

IUI affects sensorimotor skills in young rat pups. (A) *E. coli* caused an increased latency to complete surface righting on PN2–3, while IL-10/Ceftriaxone prevented the delay. (B) *E. coli* with and without IL-10/Ceftriaxone increased the time taken to complete negative geotaxis on PN2–6. (C) IL-10/Ceftriaxone improved the time taken to avoid a cliff compared to the control and *E. coli* group. (D) IL-10/Ceftriaxone decreased the amount of time spent in an open circle compared to the control and *E. coli* group (mean latency $\pm$ SEM, RM-ANOVA, * $p < .05$).

Fig. 3. Representative photomicrographs of OX-42 positive microglial cells in 70-day-old rat brain sections from saline (A) *E. coli* (B) and *E. coli* + IL-10/Ceftriaxone (C). Ox-42 immunoreactivity of amoeboid microglia was demonstrated in the saline and *E. coli* + IL-10/Ceftriaxone group, while ramified microglial were seen in the *E. coli* group (indicated by arrows). Original magnification of photomicrographs is 100 $\times$.

Fig. 4.

Intrauterine *E. coli* inoculation effects on Morris water maze. (A) Values represent means of average time taken to reach the platform (seconds) over 4 trials for a period of 5 days. No significant differences were observed between groups. (B) Values represent the ratio of time spent in target quadrant vs. quadrant opposite of target. On days 1–3, animals in the *E. coli* alone group, spent less time in the target quadrant compared to controls and animals receiving IL-10/Ceftriaxone. On days 3–5, animals in the *E. coli* + IL-10/Ceftriaxone group spent more time in the target quadrant compared to animals with IUI alone ($p < .05$). * $p < .05$ $N = 10$ – 12 in each group.

Fig. 5.

Intrauterine *E. coli* inoculation effects on LTP expression. Data is shown as percent of baseline change after LTP induction. LTP expression in *E. coli* animals showed a trend towards a reduction in the fEPSP slope compared to the saline group (A) and the IL-10/ Ceftriaxone group (B). No statistically significant differences were observed between groups ($p < .05$).

Table 1

Weights of neonatal rat pups.

	Birth weight	PN2	PN6	PN10	PN14	PN18
Saline	5.25 ± .129	6.61 ± .14*	11.21 ± .34	16.83 ± .67	24.72 ± .80	34.28 ± 1.13
<i>E. coli</i>	5.39 ± .085	6.05 ± .59	10.14 ± .33	17.31 ± .41	25.47 ± .63	35 ± .99
<i>E. coli</i> + TX	5.85 ± .132	7.58 ± .12*	13.16 ± .24*	19.86 ± .53 [†]	27.88 ± .82 [†]	35.88 ± .82

Weights are shown as means ± SEM. *E. coli* infection alone did not cause a significant weight change, but treatment after inoculation with combined IL-10/Ceftriaxone (TX) significantly increased the weight of pups (saline, $n = 30$; *E. coli*, $n = 30$; *E. coli* + IL-10/Ceftriaxone, $n = 30$).

* $p < .05$ from *E. coli*.

[†] $p < .05$ from saline.