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Full Length Research Paper

Intrauterine infection promotes brain region specific cytokine activation and hyperactivity in developing rat

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Intrauterine infection during pregnancy is associated with premature birth, periventricular leukomalacia, early activation of the fetal immune system and poor neurodevelopmental outcomes. Previous clinical studies and studies with animal models have shown increased activation of the immune system evidenced by increased levels of inflammatory cytokines, white matter damage and delays in behavioral development. Animal models of intrauterine infection with consistent behavioral results, white matter damage and immune activation have been difficult to validate. Using a rodent model of intrauterine infection, we examined neurobehavioral development and locomotor development in the resulting pups, and measured inflammatory cytokines in the striatum, frontal cortex and cerebellum. Pregnant rats at gestational day 17 were inoculated with 1×10^5 colony forming units of *Escherichia coli* or 0.1 ml of saline. Intrauterine infection led to a significant increase in the expression of interleukin 1 β, interleukin-6 and tumor necrosis factor-α. *E. coli* injection increased walking, turning and overall motor activity in rats. In summary, the results of this study indicate that *E. coli* induced intrauterine infection resulted in neuroinflammation and led to hyperactivity in basic locomotion.

Key words: Intrauterine infection, *E. coli,* neurodevelopment behavior, immune activation.

INTRODUCTION

Intrauterine infection is a major risk factor for the development of neurodevelopmental brain damage (Dammann et al., 2002; Dammann and Leviton, 1998; Nelson and Willoughby, 2000; Yoon et al., 2000) and is associated with an increased risk of the development of motor impairments similar to those seen in cerebral palsy and periventricular leukomalacia (Bell and Hallenbeck, 2002; Dammann and Leviton, 1998; Wu and Colford, 2000; Yoon et al., 2003). White matter damage, astrocytosis and cytokine activation have been demonstrated in experimental models of intrauterine infection, all of which are capable of leading to delays in brain development (Bell and Hallenbeck, 2002; Cai et al., 2000; Debillon et al., 2000). Animal models of intrauterine infection have white matter lesions similar to those seen in children with cerebral palsy, and have delays in basic neurodevelopmental task (Poggi et al., 2005; Toso et al., 2005). These experimental models show that maternal or postnatal endotoxin exposure sensitizes the immature brain and increases cytokine levels, both of which can be detrimental to posture and motor development.

Endotoxin exposure is a common and powerful stimuli for inflammatory cytokine activation in the setting of intrauterine infection (Kadhim et al., 2001). Several studies looking at the incidence of *in utero* bacterial infections have also found an increased incidence in neurodevelopmental problems in these exposed infants (Schendel, 2001; Vigneswaran et al., 2004). In addition to these clinical studies, animal studies have shown that bacterial/endotoxin-induced intrauterine infection leads to an increase in inflammatory cytokines in both the uterine tissues and the fetal brain (Bell and Hallenbeck, 2002; Cai et al., 2000; Urakubo et al., 2001). *Escherichia coli* is a gram-negative bacterium, which is often found in the urinary tract leading to urinary tract infections. In the

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setting of pregnancy, the bacteria can colonize in the uterine cavity and potentially the uterine tissues. Recent studies have shown that *E.coli* is one of the most common uropathogens, up to an incidence of 62.9% in pregnant women with infection, and that *E.coli* infection is strongly correlated with neonatal sepsis (Guiral et al., 2011; Kuhn et al., 2010; Rafal'skii et al., 2009). Even though *E.coli* is not the only bacteria associated with prenatal infection in humans, it has been isolated in 20 to 40% of prenatal infection related cerebral palsy cases (Mittendorf et al., 2001; Vigneswaran et al., 2004).

We recently reported that in a rodent model of intrauterine infection, developmental, cognitive and motor deficits are present in juvenile and adult offspring (Wallace et al., 2010a; Wallace et al., 2010b). In addition, white matter damage similar to that seen in periventricular leukomalacia is present, including astrocytosis, ventriculomegaly and changes in oligodendrocyte precursors and decreases in Purkinje cell density (Pang et al., 2005; Rodts-Palenik et al., 2004). Our goal in this study is to determine if intrauterine injection of *E. coli* alters postural and motor development in this rodent model of intrauterine infection, and if any changes are accompanied by increased cytokine production in the brain.

MATERIALS AND METHODS

Animal model

Twelve timed-pregnant Sprague Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN) were obtained at gestational day (GD) 13. Food and water were available *ad libitum* and the colony was maintained in a 12:12 h light/dark schedule. On GD 17 dams were randomly assigned to either saline (n=6), or *E. coli* (ATCC #25922, Manassas, VA) (n=6). Animals were anesthetized and inoculated with 100 µl of sterile saline or 100 µl of 1×10^5 colony forming units of *E. coli* at the bifurcation of the uterine horns (Pang et al., 2005; Rodts-Palenik et al., 2004; Wallace et al., 2010a; Wallace et al., 2010b). Dams were maintained on a standard maintenance 8640 Teklad 22/5 rodent diet from Harlan for the length of the study. All animals were observed daily and allowed to deliver without any additional experimental manipulation and experiments were carried out with the approval of the institutional animal care and use committee of the University of MS Medical Center.

Behavioral testing

After delivery, rat pups (*E .coli* = 30; control = 30) were weighed and remained with dams for the length of the study. Locomotor testing (Altman and Sudarshan, 1975; Clarac et al., 2004) began at postnatal day (PND) PND 2 and ended at PND 12. Throughout this time rat pups were separated from dams for a period not exceeding one hour a day. All testing was done between 08:00 and 11:00 h in a room specialized for behavioral testing.

Locomotor testing was analyzed under the following parameters: (1) Crawling was indicated by paddling movements of the paws which results in the pup dragging or pulling itself. (2) Walking was defined as an advanced form of crawling in which the pup can move forward or backward without its' pelvis touching the floor. (3) Pivoting was indicated by the pup making broad swipes with the paws, producing a paddling motion which results in a turn. (4) Turning was defined as an advanced form of pivoting in which the pup can turn without its' pelvis touching the floor. (5) Head raise was indicated by the pup raising its head in a vertical motion with the nose pointing up. (6) Grooming was indicated by the pup licking its' paws or pawing its head.

For locomotor testing animals were placed individually in a clear cage and their movements were video-taped for 5 min. All cages were kept under an infrared light to insure that the animals would not lose any body heat while separated from their litter mates. Video tapes were viewed after testing, using a time-sampling method in which every 10 s the pups' activity was scored, for a total of 300 sc. Tape raters were blind to treatment and inter-rater reliability was r^2=0.91.

Homogenate preparation and ELISA

At PND 16 pups were sacrificed and the brains were extracted for cytokine analysis. The frontal cortex, striatum and cerebellum, were dissected, weighed and fast-froze with dry ice and stored at -80°C. To prepare tissue for biochemical analysis, tissue was crushed under liquid nitrogen using a mortar and pestle, followed by homogenization in ice-cold phosphate buffered saline containing a commercial mix of protease inhibitors (Roche Diagnostics, Indianapolis, IN). The homogenate was centrifuged at 4°C for 10 minutes at 1600 RPM. The supernatant was extracted and used for ELISA testing. Commercially available ELISA kits for tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and interleukin 1β (IL-1β) (RnD Systems, Minneapolis, MN) were used to analyze the amount of cytokine expression for each region of interest and was carried out according to manufacturer's instructions. The immunoassay intra-assay coefficient of variation was less than 5% and the detection limits were 5 pg/ml, <5 pg/ml and 21 pg/ml, respectively. A commercially available BCA kit for total protein concentration (Pierce Chemical, Rockford, IL) was used to determine the total amount of protein in each sample. The total protein concentration was normalized for each brain region to the corresponding cytokine value.

Statistical analysis

Behavioral data was analyzed by RM-ANOVA using a general linear model created in SPSS version 14.0 for PC. Postnatal day was the repeated measure and prenatal treatment a fixed factor. Post-hoc analysis was done using Bonferroni method of analysis. To determine if intrauterine *E. coli* injection altered the appearance of any of the above behaviors (crawling, walking, etc.) Fisher's exact test using a two-tailed *p* value was used. Cytokine protein production in specific brain regions as measured by ELISAs were tested by ANOVA. Post-hoc analysis was performed by Bonferroni correction. P values < 0.05 were considered significant.

RESULTS

General newborn characteristics

All dams delivered between GD 21 to 22 and there were no statistically significant differences between survival rates in the *E .coli* group versus control (p = 0.087) or in birth weights (p=0.149) between the two groups. Surrogate dams were not used in this study, but dams in the saline groups had 1 to 2 pups taken away per litter to

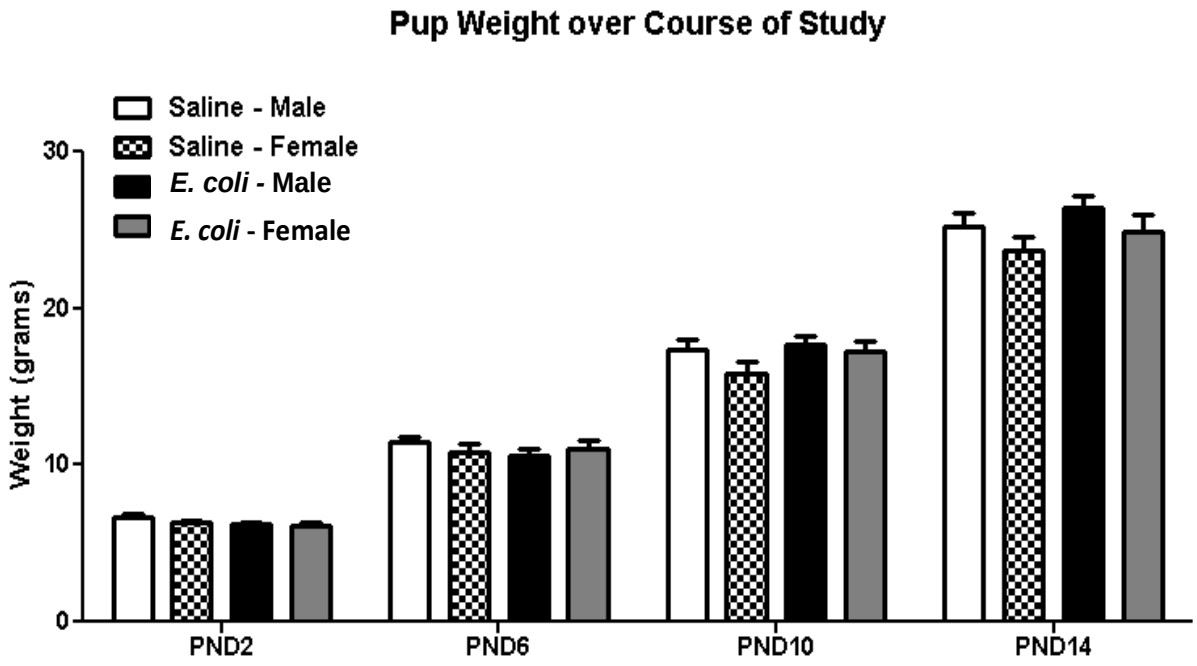

Figure 1. The effects of intrauterine infection on postnatal weight gain in pups from PNDs 2 to 14. There were no statistically significant (p=0.45) differences in postnatal weight gain between males and females within and between the groups. Data is represented as average weight (grams) ± S.E.M. recorded every 4 days. At each time point: saline males (n=16), saline females (n=14), *E. coli* males (n=13), *E. coli* females (n=17).

equalize litter sizes among treatment groups. There were no significant differences in pup weights (p = 0.45) among males or females within and between the treatment groups during the study (Figure 1).

Intrauterine infection leads to hyperactivity

Intrauterine infection did not have a gender specific effect on the development of locomotion, so all pups are reported together (saline males [n=16], saline females [n=14], *E. coli* males [n=13], *E. coli* females [n=17]; data not shown).

Intrauterine infection significantly reduced the total incidence of crawling (p = 0.05) and pivoting (p = 0.05; Figure 2A and B) while significantly increasing the incidence of walking (p = 0.05) and turning (p = 0.05) (Figure 2C and D) as compared to rat pups in the control group.

There was no significant difference in the incidence of head raises (p = 0.319) or grooming behavior (p = 0.497; data not shown) between the two groups.

There were no significant differences in the appearance of any locomotor behaviors between the two groups (Table 1).

To determine if the overall activity level of pups changed due to intrauterine infection, the total time spent moving was calculated. Intrauterine infection, significantly decreased the time spent active as compared to pups in the saline group on PND 2 (p = 0.05; Figure 3), while causing hyperactivity between PNDs 6, 9 to 11 as compared to pups in the saline group (p < 0.01 ; Figure 3).

Intrauterine infection leads to region specific increases in cytokine expression

As endotoxin administration increases the immune response, we set out to determine if fetal immune activation still persisted after the *in utero* insult. Intrauterine infection significantly increased IL-1β (15.86 ± 1.34 versus 7.26 ± 1.4 pg/ml; p = 0.001), TNF-α (40.94 ± 3.81 versus 17.38 ± 3.18 pg/ml; p <0.001) and IL-6 (11.98 ± 2.27 versus 4.75 ± 1.15 pg/ml; p = 0.008) in the frontal cortex at PND 16 (Figure 4A) as compared to the saline group.

In the striatum intrauterine infection significantly increased IL-1β (6.48 ± 1.09 versus 3.36 ± 0.65 pg/ml; p = 0.021), TNF-α (10.18 ± 2.07 versus 3.62 ± 1.51 pg/ml; p = 0.016) and IL-6 (4.27 ± 0.19 versus 1.54 ± 0.63 pg/ml; p = 0.001) as compared to the saline group (Figure 4B). Intrauterine infection also significantly increased IL-1β (16.51 ± 2.56 versus 7.8 ± 1.19 pg/ml; p = 0.005), TNF-α (33.07 ± 3.95 versus 21.51 ± 1.34 pg/ml; p = 0.010) and IL-6 (10.14 ± 0.94 versus 3.31 ± 0.54 pg/ml; p < 0.001) in the cerebellum as compared to the saline group (Figure 4C).

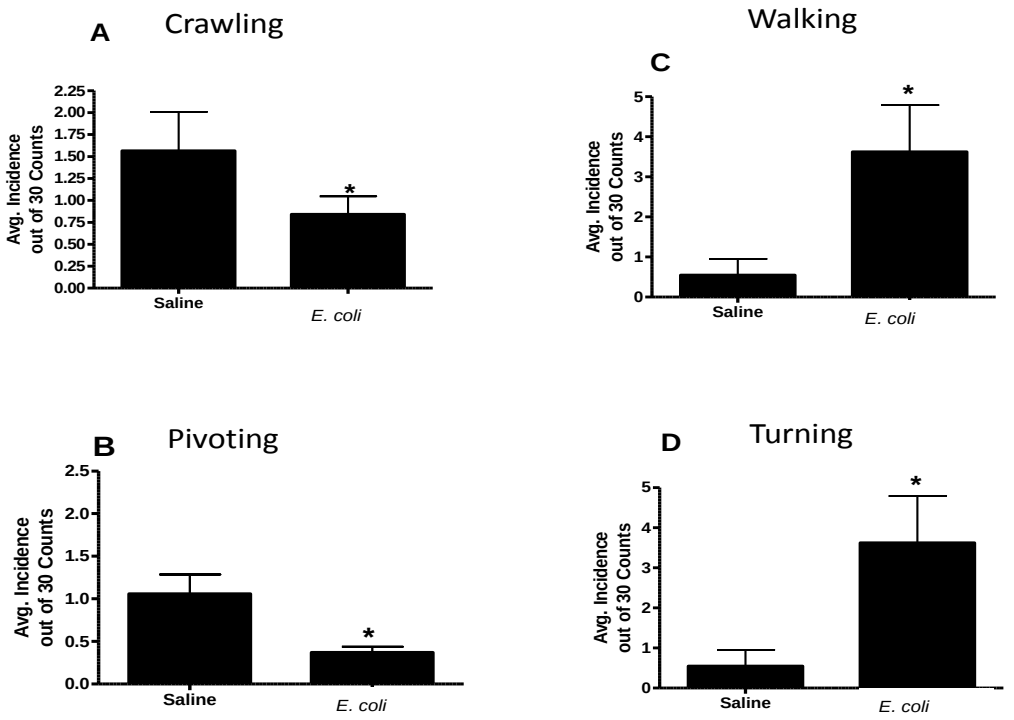

Figure 2. The effects of intrauterine infection on the development of locomotion in pups from PNDs 2 to12. Pups from the *E. coli* group demonstrated a statistically significant reduced incidence of crawling and pivoting and a statistically significant increased incidence of walking and turning as compared to the control group. These data represent an average of each form of locomotion over the indicated days. Each pup spent 5 min in a clear cage, in which their activity was recorded and scored at a later date. Every 10 s the action of locomotion was recorded, for a total recording of 30 incidents of movement. [A]Crawling, [B]Pivoting, [C]Walking, [D]Turning. Data is represented as average incidence of a behavior + S.E.M. There was no statistically significant treatment by postnatal day effect so the data was averaged for each day per treatment was analyzed using a student's *t*-test, and the data graphed as indicated in this figure. *Significantly different from corresponding saline group as indicated by two-way RM-ANOVA and post-hoc Bonferroni, corrected *t*-tests ($p < 0.01$). At each time point, saline n = 30 and *E. coli* n = 30.

Table 1. Appearance of basic locomotor skills in the development of walking.

Parameter	Saline n = 30 mean start date (range)	*E. coli* n = 3 mean start date (range)	P value
crawling	PND2 (2-12)	PND3 (3-12)	0.067
Walking	PND9 (9-12)	PND10 (9-12)	1.00
Pivoting	PND3 (2-12)	PND3 (2-12)	1.40
Turning	PND8 (8-12)	PND8 (7-12)	1.00
Head raise	PND2 (2-12)	PND2 (2-12)	1.40
Grooming	PND10 (10-12)	PND10 (10-12)	1.24

DISCUSSION

It is now well recognized that intrauterine infection can potentially lead to neurodevelopmental and neurological disorders through activation of the fetal immune system (Bell and Hallenbeck, 2002; Bell et al., 2004; Chew et al., 2006; Yoon et al., 2003). We have previously shown that *E. coli* induced intrauterine infection leads to white matter damage, astrogliosis, and increased IL-1β expression in the hippocampus and cerebellum (Rodts-Palenik et al., 2004; Wallace et al., 2010a; Wallace et al., 2010b). Furthermore, rat pups exposed *in utero* to *E. coli* have long-term sensorimotor, motor and cognitive deficits compared to rat pups from non-infected dams (Wallace,

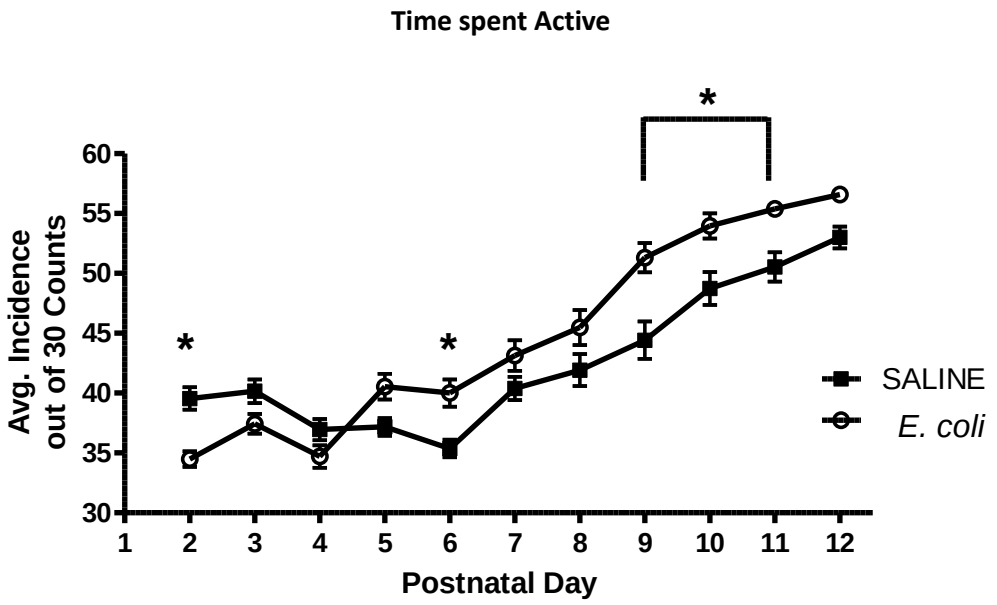

Figure 3. Intrauterine infection increases the total time spent active. *E. coli* induced-intrauterine infection increased the time spent active after PND 5, indicating that these pups were more hyperactive in comparison to pups in the saline group. Each pup spent 5 min in a clear cage from PN 2 to 12, in which their activity was recorded and scored at a later date. Every 10 s the mode of locomotion was recorded, for a total recording of 30 incidents of movement. The overall activity was assessed as the time spent motionless. Data is represented as average incidence of a motionless score + S.E.M. Significance by two way RM-ANOVA was set to $p < 0.05$. Post-hoc analysis with Bonferroni, corrected t-tests set to $p < 0.01$. *denotes days in which *E. coli* spent significantly less time motionless as compared to pups in the saline group. # denotes days in which *E.coli* spent significantly more time motionless as compared to pups in the saline group. At each time point, saline n = 30 and *E.coli* n = 30.

et al., 2010a; Wallace et al., 2010b). In the current study we demonstrate that intrauterine infection has a regional effect on cytokine activation which corresponds to increased motor activity.

The final output of locomotion depends on the development of the central nervous system and the muscular-skeletal framework. This phase of development starts during the embryological life and continues after birth (Kudo et al., 1991). In rodents there is a very precise onset of locomotive behaviors, thus making it easy to determine if prenatal or early age insults affect the onset and development of locomotor behaviors. Rat pups in the intrauterine infection group, had increased incidences of walking and turning over the observed periods of movement, and decreased incidences of crawling and pivoting. This corresponds to the overall increase in total activity seen after PND 6 which is indicative of an increase in motor activity. The significant increase in motor activity seen in this model implies that intrauterine infection does affect basic neural circuitry but not necessarily the ability to develop locomotion. Increases in pivoting are correlated with decreased striatal dopamine levels. Other investigators have found that endotoxin or pesticide administration elicits an inflammatory response in the nigrostriatal pathway capable of inducing behavioral changes (Choi et al., 2009; Lazarini et al., 2001). Furthermore, *in vivo* studies have shown that dopamine neurons and nigrostriatal development is compromised when the developing striatum was exposed to lipopolysaccharide (Snyder-Keller and Stark, 2008), suggesting that inflammation may indeed compromise motor behavior and may prove to be an important area of investigation in this model.

To determine if the changes in motor activity and development were accompanied by brain inflammation, we examined the frontal cortex, striatum and cerebellum for evidence of cytokine activation. *E. coli* injection significantly increased IL-6, IL-1β and TNF-α in all areas examined which is supported by several studies which have seen an inflammatory response due to endotoxin administration in the prenatal or early postnatal period (Bell and Hallenbeck, 2002; Bilbo et al., 2005; Cai et al., 2000). The frontal cortex is associated with the integration of voluntary motor activities, while the cerebellum is responsible for coordination, timing sequence and patterned activity of skilled or learned movements and the striatum helps integrate motor pathways (Adams and Victor, 1989; Kolb 1984; Konczak and Timmann, 2007). Despite the increase in inflame-mation in these regions, *E. coli* exposure did not affect

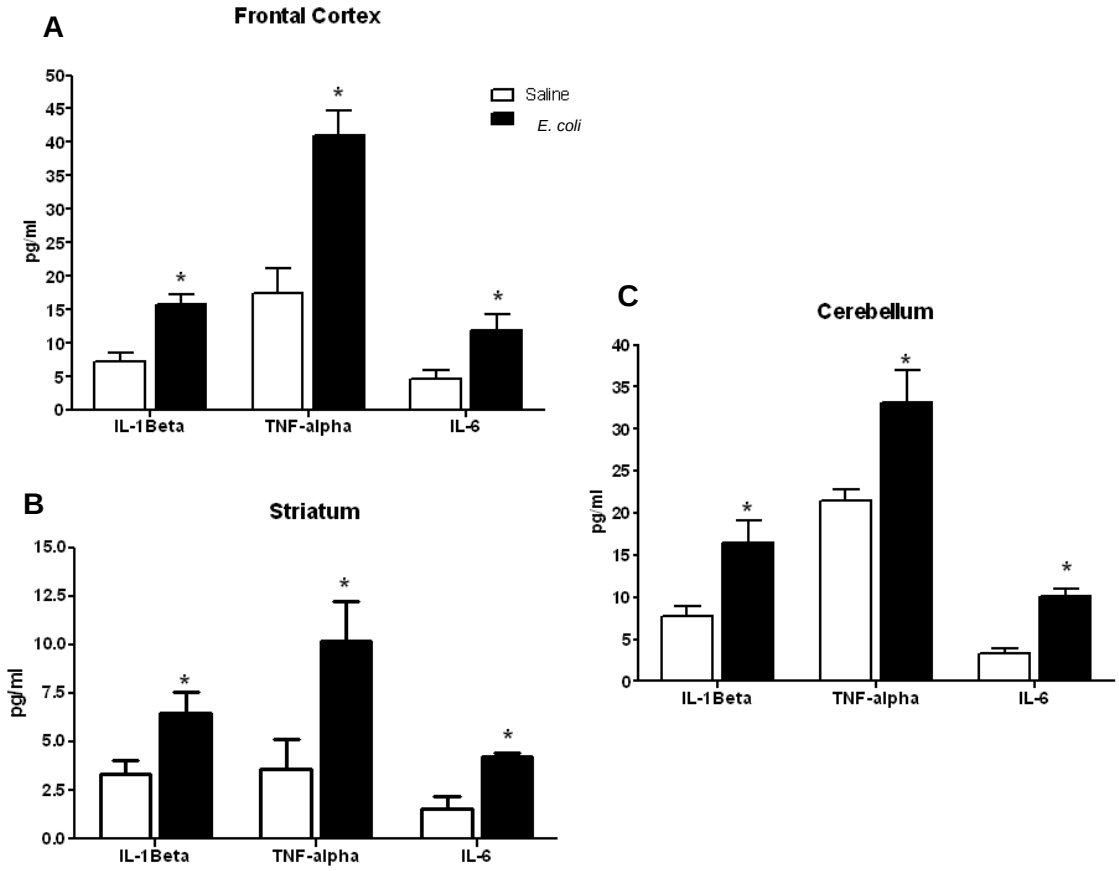

Figure 4. The effects of intrauterine infection on the inflammatory cytokine expression in the brain. IL-1Beta, IL-6 and TNF-alpha were significantly increased due to intrauterine injection of *E.coli* in all brain regions. Data is represented as mean + S.E.M. Data was analyzed using a student's t-test. *p < 0.05 significantly different as compared to corresponding saline group. Saline n = 15 and *E. coli* n = 15.

the pups' coordination or timing sequence of ambulatory skills. This would suggest that inflammation alone is not a causative factor for delays in basic ambulation.

Previous studies investigating the role of inflammatory cytokines on rodent behavior have shown that increased mRNA, plasma and neuronal levels of IL-6 are associated with animal models of autism and maternal immune infection (Pang et al., 2006; Parker-Athill and Tan, 2010; Wei et al., 2011). IL-6 plays a role in normal brain development, learning and memory through activation of the STAT (signal transducers and activators of transcription) pathway (Bauer et al., 2007; He et al., 2005). Through mechanisms that are not well understood, IL-6 can trigger the inflammatory cascade and lead to neuronal degeneration and lymphocyte migration thus leading to the development of behavioral abnormalities. IL-1β is capable of activating the hypothalamic-pituitary-adrenal axis and also works through activation of the STAT pathway. IL-1β is similar to IL-6 in that it also has a role in the normal development of the CNS, but can have long-term effects on developmental skills and cognition when activated above physiological levels (Giulian et al., 1988; Wallace et al., 2010a). TNF-α mediates and increases inflammatory cytokine activity similar to IL-1β. Several studies have also shown an immediate increase in TNF-α following intracerebral injections of lipopolysaccharide (LPS) (Cai et al., 2003; Gatti and Bartfai, 1993; Siren et al., 1992), which indicates that LPS is capable of increasing TNF-α production in the brain in response to either direct or indirect endotoxin exposure.

While there is converging evidence that there is a relationship between endotoxin infections during pregnancy and alterations in offspring behavior, the exact physiological mechanism linking the immune response to behavior is still unclear. Proinflammatory cytokines such as TNF-α, IL-1β and IL-6 act in a cascade-like fashion to induce each other and potentially affect the fetal brain when activated *in utero* (Dammann and Leviton, 1998; Luheshi, 1998), it is probable that the increase in inflammatory cytokines in the perinatal brain, is not the only factor leading to the altered behaviors in the offspring.

The peripheral immune system may also be activated, leading to further increases and activation of the inflammatory cascade. However, if this is possible it

should be noted that the increase in inflammatory cytokines did not lead to sickness behavior, which is characteristic of high levels of inflammatory cytokines.

Elevations in inflammatory cytokines in rodents can lead to a set of behaviors, primarily weight loss and lethargy, termed sickness behavior (Konsman et al., 2002). In this study we did not use surrogate dams or monitor mothers for sickness behavior, but used the ability of the pup to gain weight as a main index of health (Jen et al., 1978). Although pup weight in the *E. coli* group was not significantly less than pups in the saline group, it is possible that there were some effects of sickness behavior among the dams (Figure 1). However due to the hyperactivity seen in the *E. coli* group it would appear as if these animals did not experience any malnourishment.

E. coli did not delay the development of walking but instead increased the activity of walking and turning. The lack of impairments in the development of locomotion as determined by postural assessments indicates that intrauterine infection did not appear to delay or impair any of the neuromuscular networks needed to have successful locomotion (Clarac et al., 2004). This is despite increasing cortical, striatal and cerebellar levels of inflammatory cytokines. Our group and others have shown that endotoxin and cytokine administration does impair cognitive and sensorimotor tasks, which suggest that more complex forms of behavior may be more sensitive to inflammation (Pang et al., 2006; Toso et al., 2005; Wallace et al., 2010a; Wallace et al., 2010b). Further studies are needed to fully elucidate the mechanism(s) by which neurochemical alterations and neuroinflammation may result in behavioral deficits.

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REFERENCES

Altman J, Sudarshan K (1975). Postnatal development of locomotion in the laboratory rat. Anim. Behav., 23: 869-920.

Bauer S (2007). The neuropoietic cytokine family in development, plasticity, disease and injury. Nat. Rev. Neurosci., 8: 221-232.

Bell M, Hallenbeck J (2002). Effects of Intrauterine inflammation on developing rat brain. J. Neurosci. Res., 70: 570-579.

Bell M (2004). Determining the fetal inflammatory response in an experimental model of intrauterine inflammation in rats. Pediatr. Res., 56: 541-546.

Bilbo S (2005). Neonatal infection induces memory impairments follwoing an immune challenge in adulthood. Behav. Neurosci., 119: 293-301.

Cai Z (2000). Cytokine induction in fetal rat brains and brain injury in neonatal rats after maternal lipopolysaccharide administration. Ped. Res., 47: 64-72.

Cai Z (2003). Differential roles of tumor necrosis factor-alpha and interleukin-1beta in lipopolysaccharide-induced brain injury in the neonatal rat. Brain Res., 975: 37-47.

Chew L (2006). Microglia and inflammation: impact on development and brain injuries. Mental Retardation. Dev. Disabil., 12: 105-112.

Choi D (2009). Striatal Neuroinflammation Promotes Parkinsonism in Rats. PLos ONE,. 4: 1-11.

Clarac F (2004). The maturation of locomotor networks. Progress in Brain Res., 143: 57-66.

Dammann O (2002). Perinatal Infection, fetal inflammatory response, white matter damage and cognitive limitations in children born preterm. Mental Retard. Dev. Disabil., 8: 46-50.

Dammann O, Leviton A (1998). Infection remote from the brain, neonatal white matter damage, and cerebral palsy in the preterm infant. Semin. Pediatr. Neurol., 5: 190-201.

Debillon T (2000). Intrauterine infection induces programmed cell death in rabbit periventricular white matter. Pediatr. Res., 47: 736-742.

Gatti S, Bartfai T (1993). Induction of tumor necrosis factor-alpha mRNA in the brain after peripheral endotoxin treatment: comparison with interleukin-1 family and interleukin-6. Brain Res., 624: 291-294.

Giulian D (1988). Interleukin-1 is an astroglial growth factor in the developing brain. J. Neurosci., 8: 709-714.

Guiral E (2011). Prevalence of Escherichia coli among samples collected from the genital tract in pregnant and nonpregnant women: relationship with virulence. FEMS Microbiol. Lett., 314: 170-173.

He F (2005). A positive autoregulatory loop of Jak-STAT signaling controls the onset of astrogliogenesis. Nat. Neurosci., 8: 616-625.

Jen K (1978). Effects of undernutrition and litter size on material variables and pup development. Dev. Psychobiol., 11: 279-287.

Kadhim H (2001). Inflammatory cytokines in the pathogenesis of periventricular leukomalacia. Neurology, 56: 1278-1284.

Kolb B (1984). Functions of the frontal cortex of the rat: A comparative review. Brain Res., 320: 65-98.

Konczak J, Timmann D (2007). The effect of damage to the cerebellum on sensorimotor and cognitive function in children and adolescents. Neurosci. Behav. Rev., doi:10.1016/jneubiorev.04.014.

Konsman J (2002). Cytokine-induced sickness behavior: mechanisms and implications. Trends Neurosci., 25: 154-159.

Kuhn P (2010). Incidence and distribution of pathogens in early-onset neonatal sepsis in the era of antenatal antibiotics. Paediatr. Perinatal. Epidemiol., 24: 479-487.

Lazarini C (2001). Effects of prenatal exposure to deltamethrin on forced swimming behavior, motor activity, and striatal dopamine levels in male and female rats. Neurotoxicol. Teratol., 23: 665-673.

Luheshi G (1998). Cytokines and fever: Mechansims and sites of action. Ann. Acad. Sci., 856: 83-89.

Mittendorf R (2001). The association of coagulase-negative staphylococci isolated from the chorioamnion at delivery and subsequent development of cerebral palsy. J. Perinatol., 21: 3-8.

Nelson K, Willoughby R (2000). Infection, inflammation and the risk of cerebral palsy. Curr. Opin. Neurol., 13: 133-139.

Pang Y (2006). Role of interleukin-6 in lipopolysaccharide-induced brain injury and behavioral dysfunction in neonatal rats. Neuroscience, 141: 745-755.

Pang Y (2005). Suppression of glial activation is involved in the protection of IL-10 on maternal *E. coli* induced neonatal white matter injury. Dev. Brain Res., 157: 141-149.

Parker-Athill E, Tan J (2010). Maternal immune activation and autism spectrum disorder: interleukin-6 signaling as a key mechanistic pathway. Neurosignals, 18: 113-128.

Poggi S (2005). No phenotype associated with established lipopolysaccharide model for cerebral palsy. Am. J. Obstet. Gynecol., 192: 727-733.

Rafal'skii V (2009). Present-day beta-lactame antibiotics in the treatment of urinary infections in pregnant women. Urologiia, 5: 14-18.

Rodts-Palenik S (2004). Maternal infection-induced white matter injury is reduced by treatment with interleukin-10. Am. J. Obstetr. Gynecol., 191: 1387-1392.

Schendel D (2001). Infection in pregnancy and cerebral palsy. J. Am. Med. Associ., 56: 105-108.

Siren A (1992). Release of proinflammatory and prothrombotic mediators in the brain and peripheral circulation in spontaneously hypertensive and normotensive Wistar-Kyoto rats. Stroke, 23: 1643-1651.

Snyder-Keller A, Stark P (2008). Prenatal inflammatory effects on nigrostriatal development in organotypic cultures. Brain Res., 1233: 160-167.
Toso L (2005). Inflammatory-mediated model of cerebral palsy with developmental sequelae. Am. J. Obstetr. Gynecol., 193: 933-941.
Urakubo A (2001). Prenatal exposure to maternal infection alters cytokine expression in the placenta, amniotic fluid, and fetal brain. Schizophrenia Res., 47: 27-36.
Vigneswaran R (2004). Cerebral palsy and placental infection: a case-cohort study. BMC Pregnancy Childbirth, 27: 1.
Wallace K (2010a). Interleukin-10/Cefriaxone prevents E.coli-induced delays in sensorimotor task learning and spatial memory in neonatal and adult Sprague Dawley rats. Brain Res. Bull., 81: 141-148.
Wallace K (2010b). Prenatal infection decreases calbindin, decreases Purkinje cell volume and density and produced long-term motor deficits in Sprague-Dawley rats. Dev. Neurosci., 32: 302-312.
Wei H (2011). IL-6 is increased in the cerebellum of the autistic brain and alters neural cell adhesion, migration and synapse formation. Journal of Neuroinflammation. 19: 1.
Wu Y, Colford J (2000). Chorioamnionitis as a risk factor for cerebral palsy. JAMA, 284: 1417-1424.
Yoon B (2003). Intrauterine Infection and the development of cerebral palsy. British. J. Obstetr. Gynecol., 20: 124-127.
Yoon B (2000). Fetal exposure to an intra-amniotic inflammation and the development of cerebral palsy at the age of three years. Am. J. Obstetr. Gynecol., 182: 675-681.