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# Neonatal *Escherichia coli* infection alters glial, cytokine, and neuronal gene expression in response to acute amphetamine in adolescent rats

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ABSTRACT

Neonatal bacterial infection in rats alters the responses to a variety of subsequent challenges later in life. Here we explored the effects of neonatal bacterial infection on a subsequent drug challenge during adolescence, using administration of the psychostimulant amphetamine. Male rat pups were injected on postnatal day 4 (P4) with live *Escherichia coli* (*E. coli*) or PBS vehicle, and then received amphetamine (15 mg/kg) or saline on P40. Quantitative RT-PCR was performed on micropunches taken from medial prefrontal cortex, nucleus accumbens, and the CA1 subfield of the hippocampus. mRNA for glial and neuronal activation markers as well as pro-inflammatory and anti-inflammatory cytokines were assessed. Amphetamine produced brain region specific increases in many of these genes in PBS controls, while these effects were blunted or absent in neonatal *E. coli* treated rats. In contrast to the potentiating effect of neonatal *E. coli* on glial and cytokine responses to an immune challenge previously observed, neonatal *E. coli* infection attenuates glial and cytokine responses to an amphetamine challenge.



Early life events, including exposure to physical and psychological stressors, can have long-lasting effects on an individual's response to challenges later in life. The early postnatal period in the rat corresponds roughly to the third trimester of prenatal development in the human, and complications including infections can affect up to one-third of pregnancies [11]. Previous work has demonstrated that in adult rats that had experienced neonatal infection on postnatal day 4 (P4) with the bacterium *Escherichia coli*, an immune challenge with lipopolysaccharide (LPS) produced enhanced glial and pro-inflammatory cytokine responses in the hippocampus and plasma [3]. In contrast, adult rats treated on P4 with the same dose of *E. coli* had attenuated responses to psychological stressors, including reductions in stress-induced plasma corticosterone levels and depressive-like behavior [5]. Thus, neonatal bacterial infection can confer either vulnerability to, or protection from, a later life challenge. However, it is unknown what effects neonatal bacterial infection might have on the glial and neuroimmune changes produced by drugs of abuse.

Work in our laboratory [12] and others [20] has demonstrated that abused drugs can produce numerous effects on non-neuronal cell types including astrocytes and microglia, and that glia can modulate drug action [7]. Amphetamines, including D-amphetamine [27], are particularly potent activators of microglia in both mice [27] and humans [25]. Thus we explored the effects of neonatal *E. coli* infection on a subsequent D-amphetamine challenge during adolescence. We chose adolescence because this is the developmental period in which many recreational drug users are first exposed to drugs [24]. Quantitative RT-PCR was performed on tissue from three regions affected by drugs of abuse: the medial prefrontal cortex (mPFC), nucleus accumbens (NAcc), and CA1 subfield of the hippocampus. Previous work in our laboratory has revealed changes in morphine-induced astrocytic and microglial activation in these regions [13]. We tested the hypothesis that neonatal infection would increase the expression of amphetamine-induced glial activation markers and pro-inflammatory cytokines, as was the case after LPS challenge [5]. Real-time RT-PCR was performed to detect mRNA for the microglial membrane protein CD11b, the astroglial marker glial fibrillary acidic protein (GFAP), the pro-inflammatory cytokines interleukin (IL) IL-1β, IL-6, and tumor necrosis factor alpha (TNF-α), the anti-inflammatory cytokine IL-10, the anti-inflammatory neuroimmune regulatory molecule CD200 and, as well as the effector immediate early gene activity-regulated-cytoskeleton-associated protein (Arc), which is primarily neuronal.

Pups were derived from Sprague–Dawley rats obtained from Harlan (Indianapolis, IN) using previously published procedures [2–6]. The colony was maintained at 22 °C on a 12:12 h light:dark cycle with food and water freely available. All experiments were

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**Table 1**
Gene expression (relative to GAPDH) in adolescent rats treated neonatally with *Escherichia coli* or PBS vehicle and during adolescence with saline vehicle.

| Gene | Prefrontal cortex | | Nucleus accumbens | | Hippocampus | |
|---|---|---|---|---|---|---|
| | PBS | *E. coli* | PBS | *E. coli* | PBS | *E. coli* |
| Arc | 2.38 ± 0.45 | 3.06 ± 0.60 | 2.12 ± 0.35 | 1.76 ± 0.28 | 3.69 ± 0.59 | 6.20 ± 1.13 |
| CD11b | 3.26 ± 0.41 | 2.74 ± 0.52 | 3.39 ± 0.49 | 3.94 ± 0.92 | 2.70 ± 0.54 | 3.01 ± 0.62 |
| CD200 | 1.41 ± 0.15 | 2.04 ± 0.35 | 3.51 ± 0.39 | 5.58 ± 0.35** | 6.73 ± 3.06 | 6.75 ± 3.17 |
| GFAP | 2.70 ± 0.50 | 3.64 ± 0.86 | 3.27 ± 0.46 | 3.83 ± 0.36 | 8.55 ± 1.35 | 13.67 ± 2.37 |
| IL-1β | 2.63 ± 0.46 | 9.95 ± 3.91 | 2.06 ± 0.39 | 2.29 ± 0.65 | 7.33 ± 1.56 | 12.10 ± 3.68 |
| IL-6 | 1.63 ± 0.24 | 1.85 ± 0.30 | 3.66 ± 0.60 | 3.75 ± 0.59 | 3.94 ± 0.85 | 6.67 ± 2.72 |
| IL-10 | 4.02 ± 1.14 | 3.61 ± 1.33 | 8.19 ± 2.88 | 9.89 ± 6.26 | 12.71 ± 3.95 | 24.77 ± 7.27 |
| TNF-α | 3.48 ± 0.56 | 5.15 ± 0.91 | 4.03 ± 0.87 | 3.58 ± 0.40 | 3.11 ± 0.53 | 6.20 ± 1.23* |

Values are mean ± S.E.M. of 6–8 rats.
* $p < .05$, *E. coli* different from PBS control.
** $p < .01$, *E. coli* different from PBS control.

conducted with protocols approved by the University of Colorado Animal Care and Use Committee. Litters were culled on P4 to two females and up to eight males. Because these experiments build on phenomena that have only been tested in males [2–6] only males pups were used.

*E. coli* culture (ATCC 15746; American Type Culture Collection) vial contents were hydrated and grown overnight in 30 ml of brain–heart infusion (Difco Labs) at 37 °C and processed as previously reported [2–6].

Pups were injected subcutaneously (30G needle) on P4 with either $0.1 \times 10^6$ colony forming units (CFU) of live bacterial *E. coli* per gram body weight suspended in 0.1 ml PBS, or 0.1 ml PBS alone. All pups were removed from the mother at the same time and placed into a clean cage with bedding, injected individually, and returned to the mother as a group. Elapsed time away from the mother was less than 5 min. All pups from a single litter received the same treatment due to concerns over possible cross-contamination from *E. coli*. Injections were given between 10:00 and 10:30 h. Pups were weaned on P21 into sibling pairs and remained undisturbed until P40. To control for possible litter effects, a maximum of two pups/litter were assigned to a single experimental group. On P40, rats received a single injection of D-amphetamine (Sigma, 15 mg/ml/kg) or saline vehicle. We used the D-amphetamine dose of Moskowitz et al. [19] that was shown to produce protein disaggregation adolescent rats but is lower than those that produce frank toxicity [27]. Each rat in a cage received the same drug treatment. Rats were returned to their home cages after the injection, where they remained until killed 2 h later. Real-time RT-PCR was performed using previously published procedures [2,3,10]. cDNA sequences were obtained from GenBank at the National Center for Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov). Primer sequences were designed using an online Oligo Analysis & Plotting Tool (Qiagen) and tested for sequence specificity using the basic local alignment search tool at NCBI. The following primers were used (*gene*, forward (F) and reverse (R) sequence, and GenBank accession number):

*Arc*, F: ACAGAGGATGAGACTGAGGCAC, R: TATTCAGGCTGGGTCCTGTCAC, U19866;
*CD11b*, F: CTGGGAGATGTGAATGGAG, R: ACTGATGCTGGCTACTGATG, NM_012711;
*CD200*, F: TGTTCCGCTGATTGTTGGC, R: ATGGACACATTACGGTTGCC, NM_031518;
*GAPDH*, F: GTTTGTGATGGGTGTGAACC, R: TCTTCTGAGTGGCAGTGATG, M17701;
*GFAP*, F: AGGGACAATCTCACACAGG, R: GACTCAACCTTCCTCTCCA, AF028784;
*IL-1β*, F: GAAGTCAAGACCAAAGTGG, R: TGAAGTCAACTATGTCCCG, M98820;
*IL-6*, F: ACTTCACAGAGGATACCAC, R: GCATCATCGCTGTTCATAC, NM_012589;
*IL-10*, F: TAAGGGTTACTTGGGTTGCC, R: TATCCAGAGGGTCTTCAGC, NM_012854;
*TNF-α*, F: CTTCAAGGGACAAGGCTG, R: GAGGCTGACTTTCTCCTG, D00475.

For each experimental sample, triplicate reactions were conducted using published procedures. Gene expression was determined using the $2^{-\Delta\Delta Ct}$ method [17] relative to the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). No group differences were observed in GAPDH mRNA expression. Because of (mostly nonsignificant) variability in constitutive gene expression between the *E. coli* and PBS saline groups (Table 1) data were normalized to percent of saline control. Data were analyzed using a two-way ANOVA. When significant interactions were found, post-hoc comparisons were made using Student–Neuman–Keuls tests ($\alpha$ set at .05). When significant interactions were not found, we tested our a priori hypothesis using the more conservative Scheffe's tests ($\alpha$ set at .05).

Table 1 shows expression of all the genes assessed. One-way ANOVA revealed that neonatal *E. coli* treated rats had greater CD200 mRNA expression than neonatal PBS controls in the NAcc, $F(1,12) = 15.34$, $p < .01$. Finally, neonatal *E. coli* treated rats had greater TNF-α mRNA expression than neonatal PBS controls in the CA1, $F(1,12) = 4.88$, $p < .05$.

In the mPFC, amphetamine increased the expression of mRNA for IL-1β, IL6, TNF-α, CD200, Arc, and GFAP only in rats treated neonatally with PBS (Fig. 1). Two-way ANOVA revealed several neonatal treatment by adolescent treatment interactions: IL-1β, $F(1,24) = 6.68$, $p < .01$; IL6, $F(1,25) = 4.44$, $p < .05$; TNF-α, $F(1,24) = 4.50$, $p < .05$; and CD200, $F(1,25) = 5.33$, $p < .05$. In each of these cases post-hoc tests indicated that the PBS + amphetamine group had greater mRNA levels than all other groups. There was a near significant neonatal by adolescent treatment interaction for Arc mRNA, $F(1,25) = 3.83$, $p = .06$. A priori tests indicated that the PBS + amphetamine group had greater levels of mRNA than PBS + saline. There was a main effect of adolescent treatment on GFAP, $F(1,26) = 7.16$, $p < .05$; amphetamine increased GFAP mRNA expression. There were no significant main effects or interactions for CD11b or IL-10 (not shown).

In the NAcc, amphetamine increased the expression of mRNA for IL-1β and CD200 in rats treated neonatally with PBS, while decreasing expression of CD200 mRNA in neonatal *E. coli* treated rats (Fig. 2). There was a main effect of adolescent treatment on IL-1β mRNA, $F(1,23) = 12.99$, $p < .01$; a priori tests indicated that the PBS + amphetamine group had greater levels of IL-1β mRNA than PBS + saline. There was a neonatal by adolescent treatment interaction for CD200 mRNA, $F(1,25) = 11.36$, $p < .01$; post-hoc tests indicated that the PBS + amphetamine group had greater CD200 mRNA levels than all other groups and *E. coli* + amphetamine had lower CD200 mRNA levels than *E. coli* + saline. There was a main effect of adolescent treatment $F(1,25) = 5.14$, $p < .05$; amphetamine increased Arc mRNA expression. There were no main effects or interactions on mRNA for IL6 or GFAP (Fig. 2), or on CD11b, IL-10, or TNF-α mRNA expression (not shown).

In the CA1 region of the hippocampus, amphetamine increased the expression of mRNA for Arc and GFAP only in rats treated neonatally with PBS, and increased the expression of IL-1β mRNA overall (Fig. 3). There were neonatal by adolescent treatment interactions for GFAP, $F(1,26) = 6.10$, $p < .05$, and Arc, $F(1,26) = 7.97$, $p < .01$. Post-hoc tests indicated that the PBS + amphetamine group had greater Arc and GFAP mRNA levels than all other groups. There was also a