Environmental enrichment rescues the effects of early life inflammation on markers of synaptic transmission and plasticity

Running Title: Experience rescues the effects of prenatal inflammation

Kentner, Amanda.C[1]; Khoury, Antoine[2]; Queiroz, Erika[2]; MacRae, Molly[1]

[1]School of Arts & Sciences, Health Psychology Program, MCPHS University (formerly the Massachusetts College of Pharmacy & Health Sciences), Boston Massachusetts, United States 02115

[2]School of Pharmacy, MCPHS University, Boston Massachusetts, United States 02115

Please address all correspondence to Amanda Kentner at the above address or at any of the following coordinates: Office #617-274-3360, Fax # 617-732-2959, or via electronic mail – amanda.kentner@mcphs.edu.

Figures: 5
Number of words: Abstract (273), Body (4314).

Author Contributions: A.C.K designed the study and wrote the manuscript, A.K., E.Q., M.M ran the experiments, A.K, E.Q. & A.C.K analyzed the data.

Disclosures and Potential Conflict of Interests: None.

Abstract

Environmental enrichment (EE) has been successful at rescuing the brain from a variety of early-life psychogenic stressors. However, its ability to reverse the adverse behavioral and neural alterations induced by a prenatal maternal infection model of schizophrenia is less clear. Moreover, the specific interactions between the components (i.e. social enhancement, novelty, physical activity) of EE that lead to its success as a supportive intervention have not been adequately identified. In the current study, standard housed female Sprague-Dawley rats were administered either the inflammatory endotoxin lipopolysaccharide (LPS; 100ug/kg) or pyrogen-free saline (equivolume) on gestational day 15. On postnatal day 50, offspring were randomized into one of three conditions: EE (group housed in a large multi-level cage with novel toys, tubes and ramps), Colony Nesting (CN; socially-housed in a larger style cage), or Standard Care (SC; pair-housed in standard cages). Six weeks later we scored social engagement and performance in the object-in-place task. Afterwards hippocampus and prefrontal cortex (n = 7-9) were collected and evaluated for excitatory amino acid transporter (EAAT) 1-3, brain-derived neurotrophic factor (BDNF), and tropomyosin receptor kinase B (TrkB) gene expression (normalized to GAPDH) using qPCR methods. Overall, we show that gestational inflammation downregulates genes critical to synaptic transmission and plasticity, which may underlie the pathogenesis of neurodevelopmental disorders such as schizophrenia and autism. Additionally, we observed disruptions in both social engagement and spatial discrimination. Importantly, behavioral and neurophysiological effects were rescued in an experience dependent manner. Given the evidence that schizophrenia and autism may be associated with infection during pregnancy, these data have compelling implications for the prevention and reversibility of the consequences that follow immune activation in early in life.

Key Words: Animal model; environmental enrichment; social housing; experience;

inflammation; prenatal; neurodevelopment; behavior; memory; hippocampus; prefrontal cortex;

BDNF; TrkB; excitatory amino acid transporter

Introduction

Prenatal infection is associated with an elevated risk for neurodevelopmental disorders in human offspring and the appearance of related neurophysiological and behavioral markers in laboratory animals such as rats and mice (reviewed in Boska, 2010). In rodents, neurophysiological markers of inflammation-induced programming include changes in neurotransmitter/receptor level and binding (Baharnoori et al., 2013; Meyer et al., 2008; Winter et al., 2009), oxidative stress (Lanté et al., 2007, 2008), hypothalamic-pituitary-adrenal (HPA) axis dysregulation (Connors et al., 2014) and immune functioning (Borrell et al., 2002); each have been implicated in the pathogenesis of schizophrenia, autism, and/or cerebral palsy (Boska, 2010; Chiappelli et al., 2016; Gu et al., 2015; Smesny et al., 2015; Tonni et al., 2014). Following prenatal inflammation, programming effects are further manifested through behavioral changes such as decreased prepulse inhibition (Fortier et al., 2007), learning impairments and disruptions in social behavior (Connors et al., 2014; Howland et al., 2012;Taylor et al., 2012), all of which are markers of neurodevelopmental brain disorders such as those noted above.

One animal model of prenatal infection is activation of Toll-like receptor (TLR) 4 by administration of the gram negative endotoxin lipopolysaccharide (LPS) during pregnancy. When TLRs are activated they stimulate the synthesis and release of proinflammatory cytokines such as interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α, which are measureable in the maternal circulation following LPS challenge (Ashdown et al., 2006). The release of these mediators result in the production of prostaglandins and additional cytokines in the brain (Maier et al., 1998). Further consequences of this TLR4 activation include acute signs of maternal immune activation (MIA) such as piloerection, lethargy, and reduced feeding and body weight (Connors et al., 2014; French et al., 2013). This transient sickness response during pregnancy is

associated with disruptions in offspring neurodevelopment resulting in altered glutamatergic and GABAergic neuronal systems, and associated behaviors (Baharnoori et al., 2013; Lanté et al., 2007; Lanté et al., 2008; Lowe et al., 2008; Müller & Schwarz, 2006; Romero et al., 2010; Wishhof et al., 2015).

Clinical investigations have shown disrupted inhibitory and excitatory neurotransmitter profiles to be related to the pathogenesis of schizophrenia and autism (Eastwood & Harrison, 2005; Yuan et al., 2015). Sophisticated control of extracellular glutamate concentrations is integral to the prevention of excitotoxicity and neuronal cell death (reviewed by Yi & Hazell, 2006). Five subtypes of glutamate transporters have been identified and are responsible for clearance of glutamate, terminating its action. Of the transporters, excitatory amino acid transporter (EAAT) 1 and 2 are primarily expressed on glial cells, while EAAT3, EAAT4, and EAAT5 are localized to neurons (see Takahashi et al., 2015; Jensen et al., 2015). Notably, EAAT2 expression is significantly decreased in the dorsolateral prefrontal cortex (DLPFC) and parahippocampus of schizophrenic patients (Ohnuma et al., 1998; Ohnuma et al., 2000). Moreover, there is evidence that EAAT2 glycosylation is reduced in schizophrenia subjects versus controls (Bauer et al., 2010). Dysregulation of EAAT1 and EAAT3 have also been reported in schizophrenic as well as autistic patients (Purcell et al., 2001; Rao et al., 2012; Shan et al., 2013; The Autism Genome Project Consortium, 2007). Therefore, in the present study we sought to characterize the expression of the parallel rodent transporter genes in an LPS model of MIA.

Resiliency against early-life adversity can be mediated by experience. For example, environmental enrichment (EE), an enhanced living condition filled with novelty and increased opportunities for social exploration and activity, offers mitigative effects against challenges

encountered during early development (Francis et al., 2002; Kentner, 2015). Following repeated maternal separation, EE housing attenuated the heightened restraint-induced plasma corticosterone response and anxiety-like behavior associated with this early-life stressor (Francis et al., 2002). EE also reversed impairments in social interaction, dysregulated corticosterone secretion, and some indicators of immunosuppression associated with prenatal stress (Laviola et al., 2004; Morley-Fletcher et al., 2003). Moreover, EE is protective against HPA axis and social disruptions following MIA (Connors et al., 2014). Importantly, schizophrenia symptoms typically present in late adolescence or early adulthood (reviewed by Insel, 2010). Moreover, measureable social, cognitive, and physiological disruptions following laboratory-induced MIA are apparent during the adolescent period (Connors et al., 2014; Taylor et al., 2012). Therefore we chose later adolescence as the time to expose our animals to enrichment in order to evaluate its potential to rescue the neurodevelopmental effects of this inflammatory model.

The mechanisms by which EE compensates against the effects of early adversity are a current line of investigation. EE increases brain-derived neurotrophic (BDNF) levels and concomitantly amplifies excitatory action through the formation of glutamatergic synapses, dampening the ratio of inhibitory/excitatory neurotransmitter systems, which has been tied to enhanced synaptic plasticity (see Sale et al., 2014). Notably, prenatal LPS administration reduces BDNF expression throughout the hippocampus (Lin & Wang, 2014; Williamson et al., 2012) as do acute and chronic stressors in this and other limbic structures (Roceri et al., 2004; Hill et al., 2014; Matini & Valverede, 2012). Furthermore, evidence points to lower levels of plasma BDNF in schizophrenic patients (Zhang et al., 2015). Although this neurotrophin does not directly cause disruptions in mood, and associated neuropathology, it has influences on the stress-related circuitry that underlie these vulnerabilities (see Castren et al., 2007). Importantly, elevated

levels of BDNF have been tied to resiliency against early-life stressors, mediated through the serotonergic system (see Homburg et al., 2014). As such, we sought to examine whether a developmental BDNF deficit is present in a MIA rat model and if EE could reverse this effect.

Therefore the purpose of the following investigation was to examine the influence of TLR4 activation on the expression of hippocampal and prefrontal cortex levels of a) EAAT1-3, b) BDNF, and c) associated behavioral and cognitive disruptions in a prenatal LPS model of schizophrenia/autism. Moreover, we sought to determine whether environmental experience (social and/or novel enrichment) could abate these inflammation-induced developmental disruptions.

1. Experimental Procedures

1.1. Animals and gestational treatment

Pregnant Sprague-Dawley rats were obtained from Charles River (Wilmington, MA) and arrived on the morning of gestational day (G)12. Rats were immediately pair-housed in standard cages and maintained at 20°C on a 12 h light/dark cycle (0700-1900 light) with *ad libitum* access to food and water. Experimental procedures were approved by the MCPHS University Institutional Animal Care and Use Committee and were carried out in compliance with the Association for Assessment and Accreditation of Laboratory Animal Care. A flowchart of the study procedures is located in Figure 1.

On G15 (between 9:00-10:00 hrs) pairs were separated into clean cages. Immediately following an initial baseline 'sickness' evaluation of all animals, dams were given an i.p. injection of either LPS (n=12; Escherichia coli, serotype 026:B6; L-3755, Sigma, St. Louis, MO; 100 ug/kg) in pyrogen-free saline, or an equivalent volume of pyrogen-free saline (n=12)

between 11:30-12:30 hrs. Gestational day 15 was chosen as the time of inflammatory challenge since previous investigations have shown MIA on this day to elicit long term changes in offspring behavior (Howland et al., 2012; Oskviq et al., 2012) specific to our cognitive and social measures of interest. To ensure the sickness-inducing effects of gestational LPS, sickness indicators were assessed by blinded observers as described previously (Connors et al., 2014). Dams were evaluated on measures of ptosis (droopy eyelids), piloerection (ruffled, unkempt coat appearance), and lethargy. Each was scored on a three point scale (none = 0, mild = 1, or severe = 2). Based on the presence and magnitude of these measures, rats were assigned a total composite sickness score at each time point evaluated (see Connors et al., 2014; adapted from Hayley et al., 2002; Kentner et al., 2007). Food and water intake were assessed at 4 and 24 hrs post inflammatory challenge. Differences in 24-hr body weight gain were also recorded.

On postnatal day (P)3 [day of birth = P1] all litters were adjusted to 10 pups with the male-to-female ratio being equal wherever possible. Animals were weaned (P22) into same-sex pair-housed standard cages (i.e. one LPS and one saline treated rat per cage). Given previous work showing early-life inflammation to have a preferential vulnerability in males (Connors et al., 2014; Bilbo et al., 2012), and since the purpose of the current study was to evaluate the role of experience in the reversal of disruptions that follow MIA, we evaluated males only.

On P50 three male rats from each of 16 litters (n= 8 saline; n = 8 LPS) were equally allocated across three conditions: one sibling was randomized into Standard Care (SC; standard cage with tube, chew bone, and Nestlets©; housed 2 per cage), one placed into social enrichment - Communal Nesting (CN; larger style one-level cage with tube, chew bone and Nestlets©; housed 4 per cage), and the third male from each litter was randomized into EE (large multi-level cage with novel toys, tubes, chew bone, Nestlets© and ramps; Critter Nation, Muncie

IN; housed 4 per cage). In order to maintain the novelty of the EE condition toys and tubes were changed twice a week. Please note that during the randomization process, all animals/groups were housed in clean cages with novel cage mates. Two EE rats (one saline and one LPS) became aggressive and were immediately removed from the study. Additional SC animals came from two extra litters . Overall, there was n=9 for SC, n=8 for CN, and an n=7 for EE. Eight SC-housed saline treated rats were retained as stimulus animals for the social interaction test (described below). Extra litters (and siblings) were transferred to other protocols for research and/or teaching purposes.

1.2. Offspring behavior

Six weeks following placement into either SC, CN or EE, rats were habituated to a black test arena (40 cm x 40 cm x 28 cm), across three separate days, for ten minutes daily. Social behavior and spatial memory were evaluated in a counter-balanced manner. Tests were digitally recorded and manually scored using the software ODLog™ 2.0 (http://www.macropodsoftware.com/) by blinded observers.

Social Interaction: Animals were paired with a novel same sex and age SC housed conspecific (n=8) for ten minutes as previously described (MacRae et al., 2015b). Rats were evaluated on the frequency and duration of allogrooming, mounting/crawling, and approaching/following. Based on previous observations showing shorter durations of contact directed towards animals treated with LPS in early-life (Connors et al., 2014; MacRae et al., 2015a, 2015b), composite scores were calculated to determine the total duration of time spent *initiating* versus *receiving* social contact, in addition to total contact time.

Object-in-place: Rats were given five minutes to explore the arena and four novel objects

(one placed in each corner). Rats were returned to the arena following a 1-hour delay at which time objects had been replaced with their identical copies. In this phase the position of two objects switched location. After five minutes of exploration a discrimination ratio was calculated [(total time exploring moved objects – total time exploring permanent objects)/(total time exploring both objects)] as previously described (Connors et al., 2014; Howland et al., 2012).

1.3. Brain collection and analysis

Immediately following the behavioral tests rats were anesthetized with a mixture of Ketamine/Xylazine (40-80 mg/kg, i.p/5-10 mg/kg, i.p) and perfused intracardially with a phosphate buffer solution. Brains were removed, prefrontal cortex and hippocampus dissected, frozen on dry ice, and stored at −75 °C until processing.

1.4. RT-PCR

RNA (n=7-9) was isolated using RNeasy (Qiagen) and resuspended in RNase-free water. Concentration was measured on a spectrophotometer (Synergy™ HT, Biotek) and cDNA transcribed using Roche cDNA synthesis kit with 175 ng/uL of RNA. Changes in excitatory amino acid transporter (EAAT)1 (*Slc1a3*; Rn01402419_g1), EAAT2 (*Slc1a2*; Rn00691548_m1), EAAT3 (*Slc1a1*; Rn00564705_m1), vesicular glutamate transporter (VGLUT)1 (*Slc17a7*; Rn01462431_m1), N-methyl-D-aspartate (NMDA) 2B receptor subtype (*Grin2b*; Rn00680474_m1), brain-derived neurotrophic factor (*Bdnf*; Rn00560868_m1), and neurotrophic tyrosine kinase receptor, type 2 (Trk)B (*Ntrk2*; Rn00820626_m1) were measured using quantitative real-time PCR with Taqman Fast Advanced Mastermix (ThermoFisher

Scientific). Glyceraldehydes-3-phosphate dehydrogenase (*Gapdh*; Rn99999916_s1) was measured for each sample, as an endogenous control. Triplicate samples were prepared in a 96-well plate and analyzed (Applied Biosystems StepOnePlus Real-Time PCR System). The cycle number at threshold (CT value) was used for calculations of relative amount of mRNA molecules by using the standard curve method. Standard curves for each gene of interest were created by serial dilution of the RT products from control samples. Semiquantitative analysis of gene expression was normalized in relation to Gapdh and data presented as mean expression relative to SC-saline treated controls.

1.5. Statistical analysis

To compute sample size 'N' as a function of the power level (80%), an alpha level of 0.05, and our effect size estimates, we utilized an *a priori* power analysis to determine that n=8 was sufficient for most measures evaluated using multi-level ANOVA. Both BDNF and EAAT3 mRNA analyses were underpowered as they required n=13 animals. Composite maternal sickness indicator scores, and food and water intake were evaluated using repeated measures ANOVAs. Maternal body weight gain between G15 and G16 was evaluated by one-way ANOVA. All other treatment group comparisons, except for the object-in-place test, were made using two-way ANOVAs. In the case of the object-in-place test, discrimination ratios for each group were evaluated using one single group t-tests where '0' was used as a comparison value to indicate equal, or chance, performance in exploring the two objects (see Connors et al., 2014; Howland et al., 2012). LSD post hocs, pairwise t-tests, and Bonferroni alpha adjustments were used as appropriate (Howell, 2002).

2. Results

2.1. *Maternal sickness and postnatal body weight*

Repeated two-way ANOVA revealed a significant time by gestational treatment interaction on the sickness indicator variable ($F(8,184)=7.744$, $p=0.0001$; Figure 2A). Observable sickness indicators were not expected until approximately 105 minutes following LPS injection (Connors et al., 2014) so an adjusted alpha criterion of 0.01 was utilized for follow-up tests. Post hoc analyses showed elevated sickness in LPS compared to saline dams 135 ($t(23)=2.833$, $p=0.009$) and 150 ($t(23)=2.991$, $p=0.007$) minutes after challenge (Figure 2A). There were also significant time by gestational treatment interactions on both food ($F(1,23)=15.646$, $p=0.001$) and water ($F(1,23)=6.774$, $p=0.016$) intake. Follow-up tests confirmed that LPS treated dams had larger reductions of food (4 hrs – $t(23)=-2.965$, $p=0.007$; 24 hrs – $t(23)=-4.427$, $p=0.0001$; Figure 2B) and water (24 hrs – $t(23)=-2.399$, $p=0.025$; Figure 2C) intake compared to controls. A significant main effect of gestational treatment was found on body weight gain between G15 and G16 ($F(1,23)=19.912$, $p=0.0001$; Figure 2D); LPS rats gained less weight compared to saline-treated dams. Combined, these maternal data confirm that LPS induced sickness.

With respect to offspring body weights, there were no main effects or interactions for either housing or time ($p>0.05$), however as was expected there was a significant main effect of time ($p<0.0001$) in which all animals gained weight across the course of the study.

2.2. *Social interaction*

There was a significant main effect of gestational treatment ($F(1,42)=4.610$, $p=0.038$; Figure 3A) with LPS animals having lower levels of total time in social contact; this was not rescued by any enrichment housing condition ($p>0.05$; Figure 3A). With respect to the duration of time

spent in initiated contact there was a main effect of housing ($F(2,41)=10.213$, $p=0.0001$; Figure 3B) in that CN ($p=0.003$) and EE ($p=0.0001$) rats initiated significantly longer durations of contact than SC offspring. There was also a main effect of gestational treatment on the duration of time spent receiving contacts ($F(1,42)=11.545$, $p=0.001$; Figure 3C).

2.3. Object-in-place

Saline treated SC ($t(8)=3.371$; $p=0.01$), CN ($t(7)=2.835$;$p=0.025$), and EE ($t(6) = 4.401$; $=0.005$) rats had intact object-in-place memory (Figure 3D). In contrast, both SC ($t(8)=0.359$; $p=0.729$) and EE ($t(6)=2.288$; $p=0.062$) LPS- rats were disrupted on this measure (Figure 3D). However, CN-LPS animals ($t(7)=4.663$; $p=0.002$; Figure 3D) had intact spatial discrimination memory.

2.4.*RT-PCR*

2.4.1. *Glutamatergic Markers*

MIA offspring had lower levels of hippocampal ($F(1,42) =7.457$, $p =0.009$; Figure 4A), but not prefrontal cortex ($p>0.05$; Figure 4B) EAAT3 mRNA compared to saline animals. Evaluation of transcriptional changes in hippocampal EAAT2 (Figure 4C), revealed no effect of either gestational treatment or housing ($p>0.05$; data not shown). However, Two-way ANOVA revealed a gestational treatment by housing interaction in the expression of EAAT2 in the prefrontal cortex ($F(2,41)=4.101$, $p=0.024$; Figure 4D). SC-LPS males had significantly lower levels of prefrontal EAAT2 than saline exposed offspring ($t(16)=-3211$, $p=0.005$;Figure 4D). In contrast there were no significant differences between gestational saline or LPS treated rats housed in either CN or EE conditions ($p>0.05$; Figure 4D). CN-LPS treated rats had significantly

higher levels of prefrontal EAAT2 than SC-LPS males (t(15)=3.369, p=0.004; Figure 4D) whereas EE-LPS rats did not show the same protection from MIA (p>0.05; Figure 4D). There were no effects of either hippocampal or prefrontal cortex related expression of EAAT1, NR2B, or VGLUT (p>0.05; data not shown).

2.4.2. BDNF and TrkB

With respect to BDNF gene expression a two-way ANOVA revealed a significant gestational treatment by housing interaction in hippocampus (F(2,42)=4.213, p=0.021; Figure 5A) but not prefrontal cortex (p>0.05; Figure 5B). Follow-up tests revealed that SC-LPS rats had lower levels of hippocampal BDNF gene compared to saline treated rats (t(16)=-3.212, p=0.005). Notably, both CN (t(15)=2.648, p=0.018) and EE (t(14)=4.836, p=0.0001) MIA animals had higher levels of BDNF mRNA in this region compared to SC-LPS animals (Figure 5A). As a group, EE rats had higher levels of BDNF gene expression compared to SC rats (p=0.009).

ANOVA failed to show significant interactions or main effects in the hippocampus for the TrkB receptor gene (p>0.05; Figure 5C). However, a significant two-way ANOVA demonstrated a gestational treatment by housing effect for TrkB expression in the prefrontal cortex (F(2,41)=4.349, p=0.019; Figure 5D). Again, gene expression was lower in SC animals treated with LPS compared to saline (t(16)=-2.981, p=0.009). Here too, EE housing rescued the effects of gestational inflammation (t(14)=5.109, p=0.001). Employing the Bonferonni alpha adjustment for multiple comparisons, CN-LPS treated rats did not differ in prefrontal TrkB mRNA compared to CN-saline treated controls (t(14)=2.282, p=0.039; Figure 5D). Moreover, in EE rats TrkB expression was higher than in SC animals (p=0.021).

3. Discussion

We show that gestational LPS induces the downregulation of genes critical to synaptic transmission and plasticity, which may underlie the pathogenesis of neurodevelopmental disorders such as schizophrenia and autism (Bauer et al., 2010; Ohnuma et al., 1998; Ohnuma et al., 2000;Zhang et al., 2015). The most novel aspect of this study is our observation that prenatal inflammation reduced EAAT2 gene expression in prefrontal cortex, an effect that was rescued in an experience dependent manner. Additionally, we demonstrate that MIA is associated with a respective downregulation of BDNF and TrkB receptor genes in hippocampus and prefrontal cortex, which are also abated by environmental complexity. Moreover, we show that our prenatal infection model results in both social and spatial discrimination disruptions with only the latter being remediated by experience, in this case by CN. In terms of social behavior, similar to previous findings (Connors et al., 2015; MacRae et al., 2015a; MacRae et al., 2015b), male animals treated with LPS in early-life had reduced durations of contact directed towards them. Preliminary data suggest that this effect may be mediated through changes in olfactory scent cues following early-life inflammation (MacRae et al., 2015a). Although we did not observe EE to rescue the effects of inflammation on social engagement, we have previously shown early-life EE exposure to protect against these disruptions (Connors et al., 2014). Notably, early-life physical activity promotes beneficial changes in gut bacteria (see Milka & Fleshner, 2015) which may suggest a mechanism for EE's protective effects against reduced social contact. Together this work suggests that the benefit of some interventions are dependent on timing of exposure.

Disruption of glutamate signalling is thought to be part of the etiology underlying some neurodevelopmental disorders such as autism and schizophrenia (Chiocchetti et al., 2014 Schwartz et al., 2012). Given its central role in neurotransmission and clinical reports of its

involvement in schizophrenia (Eastwood & Harrison, 2005; Yuan et al., 2015), the glutamatergic transporter system may extend upon the receptor hypofunction hypothesis in brain disease (Bellesi et al., 2009). Previous investigators have measured elevated levels of glutamate in the medial prefrontal and anterior cingulate cortices, among other regions, of never medicated schizophrenia patients (Kegeles et al., 2012 ; reviewed by Poels et al., 2014a; Poels et al., 2014b) and report normal glutamate levels in the medicated state (Kegeles et al., 2012; reviewed by Poels et al., 2014a). To this end we were particularly interested in evaluating the gene expression of EAAT1-3 in our MIA model as these transporters are critically involved in uptake of this transmitter. Overall, we observed a selective downregulation of EAAT2 mRNA in the prefrontal cortex of male offspring. Although EAAT2 is primarily found on astrocytes (see Lauriat and McInnes, 2008; Takahashi et al., 2015; Jensen et al., 2015) it is important to note that splice variants of this transporter have been detectable on neurons throughout the brain (Chen et al., 2002; Lauriat and McInnes, 2007). Regardless, downregulation of EAAT2 does correspond with an elevated level of glutamate as this transporter is responsible for 90% of glutamate uptake (Danbolt, 2001).

Behaviorally, over expression of EAAT2 in the frontal cortex, hippocampus, and striatum leads to impaired prepulse inhibition of the startle reflex in rats, a task used to evaluate information processing which is reduced in schizophrenic patients (Bellesi et al., 2009; Bellesi & Conti, 2010). In a transgenic animal model of Alzheimer's disease, reduced expression of EAAT2 led to a series of cognitive deficits including poorer spatial learning memory in the T-maze (Takahashi et al., 2015). Additionally, prefrontal executive functioning and working memory were inferior in schizophrenic patients carrying the G, versus T, allele of the EAAT2 SNP rs4354668 (−181 T/G). This particular SNP variation is associated with lower EAAT2 and

higher glutamate concentrations (see Spangaro et al., 2012). Indeed, both low expression and glycosolation of EAAT2 have been observed in schizophrenic patients (Bauer et al., 2010; Ohnuma et al., 1998; Ohnuma et al., 2000). With respect to MIA we observed disrupted social engagement and object-in-place discrimination, common impairments in inflammatory models of schizophrenia and autism (Connors et al., 2014; Howland et al., 2012; MacRae et al., 2015; Taylor et al., 2012). Notably, only the disruption on the latter measure followed the pattern of prefrontal EAAT2 mRNA expression. Specifically, SC and EE animals challenged *in utero* with LPS had decreased transporter expression and impaired spatial memory. Moreover, only CN restored prefrontal EAAT2 and rescued object-in-place discrimination, a task mediated in part by the prefrontal cortex (Warburton et al., 2013). Future work will need to further delineate the role of EAAT2 in spatial memory.

EE protocols that offer cognitive benefits against challenges are often confounded by access to running wheels which cannot be separated from other elements of the enriched experience (reviewed by Kentner, 2015). In our inflammatory models a) we have not included running wheels and b) EE exposure has not protected against (Connors et al., 2014; MacRae et al., 2015) or reversed impairments in spatial discrimination. Therefore we considered the possibility that increased levels of physical activity, associated with EE protocols utilizing running wheels, mediate the rescue of cognitive function reported by others (e.g. Huang et al., 2006; Jurgens & Johnson, 2012). However, since CN offered remediation following prenatal LPS it appears that later life interventions, independent of exercise, may induce experience mediated plasticity related to spatial discrimination outcomes.

Although EE housing has the potential to be stressful, obstructing its rehabilitative potential on some measures, in our hands EE has not affected biological indicators of chronic stress, either

basally or in response to a restraint stressor (Connors et al., 2015; MacRae et al., 2015). However, animals had been maintained in complex housing from breeding until the end of the study whereas in the current work rats were placed into EE housing at adolescence. This elicited changes in the established social order as evidenced by the aggression expressed by two rats removed from the study. Moreover, we did not differentiate between dominant and subordinate categories among the remaining animals and it is possible that EE could have been unfavourable for some. Indeed, there is evidence that social disturbances amongst cage mates may negatively affect study outcomes on measures of stress and immunity in mice (McQuaid et al., 2012; McQuaid et al., 2013a; McQuaid et al., 2013b). In contrast to EAAT2 expression and spatial discrimination, EE did rescue the inflammatory induced reduction in BDNF and TrkB mRNA (described below) suggesting that this complex condition was not stressful enough to fully prevent benefit. Therefore it is important to address the components of this rearing condition (i.e. novelty, physical activity, social engagement) and how they may individually, or in concert, counteract or augment impairments following early life adversity.

Clinical evidence suggests a role for EE in the rehabilitation of autism, cerebral palsy, and schizotypical personality behaviors (Raine et al., 2003; Morgan et al., 2015; Woo et al., 2015). Future work must establish the appropriate protocols and mechanisms by which EE exerts its benefits in these neurodevelopmental disorders. One line of interest is the role of BDNF which is increased in the brain following CN and EE (Branchi et al., 2006; Rosi et al., 2006; Williamson et al., 2012). Moreover, its role in synaptic plasticity and resiliency (Sale et al., 2014; Homburg et al., 2014), especially in the context of enrichment, highlight its potential to mediate rehabilitation and neuroprotection . Notably, BDNF increases mRNA expression of GLUR1 via its action with the TrkB receptor, at least acutely (Caldeira et al., 2007). We have previously

demonstrated elevated GLUR1 in the prefrontal cortex of enriched rats (Connors et al., 2015). Combined with the current observation of upregulated TrkB receptor in this region, our work aligns with evidence suggesting that BDNF underlies the effects of EE, regulating the excitatory/inhibitory balance of the brain (see Sale et al., 2014) .

Altogether, our findings corroborate evidence that early-life infection can interfere with normal brain development and impart long-term programming effects on neural systems that underlie disorders such as autism and schizophrenia. Moreover, our data provide compelling implications for the role of the environment in rescuing and promoting resiliency against adversities such as early-life infection.

Acknowledgements

We would like to thank the MCPHS School of Pharmacy and the School of Arts & Sciences for their departmental support.

Funding and Disclosure

The authors declare no conflict of interest.

Figure Legends

Figure 1. Flow chart of study procedures.

Figure 2. Maternal Sickness Indicators. (A) total composite score for all sickness indicators evaluated (piloerection, ptosis, and lethargy) at 60, 75, 90, 105, 120, 135, 150, and 180 min post maternal immune activation. Dams treated with LPS had significantly higher sickness scores at 135 and 150 minutes compared to saline treated controls. Total (B) food and (C) water intake 4 hr and 20 hr after challenge, and (D) 24 hr body weight change. LPS treated animals had significant reductions in food and water intake and slower weight gain compared to saline treated dams. Data are based on means (±SEM); saline (white bars; n =12) vs. LPS (black bars; n = 12); *p < 0.05; **p < 0.01.

Figure 3. Behavioral observations for male offspring following six weeks of housing in standard conditions (SC), colony nesting (CN), or environmental enrichment (EE). Social interaction data show duration (seconds) spent (A) in total contact, (B) initiating contact, and (C) receiving contacts. Object-in-place (D) discrimination ratios show that all animals exposed to saline (white bars) on gestational day 15 had intact spatial discrimination ability while LPS (black bars) treated animals in SC and EE housing did not. Male CN-LPS rats had intact discrimination ratios suggesting social housing can reverse inflammatory mediated spatial disruptions in this task. *p < 0.05; **p < 0.01, indicates intact spatial discrimination ability. SC-Saline, n = 9; SC-LPS, n= 9; CN-Saline, n=8; CN-LPS, n =8; EE-Saline, n=7; EE-LPS, n=7.

Figure 4. Early inflammatory programmed changes in (left) hippocampal and (right) prefrontal cortex (A,B) EAAT3 and (C,D) EAAT2 gene expression. Data represent mean expression (±SEM) relative to SC-saline controls; saline (white bars) vs. LPS (black bars); *p < 0.05; **p < 0.01; SC-Saline, n = 9; SC-LPS, n= 9; CN-Saline, n=8; CN-LPS, n =7-8; EE-Saline, n=7; EE-LPS, n=7.

Figure 5. Early inflammatory programmed changes in (left) hippocampal and (right) prefrontal cortex (A,B) BDNF, and (C,D) TrkB gene expression. Data represent mean expression (±SEM) relative to SC-saline controls; saline (white bars) vs. LPS (black bars); *p < 0.05; **p < 0.01; SC-Saline, n = 9; SC-LPS, n= 9; CN-Saline, n=8; CN-LPS, n =7-8; EE-Saline, n=7; EE-LPS, n=6-7.

References

Ashdown A, Dumont Y, Ng M, Poole S, Boksa P, Luheshi, GN (2006): The role of cytokines in mediating effects of prenatal infection on the fetus: implications for schizophrenia. Molecular Psychiatry 11: 47-55.

Baharnoori M, Bhardwaj SK, Srivastava LK (2013): Effect of maternal lipopolysaccharide administration on the development of dopaminergic receptors and transporter in the rat offspring. PLoS ONE 8: e54439.

Bauer D, Haroutunian V, Meador-Woodruff JH, McCullumsmith RE (2010): Abnormal glycosylation of EAAT1 and EAAT2 in prefrontal cortex of elderly patients with schizophrenia. Schizophr Res 117: 92–98.

Bellesi, M., Conti, F (2010): The mGluR2/3 agonist LY379268 blocks the effects of GLT-1 upregulation on prepulse inhibition of the startle reflex in adult rats. Neuropsychopharmacology 35: 1253–1260.

Bellesi, M., Melone, M., Gubbini, A., Battistacci, S., Conti, F (2009): GLT-1 upregulation impairs prepulse inhibition of the startle reflex in adult rats. Glia 57: 703–713.

Bilbo, SD, Smith SH, Schwarz JM (2012): A lifespan approach to neuroinflammatory and cognitive disorders: a critical role for glia. J.Neuroimmune Pharmaco 7: 24–41.

Boksa P (2010): Effects of prenatal infection on brain development and behavior: A review of findings from animal models. Brain Behav Immun 24:881–897.

Borrell, J, Vela JM, Arévalo-Martin A, Molina-Hogado E, Guaza C (2002): Prenatal immune challenge disrupts sensorimotor gating in adult rats: implications for the etiopathogenesis of schizophrenia. Neuropsychopharmacology 26: 204-215.

Branchi I, D'Andrea I, Fiore M, Di Fausto V, Aloe L, Alleva E (2006): Early social enrichment shapes social behavior and nerve growth factor and brain- derived neurotrophic factor levels in the adult mouse brain. Biol. Psychiatry 60: 690–696.

Caldeira MV, Melo CV, Pereira DB, Carvalho R, Correia SS, Backos DS, Carvalho AL, Esteban JA, Duarte CB (2007) Brain-derived neurotrophic factor regulates the expression and synaptic delivery of alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor subunits in hippocampal neurons. The Journal of Biological Chemistry 282: 12619–12628.

Castren E, Voikar V, Rantamaki T (2007): Role of neurotrophic factors in depression. Current Opin Pharmacol 7: 18–21.

Chen W, Aoki C, Mahadomrongkul V, Gruber CE, Wang GJ, Blitzblau R. et al. (2002): Expression of a variant form of the glutamate transporter GLT1 in neuronal cultures and in neurons and astrocytes in the rat brain Journal of Neuroscience 22: 2142–2152.

Chiappelli J, Shi Q, Kodi P, Savransky A, Kochunov P, Rowland LM, Nugent KL, Hong LE (2016): Disrupted glucocorticoid-immune interactions during stress response in schizophrenia. Psychoneuroendocrinology 63: 86-93.

Chiocchetti AG, Bour HS, Freitag CM (2014): Glutamatergic candidate genes in autism spectrum disorder: an overview. Journal of Neural Transmission, 121, 9, 1081-1106.

Connors EJ, Shaik AN, Migliore MM, Kentner AC (2014): Environmental enrichment mitigates the sex-specific effects of gestational inflammation on social engagement and the hypothalamic pituitary adrenal axis-feedback system. Brain, Behavior, and Immunity 42: 178-190.

Connors EJ, Migliore MM, Shaik AN, Kentner AC (2015): Environmental enrichment models a naturalistic form of maternal separation and shapes the anxiety response patterns of offspring. Psychoneuroendocrinology 52:153–167.

Danbolt NC (2001): Glutamate uptake. Progress in Neurobiology 65: 1-105.

Dougherty KD, Dreyfus CF, Black IB (2000): Brain-derived neurotrophic factor in astrocytes, oligodendrocytes, and microglia/macrophages after spinal cord injury. Neurobiol Dis 7: 574–585.

Eastwood SL, Harrison PJ (2005): Decreased expression of vesicular glutamate transporter 1 and complexin II mRNAs in schizophrenia: further evidence for a synaptic pathology affecting glutamate neurons. Schizophrenia Research 73: 159-172.

Fortier ME, Luheshi GN, Boksa P (2007): Effects of prenatal infection on prepulse inhibition in the rat depend on the nature of the infectious agent and the stage of pregnancy. Behav Brain Res 181: 270–277.

Francis DD, Diorio J, Plotsky PM, Meaney MJ (2002) Environmental enrichment reverses the effects of maternal separation on stress reactivity. J Neuroscience 22: 7840-7843.

Fremeau RT, Burman J, Qureshi T, Tran C H, Proctor J, Johnson J, et al. (2002): The identification of vesicular glutamate transporter 3 suggests novel modes of signaling by glutamate. Proc. Natl. Acad. Sci. U S A 99:14488–14493.

Fremeau RT, Troyer MD, Pahner I, Nygaard GO, Tran CH, Reimer RJ, Bellocchio EE, Fortin D, Storm-Mathisen J, Edwards R H (2001): The expression of vesicular glutamate transporters define two classes of excitatory synapse. Neuron 31: 247-260.

French S, Chester EM, Demas GE (2013): Maternal immune activation affects litter success, size and neuroendocrine responses related to behavior in adult offspring. Physiology &

Behavior 119: 175-184.

Gu F, Chauhan V, Chauhan A (2015): Glutathione redox imbalance in brain disorders. Curr Opin Clin Nutr Metab Care 18:89–95.

Insel, T.R. (2010). Rethinking schizophrenia. Nature, 468, 187-193.

Hayley S, Kelly O, Anisman H (2002): Murine tumor necrosis factor-alpha sensitizes plasma corticosterone activity and the manifestation of shock: modulation by histamine. J. Neuroimmunol 131: 60–69.

Hill RA, Klug M, Kiss Von Soly S, Binder MD, Hannan AJ, van den Buuse M.(2014): Sex-specific disruptions in spatial memory and anhedonia in a "two hit" rat model correspond with alterations in hippocampal brain-derived neurotrophic factor expression and signaling. Hippocampus 24:1197-211.

Homburg JR, Molteni R, Calabrese F, Riva MA (2014): The serotonin–BDNF duo: Developmental implications for the vulnerability to psychopathology. Neuroscience and Biobehavioral Reviews 43: 35-47.

Howell DC (2002): Statistical Methods for Psychology, 5th ed. Duxbury Press, Belmont, Canada.

Howland JG, Cazakoff BN, Zhang Y (2012): Altered object-in-place recognition memory, prepulse inhibition, and locomotor activity in the offspring of rats exposed to a viral mimetic during pregnancy. Neuroscience 201: 184–198.

Huang FL, Huang K-P, Wu J, Boucheronn C (2006): Environmental enrichment enhances neurogranin expression and hippocampal learning and memory but fails to rescue the impairments of neurogranin null mutant mice. The Journal of Neuroscience 26: 6230–623.7

Jensen AA, Fahlke C, Bjørn-Yoshimoto WE, Bunch L (2015): Excitatory amino acid transporters: recent insights into molecular mechanisms, novel modes of modulation and new therapeutic possibilities. Current Opinion in Pharmacology 20: 116-123.

Jurgens HA, Johnson RW (2012): Environmental enrichment attenuates hippocampal neuroinflammation and improves cognitive function during influenza infection. Brain, Behavior, and Immunity 26: 1006-1016.

Kegeles LS, Mao X, Stanford AD, Girgis R, Ojeil N, Xu X, Gil R, Slifstein M, Abi-Dargham A, Lisanby SH, Shungu DC (2012): Elevated prefrontal cortex gamma-aminobutyric acid and glutamate- glutamine levels in schizophrenia measured in vivo with proton magnetic resonance spectroscopy. Arch Gen Psychiatry 69: 449–459.

Kentner AC (2015) Neuroprotection and recovery from early-life adver- sity: considerations for environmental enrichment. Neural Regen Res 10:1545-1547.

Kentner AC, James JS, Miguelez M, Bielajew C(2007): Investigating the hedonic effects of interferon-a on female rats using brain-stimulation reward. Behav. Brain Res 177: 90–99.

Kim KC, Kim P, Go HS, Choi CS, Park JH, Kim HJ, Jeon SJ, et al. (2013): Male specific alteration in excitatory post-synaptic development and social interaction in pre-natal valproic acid exposure model of autism spectrum disorder. Journal of Neurochemistry 124: 832-843.

Konradi C, Heckers S (2003): Molecular aspects of glutamate dysregulation: implications for schizophrenia and its treatment, Pharmacology & Therapeutics 97: 153–179.

Lanté F, Meunier J, Guiramand J, Maurice T, Cavalier M, de Jesus Ferreira MC, Aimar R, Cohen-Solal C, Vignes M, Barbanel G (2007): Neurodevelopmental damage after prenatal infection: role of oxidative stress in the fetal brain. Free Radic. Biol. Med.

42:1231–1245.

Lanté F, Meunier J, Guiramand J, De Jesus Ferreira MC, Cambonie G, Aimar R, Cohen-Solal C, Maurice T, Vignes M, Barbanel G (2008): Late N-acetylcysteine treatment prevents the deficits induced in the offspring of dams exposed to an immune stress during gestation. Hippocampus 18: 602–609.

Lauriat TL, McInnes LA (2007): EAAT2 regulation and splicing: relevance to psychi atric and neurological disorders, Molecular Psychiatry 12: 1065–1078.

Laviola G, ReaM, Morley-Fletcher S, DiCarlo S, Bacosi A, DeSimone R, Bertini M, Pacifici R. (2004): Beneficial effects of enriched environment on adolescent rats from stressed pregnancies. Eur J Neurosci 20: 1655–1664.

Lin Y-U, Wang S (2014): Prenatal lipopolysaccharide exposure increases depression-like behaviors and reduces hippocampal neurogenesis in adult rats. Behavioural Brain Research 259: 24-34.

Lowe GC, Luheshi GN, Williams S (2008): Maternal infection and fever during late gestation are associated with altered synaptic transmission in the hippocampus of juvenile offspring rats. Am J Physiol Regul Integr Comp Physiol 295: R1563-R1571.

MacRae, M. Kenkel, W., Kentner, A.C. (2015a). Social rejection following neonatal inflammation is mediated by olfactory scent cues. Brain, Behavior, and Immunity, 49, 43-49.

MacRae M, Macrina T, Khoury A, Migliore MM, Kentner AC (2015b): Tracing the trajectory of behavioral impairments and oxidative stress in an animal model of neonatal inflammation. Neuroscience 298: 455-466.

Maier SF, Goehler LE, Fleshner M, Watkins LR (1998): The role of the vagus nerve in cytokine-

to-brain communication. Ann NY Acad Sci 840: 289–300.

Martini M., Valverde O (2012): A single episode of maternal deprivation impairs the motivation for cocaine in adolescent mice. Psychopharmacology 219:149, 158.

McQuaid RJ, Audet M-J, Anisman H (2012): Environmental enrichment in male CD-1 mice promotes aggressive behaviors and elevated corticosterone and brain norepinephrine activity in response to a mild stressor. Stress 15: 354-360.

McQuaid RJ, Audet M-C, Jacobson-Pick S, Anisman H (2013): Environmental enrichment influences brain cytokine variations elicited by social defeat in mice. Psychoneuroendocrinology 38: 987—996.

McQuaid RJ, Audet M-C, Jacobson-Pick S, Anisman H (2012b): The differential impact of social defeat on mice living in isolation or groups in an enriched environment; Plasma corticosterone and monoamine variations. Int J Neuropsychopharmacol 16 351–363.

Meyer U, Nyffeler M, Yee BK, Knuesel I, Feldon J (2008): Adult brain and behavioral pathological markers of prenatal immune challenge during early/middle and late fetal development in mice. Brain Behav Immun 22: 469–486.

Milka, A., Fleshner, M. (in press). Early life exercise may promote lasting brain and metabolic health through gut bacterial metabolites. Immunology and Cell Biology, doi: 10.1038/icb.2015.113.

Morgan C, Novak I, Dale RC, Badawi N (2015): Optimising motor learning in infants at high risk of cerebral palsy: a pilot study. BMC Pediatrics 15:30.

Morley-Fletcher S, Rea M, Maccari S, Laviola G (2003): Environmental enrichment during adolescence reverses the effects of prenatal stress on play behaviour and HPA axis reactivity in rats. Eur J Neurosci 18: 3367–3374.

Müller N, Schwarz M. (2006): Schizophrenia as an inflammation mediated dysbalance of glutamatergic neurotransmission. Neurotoxicity Research 10: 131-148.

Navarro D, Alvarado M, Navarrete F, Giner M, Obregon MJ, Manzanares J, Berbel P (2015): Gestational and early postnatal hyperthyroidism alters VGlut1 and VGAT bouton distribution in the neocortex and hippocampus, and behavior in rats. Frontiers in Neuroanatomy 9: 9.

Noda M (2015): Possible role of glial cells in the relationship between thyroid dysfunction and mental disorders. Frontiers in Cellular Neuroscience 9:194.

Ohnuma T, Augood SJ, Arai H, McKenna PJ, Emson PC (1998): Expression of the human excitatory amino acid transporter 2 and metabotropic glutamate receptors 3 and 5 in the prefrontal cortex from normal individuals and patients with schizophrenia. Brain Research: Molecular Brain Research 56: 207-217.

Ohnuma T, Tessler S, Arai H, Faull RL, McKenna PJ, Emson PC (2000): Gene expression of metabotropic glutamate receptor 5 and excitatory amino acid transporter 2 in the schizophrenic hippocampus. Brain Research: Molecular Brain Research 85: 24-31.

Oskviq DB, Elkahloun AG, Johnson KR, Phillips TM, Herkenham, M. (2012). Maternal immune activation by LPS selectively alters specific gene expression profiles of interneuron migration and oxidative stress in the feus without triggering a fetal immune response. Brain, Behavior, and Immunity, 26, 623-634.

Ozawa K., Hashimoto K, Kishimoto T, Shimizu E, Ishikura H, Iyo M, (2006). Immune activation during pregnancy in mice leads to dopaminergic hyperfunction and cognitive impairment in the offspring: a neurodevelopmental animal model of schizophrenia. Biol. Psychiatry 59: 546– 554.

Paoletti P Bellone C Zhou Q (2013): NMDA receptor subunit diversity: impact on receptor properties, synaptic plasticity and disease. Nat Rev Neurosci 14:383–400.

Plitman E, Nakajima S, de la Fuente-Sandoval C, Gerretsen P, Chakravarty MM, Kobylianskii J, Chung JK, Caravaggio F, Iwata Y, Remington G, Graff-Guerrero A (2014): Glutamate-mediated excitotoxicity in schizophrenia: a review. Eur Neuropsychopharmacol 24: 1591-1605.

Poels EM, Kegeles LS, Kantrowitz JT, Javitt DC, Lieberman JA, Abi-Dargham A, Girgis RR (2014a): Glutamatergic abnormalities in schizophrenia: a review of proton MRS findings. Schizophr Res 152: 325–332.

Poels EM, Kegeles LS, Kantrowitz JT, Slifstein M, Javitt DC, Lieberman JA, Abi-Dargham A, Girgis RR (2014b) Imaging glutamate in schizophrenia: review of findings and implications for drug discovery. Mol Psychiatry 19: 20–29.

Purcell AE, Jeon OH, Zimmerman AW, Blue ME, Pevsner J (2001) Postmortem brain abnormalities of the glutamate neurotransmitter system in autism. Neurology 57: 1618-1628.

Raine A, Mellingen K, Liu J, Venables P, Mednick AA (2003): Effects of environmental enrichment at ages 3–5 years on schizotypical personality and antisocial behavior at ages 17 and 23 years. Am J Psychiatry 160:1627–1635.

Rao JS, Kellom M, Reese EA, Rapoport SI, Kim H-W (2012): Dysregulated glutamate and dopamine transporters in postmortem frontal cortex from bipolar and schizophrenic patients. Journal of Affective Disorders 136: 63-71.

Roceri M, Cirulli F, Pessina C, Peretto P, Racagni G, Riva MA, (2004): Postnatal repeated maternal deprivation produces age-dependent changes of brain- derived neurotrophic

factor expression in selected rat brain regions. Biol Psychiatry 55: 708–714.

Romero E, Guaza C, Castellano B, Borrell J (2010): Ontogeny of sensorimotor gating and immune impairment induced by prenatal immune challenge in rats: implications for the etiopathology of schizophrenia. Mol Psychiatry 15: 372– 383.

Rossi C, Angelucci A, Costantin L, Braschi C, Mazzantini M, Babbini F, Fabbri ME, Tessarollo L, Maffei L, Berardi N, Caleo M (2006): Brain-derived neurotrophic factor (BDNF) is required for the enhancement of hippocampal neurogenesis following environmental enrichment. Eur J Neurosci 24: 1850–1856.

Sale A, Bernardi N, Maffei L (2014): Environment and brain plasticity: towards an endogenous pharmacotherapy. Physiol Rev 94: 189-234.

Shan D, Lucas EK, Drummon JB, Haroutunian V, Meador-Woodruff JH, McCullumsmith RE (2013): Abnormal expression of glutamate transporters in temporal lobe areas in elderly patients with schizophrenia. Schizophrenia Research 144: 1-8.

Sheldon AL, Robinson MB (2007): The role of glutamate transporters in neurodegenerative diseases and potential opportunities for intervention. Neurochem Int 51: 333–355.

Schwartz TL, Sachdeva S, Stahl SM. (2012:. Glutamate neurocircuitry: theoretical underpinnings in schizophrenia. Front Pharmacol 3: 195.

Smesny S, Gussew A, Biesal NJ, Schack S, Walther M, Rzanny R, Milleit B, Gaser C, Sobanski T, Schultz CC, Amminger P, Hipler U-C, Sauer H, Reichenbach J (2015): Glutamatergic dysfunction linked to energy and membrane lipid metabolism in frontal and anterior cingulate cortices of never treated first-episode schizophrenia patients. Schizophrenia Research 168: 322-329.

Spangaroa M, Bosiaa M, Zanolettib A, Bechia M, Cocchia F, Pirovanob P, Lorenzia C,

Bramantic P, Benedettia F, Smeraldia E (2012): Cognitive dysfunction and glutamate reuptake: Effect of EAAT2 polymorphism in schizophrenia. Neuroscience Letters 522: 151– 155.

Cavallaro R, Steiner AA, Ivanov AI, Serrats J, Hosokawa H, PhayreAN, Robbins JR, Roberts JL, Kobayashi S, Matsumura K, Sawchenko PE, Romanovsky AA (2006): Cellular and molecular bases of the initiation of fever. PLoS Biol 4:e284.

The Autism Genome Project Consortium (2007): Mapping autism risk loci using genetic linkage and chromosomal rearrangements. Nature Genetics 39: 319-328.

Takahashi K, Foster JB, Lin C-LG (2015): Glutamate transporter EEAT2: regulation, function, and potential as a therapeutic target for neurological and psychiatric disease. Cell Mol Life Sci 72: 3489-3506.

Takahashi K, Kong Q, Lin Y, Stouffer N, Schulte DA, Lai L, Liu Q, Chang L-C, Dominquez S, Xing X, Cuny GD, Hodgetts KJ, Glicksman MA, Glenn Lin C-L (2015): Restored glial glutamate transporter EAAT2 function as a potential therapeutic approach to Alzheimer's Disease. Journal of Experimental Medicine 212: 319-332.

Taylor PV, Veenema AH, Paul MJ, Bredewold R, Isaacs S, de Vries GJ (2012): Sexually dimorphic effects of a prenatal immune challenge on social play and vasopressin expression in juvenile rats. Biology of Sex Differences 3: 15.

Tonni G, Leoncini S, Signorini C, Ciccoli L, De Felice C (2014): Pathology of perinatal brain damage: background and oxidative stress markers. Arch Gynecol Obstet 290: 13-20.

Warburton EC, Barker GRI, Brown MW (2013): Investigations into the involvement of NMDA mechanisms in recognition memory. Neuropharmacology 74: 41–47.

Williamson LL, Chao A, Bilbo SD (2012): Environmental enrichment alters glial antigen

expression and neuroimmune function in the adult rat hippocampus. Brain, Behavior, and Immunity 26: 500-510.

Wischhof L, Irrsack E, Osorio C, Koch M (2015): Prenatal LPS-exposure – a neurodevelopmental rat model of schizophrenia – differentially affects cognitive functions, myelination, and parvalbumin expression in male and female offspring. Progress in Neuro-Psychopharmacology and Biological Psychiatry 57: 17-30.

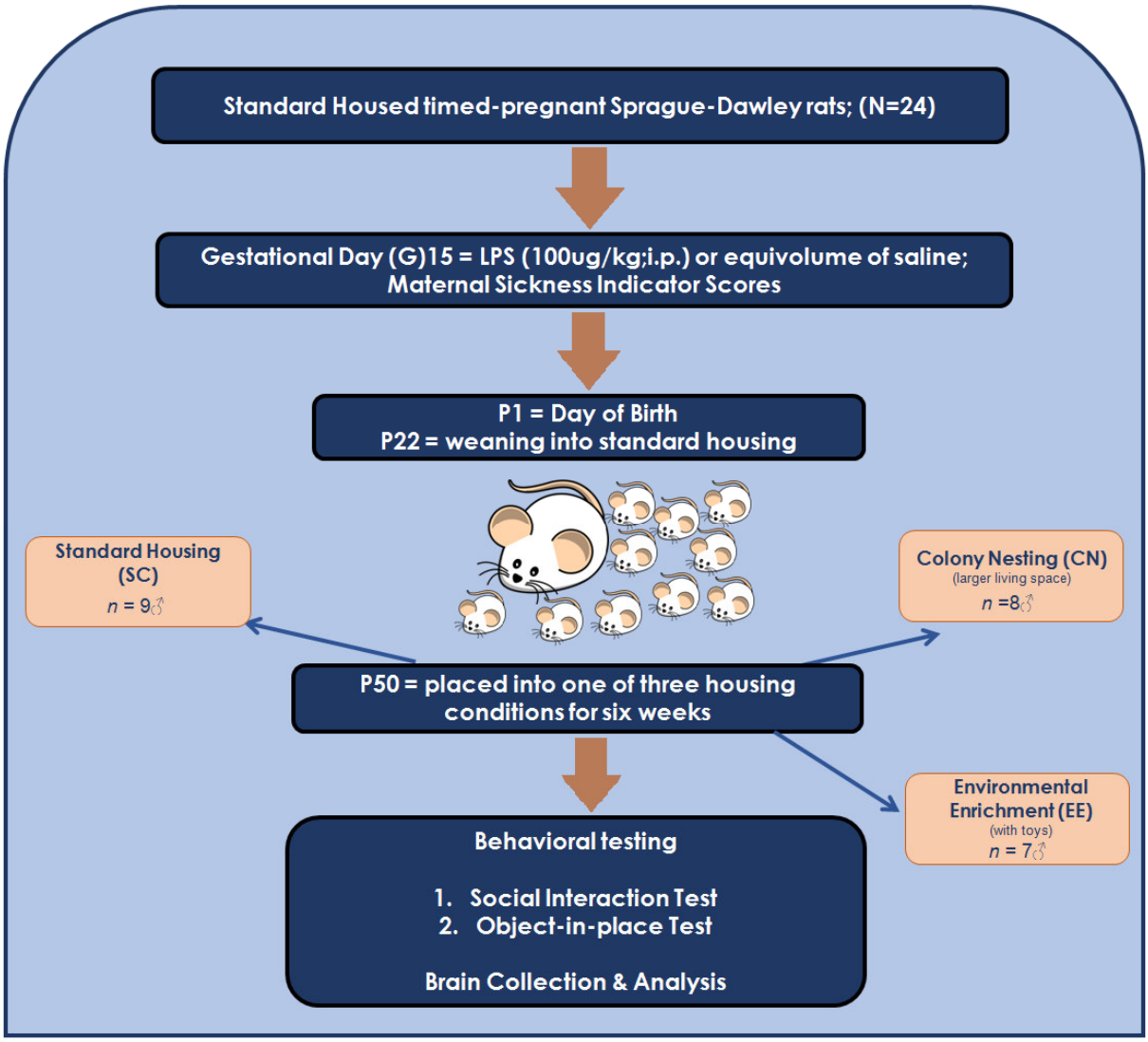
Winter C, Djodario-Irani A, Sohr R, Morgenstern R, Feldon J, Juckel G, Meyer U (2009): Prenatal immune activation leads to multiple changes in basal neurotransmitter levels in the adult brain: implications for brain disorder of neurodevelopmental origin such as schizophrenia. International Journal of Neuropsychopharmacology 12: 513-524.

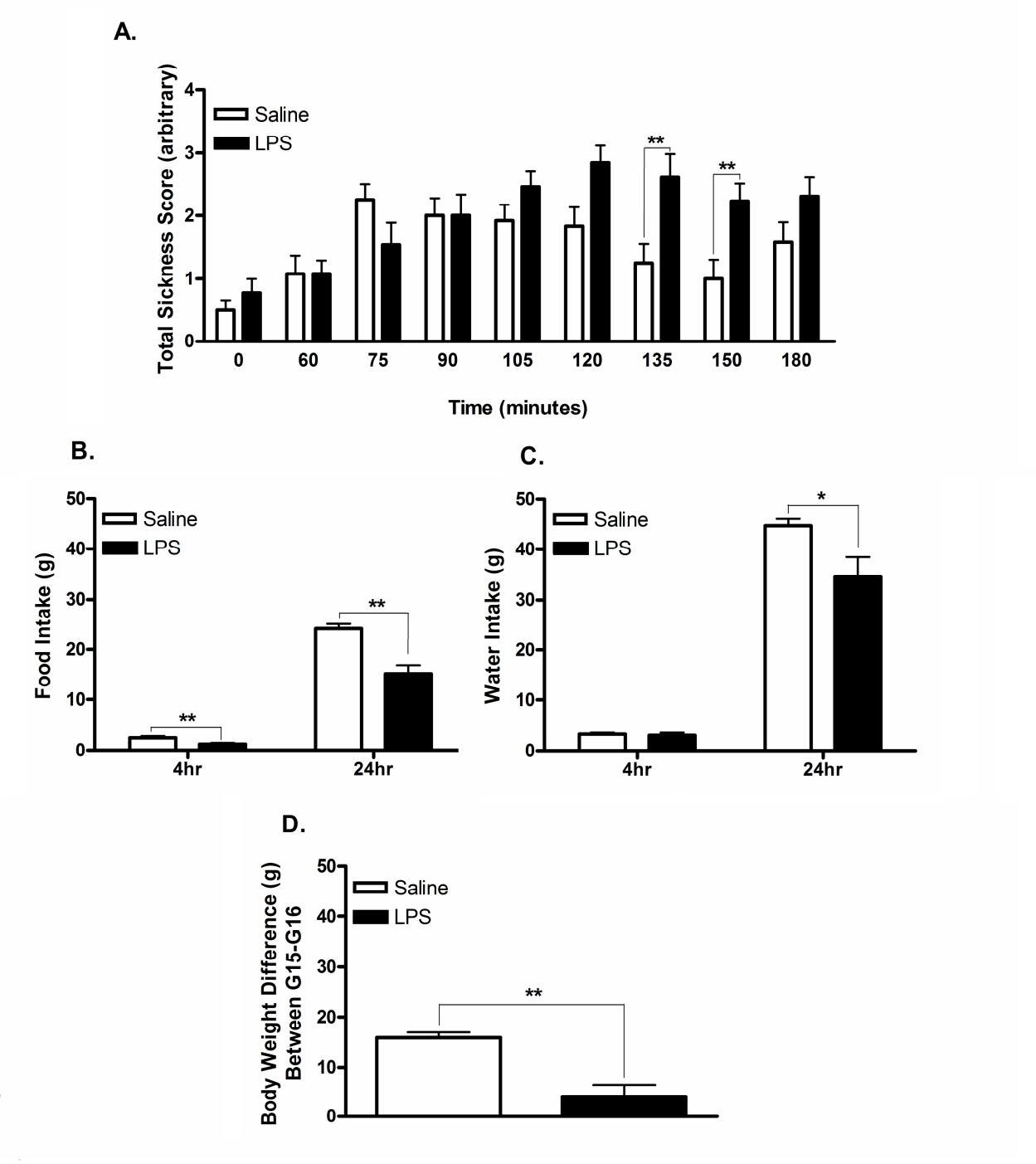
Woo CC, Donnelly JH, Steinberg-Epstein R, Leon M (2015): Environmental enrichment as a therapy for autism: a clinical trial replication and exten- sion. Behav Neurosci 129:412-422.

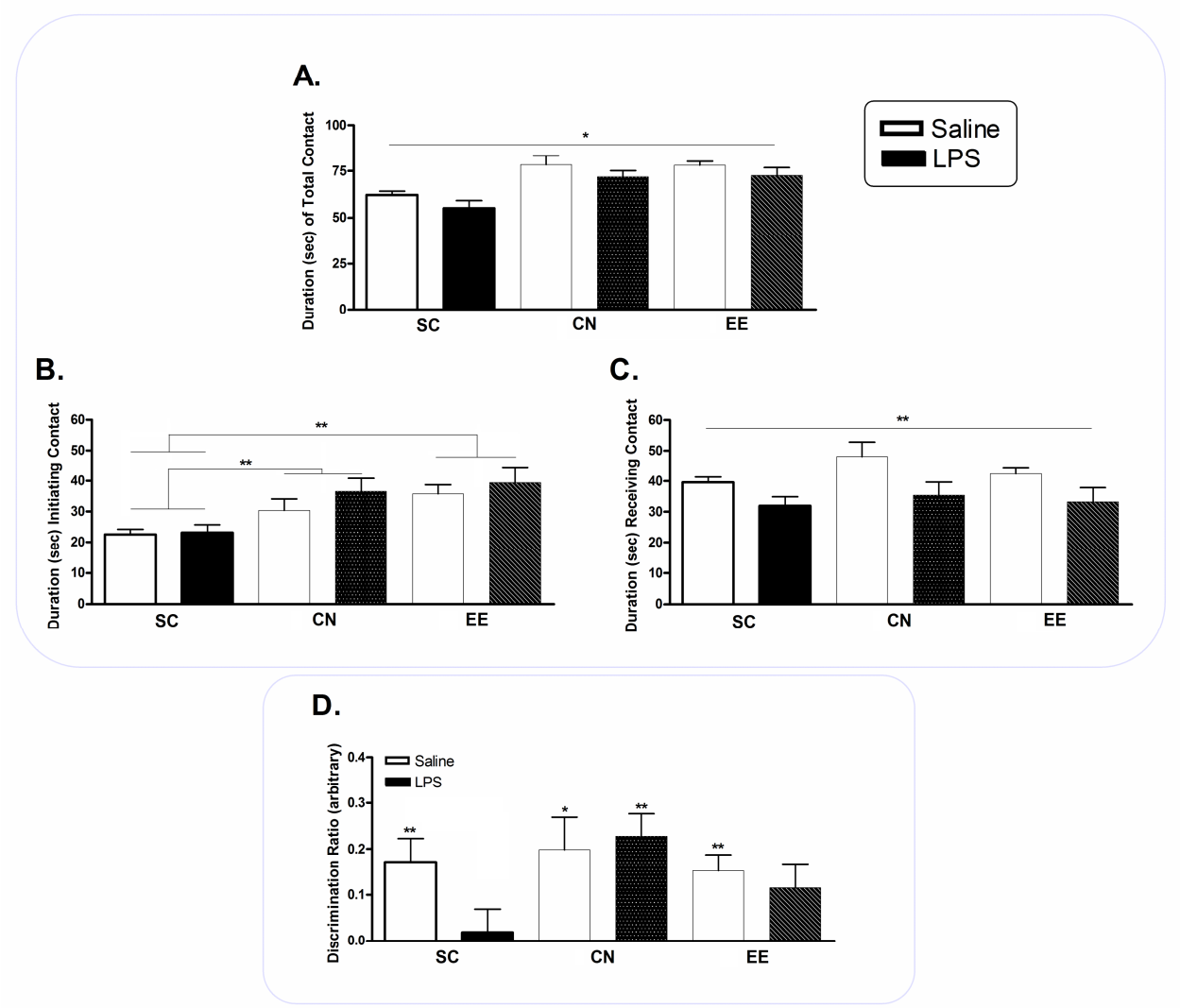
Yi JH, Hazell AS (2006): Excitotoxic mechanisms and the role of astrocytic glutamate transporters in traumatic brain injury. Neurochem Int 48: 394–403.

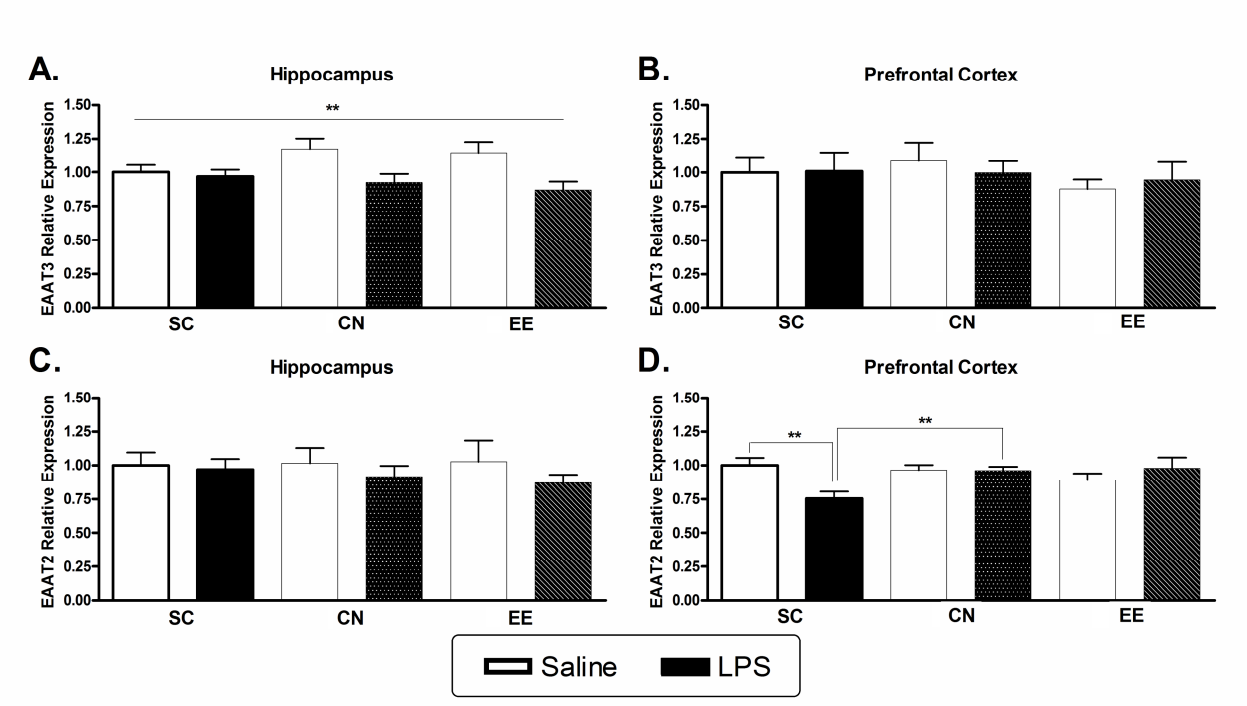
Yuan H, Low C-M, Moody OA, Jenkins A, Traynelis SF (2015): Ionotropic GABA and glutamate receptor mutations and human neurologic diseases. Molecular Pharmacology 88: 203-217.

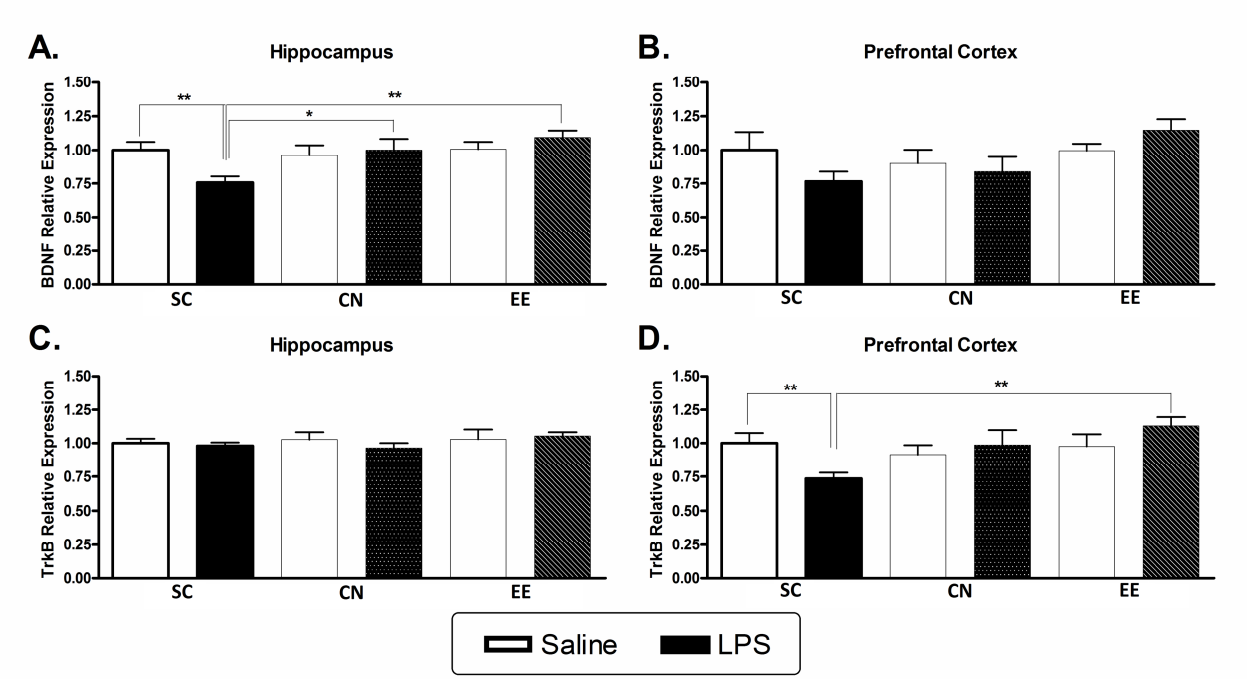
Zhang XY, Tana Y-L, Chena D-C, Tana S-P, Yanga F-D, Wub HW, Zunta-Soares GB, Huang, X –F, Kosten TR, Soares JC (2015): Interaction of BDNF with cytokines in chronic schizophrenia. Brain, Behavior, and Immunity, in press.











Highlights

- LPS-induced maternal immune activation reduced gene expression of EAAT2, BDNF and TrkB
- Environmental enrichment reversed the effect of early-life inflammation on hippocampal BDNF and prefrontal TrkB
- Social enrichment reversed accompanying spatial disruptions and downregulation of prefrontal EAAT2
- These data have compelling implications for the reversibility of early-life adversity