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Effects of Early Adolescent Environmental Enrichment on Cognitive Dysfunction, Prefrontal Cortex Development, and Inflammatory Cytokines After Early Life Stress

ABSTRACT: *Early postnatal stress such as maternal separation causes cognitive dysfunction later in life, including working memory deficits that are largely mediated by the prefrontal cortex. Maternal separation in male rats also yields a loss of parvalbumin-containing prefrontal cortex interneurons in adolescence, which may occur via inflammatory or oxidative stress mechanisms. Environmental enrichment can prevent several effects of maternal separation; however, effects of enrichment on prefrontal cortex development are not well understood. Here, we report that enrichment prevented cognitive dysfunction in maternally separated males and females, and prevented elevated circulating pro-inflammatory cytokines that was evident in maternally separated males, but not females. However, enrichment did not prevent parvalbumin loss or adolescent measures of oxidative stress. Significant correlations indicated that adolescents with higher oxidative damage and less prefrontal cortex parvalbumin in adolescence committed more errors on the win-shift task; therefore, maternal separation may affect cognitive dysfunction via aberrant interneuron development.* © *2015 Wiley Periodicals, Inc.* Dev Psychobiol 58:482–491, 2016.

Keywords: *early life stress; adolescence; oxidative stress; cytokines; win-shift; rat*

INTRODUCTION

Early life exposure to stressful situations impairs cognitive processes later in life (Brenhouse & Andersen, 2011); in humans, childhood adversity leads to significant increases in vulnerability to psychiatric disorders, including depression, drug abuse, and schizophrenia (Kessler, Davis, & Kendler, 1997; Teicher, Tomoda, & Andersen, 2006). Importantly, many disorders that are attributable to early life stress emerge after a delay—usually in adolescence (Andersen & Teicher, 2008). Exposure to early life stress might, therefore, yield a vulnerable population with neurodevelopmental deficits that could particularly benefit from intervention during a critical period.

Daily repeated removal of rat pups from their mothers (e.g., maternal separation [MS]) during the neonatal period is an ethologically relevant rodent model of early life stress (Lehmann & Feldon, 2000). Several preclinical studies report effects of MS on the prefrontal cortex (PFC) that manifest in adolescence or adulthood but not earlier in development (Chocyk, Dudys, Przyborowska, Mackowiak, & Wedzony, 2010;

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Wilber, Southwood, & Wellman, 2009), which may be due to the late and protracted developmental profile of the PFC (Andersen & Teicher, 2008). Specifically, we have shown that MS leads to adolescent decreases in parvalbumin (PVB)-containing interneurons in the PFC (Brenhouse & Andersen, 2011; Holland, Ganguly, Potter, Chartoff, & Brenhouse, 2014). These decreases were specific to the PFC, as they were not observed in the hippocampus, where several other effects of MS have been found (Brenhouse & Andersen, 2011). Loss of PVB interneurons is important because decreases in PVB are noted in the PFC of postmortem tissue samples from schizophrenia patients, and proper functioning of PVB interneurons is necessary for cognitive processes such as working memory (Lewis, Hashimoto, & Volk, 2005; Wilson, O'Scalaidhe, & Goldman-Rakic, 1994). Specifically, regulated PFC excitability via interneurons has been implicated in spatial working memory as assessed by the win-shift working memory task (Enomoto & Floresco, 2009), which is disrupted after MS (Brenhouse & Andersen, 2011).

PVB interneurons are particularly vulnerable to oxidative damage during development (Cabungcal, Steullet, Kraftsik, Cuenod, & Do, 2013; Morishita, Cabungcal, Chen, Do, & Hensch, 2015), purportedly through generation of reactive oxygen species via NADPH oxidase (NOX) enzymes (Schiavone et al., 2009); therefore, the loss of PVB after MS may occur via oxidative damage (see Schiavone, Colaianna, & Curtis, 2015). Consistent with this hypothesis, MS exposure increases adolescent PFC levels of cyclooxygenase-2, which is an enzyme expressed during pro-inflammatory activity and is a contributor to oxidative stress; blocking cyclooxygenase-2 activity during early adolescence protects against PVB loss after MS (Brenhouse & Andersen, 2011). Moreover, stress has been repeatedly reported to yield oxidative damage in the PFC (Tagliari et al., 2010; Zlatkovic et al., 2014) and to provoke a pro-inflammatory phenotype in rodent models of depression and cognitive impairment (Sahin et al., 2015; Wieck, Andersen, & Brenhouse, 2013). Therefore, we hypothesized that MS leads to a pro-inflammatory phenotype and increased oxidative stress in the PFC with subsequent loss of PFC PVB and working memory deficits.

Environmental enrichment (EE) following MS represents one promising avenue to reverse some effects of early life stress because adolescent EE prevents several cognitive and affective consequences of MS (Francis, Diorio, Plotsky, & Meaney, 2002; Hui et al., 2011; Vivinetto, Suarez, & Rivarola, 2013). Protective effects of EE have been most readily studied in the hippocampus, with compelling evidence that EE prevents against MS-induced decreases in N-acetylaspartate—a mitochondrial protein associated with energy production (Hui et al., 2011). Although fewer investigations have studied the effects of EE on the PFC after early life stress, recent data demonstrate that rearing in an enriched environment can protect against adolescent PFC oxidative stress after a neonatal inflammatory insult (MacRae, Macrina, Khoury, Migliore, & Kentner, 2015). Furthermore, in typically developing and aged animals and humans, EE has anti-inflammatory (Arranz et al., 2010) and anti-oxidant effects (Sampedro-Piquero, Zancada-Menendez, Begega, Rubio, & Arias, 2013). We hypothesized that EE would prevent adolescent cognitive deficits and PVB loss in the PFC after MS via these protective mechanisms.

Here, we investigated the effects of MS and subsequent early adolescent EE on adolescent cognitive performance in the 8-arm win-shift task and on PFC PVB in males and females. Circulating cytokines and evidence for oxidative stress in the PFC were measured to determine whether MS and EE had interactive effects on inflammatory or oxidative dysregulation.

METHODS

All experiments were performed in accordance with the 1996 Guide for the Care and Use of Laboratory Animals (NIH) with approval from the Institutional Animal Care and Use Committee at Northeastern University. The timeline for all experimental procedures is illustrated in Figure 1.

Subjects

Twenty gestational Day 15 Sprague Dawley dams acquired from Charles River Laboratories (Wilmington, MA) were used in this study. Pregnant rats were housed individually in a 12h/12h light-dark cycle (light period 0700–1900) with food and water provided ad libitum. Day of birth was designated postnatal Day (P) 0. At P1, litters were culled to 10 pups. Sex distribution was balanced within litters as close to five males/five females as possible. From each MS or Con litter, no more than one pup per sex and housing condition was assigned, in order to avoid litter effects.

Maternal Separation

Maternally separated (MS) pups were removed from their home cages for 4 hr/day from P2 to 20. During separations, each pup was placed in an individual cup containing home cage bedding. From P2 to 12, separation cups were placed in a warm water bath maintained at 37°C. From P12 to 20, once rat pups were capable of thermoregulation, separation containers were at room temperature. Control (Con) litters remained with their dams until weaning at P21 except for

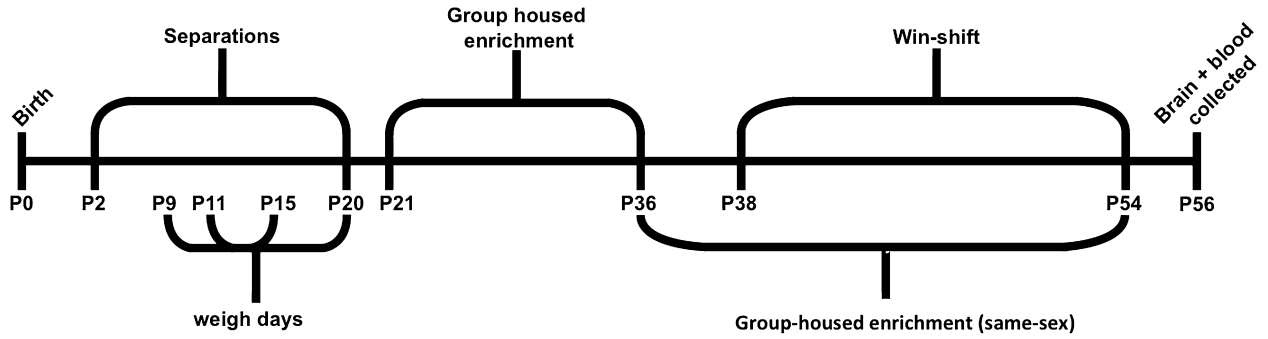

FIGURE 1 Timeline of experimental design.

weighing (<5 min separation from dam) at P9, P11, P15, and P20.

Early Adolescent Environmental Enrichment

Subjects were weaned at P21. Nonenriched (NE) animals were pair housed and matched for sex and group in standard animal facility clear polypropylene cages. Animals receiving environmental enrichment (EE) were moved to a clear polypropylene $80 \times 60 \times 23\,cm^3$ group cage. Six animals (three males, three females) matched for group were placed together into each EE cage. Enrichment materials, which included toys, platforms, tunnels, and enrichment bedding, were placed in the enrichment cages. The enrichment materials were changed weekly. At P36 and for the duration of behavioral testing, same-sex threesomes were moved from their group enrichment cages to smaller enrichment cages, in order to facilitate food restriction for the purposes of the win-shift paradigm (explained below). Animals were retained with their previous cage-mates to avoid any changes in social hierarchy. These EE conditions were adapted from commonly used postweaning EE paradigms that have been shown to buffer or protect against perinatal stress or low maternal care (e.g., Bredy, Zhang, Grant, Diorio, & Meaney, 2004; Vivinetto et al., 2013).

8-Arm Radial Maze Win-Shift

All subjects were food-restricted to 90% of free-fed body weight beginning on P36. An 8-arm radial arm maze was used in a win-shift task to measure short- and long-term spatial working memory. On P38-P39, rats were habituated to an 8-arm maze for 5 min. After each habituation session, sucrose pellets (TestDiet, Richmond, IN) were introduced into their home cage. Rats were then trained in the win-shift task, as described previously (Enomoto & Floresco, 2009). Briefly, in Phase 1, rats were introduced into the center of the maze and given 5 min to consume sucrose pellet food rewards from four open arms, with the other four arms closed. After a 5-min delay period in the home cage, rats were reintroduced in Phase 2 to the maze with all eight arms open but with rewards only available in the arms that had previously been blocked. An entry into an arm that did not contain a reward was marked as error. After reaching criterion of two consecutive days with $\leq$1 error in Phase 2, subjects were tested for three consecutive days with varying delay times between Phases 1 and 2 (5 min, 30 min, 3 hr). Delay sequence was counterbalanced across subjects. All subjects reached criterion within 14 days, and completed testing by P56. The maze was cleaned with 30% ethanol between each test and the configuration of open and closed doors changed daily.

Tissue Collection

At P56, animals were sacrificed via rapid decapitation between 1,200 and 1,400. Trunk blood was collected in EDTA coated microcentrifuge tubes. Blood plasma was collected and frozen after blood samples were spun at 1,000 RCF for 10 min. The medial PFC was immediately dissected using a brain block on ice and according to rat brain atlas landmarks (Paxinos & Watson, 1986) to include prelimbic and infralimbic regions and stored in an $-80^{\circ}C$ freezer. Brain tissue was later processed for westerns by homogenizing the tissue in 250 µl of 1% SDS in water. Homogenates were centrifuged at 4°C at 16,000 RCF for 10 min. Supernatants were placed in aliquots and stored at $-80^{\circ}C$. A Lowry's assay was run on all samples to determine protein concentration.

Western Blots

Thirty milligram of protein from each sample were separated by SDS–PAGE for 1 hr at 150 V through Novex® 4–12% Bis–Tris Protein Gels (Life Technologies, Carlsbad, CA). Blots were transferred onto a nitrocellulose membrane at 20 V overnight at 4°C. After transfer, the nitrocellulose membranes were blocked in a 5% milk solution in .1 M Tris-Buffered Saline containing Tween 20 (TBS-T) for 1 hr. The membranes were then incubated in 1:5,000 rabbit monoclonal anti-NADPH oxidase 2 (NOX2; Abcam AB129068, Cambridge, MA) to measure evidence of oxidative stress, 1:1,000 rabbit monoclonal anti-parvalbumin (Sigma P3088, St. Louis, MO), and 1:10,000 mouse monoclonal C4 anti-actin (loading control; MPBio 08691001, Solon, OH) diluted in the blocking solution, for 1.5 hr. Membranes were then rinsed in TBS-T and incubated in 1:2,000 anti-rabbit IgG peroxidase labeled horse (PI-1000 Vector, Burlingame, CA) and 1:2,000 anti-mouse IgG peroxidase labeled goat (PI-2000 Vector, Burlingame, CA) diluted in blocking solution, for 1 hr. Membranes

were developed with Western Lighting® ECL (PerkinElmer NEL1030001EA, Waltham, MA) and imaged with ChemiDoc XRS (BioRad, Hercules, CA) for analysis with ImageLab® (BioRad).

Carbonylated Protein Analysis for Evidence of Oxidative Damage

Oxidative damage to proteins in the PFC was measured by semi-quantitative analysis of carbonylated proteins, using an Oxidative Protein Western Blot Detection Kit (Abcam ab178020, Cambridge, MA). The manufacturers instructions were followed for sample preparation and analysis. Samples were diluted to 3 μg/μl. Proteins were derivatized with 2,4-dinitrophenylhydrazine (DNPH), which leads to formation of a stable dinitrophenyl (DNP) hydrazone product in the presence of carbonyl groups. Derivitized samples were subjected to SDS–PAGE and transferred overnight onto nitrocellulose membranes at 20 V at 4°C. Membranes were incubated with antibodies to DNP (1:5,000 rabbit anti-2,4-dinitrophenol) and then secondary antibodies (1:5,000 anti-rabbit IgG HRP conjugated goat). The membranes were developed with Western Lighting® ECL (PerkinElmer NEL1030001EA), imaged with a ChemiDoc XRS (BioRad), and analyzed with ImageLab® (BioRad). Here, we analyzed the optical density of each entire lane in order to capture all carbonylated proteins, regardless of size (Dalle-Donne, Rossi, Giustarini, Milzani, & Colombo, 2003).

Cytometric Bead Array Analysis of Circulating Cytokines

Plasma samples were collected and stored at −80°C for later analysis. The samples were thawed and processed on the same day. Plasma levels of IL-4, IL-10, and TNFα were measured using Cytometric Bead Array (CBA) Rat Flex Sets according to the manufacturer's instructions (BD Biosciences, San Jose, CA). Acquisition was performed with the BD LSR II flow cytometer at the Beth Israel Deaconess Medical Center, Boston, MA. Quantitative results were generated using the FCAP Array v.3.0 software (BD Biosciences) with detection limits of 3.4 pg/ml for IL-4, 19.4 pg/ml for IL-10, and 27.7 pg/ml for TNF-α.

Data Analysis

Three-way (Sex × Rearing × Housing) ANOVAs were performed with IBM SPSS Statistics V.22 to compare effects on all measures. "Rearing" refers to MS versus Con, whereas "Housing" refers to EE versus NE. Bonferroni correction for multiple comparisons were performed using GraphPad Prism where appropriate. Multiplicity-corrected *p*-values are reported for Bonferroni tests. To control for protein loading variability in PVB and NOX2 Western blots, optical densities determined with ImageLab for each protein band were normalized to the optical density of the actin band within each lane. Correlations between protein levels and behavior were analyzed using Pearson's correlation. Interaction of MS with the PVB: Win-shift error relationship (Fig. 3) was analyzed using multiple regression analysis with Rearing as an intervening variable within IBM SPSS Statistics V.22.

RESULTS

Effects of MS and EE on Win-Shift Performance

Performance during testing after 5 or 30 min delays between Phases 1 and 2 were not different between sex or group (data not shown). After a 3 hr delay, while no Sex × Rearing × Housing interaction was found ($p = .897$), a Rearing × Housing interaction ($F[1,57] = 4.4$; $p = .04$) revealed that MS + NE males (adjusted Bonferroni $p = .033$) and females (adjusted Bonferroni $p = .05$) committed more overall errors than Con + NE, which was prevented in MS + EE subjects. For the purposes of illustration, data are collapsed across sex in Figure 2A while separate measures for males and females are illustrated in Figure 2B.

Effects of MS and EE on PFC PVB, and Relationship to Win-Shift Performance

Consistent with previous reports (Holland et al., 2014), we observed that MS-exposed males, but not females, displayed decreased PFC PVB in adolescence. While no Sex × Rearing × Housing interaction was found ($p = .685$), a Sex × Rearing interaction ($F[1,65] = 5.24$; $p = .025$) revealed a decrease of PVB in MS + NE and MS + EE males compared to Con + NE males (adjusted

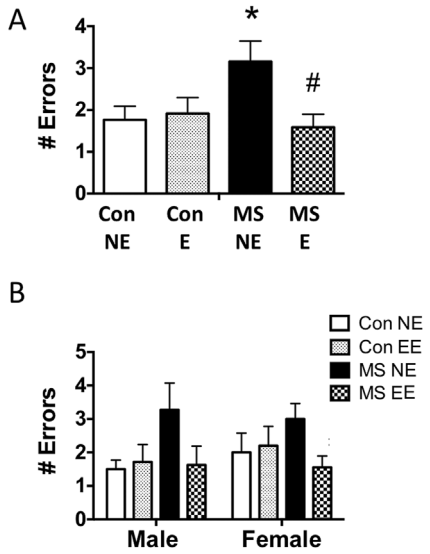

FIGURE 2 Effects of maternal separation (MS) and environmental enrichment (EE) on win-shift performance. (A) Number of errors after a 3 hr delay between Phases 1 and 2, with both sexes combined ($n = 16$–19/group; no significant interaction of sex was observed). Means ± SEM are shown. $^{*}p < .05$ difference from Con-NE. $^{\#}p < .05$ difference from MS-NE. (B) Separate graphical representations of MS and EE effects on win-shift performance in males ($n = 8$–10) and females ($n = 7$–9).

Bonferroni $p = .042$), while no significant differences were noted in females ($p > .1$). Thus, although MS caused PVB loss, EE did not prevent PVB loss in MS-exposed males (Fig. 3A). However, Pearson's correlation analyses revealed that MS exposure differentially impacted the relationship between PFC PVB and win-shift performance in all subjects (Fig. 3C). In Con subjects, higher levels of PVB correlated with more errors on the win-shift task (Pearson's $r = .444$; $R^2 = .198$; $p = .0296$). In MS subjects, however, the significant relationship between PVB and win-shift errors was reversed: higher levels of PVB correlated with fewer errors on the win-shift task (Pearson's $r = -.391$; $R^2 = .153$; $p = .0398$). The impact of MS on this relationship was revealed by a significant interaction of rearing in a regression analysis between PVB and win-shift errors ($p = .035$). Sex was not an intervening factor in these analyses.

Effects of MS and EE on Circulating Pro-Inflammatory Cytokines

Males and females were differentially affected by the adolescent consequences of MS on TNFα levels, resulting in a Sex × Rearing × Housing interaction ($F[1,1,59] = 2.7$; $p = .031$; Fig. 4A). In males but not females, MS + NE subjects expressed higher circulating TNFα than CON + NE (adjusted Bonferroni $p = .010$), which was prevented in MS + EE males. As the impact of pro-inflammatory cytokines is tempered by expression of anti-inflammatory cytokines, we measured the ratio of TNFα to IL-10 and IL-4 (anti-inflammatory cytokines). No effects on IL-4 (data not shown) or IL-10 (Fig. 4B) were observed. However, although a 3-way interaction only reached trend-level significance ($p = .06$), a significant Rearing × Housing interaction in males ($F[1,26] = 4.338$; $p = .0472$) but not females revealed that MS + NE males expressed a higher TNFα/IL-10 ratio than Con + NE (adjusted Bonferroni $p = .015$), whereas MS + EE were not different from Controls (Fig. 4C).

Effects of MS and EE on PFC Oxidative Stress, and Relationship to Win-Shift Performance

Neither MS nor EE affected levels of carbonyl groups or NOX2 in the adolescent PFC of males or females (Fig. 5A and C). However, subjects with higher levels of carbonyated proteins in the PFC committed more win-shift errors, regardless of sex, MS, or enrichment (Fig. 5B; Pearson's $r = .387$; $R^2 = .150$; $p = .005$).

DISCUSSION

The results of this study demonstrate that EE following early life stress can prevent spatial working memory deficits in adolescence as well as heightened circulating

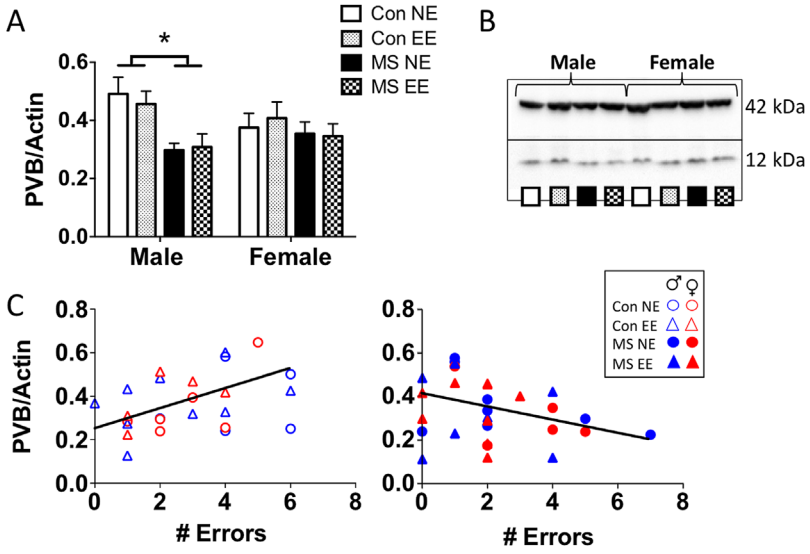

FIGURE 3 Effects of maternal separation (MS) and environmental enrichment (EE) on prefrontal cortex parvalbumin (PVB). (A) Means ± SEM for levels of PFC PVB (relative to actin) in males ($n = 7–8$) and females ($n = 6–7$) are shown. $^*p < .05$ difference between MS and controls (Con). (B) Representative bands for actin (42 kDa) and PVB (12 kDa). (C) Scatter plots showing correlation between PVB levels and win-shift performance in Con (left; $n = 30$) and MS (right; $n = 30$) subjects.

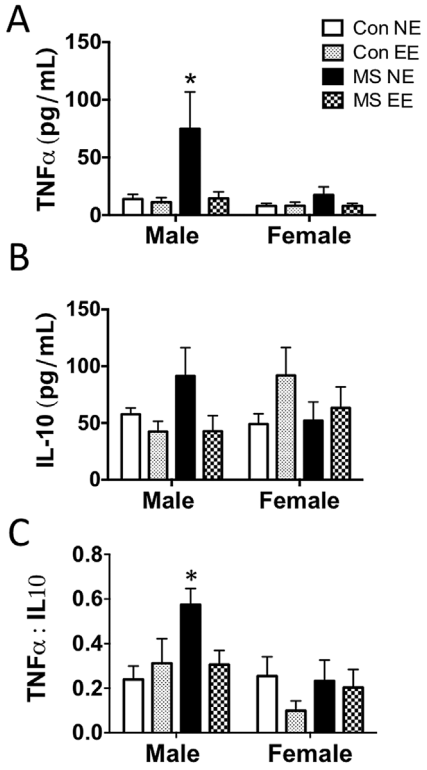

FIGURE 4 Effects of maternal separation (MS) and environmental enrichment (EE) on (A) circulating TNFα ($n = 7$ −9), (B) IL-10 ($n = 7$–9) and (C) TNFα/IL-10 ratio ($n = 7$–8). Means ± SEM are shown. $^{*}p < .05$ difference from Con-NE.

pro-inflammatory cytokines in adolescent males. The positive relationship we observed between plasma pro-inflammatory ratios and win-shift deficits in adolescence lends support to the hypothesis that cognitive dysfunction is mediated by immune mechanisms, and that EE prevents cognitive deficits through protection of inflammatory processes. Although we did not observe effects of MS or EE on the measures of oxidative stress or oxidative damage that were employed here, another correlation between higher levels of carbonlyated proteins in the PFC and win-shift deficits further suggests that cognitive dysfunction may be a consequence of damage via oxidative stress mechanisms.

Our hypothesis that EE would prevent PVB loss after MS by preventing a pro-inflammatory profile and PFC oxidative stress was not supported. Although MS yielded lower levels of PVB in male adolescents as previously observed (Brenhouse & Andersen, 2011; Holland et al., 2014; Wieck et al., 2013), EE during early adolescence did not prevent PVB loss. Further, PVB levels were not correlated with levels of TNFα, TNFα/IL-10, or with measures of oxidative stress. Therefore, these data fail to support the hypothesis that MS-attributable PVB loss during adolescence occurs via adolescent inflammation or oxidative stress. However, previous studies have revealed that early insults decrease anti-oxidant processes, such as reduced glutathione levels (Carboni et al., 2010). Therefore, the ratio of oxidative stress to anti-oxidant may uncover MS effects that were not observed here. Indeed, as previously described, rearing in an enriched environment can protect against adolescent PFC oxidative stress after a neonatal inflammatory insult (MacRae et al., 2015). We also note that the PFC tissue analyzed was not free of blood contamination, which potentially interfered with oxidative stress measures. Additionally, PVB interneuron development displays distinct critical periods of vulnerability to oxidative stress (Cabungcal et al., 2014; Do, Cuenod, & Hensch, 2015; Morishita et al., 2015); therefore, it is possible that MS yields neuro-immune or oxidative damage to PVB interneurons that could have been detected an earlier time-point than late adolescence, when PVB loss had already occurred. We also highlight that late adolescent PVB loss was only seen in MS-exposed males but not females. This sex difference was consistent with our previous report that MS yields an earlier effect on PFC PVB in females compared to males, wherein PVB loss after MS was evident in juvenile females but normalized by adolescence (Holland et al., 2014). Taken together, the developmental mechanisms underpinning MS-attributable PVB loss are not well understood; however, our lack of support for an immune-oxidative stress mechanism may be due to the late assessment of these measures in the current study.

Cognitive impairment in MS-exposed adolescents was prevented by EE, whereas PVB loss was not. However, PVB levels were significantly correlated with win-shift performance, supporting the strong evidence that PVB interneuron function is critical for cognitive functions such as working memory (Lewis et al., 2005; Wilson et al., 1994). Additionally, we observed a surprising impact of MS on the relationship between PFC PVB levels and cognitive performance. Control subjects expressing higher levels of PVB committed more errors on the win-shift task, whereas MS-exposed subjects displayed a negative relationship between PVB and win-shift errors. Recent work by Hensch and colleagues has revealed that PVB-containing interneurons play an important role in the establishment of critical periods for development and plasticity (Takesian & Hensch, 2013; Yang, Lin, & Hensch, 2012); therefore, we hypothesize that in typically developing animals, less pruning of PVB interneurons may represent a relative lack of plasticity in the PFC and a relative cognitive impairment. However, MS-exposed

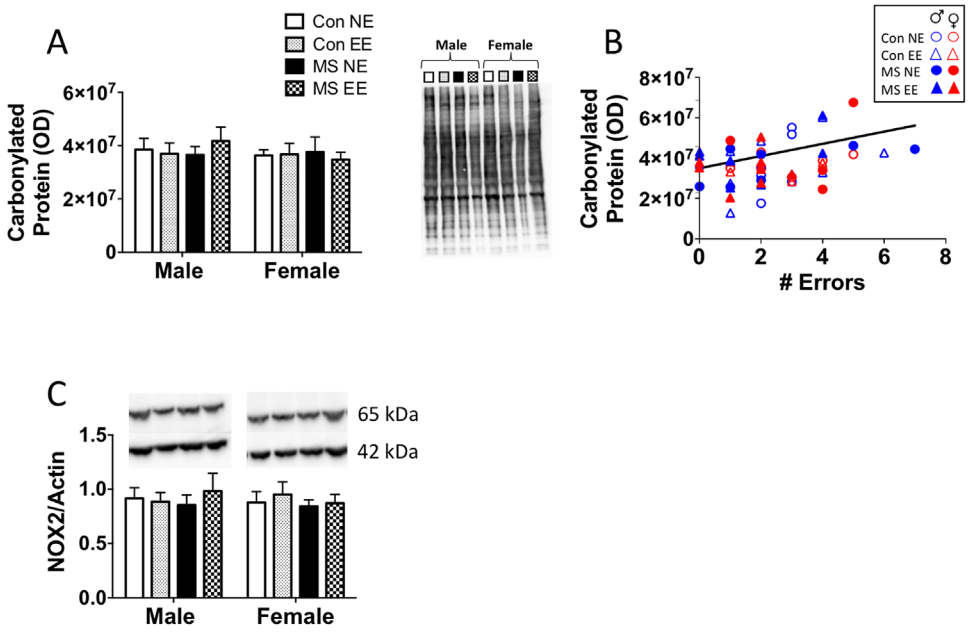

FIGURE 5 Effects of maternal separation (MS) and environmental enrichment (EE) on oxidative stress and oxidative damage in the prefrontal cortex. (A) Levels of carbonyl groups in the adolescent PFC were not affected by rearing or treatment condition ($n = 7$–9). Inset shows representative lanes from Western blots. (B) Scatter plot showing correlation between carbonyl groups and win-shift performance in all subjects ($n = 57$). (C) Adolescent PFC NOX2 (relative to actin) was not affected by MS or EE ($n = 7$–9). Inset shows representative bands for NOX2 (65 kDa) and actin (42 kDa). Means ± SEM are shown.

animals display a greater loss of PVB than controls (Fig. 3), likely to the point of a deficit that yields cognitive dysfunction. Indeed, hypofunction of PFC GABA signaling has been shown to increase spatial working memory errors, which require prospective foraging similar to phase 2 of the win-shift task (Auger & Floresco, 2014). Other developmental manipulations such as neonatal ventral hippocampal lesions have also been shown to disrupt both PVB interneuron signaling within the PFC (Tseng et al., 2008) and performance on the win-shift task (Brady, Saul, & Wiest, 2010). However, the inability for EE to protect PFC PVB interneurons may speak to the nature of the enrichment used or a need for enrichment *during* MS, when PVB development may be most vulnerable.

Although the link between MS and PVB loss requires more investigation, prolonged or delayed effects of early life stress on circulating inflammatory mediators have been repeatedly observed in humans and animals (e.g., Dennison, McKernan, Cryan, & Dinan, 2012, see Ganguly & Brenhouse, 2015 for review) and appear from our data to mediate behavioral dysfunction (Fig. 4). Interestingly, TNFα was only affected in MS-exposed males, but not in females. Few studies to date have directly investigated the sex-specific effects of early life stress on immune programming. We have reported that males but not females expressed heightened cyclooxygenase-2 in the PFC after MS (Holland et al., 2014), suggesting that males may be more vulnerable than females to the inflammatory consequences of early life stress. Further supporting this hypothesis are the reports that males are more sensitive than females to the long-term effects of early-life immune challenges (Bilbo, Smith, & Schwarz, 2012; MacRae et al., 2015; Schwarz & Bilbo, 2012), and that the effect of MS on later endotoxin-induced sickness behavior is more severe in males compared to females (Avitsur & Sheridan, 2009). The developmental mechanisms underpinning these sex-dependent effects are not known. However, during puberty testosterone has been shown to promote an anti-inflammatory profile in males (Zhang, Xing, He, & Wang, 2007); therefore, the observed pro-inflammatory profile in MS-exposed adolescent males suggests that testosterone modulation of cytokines may be diminished in these rats. Indeed, adolescent male rats exposed to MS have been shown to display reduced testosterone levels (Bodensteiner, Christianson, Siltumens, & Krzykowski, 2014). Although we did not assess gonadal

hormones in the present study, the differential influence of hypothalamic–pituitary–gonadal axis development on immune function in males and females may contribute to sex-dependent effects of MS and requires further study.

EE prevented the MS-induced pro-inflammatory profile in adolescent males. The protective effect of EE further supports an anti-inflammatory effect of EE that has been observed in typically developing (Arranz et al., 2010) (Singhal, Jaehne, Corrigan, & Baune, 2014) and developmentally challenged (Connors, Shaik, Migliore, & Kentner, 2014) animals. Additionally, the protective effects of EE on inflammatory cytokines co-occurred with protection against cognitive deficits in the present study. These findings corroborate clinical reports that children taken from Romanian institutions by age 2 and raised with proper care showed improvement in cognitive function later in childhood (Rutter, 1998). Therefore, our data support and highlight the usefulness of an enriched environment for prevention of posttraumatic sequelae.

The EE paradigm used here offered access to larger social groups, novelty, and increased access to activity during the postweaning, peri-adolescent period. EE of this type during the peri-adolescent period has previously been shown to be more effective than physical enrichment alone, without a social component (Elliott & Grunberg, 2005). Other mechanisms of enrichment, such as early handling during the preweanling period, also reportedly buffer the effects of later stress exposure with regards to inflammation and cognition (Bilbo et al., 2007; George, Stout, Tan, Knox, & Liberzon, 2013). Taken together with the current report, both perinatal and postweaning environmental conditions appear to affect later physiological and psychological development.

In conclusion, the current study illustrated that MS can impair cognitive and inflammatory processes that co-occur with PFC PVB loss. Although neither MS nor EE affected measures of oxidative stress in the adolescent PFC, evidence for oxidative damage was correlated with cognitive function; therefore, more work is needed to determine whether MS affects oxidative stress during early development to yield PVB loss and/or behavioral impairments.

NOTES

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