Early life stress disrupts social behavior and prefrontal cortex paravalbumin interneurons at an earlier time-point in females than in males

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Abstract

Exposure to early life stress (ELS) increases susceptibility to psychiatric disorders later in life. However, the mechanisms through which ELS leads to increased vulnerability to mental illness, which affects males and females differently, is largely unknown. ELS alters the function of the prefrontal cortex (PFC), a brain region that mediates emotional and cognitive functions. PFC dysfunction after ELS could be caused in part by the loss of interneurons that express the protein paravalbumin (PVB). Loss of PVB could underlie several behavioral deficits, such as social interaction. In this study, we investigated sex and ELS exposure interact to affect both PFC paravalbumin and social interaction over development. We also measured the inflammatory mediator cyclooxygenase-2 (COX-2), which plays an important role in oxidative stress and neuronal damage, in an effort to elucidate the mechanism behind PVB loss after ELS. After ELS, females exhibited an earlier change in social behavior and expressed PVB loss earlier in development than did males. Additionally, ELS males displayed more COX-2 (indicating inflammation or oxidative stress) in adolescence, while no changes were seen in females. These divergent profiles in male and female rats display sexually dimorphic responses to ELS.

Introduction

Individuals that are exposed to ELS are vulnerable to psychiatric disorders such as schizophrenia, anxiety, and depression, which persist throughout adulthood.1-3 Consequences of ELS often do not emerge until adolescence or early adulthood.4 The maternal separation paradigm of ELS in rodents leads to deficits in PFC-mediated behaviors.5-7 PVB interneurons have been suggested to be vulnerable to oxidative stress.8-9 COX-2 expression is increased in the prefrontal cortex in mice after ELS.8-9 As males and females respond differently to stress, investigating sex differences after ELS on brain development and social behavior provides insight into how rats respond differently to their environments.

Methods

Subjects: Pregnant female multiparous Sprague-Dawley rats were obtained from Charles River Laboratories 4 postnatal day (P1). Litters were randomly assigned to either a maternal separation group (ELS group) or a control group (CON group), whereby standard facility rearing was maintained. Pups in the ELS group were isolated for 4 h per day in a thermoneutral environment between P9-20. Rats were housed with food and water available ad libitum in constant temperature and humidity conditions on a 12-h light-dark cycle (light period 0700-1900).

Tissue Collection: Separate groups of rats were rapidly decapitated at either P25-27 (juvenile: n=7-8/group) or P42-45 (adolescence: n=7-8/group). The prefrontal cortex was dissected, flash frozen on dry ice, and stored at −80°C until Western blot analysis.

Social Interaction: Separate runs of rats were tested for social interaction at either P25 or at P40. ELS or CON subjects were marked and placed individually into a Plexiglas open field arena (100cm x 100cm) for 10 minutes to habituate to the environment. A naive conspecific of same sex and equal age was then placed into the arena for a separate 10 minute habituation period. The ELS or CON animal was then introduced into the arena facing away from the conspecific, at opposite sides of the arena. Both rats were monitored for 30 minutes by a CCTV camera suspended directly above the arena, interfaced with EthoVision to analyze nose-to-nose contacts, locomotion, distance between subjects, approach (moving towards conspecific), and avoidance (moving away from conspecific). Cumulative duration, frequency, and latency to first of nose-to-nose and nose-to-tail contacts were measured using a minimum distance of 5cm set as the threshold.

Western Blotting: Tissue was processed for IOD-PAGE and blots were incubated in primary antibody (1:100 rabbit polyclonal anti-PVB [PA1-933, Thermo Scientific] or 1:500 rabbit polyclonal anti-COX-2 [23602, Millipore]) overnight at 4°C. Blots were then incubated in secondary antibody (1:1000 Peroxidase goat anti-rabbit (IgG) antibody [P-1600, Boster]) in PBS for 1 hour at room temperature and visualized using 0.02% 3,3′-diaminobenzidine tetrahydrochloride (DAB, Thermo Scientific) at pH 6.5 for 15 minutes at 50°C. Blots were re-blocked and probed with anti-tubulin (1:2000 rabbit monoclonal anti-Tubulin [T4026, Sigma-aldrich]) and anti-mouse secondary antibody (1:2,000 Peroxidase horse anti-mouse IgG antibody [PI-2000 Vector Laboratories]). SeeBlue Plus 2 (LC5925, Life Technologies) are standard controls for molecular weight determination.

Data Analysis: Latencies, durations, frequencies and distances collected during the social interaction assessment were compared between groups using three-way analysis of variance (ANOVA) with Sex, Group (ELS, CON), and Age as interacting factors. Two- and three-way interactions were followed up with Bonferroni post-hoc tests or with Sidak-Bonferroni post-hoc tests when homogeneity of variances could not be assumed. Optical densities of each Western blot band were normalized to tubulin, and two-way ANOVAs followed by post-hoc tests compared Group and Age effects on male and female PVB and COX-2 levels in the PFC.

Results

ELS males show behavioral deficits during adolescence, while ELS females show the deficits in juvenility

ELF males had decreased PVB during adolescence, while ELS females had decreased PVB during juvenility

Figure 1: Duration of (A) latency for (B) engaging in nose-to-nose contact with a conspecific is affected by ELS. locomotion (C) did not differ between any group at either age.

Conclusions

• Males subjected to ELS displayed social interaction changes that first appeared in adolescence (Figure 1), which coincided with a decrease in PFC PVβ during adolescence (Figure 3).

• Females presented social interaction changes during juvenility (Figure 1), which is when their PVB levels were decreased (Figure 3).

• COX-2 changes occurred in ELS males, but not females. This could be due to sex differences in the mechanisms that control the frame that causes PFC deficits (Figure 3).

• While we previously reported that increased PFC COX-2 was directly correlated to decreased PFC PVβ, the cause of PVB death after ELS is still unknown

References

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