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Short communication

Sex-specific effects of early life stress on social interaction and prefrontal cortex dendritic morphology in young rats

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HIGHLIGHTS

- Maternal separation has sex-dependent effects on social interaction at P25.
- Maternal separation results in dendritic hypertrophy in infralimbic cortex of females, but not males.
- There is a positive relationship between latency to approach a conspecific and infralimbic apical dendritic length in females, but not males.
- There are sexually dimorphic effects of maternal separation across different time points in development.

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ABSTRACT

Early life stress has been linked to depression, anxiety, and behavior disorders in adolescence and adulthood. The medial prefrontal cortex (mPFC) is implicated in stress-related psychopathology, is a target for stress hormones, and mediates social behavior. The present study investigated sex differences in early-life stress effects on juvenile social interaction and adolescent mPFC dendritic morphology in rats using a maternal separation (MS) paradigm. Half of the rat pups of each sex were separated from their mother for 4 h a day between postnatal days 2 and 21, while the other half remained with their mother in the animal facilities and were exposed to minimal handling. At postnatal day 25 (P25; juvenility), rats underwent a social interaction test with an age and sex matched conspecific. Distance from conspecific, approach and avoidance behaviors, nose-to-nose contacts, and general locomotion were measured. Rats were euthanized at postnatal day 40 (P40; adolescence), and randomly selected infralimbic pyramidal neurons were filled with Lucifer yellow using iontophoretic microinjections, imaged in 3D, and then analyzed for dendritic arborization, spine density, and spine morphology. Early-life stress increased the latency to make nose-to-nose contact at P25 in females but not males. At P40, early-life stress increased infralimbic apical dendritic branch number and length and decreased thin spine density in stressed female rats. These results indicate that MS during the postnatal period influenced juvenile social behavior and mPFC dendritic arborization in a sex-specific manner.

1. Introduction

Exposure to early life adversity or stress increases vulnerability to psychiatric disorders later in life, including depression, drug abuse, and schizophrenia [1–5]. Maternal separation (MS) in rodents is a well-established animal model of early life stress that induces long-lasting alterations at behavioral, immunological, neuroanatomical, biochemical, and molecular levels [6,7]. In particular, the late-developing medial prefrontal cortex (mPFC) is especially vulnerable, exhibiting compromised connectivity [8] and function [9,10] after MS exposure. Although there has been mounting evidence that early life experience can strongly alter structural plasticity of the mPFC, the direction of dendritic remodeling depends on the type and timing of stress (prenatal, perinatal, or postnatal), the region of mPFC (anterior cingulate, prelimbic, or infralimbic), and the age at which the brain is examined (juvenile, adolescence, or adulthood). Generally, prenatal [11–16] and postnatal [9,11,17–20] stress exposure results in atrophy of apical dendrites of pyramidal neurons within the mPFC of males. Spine density in the mPFC also tends to decrease as a result of early life stress [9,21]; however, Bock and colleagues [18] reported increased spine density within the anterior cingulate of male rats after MS during P14-P16, and others reported increased Cg3 spine density

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in adult males and females after MS during P3-P21 [17,19]. The effects of MS in adolescent females have not been investigated.

Early adversity has been shown to induce changes in social behaviors—including avoidance, fear, and decreased social interaction [22,23]—of which the mPFC is a substrate. Since the size and shape of dendritic arbors can influence the functional properties of neurons [24], it is important to understand how early life stress can influence structure-function relationships in the mPFC. Morphological changes in the mPFC are linked to early social and play experiences [25] and immediate early gene expression in the mPFC after isolation rearing and social interaction is region-dependent [26]. In humans, social dysfunction is also highly comorbid with stress-linked psychopathology; therefore, investigating deficits in social interaction is critical for understanding how stress can disrupt normal development.

Importantly, several of these behavioral and morphological stress-linked changes have been shown to occur in a sex-dependent manner. There are sex differences in dendritic maturation of the mPFC, with dendritic ramification occurring earlier in female rats [15]. Sex differences in morphological effects of early life stress have been less well studied, and the few that did include females found conflicting results [11,14], most likely due to differences in stress paradigms and developmental windows. Therefore, the goal of the current study was to better understand the effects of a MS early life stress paradigm on the structure and function of an understudied region of the mPFC, the infralimbic (IL) cortex. We report here that early-life stress differentially affected juvenile social behavior and adolescent IL dendritic morphology in a sex-specific manner.

2. Materials and methods

2.1. Subjects and maternal separation

Gestational day 15 pregnant Sprague-Dawley rats were ordered from Charles River Laboratories (Wilmington MA). Litters were culled to 10 pups with as close to five males and five females as possible on postnatal day (P) 1. The dams and pups were fed and given water *ad libitum* throughout gestation and development. 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. Only one male and one female per litter were used to avoid litter effects.

Maternally separated (MS) rats were separated for 4 h/day from P2-P20 in a thermo- regulated environment kept at 37 °C. Apart from weighing—where pups were removed from their home cage for 5 min at days P9, P11, P15, P20—control unstressed (CON) rats were not disturbed until weaning. Pup weights were not different between groups. During weaning, pups were rehoused with a cagemate matched for age, sex, and group. Subjects were not disturbed after weaning besides behavioral testing until sacrifice.

2.2. P25 social interaction

Social interaction was assessed at P25, an age at which subjects have been weaned but have not yet begun to enter puberty and therefore were not influenced by differential pubertal development in males and females [27]. Additionally, we previously reported effects of MS on juvenile social behavior at P25 in females, but not males [28]. Individual MS or CON rats (n = 12/group/sex) were marked and placed into a Plexiglas open field arena (100 cm × 100 cm) for 10 min to habituate. A naïve conspecific of same sex and equal age was marked then placed into the arena for a separate 10-min habituation period. The MS or CON rat was then reintroduced to the arena facing away from the conspecific, at opposite sides of the arena. Both rats were monitored for 30 min by a CCTV camera (Panasonic WV- CP500, Secaucus NJ) suspended directly above the arena. The camera was interfaced with EthoVision (v9.0; Noldus Information Technology, Leesburn VA), which digitally analyzed nose-to-nose contacts, nose-to-tail contacts, locomotion, distance between subjects, moving towards conspecific, and moving away from conspecific. The Noldus software differentiated between CON/MS and conspecific rats through color discrimination of the colored marks given to the rats prior to their habituation. Cumulative duration, frequency, and latency to first of nose-to-nose and nose-to-tail contacts were measured using a minimum distance of 5 cm set as the threshold for contacts. The cumulative duration of time subjects exhibited locomotion was recorded with a minimum velocity of 2 cm/s set as the threshold. The mean distance between subjects for the social interaction trial was also measured. The frequency and cumulative duration of approach and avoidance was measured with a threshold of 50 cm set as the minimum distance required for approach or avoidance behavior. The arena was cleaned with 30% ethanol between each test.

2.3. P40 morphology analysis

2.3.1. Euthanasia and tissue preparation

Animals were anesthetized and sacrificed by transcardial perfusion of 4% paraformaldehyde in 0.1 mol/Lphosphate buffer (phosphate-buffered saline, pH 7.4) at P40. This age was chosen due to the vulnerability of the PFC at this age to earlier developmental insults, which has been proposed to result from transient developmental events that span from synaptic rearrangement to receptor overproduction and pruning (reviewed in [29]). Brains were extracted and post-fixed in paraformaldehyde for 6 h and then placed in 0.1% sodium azide in phosphate-buffered saline at 4 °C for storage.

2.3.2. Dendritic morphology analysis

We collected 250-μm, IL-containing sections using a vibrating microtome (Leica Microsystems, Inc, Buffalo Grove, Illinois). IL neurons were visualized through a DAPI filter (Zeiss Microscopy, Thornwood, New York) on a Zeiss Axio Examiner A.1 microscope (Zeiss Microscopy). Iontophoretic microinjections of fluorescent dye Lucifer yellow were performed into IL layer II/III pyramidal neurons using a DC current of 1–6 nA for 5–10 min, until distal processes were filled with dye and no further loading could be observed. Sections were mounted and placed in a coverslip in VECTASHIELD (Vector Laboratories, Burlingame, California), and filled neurons were selected for imaging and analysis based on completeness criteria described previously [27,28]. Three to eight neurons per animal (control male: 32 neurons from 6 animals; MS male: 36 neurons from 6 animals; control female: 40 neurons from 7 animals; MS female: 33 neurons from 7 animals) were included in the analysis. For off-line tracing of apical dendrites, multiple Z-stacks of whole neurons were acquired using an Olympus FV1000 confocal microscope (Optical Analysis Corporation, Nashua, New Hampshire) with a 60 × lens and a step-size of 1 μm. Montages of all images covering the apical dendrite of one neuron were created and traced using Neurolucida (MBF Bioscience, Williston, Vermont). Apical length, branch number, and Sholl analyses were performed with Neurolucida Explorer (MBF Bioscience). For spine analysis, spine segments from apical dendrites were selected in 50-μm increments from the cell body, and approximately eight segments per neuron were sampled. Z-stacks were acquired using a 100 × lens with a zoom of 3.3, NA 1.4, and step size of 0.08 μm. Raw Z-stacks were deconvolved with AutoQuant (Media Cybernetics, Rockville, Maryland) and analyzed for spine number and shape (thin, stubby, or mushroom) using NeuronStudio software (Com-