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# Early life stress leads to developmental and sex selective effects on performance in a novel object placement task

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ABSTRACT

Disruptions in early life care, including neglect, extreme poverty, and trauma, influence neural development and increase the risk for and severity of pathology. Significant sex disparities have been identified for affective pathology, with females having an increased risk of developing anxiety and depressive disorder. However, the effects of early life stress (ELS) on cognitive development have not been as well characterized, especially in reference to sex specific impacts of ELS on cognitive abilities over development. In mice, fragmented maternal care resulting from maternal bedding restriction, was used to induce ELS. The development of spatial abilities were tracked using a novel object placement (NOP) task at several different ages across early development (P21, P28, P38, P50, and P75). Male mice exposed to ELS showed significant impairments in the NOP task compared with control reared mice at all ages tested. In female mice, ELS led to impaired NOP performance immediately following weaning (P21) and during peri-adolescence (P38), but these effects did not persist into early adulthood. Prior work has implicated impaired hippocampus neurogenesis as a possible mediator of negative outcomes in ELS males. In the hippocampus of behaviorally naïve animals there was a significant decrease in expression of Ki-67 (proliferative marker) and doublecortin (DCX-immature cell marker) as mice aged, and a more rapid developmental decline in these markers in ELS reared mice. However, the effect of ELS dissipated by P28 and no main effect of sex were observed. Together these results indicate that ELS impacts the development of spatial abilities in both male and female mice and that these effects are more profound and lasting in males.


## 1. Introduction

Early in life, parental interactions can serve as a buffer against the negative consequences of stress on physiology and learning (Stanton and Levine, 1990; Kirschbaum et al., 1995; Heinrichs et al., 2003; Shionoya et al., 2007; Taylor et al., 2008; Gee et al., 2014; van Rooij et al., 2016). However, if rearing conditions are suboptimal, parental stress or disruptions in the quality or reliability of care can be rapidly transmitted to the offspring and serve as a primary source of stress, driving changes in development and neurobehavioral outcomes (Levine, 1967; Rosenfeld et al., 1991; Suchecki et al., 1993; Liu et al., 1997; Avishai-Eliner et al., 2001; Rice et al., 2008; Raineki et al., 2010; Roth et al., 2013; Molet et al., 2014; Bath et al., 2016; Heun-Johnson and Levitt, 2016). Significant disruptions in the quality of early life care impact neural structure and functional plasticity of the brain (Teicher et al., 2006; Chen et al., 2008, 2013) and have been identified as potential catalysts for negative health outcomes, including disturbance in cognitive development. For example, in humans, institutionalized rearing or abusive early environments have been associated with the development of significant impairments in general cognitive functioning with specific deficits identified in memory recall (Bremner and Narayan, 1998) and short term memory (Bremner et al., 2000). These same

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experiences are associated with regional effects on brain development, with significant reductions in hippocampal volume and cortical thinning in frontal regions associated with memory function, attention, and spatial abilities (Bremner et al., 1997).

Significant sex disparities have been identified for stress-associated pathology, with females being twice as likely as males to develop PTSD, depression, and anxiety disorders (Weissman et al., 1996; Breslau et al., 1997a, 1997b; Felitti et al., 1998; Gater et al., 1998; Burt and Stein, 2002; Kuehner, 2003; Keita, 2007; Breslau, 2009; De Munck et al., 2009; Hankin, 2009; Olino et al., 2010; Pratchett et al., 2010). However, whether similar sex disparities exist for stress-associated cognitive disturbance have not been as well characterized. Recent studies in animal models have provided mixed results, but have identified sex, developmental status, and timing of the stressor, as potential variables contributing to risk for cognitive outcomes. For example, some studies have reported significant impairment following various forms of ELS on spatial learning in female rats (Marco et al., 2013; Wang et al., 2016), while others found a male bias in impairment in both mice and rats (Barha et al., 2007; Mueller and Bale, 2007; Salomon et al., 2011; Schulz et al., 2011; Wang et al., 2011; Naninck et al., 2015). Other studies from both mice and rats failed to identify sex differences in performance (Benoit et al., 2015; Nazeri et al., 2015), while further studies in rats found ELS to be associated with improved cognitive performance (Barha et al., 2007; Zuena et al., 2008; Uysal et al., 2012; Barbie-Shoshani et al., 2016). Thus, considerable confusion exists with regard to the effects of ELS on cognitive functioning, possibly due to the varied forms of stress, timing of stress implementation, and age at testing. It should be noted that the majority of studies focused on assessing outcomes at one or two time points in development, typically late adolescence or adulthood, or failed to take into account possible developmental changes in performance on cognitive measures.

Here, the effect of ELS, in the form of maternal bedding restriction from P4-P11 (Rice et al., 2008; Bath et al., 2016), was tested on the development of spatial learning in male and female mice across early development. To do this, ELS and control reared mice were tested on a novel object placement (NOP) task, at postnatal days 21, 28, 38, 50, and/or 75. This allowed for assessment of time points that approximate childhood (P21), the pre-adolescent period (P28), the peri-adolescent period (P38), early adulthood (P50), and adulthood (P75) in mice. To minimize potential practice effects and diminish the contribution of any single litter on the overall results, a large number of litters were sampled (26), and no mouse was tested at more than 2 developmental time points. Significant sex disparities in risk for cognitive outcomes following ELS were observed. Males showed early emergence and persistent impairments in performance on the NOP task, while females showed an earlier but transient impairment in NOP performance. The current data suggest that stress may have sex and developmental selective effects on the emergence of cognitive disturbance, and such factors may be critical in understanding the contribution of stress and sex to developmental pathology.

## 2. Methods

### 2.1. Subjects

Breeding stock of male and female C57Bl/6N mice were acquired from Charles River labs and all mice used for the current studies were derived from litters that had been bred in house. Animals were maintained under normal housing conditions on a 12h:12h light cycle with *ad libitum* access to food and water. Pups were weaned and sex segregated at 21 days of age. All animal procedures were approved by the Brown University Institutional Animal Care and Use Committee and consistent with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

### 2.2. Fragmented maternal care

Four days following the birth of a litter, the dam and pups in the fragmented maternal care condition were transferred from their standard home cage, to a home cage with a wire mesh floor and a $2 \times 4$ cm cotton nestlet as their only source of bedding (as described in Bath et al., 2016). Mice continued to have *ad libitum* access to food and water. Dam and litters remained in these modified housing conditions for seven days, and were then returned to standard housing, containing cob bedding and a $4 \times 4$ cm nestlet. Control mice were left undisturbed throughout these procedures. Litters were composed of both male and female pups, and litters ranged in size from 5 to 8 pups per litter. Pups were not culled and natural variation in sex distributions were allowed. Pups were derived from 26 different litters with a range of sex distributions. Previous work in both mice and rats have shown that the bedding restriction manipulation leads to a fragmentation in maternal care and elevations in stress hormones in the dam immediately following the stressor (Avishai-Eliner et al., 2001; Rice et al., 2008; Bath et al., 2016; Heun-Johnson and Levitt, 2016; Molet et al., 2016). In mice and rats, ELS housing leads to an increase in the number of departures by the dam from the nest, but no change in the duration or total time spent licking and grooming or arched back nursing (Heun-Johnson and Levitt, 2016; Molet et al., 2016). Here, detailed assessment of maternal behavior was not carried out. Instead, successful replication of core features of this paradigm were used to verify the efficacy of the manipulation, including diminished weight gain of pups, an effect that was observed in both male and female mice (Fig. 1).

### 2.3. Open field task

At 20 days of age, prior to beginning the novel object placement task, all mice received an initial exposure to the open field testing apparatus (the same apparatus used for NOP testing). Open field testing occurred between the hours of 9AM and 12PM, under approximately 15–20Lux of light and lasted for a total of 7 min. During this time, mice were video recorded and their activity was tracked with the aid of digital tracking software (Noldus Ethovision XT 8.5). To determine if ELS or sex significantly impacted locomotion within the testing apparatus, total distance traveled was quantified. Percent time in the center of the open field was used to test if ELS altered anxiety-like behavior.

### 2.4. Novel object placement task

One day prior to testing, mice were again habituated to the empty open field for 7 min to acclimate them to both handling as well as the testing environment. Testing occurred between the hours of 9AM and 12PM, under approximately 15–20Lux of light. A total of 26 different litters of mice were used. At each age, groups included mice from a minimum of at least 4 different litters. Testing consisted of an exploration phase (T1, exploration trial) and a recognition phase (T2, recognition trial). In the exploration trial, mice were placed in the open field with two identical objects (Supplemental Fig. 1) for 5-min. Investigation of the objects was timed using automated tracking software (Noldus Ethovision XT 8.5), with investigation defined as the subject's nose being directed at and within 1 inch of the object. After the T1 exploration phase, the subject was removed from the open field for 25 min. ITI duration was chosen based on prior work in rats, which found that 21 day old rats could complete the task with short $<$ 1hr, but not long