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Type of early life adversity confers differential, sex-dependent effects on early maturational milestones in mice

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ABSTRACT

Early life adversity (ELA) increases risk for negative health outcomes, with sex disparities in prevalence and form of ELA experienced and risk for neuropsychiatric pathology. ELA comes in many forms (e.g. parental neglect/loss, limited access to resources) but whether disparate forms of ELA have common effects on outcomes, and if males and females are equally affected, remains unknown. Epidemiological studies often fail to accurately account for differences in type, timing, and duration of adversity experienced. Rodent models allow precise control of many of these variables. However, differences in the form of ELA, species, strain, housing, and testing paradigms used may contribute to differences in outcomes leading to questions of whether differences are the result of the form of ELA or these other variables. Here, we directly compared two mouse models of ELA, maternal separation (MS) and limited bedding (LB) in males and females on development of the body, motor and visual milestones, stress physiology, and anxiety-like behavior. LB affected timing of early milestones, somatic growth, and stress physiology in both sexes, yet only females showed later anxiety-like behaviors. MS rearing affected males and females similarly in early milestone development, yet only males showed changes in stress physiology and anxiety-like outcomes. These studies provide a platform to directly compare MS and LB models within one lab. The current work advances our understanding of the unique features of ELA that shape early neurodevelopmental events and risk for later pathology, increasing the translational relevance of these ELA models.

1. Introduction

Early experiences play a significant role in guiding the timing and trajectory of neuronal and behavioral maturation. Attentive parental care can buffer against deleterious effects of stress (Iwata et al., 2007; Nawaz et al., 2018) and is associated with improved cognitive functioning, including improved performance on learning and memory measures (Del Arco et al., 2007; Hullinger et al., 2015). Conversely, diminished quality or quantity of care, abuse, or reduced access to resources experiences (e.g. early life adversity) and enriching experiences have been associated with increased risk for psychopathology (Dong et al., 2003; Dube et al., 2001; Felitti et al., 1998; Nelson et al., 2011), altered brain maturation (Gee et al., 2013; Honeycutt et al., 2020), impaired performance on learning tasks (Goodwill et al., 2018b), delayed sexual maturation (Manzano Nieves et al., 2019), and impaired regulation of the stress response (Sanchez, 2006; Sanchez et al., 2010). Early life adversity (ELA) is highly prevalent, however the type, timing, and duration of these experiences are often unique across individuals (Anda et al., 2006). In addition, sex and genetic background can contribute to differences in later risk for negative outcomes (Bath, 2020). Thus, it is important to better understand how unique types of adversity may shape neural and behavioral developmental trajectories. Controlling for these key variables will advance our understanding of the contribution of those experiences to vulnerability or resilience to pathological outcomes later in life. Given the rapid development and precise control of genetic and environmental parameters, rodent models of ELA provide an important testbed to determine the impact of different forms of adversity on brain and behavioral maturation.

Here, the effects of two commonly employed forms of ELA in rodents, limited bedding (LB) and maternal separation (MS), were compared with regard to their effects on the trajectory of multiple measures of sensory, motor and affective development, as well as neural maturation in male and female mice. These models of ELA have provided evidence for increased risk for later life negative outcomes, that may be the consequence of altered trajectory of basic neurodevelopmental programs and/or changes in the timing of regional brain development. The consequences of altered development could have lasting consequences on core systems involved in sensory, motor, learning, and

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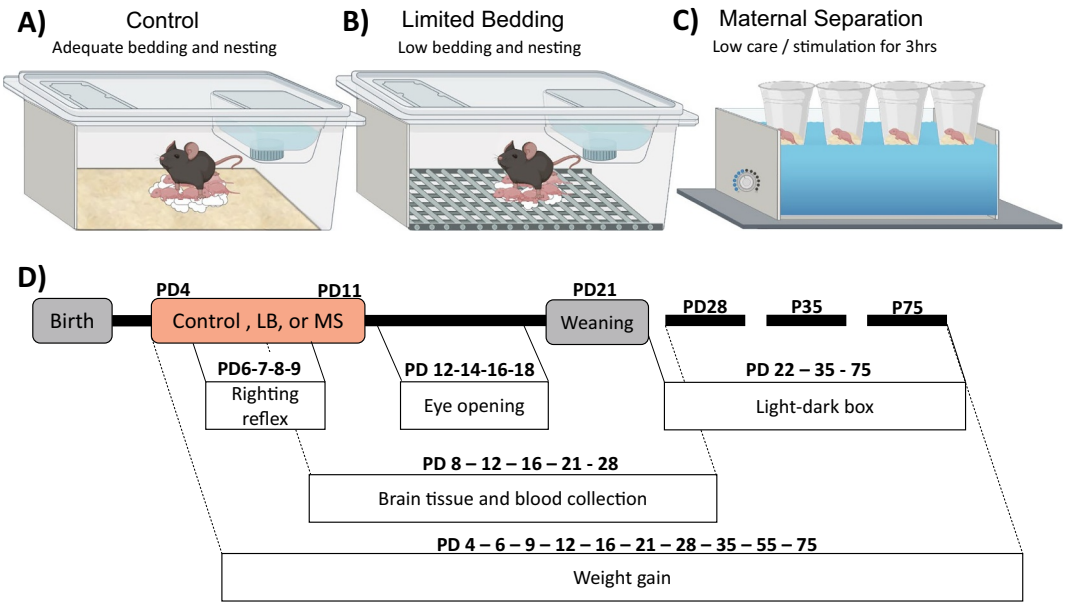

Fig. 1. Experimental design. At birth, litters were assigned to one of three treatment groups. Control reared litters were maintained in standard housing conditions with adequate bedding and 4 × 4 cm cotton nestlet (A). Limited bedding reared pups and dam were placed in a cage with a wire mesh floor, no bedding, and a 3 × 4 cm cotton nestlet from PD4-PD11 (B). Maternal separation reared pups and dam were housed in a standard home cage with bedding and 4 × 4 cotton nestlet. From PD4-PD11, maternal separation pups were removed daily from their home cage for 3 h a day into cups placed in a water bath maintained at 35–37 °C (C). A variety of early developmental outcomes were measured (D). Weight gain was measured from PD4-PD75. Righting reflex was tracked daily from PD6-PD9 to assess the trajectory of motor milestone development. Eye opening was tracked daily from PD12-PD19 to assess the trajectory of visual development. Brain tissue and blood were collected at PD8, PD12, PD16, PD21 and PD28 to assess effects of ELA on the timing of motor cortex maturation and on the trajectory of basal corticosterone levels. Finally, anxiety-like behaviors were assessed via light-dark box task at PD22, PD35, PD75.

emotional regulation (Bath et al., 2016; Ganguly and Brenhouse, 2015; Gee et al., 2013; Manzano Nieves et al., 2019). The two models were designed to parallel forms of adversity experienced by humans, which have been shown to differentially affect outcomes (Fisher et al., 2010). The LB model was designed to simulate stress associated with loss of resources, such as extreme poverty, homeless, or refugee settings. The MS model was designed to simulate stress associated with loss or absence of parental care, such as institutional rearing and parental separation. Although each of these forms of ELA ostensibly alter dam:pup interactions, the type of adversity experienced are different and may have unique consequences on developmental processes (Peña et al., 2019). Further, as these procedures are often carried out by different labs, variability in protocol administration, testing context, animal care, species, and colony composition makes comparison or generalization of results across the different paradigms difficult. Here, the two ELA manipulations were carried out within the same lab, using the same breeding colony of mice, the same investigator, and same testing apparatus, to allow for direct comparison of the effects of each of these experiences on early neurodevelopment. In addition, because adversity has been shown to affect males and females differently, both males and females were included and analyzed in parallel (Coley et al., 2019; Ganguly et al., 2019; Gobinath et al., 2015; Goodwill et al., 2018a; Goodwill et al., 2018b; Honeycutt et al., 2020; Oyola and Handa, 2017).

The impact of early experience had unique consequences on different systems, that depended on both the type of ELA and sex of the animal. Measures of early motor development were assessed by tracking righting reflex behavior and showed a developmental delay in response to both forms of ELA, with males and females being similarly delayed. For measures of early visual development (assessed by the timing of eye opening), both forms of ELA resulted in a delayed trajectory in males and females. However, the effects of LB were more robust than those observed for MS. For both somatic development (assessed by weight gain) and the maturation of HPA reactivity (assessed by basal corticosterone levels), the effects of LB were similar across sex, yet MS rearing led to sex-specific effects with males being more affected than females. Finally, effects of ELA on anxiety-like outcomes (assessed by light-dark box behavior) were found in LB females (with effects throughout development) and in MS males (with effects emerging in adulthood). This work directly compared, within a lab, the early developmental changes in two rodent models of ELA within one study and provides compelling evidence for sex- and experience- dependent effects of ELA. Overall, this work advances our understanding of the types of developmental mechanisms impacted in response to early adversity and highlights the potential unique contribution of each form of ELA on development and behavioral outcomes.

2. Methods

2.1. Mice and housing

C57BL/6N mice were bred in house and maintained on a 12 h:12 h light cycle (lights on at 7 AM) with ad libitum access to food and water. Mice were housed in 31 × 12 × 14 cm cages with cob bedding and 4 × 4 cm cotton nestlet. Litters were composed of both male and female pups and ranged from 3 to 8 pups per litter. A total of 113 litters were used for the studies described here. Virgin dams were not used for the current studies. Pups were weaned and sex segregated at postnatal day 21 (PD). All animal procedures were approved by the Brown University Institutional Animal Care and Use Committee and consistent with the guide for the care and use of animals in research.

2.2. Limited bedding (LB) manipulation

An experienced breeder female and male were pair-housed in a standard home cage with bedding and a 4 × 4 cm cotton nestlet for nest building. Four days following the birth of a litter (PD4), the dam and pups were transferred from their standard home cage, to a cage with a wire mesh floor, no bedding, and a 3 × 4 cm cotton nestlet (Fig. 1B).

Mice remained in the LB housing conditions for seven days. On PD11, the dam and pups were returned to their standard housing conditions where they remained until weaning at PD21.

2.3. Maternal separation (MS) manipulation

A breeder female and male were housed in a standard home cage with cob bedding and a 4 × 4 cm cotton nestlet. From PD4 to PD11, the pups were removed daily from their home cage for 3 h per day between the hours of ~9 AM and ~12 PM (Fig. 1C). During this separation, the pups were placed in individual cups that contained soiled home cage bedding and that were secured in cup holders in a circulating water bath maintained at 35–37 °C to support pup thermoregulation.

2.4. Developmental assays

The trajectory of early developmental outcomes were measured in both male and female control, LB, and MS pups to assess the impact of early life experience on these measures (see Fig. 1D for timeline of data collection).

2.4.1. Weight gain

Total weight of each animal was measured and recorded at PD4, PD6, PD9, PD12, PD16, PD21, PD28, PD35, PD55, and PD75. Measurements were taken by briefly removing the pup from the dam, placing it in a weighing cup, and then returning it to the dam within ~1 min of starting the manipulation.

2.4.2. Eye opening

To assess ELA effects on visual development, timing of eye opening was assessed beginning at PD12 and repeated every day until PD19. To do this, mice were individually identified by tail markings, briefly removed from the nest and visually inspected for presence of open eyes. Eye opening scores for individual animals were recorded as follows: both eyes closed = 0; half eye open = 0.5; both eyes half open = 1; one eye fully open and another eye fully closed = 1; one eye fully open and another eye half open = 1.5; two eyes fully open = 2. Following assessment pups were rapidly returned to the nest. Completion of this procedure for a single litter took an average of ~1–2 min.

2.4.3. Righting reflex

Pups were tested in the righting reflex task to assess the impact of ELA on the trajectory of motor development. Righting reflex behavior was measured daily from PD6 to PD9. For the task, a single pup was removed from the litter, placed on a flat surface on its back and their behavior was digitally recorded. The amount of time to right itself to a standing position (all four paws in contact with the ground) was measured. Pups were given a maximum of 60 s to successfully right themselves or were returned to the prone position. Following a successful right, or 60 s, mice were tested on the task again. Each pup was subjected to five consecutive trials on each day and data from individual pups were tracked independently to assess change in performance over time. Following testing, each pup was returned to the litter and the next pup was tested. The average time to right across the five trials for each day was recorded for each subject.

2.4.4. Light-dark box

Effects of ELA on development of a single form of anxiety-like behavior was assessed using the light-dark box task. The light-dark box apparatus was a 58 × 22 cm box that was divided in two equal sides connected by a small 5 × 5 cm opening. The dark side of the box was completely covered and unilluminated (~10 lx) and the light side was open and illuminated with approximately 2000 lx. At the start of the trial, the mouse was placed in the dark side of the box. Noldus Ethovision XT 11 was used to track the latency, duration, and frequency of entries to the light-side during the seven-minute task.

2.5. Physiological and neurodevelopmental assays

2.5.1. Corticosterone ELISA

Basal corticosterone (CORT) levels were assessed at several time points across early development to determine the impact of the different forms of ELA on HPA-axis development. All collection procedures occurred between 08:30–09:30 AM when baseline CORT is at its lowest (Kakihana and Moore, 1976). Trunk blood was collected by rapid decapitation at PD8, PD12, PD16, PD21 and PD28. To eliminate cohort effects or dramatic reduction in litter size, a maximum of two pups/sex were taken per litter at each developmental time point, and each litter was maintained at ~70% of initial size to eliminate potential stress associated with diminishing litter size. This led to a random sampling of pups from ~3–5 litters/timepoint. After collection, blood was allowed to clot for 1 h at room temperature and was then centrifuged at 10,000 rpm for 10 min. Serum (~50–150 µl) was collected and immediately stored at −20 °C for later analysis. Total CORT levels from serum (25× diluted) were determined using a competition-based ELISA (AssayMax, Corticosterone ELISA kit, AssayPro, St. Charles, MO; CAT: EC3001-1) using the manufacturer's protocol. This kit reports a sensitivity of up to 0.3 ng/ml, with a 5–7% intra-assay reliability, and <2% cross reactivity with steroid and stress related hormones. All samples across groups were run on a single plate with duplicates run across plates. ELISA plates were read to determine optical density on an OpsysMR plate reader (Dynex Technologies) at 450 nm. The mean optical density for each sample was calculated based on a standard curve, generated from the standardized CORT dilutions (100 ng/ml, 25 ng/ml, 6.25 ng/ml, 1.563 ng/ml, 1.563 ng/ml, 0.391 ng/ml, 0 ng/ml) and a curve was created using a four parameter logistic regression fit method to obtain the concentration of CORT (ng/ml) within each sample. Each sex was run on its own 96-well plate and quantified using the standard curve within that plate.

2.5.2. Brain tissue extraction, RNA purification, and cDNA synthesis

Brain punches were collected from the motor cortex in order to assess the maturational profile of this region during the period that righting reflex and locomotor activity was developing. The same cohort of mice was used for trunk blood and brain collection. The full brain was immediately extracted after blood collection and placed on dry ice. Brain punches were hand-dissected using Allen Brain Atlas as a guide (Paxinos and Franklin, 2001). Motor cortex punches were homogenized in RNAzol (Molecular Research Center, Cincinnati, OH) and stored at −20 °C until processing. Total RNA was isolated using the manufacturer's protocol. Briefly, DNA and protein were precipitated using isopropanol and RNA was precipitated using 75% ethanol. After precipitation, the RNA pellet was washed 3× in 75% ethanol, solubilized in 30 µl of RNAsecure resuspension solution (Thermofisher Scientific, CAT: AM7010) and stored in −20 °C until further processing. Single stranded cDNA was obtained using New England Biolabs MmULV protocols (NEB, Ipswitch, MA). cDNA was solubilized in 25 µl of 10 mM Tris-HCl, pH 7.5. 1 mM EDTA.

2.5.3. Realtime qPCR

Predesigned and pre-validated Taqman assays from Applied Biosystems (Life Technologies, Norwalk, CT) were run in multiplex with the housekeeping gene (18 s rRNA). For each plate, relative gene expression of doublecortin (*dcx)* (corrected for 18-s as loading control) was calculated based upon a standard curve created from pooled and serially diluted cDNA samples. Each sex was run on its own 382-well plate and quantified relative to the standard curve made from the cDNA samples within that plate. All gene expression profiling was completed using CFX384 RT-qPCR system (Biorad, Hercules, CA).

2.6. Statistics

A 3-way ANOVA (treatment × age × sex) was used to assess

interaction effects in weight gain, eye opening, and righting reflex. Subsequent 2-way ANOVAs were then carried out in males and females separately to test for main effects of treatment and age and their interactions on developmental measures of weight gain, eye opening, righting reflex. Due to methodological constraints on the number of samples we could include on each reverse transcription, qPCR, and ELISA plate, we did not feel confident in making direct comparisons of results between the sexes for gene expression, and basal corticosterone levels. Observed differences could be due to true sex differences, or technical differences due to the need to carry out assessment of male and female samples at separate time points and on different plates. Thus, a 2-way ANOVA was used to assess males and females separately for those measures. For measures of anxiety-like behavior, a 2-way ANOVA was used at each developmental time point separately (juvenile, adolescent, adult) to test for main effects of treatment, sex, and their interaction. Significant main effects (alpha = 0.05) were followed up with post-hoc tests using Tukey's LSD to correct for multiple comparisons. Effect sizes were estimated by calculating the value of Partial η^2 (SSeffect/SStotal + SSerror).

3. Results

3.1. ELA alters the trajectory of weight gain

A 3-way ANOVA revealed a significant treatment × sex × age interaction ($F_{(18, 5006)} = 4.074$, $p < 0.001$, partial $\eta^2 = 0.015$), in addition to a significant main effect of treatment ($F_{(2, 5006)} = 376.051$, $p < 0.001$, $\eta^2 = 0.132$), sex ($F_{(1, 5006)} = 586.519$, $p < 0.001$, $\eta^2 = 0.106$), and age ($F_{(9, 5006)} = 9519.335$ $p < 0.001$, $\eta^2 = 0.945$). Follow-up 2-way ANOVA's were analyzed per treatment group to assess effects of sex. A significant main effect of sex was observed for weight gain in all treatment groups (2-way sex × age ANOVA; Control: $F_{(1, 1688)} = 207.4$, $p < 0.001$, $\eta^2 = 0.006$; MS: $F_{(1, 1175)} = 221.8$, $p < 0.001$, $\eta^2 = 0.009$; LB: $F_{(1, 1926)} = 126.0$, $p < 0.001$, $\eta^2 = 0.004$). As the focus of this work was to assess the impact of ELA treatment on outcomes, for clarity of presentation, the data was separated by sex and analyses were carried out separately to determine LB and MS effects on weight gain.

3.1.1. Males

A significant main effect of treatment was observed on weight gain in males (2-way ANOVA, $F_{(2, 2361)} = 200.4$, $p < 0.001$, $\eta^2 = 0.009$), and both an effect of age ($F_{(9, 2361)} = 4313$, $p < 0.001$, $\eta^2 = 0.922$) and a treatment × age interaction ($F_{(18, 2361)} = 12.75$, $p > 0.001$, $\eta^2 = 0.005$; Fig. 2A) were found. Both LB and MS groups were significantly different from controls ($p < 0.001$, and $p = 0.010$; respectively) and each other ($p < 0.001$; Fig. 2A). Importantly, no effect of group was found for weight at PD4, before the ELA manipulation began ($p > 0.05$). LB mice showed a reduction in weight compared to control pups, an effect that emerged two days after the initiation of LB (PD6, $p = 0.048$; Fig. 2A) and persisted throughout development ($p < 0.05$ at all timepoints). In contrast to LB, MS and control mice did not statistically differ from each other in weight from PD4-PD28 ($p > 0.05$; Fig. 2A). However, MS mice showed a momentary increase in weight compared to controls at PD35 and PD55 ($p < 0.001$ and $p = 0.029$, respectively), with weight returning to control levels by PD75 ($p = 0.341$; Fig. 2A). Here, the type of adversity had differing effects on the trajectory of weight gain, with LB mice showing reduced weight throughout development and MS mice having increased weight compared to controls during late adolescence.

3.1.2. Females

Consistent with male weight gain results, for females a main effect of treatment was found for weight gain (2-way ANOVA, $F_{(2, 2598)} = 162.2$, $p < 0.001$, $\eta^2 = 0.006$; Fig. 2B). A significant effect of age ($F_{(9, 2598)} = 4749$, $p < 0.001$, $\eta^2 = 0.907$) and a treatment × age interaction ($F_{(18, 2598)} = 8.05$, $p < 0.001$, $\eta^2 = 0.003$; Fig. 2B) were also found. As with males, LB mice showed a reduction in weight compared to control mice ($p < 0.001$), however MS mice did not differ in weight compared to controls ($p = 0.931$). While, LB and MS groups did not differ in weight from controls at P4, immediately prior to experimental manipulation (PD4, $p > 0.05$), female LB mice weighed significantly less than both control and MS reared two days after initiation of LB (PD6 $p = 0.02$) and persisted across all developmental time points measured ($p < 0.05$ at all timepoints; Fig. 1B). However, unlike the male results, the weight of MS female mice did not differ from controls ($p = 0.931$; Fig. 2B), and no effects of MS rearing were observed for any time point measured ($p > 0.05$ at all time points). Thus, the type of adversity experienced, and sex of the animal impacted trajectories of weight gain compared with control reared mice.

3.2. ELA impacts maturational timing of visual development

3.2.1. Eye opening

At birth, the eyes of mouse pups are closed and remain closed during the ~first week and a half of life. Eye opening represents an important milestone, triggering significant organizational effects on visual cortex maturation to support later vision, stereopsis, navigation and interaction with the environment. To assess the effect of ELA on the trajectory of eye opening, the degree of eye opening (see methods) was scored from PD12 until all animals showed complete eye opening (e.g. PD19). A 3-way repeated measures ANOVA (age × treatment × sex) revealed a between-subject effect of treatment ($F_{(2, 409)} = 59.016$, $p < 0.001$, $\eta^2 = 0.224$), and of sex ($F_{(1, 409)} = 3.383$, $p = 0.018$, $\eta^2 = 0.014$) and a within-subject effect of age ($F_{(3.628, 1483)} = 1567.051$, $p < 0.001$, $\eta^2 = 0.793$), age × treatment ($F_{(7.256, 1483)} = 19.689$, $p < 0.001$, $\eta^2 = 0.088$), age × sex ($F_{(3.628, 1483)} = 3.958$, $p < 0.001$, $\eta^2 = 0.010$), but not an age × treatment × sex interaction ($F_{(7.256, 1483)} = 0.097$, $p = 0.687$, $\eta^2 = 0.003$). As the focus of this work was to assess the impact of ELA treatment on outcomes, data are presented for males and females separately and further analysis was carried out to determine LB and MS effects on eye opening in males and females separately.

3.2.1.1. Males. In males, a main effect of treatment on eye opening score was observed (2-way ANOVA, $F_{(7, 1407)} = 465.8$, $p < 0.001$, $\eta^2 = 0.019$). An effect of age (2-way ANOVA, $F_{(3.592, 718.4)} = 749.1$, $p < 0.01$, $\eta^2 = 0.659$) and a treatment × age interaction (2-way ANOVA, $F_{(14, 1400)} = 9.354$, $p < 0.001$, $\eta^2 = 0.016$; Fig. 3A) were also observed. Interestingly, the trajectory of eye opening differed for each group. Eye opening in both LB and MS mice were significantly different from controls ($p < 0.001$ and $p = 0.032$, respectively) and LB mice were also significantly different from MS mice ($p = 0.003$; Fig. 3A). Post-hoc analysis at each PD showed that LB mice had lower (e.g. delayed) eye-opening scores compared to controls at all time points from PD12-PD17 ($p < 0.05$) and lower scores compared to MS from PD14-PD15 ($p < 0.05$; Fig. 3A). In addition, MS mice showed reduced eye-opening scores compared to controls on PD14 ($p = 0.034$) and PD16 ($p = 0.011$; Fig. 3A). No significant difference between the groups were seen at PD19 ($p > 0.05$; Fig. 3A), when all mice had reached completion of eye opening. Both forms of adversity resulted in delayed eye opening in males, with LB manipulation showing a more robust effect on this measure.

3.2.1.2. Females. The effect of ELA on the trajectory of eye-opening in females was similar to those observed in males. Eye opening scores revealed a main effect of treatment (2-way ANOVA, $F_{(2, 209)} = 47.65$, $p < 0.001$, $\eta^2 = 0.044$), age ($F_{(3.546, 741)} = 824.5$, $p < 0.001$, $\eta^2 = 0.669$) and treatment × age interaction ($F_{(14, 1463)} = 11.11$, $p < 0.001$, $\eta^2 = 0.018$; Fig. 3B). Again, the type of ELA resulted in different developmental trajectories, with LB and MS mice both being significantly different from control ($p < 0.001$, $p = 0.004$, respectively)

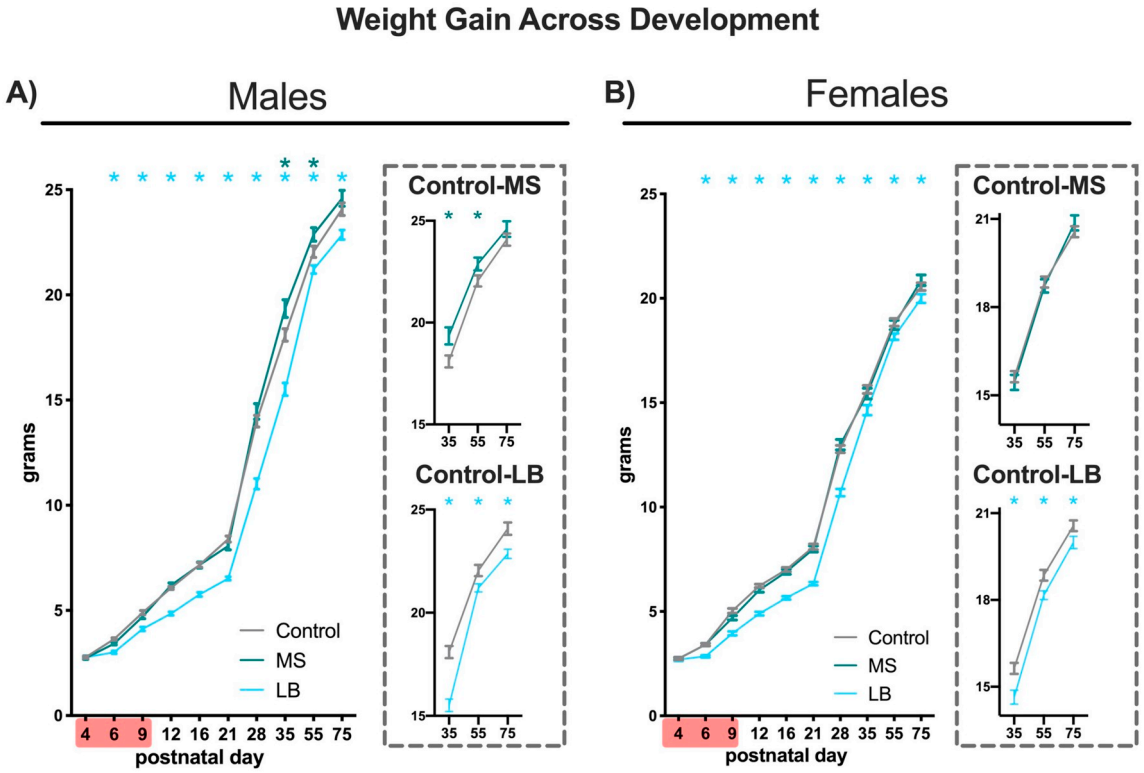

Fig. 2. ELA in the form of LB, but not MS, affects physical growth. Plots of the mean weight for male (A) and female mice (B) reared in control (gray-closed), MS (green-open) or LB (blue-closed) conditions. Insets on the right represent the same data as graphs on the left between ages P35-75. Red highlighter on x-axis indicates ELA being experienced. Green * indicates significant difference between MS-Control at that time point. Blue * indicates significant difference between LB-Control at that time point. n/group = 65–75, all plots depict mean and standard error of the mean. *Tukey's LSD $p < 0.05$

and each other ($p < 0.001$; Fig. 3B). LB mice had lower eye-opening scores compared to controls at all time points from PD12-PD18 ($p < 0.05$) and compared to MS from PD12-PD18 ($p < 0.05$; Fig. 3B). Additionally, MS mice had lower eye-opening scores compared to controls at PD12 ($p = 0.029$) and from PD14-PD16 ($p < 0.05$; Fig. 3B). No significant differences between the groups were seen at PD19 ($p > 0.05$; Fig. 3B), when all mice had completed eye opening. These results suggest that ELA delayed eye opening in females and the type of adversity determined the degree of delay.

3.3. ELA impacts behavioral measures of motor maturation

3.3.1. Righting reflex

As in humans, motor development in mice can be assessed by tracking the trajectory of motor milestones or emerging expertise in motor development. In mice, proficiency in the ability to right from a supine to a prone position (righting reflex) improves significantly during the first week of life. The maturation of this behavior is crucial for the pup to begin interacting with the environment and seek parental care when needed. To assess the impact of ELA on the trajectory of righting reflex maturation, the duration of time it took mice to right themselves was measured daily from PD6 to PD9. A 3-way repeated measures ANOVA (age × treatment × sex) revealed a between-subject effect of treatment ($F_{(2, 129)} = 17.648$, $p < 0.001$, $\eta^2 = 0.215$), and of sex ($F_{(1, 129)} = 3.726$, $p = 0.028$, $\eta^2 = 0.028$) and a within-subject effect of age ($F_{(2.3, 296.8)} = 132.365$, $p < 0.001$,$\eta^2 = 0.506$), age × treatment ($F_{(4.603, 296.8)} = 6.417$ $p < 0.001$, $\eta^2 = 0.090$), but not age × sex ($F_{(2.30,296.8)} = 1.417$, $p = 0.236$, $\eta^2 = 0.011$), or age × treatment × sex interaction ($F_{(4.603, 296.8)} = 1.322$, $p = 0.258$, $\eta^2 = 0.020$). Next, we assessed the impact of ELA on righting reflex in males and females separately.

3.3.1.1. Males. For males, a main effect of treatment (2-way ANOVA, $F_{(2, 64)} = 6.669$, $p < 0.002$, $\eta^2 = 0.057$), age ($F_{(2.84, 146.5)} = 448$, $p < 0.001$, $\eta^2 = 0.267$), and a treatment × age interaction ($F_{(6, 192)} = 2.171$, $p = 0.47$, $\eta^2 = 0.025$; Fig. 3C) were found. Post-hoc tests showed that both LB and MS rearing groups were significantly different from controls ($p < 0.01$) but not from each other ($p = 0.803$; Fig. 3C). Post-hoc comparisons within age were analyzed to assess the effect of ELA on righting behavior across early development. LB male mice took longer to right compared to controls at PD6 ($p = 0.052$), PD7 ($p = 0.019$), and PD8 ($p = 0.012$; Fig. 3C). By PD9, both LB and control male mice were significantly faster to right than prior time points and no longer different from controls (LB vs Control, $p = 0.271$; Fig. 3C). MS mice showed a similar trajectory. At PD6, MS mice showed a trend toward a longer righting reflex time compared to controls ($p = 0.060$;) and remained slower than controls at PD7 ($p = 0.01$), and PD8 ($p = 0.014$; Fig. 3C). By PD9, MS mice also showed a rapid righting reflex and were not different from controls ($p = 0.142$; Fig. 3C).

3.3.1.2. Females. In females, a main effect of treatment (2-way ANOVA, $F_{(2, 66)} = 11.73$, $p < 0.001$, $\eta^2 = 0.096$), age ($F_{(2, 329)} = 96.95$, $p < 0.001$, $\eta^2 = 0.347$) and treatment × age interaction ($F_{(6, 198)} = 6.365$, $p < 0.001$, $\eta^2 = 0.045$; Fig. 3D) were found. Righting reflex in both LB ($p < 0.001$) and MS ($p < 0.001$) reared groups differed from control reared mice but did not differ from each other (LB vs MS; $p = 0.943$; Fig. 3D). Both LB and MS reared mice took longer to right themselves at PD6 ($p < 0.001$ and $p = 0.010$, respectively), PD7 ($p = 0.001$ and $p = 0.011$, respectively) and PD8 ($p = 0.01$ and $p = 0.053$, respectively; Fig. 3D). At PD9, mice showed rapid righting reflex and were not different from controls (LB: 0.163, MS: 0.243 Fig. 3D).

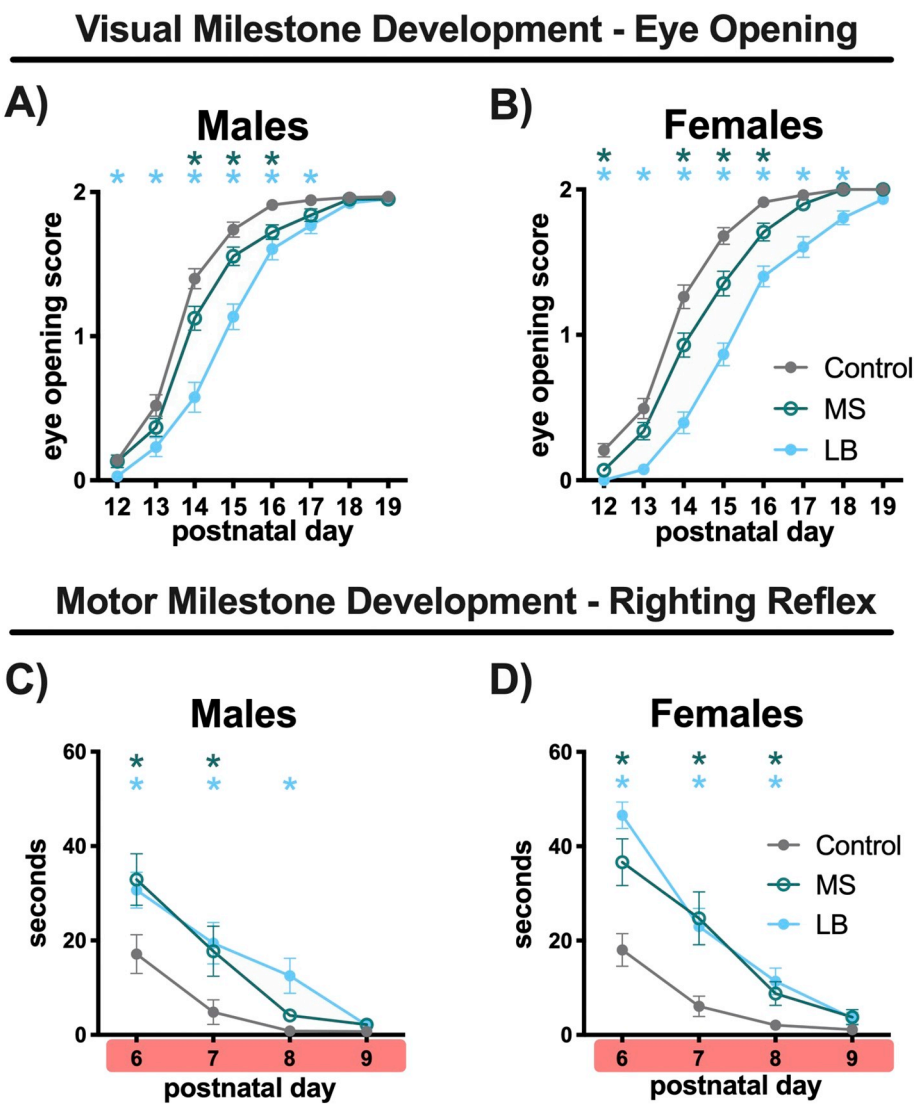

Fig. 3. Both forms of ELA delay the maturation of motor behavior and signs of visual development. Plots of mean eye-opening scores measured on PD12-PD19 for male (A) and female mice (B) reared in control (gray-closed), MS (green-open) or LB (blue-closed) conditions. Plots of the mean duration of time (s) it took mice to right themselves on PD6-PD9 in male (C) and female mice (D) reared in control (gray-closed), MS (green-open) or LB (blue-closed) conditions. Red highlighter on x-axis indicates ELA being experienced. Green * indicates significant difference between MS-Control at that time point. Blue * indicates significant difference between LB-Control at that time point. Eye opening: 65–80/group, sampled from 23 to 28 litters. Eye opening: 21-23/group, sampled from 5 to 7 litters. All plots depict mean and standard error of the mean. *Tukey's LSD $p < 0.05$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.4. Type of ELA alters expression of marker of immature neurons in the motor cortex

As motor cortex matures, there is a decrease in the expression of markers of immature neurons. To determine the effects of ELA on the maturation of motor cortex, gene expression for a marker of immature neurons (doublecortin- *dcx*) was measured at multiple time points from PD12-PD21.

3.4.1. Males

In males, there was a main effect of treatment (2-way ANOVA, $F_{(2, 60)} = 2.984$, $p = 0.053$, $\eta^2 = 0.023$) and age ($F_{(3, 60)} = 61.97$, $p \leq 0.001$, $\eta^2 = 0.717$) for *dcx* expression. In addition, a trending treatment × age interaction was observed ($F_{(6, 60)} = 2.029$, $p = 0.075$, $\eta^2 = 0.047$; Fig. 4A). Subsequent analysis of *dcx* expression at each PD showed that both LB and MS mice had increased expression of *dcx* compared to controls at PD8 ($p < 0.001$ and $p = 0.003$, respectively) and that LB did not differ from MS levels ($p = 0.734$; Fig. 4A). In addition, no detectable differences in *dcx* expression were seen at PD12, PD16, and PD21. *Dcx* expression was nearly undetectable at PD12, PD16, and PD21.

3.4.2. Females

In females, there was a trend level main effect of treatment on *dcx* expression (2-way ANOVA, $F_{(2, 60)} = 2.740$, $p = 0.072$, $\eta^2 = 0.021$), a main effect of age (2-way ANOVA, $F_{(3, 60)} = 54.83$, $p < 0.001$, $\eta^2 = 0.653$) and a tending treatment × age interaction (2-way ANOVA, $F_{(6, 60)} = 2.105$, $p = 0.065$, $\eta^2 = 0.050$; Fig. 4B). As age specific effects were anticipated, with diminishing differences between groups as animals aged, planned post-hoc analyses were performed. LB female mice had higher levels of *dcx* expression at PD8 compared to controls ($p < 0.001$) while MS were not significantly different compared to controls at PD8 ($p = 0.133$; Fig. 4B). In addition, no effect of treatment on *dcx* level was found at PD12, PD16, and PD21.

3.5. Type of ELA drives differential developmental patterns of basal stress hormone levels

3.5.1. Corticosterone

The HPA-axis undergoes a protracted development, with multiple reports showing a hypo-responsive period during early development resulting from a diminished ability to synthesize or secrete CORT between P2-10/14 in rodents (Sapolsky and Meaney, 1986; Schmidt et al., 2003). As neural and adrenal tissues mature, HPA-axis begins to show the prototypical response to stressors, as evidenced by elevated basal and stress-induced levels of CORT in the brain and periphery. Here, the effects of ELA on the trajectory of basal CORT levels in blood serum were assessed as an indirect measure of HPA maturation.

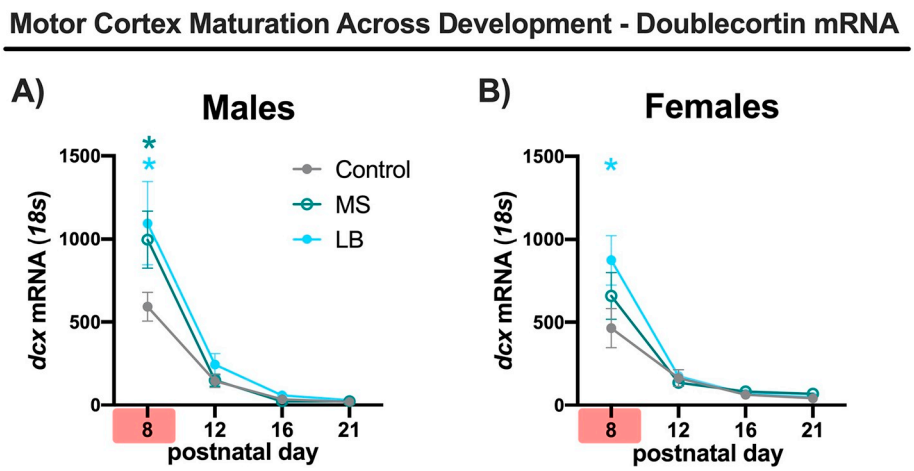

Fig. 4. ELA alters the maturation of the motor cortex. Plots of mean doublecortin mRNA expression relative to control gene (18 s) at PD8, PD12, PD16, PD21 for male (A) and female mice (B) reared in control (gray-closed), MS (green-open) or LB (blue-closed) conditions. Red highlighter on x-axis indicates ELA being experienced. Green * indicates significant difference between MS-Control at that time point. Blue * indicates significant difference between LB-Control at that time point. n/group = 5–6, all plots depict mean and standard error of the mean. *Tukey's LSD $p < 0.05$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

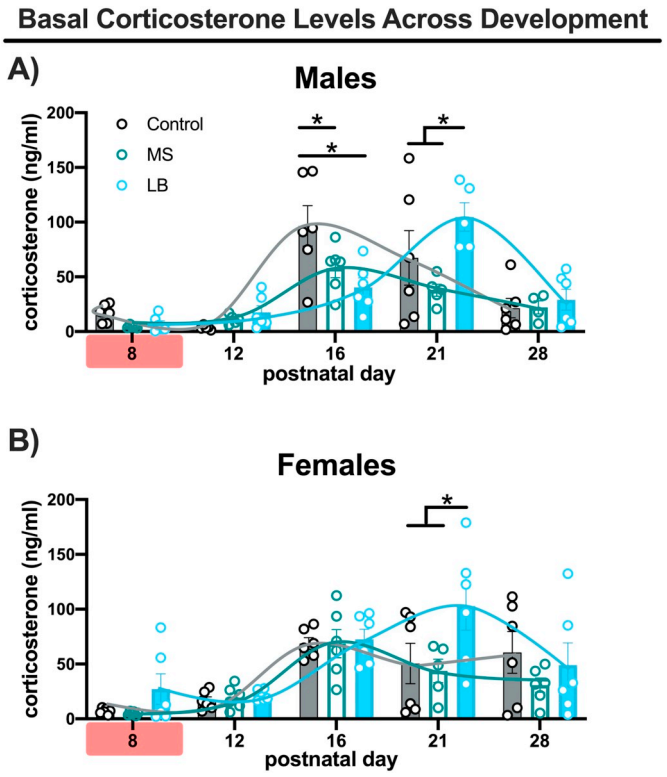

Fig. 5. Type of ELA alters basal corticosterone levels. Bar graphs depict mean corticosterone levels at PD8, PD12, PD16, PD21, PD28 in male (A) and female (B) mice reared in control (gray-filled), MS (green-unfilled) or LB (blue-filled) conditions. Curves represent summary data. Red highlighter on x-axis indicates ELA being experienced. All bars depict mean, standard error of the mean and individual data points. *Tukey's LSD $p < 0.05$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.5.1.1. Males. Baseline CORT levels in males showed a trending effect of treatment (2-way ANOVA, $F_{(2, 68)} = 2.509$, $p = 0.088$, $\eta^2 = 0.026$), an effect of age ($F_{(4, 68)} = 22.01$, $p < 0.001$, $\eta^2 = 0.464$) and a treatment × age interaction ($F_{(8, 68)} = 3.626$, $p = 0.001$, $\eta^2 = 0.153$; Fig. 5A). CORT levels were nearly absent in all treatment groups at PD8 and PD12. At P16, CORT levels in controls showed a dramatic increase and were elevated compared to LB and MS mice at this age ($p < 0.001$ and $p = 0.029$, respectively; Fig. 5A). Importantly, LB male mice showed a similar increase in CORT at PD21 and had elevated CORT levels compared to both control ($p = 0.048$) and MS reared mice ($p < 0.001$; Fig. 5A). Based upon this data, control mice showed maturational elevations in circulating basal CORT around P16, with LB males showing a delayed elevation in developmental CORT expression and MS animals showing a general blunting in developmental elevations of CORT at the time points measured (inset).

3.5.1.2. Females. For baseline CORT levels in females, a main effect of treatment (2-way ANOVA, $F_{(2, 74)} = 3.835$, $p = 0.026$, $\eta^2 = 0.051$) and age ($F_{(4, 74)} = 13.44$, $p < 0.001$, $\eta^2 = 0.364$) were found, but no treatment × age interaction ($F_{(8, 74)} = 1.403$, $p = 0.209$, $\eta^2 = 0.076$; Fig. 5B). As with males, CORT levels in females were low at PD8 and PD12. LB rearing led to elevated basal CORT levels at PD21 compared to both control ($p < 0.001$) and MS mice ($p = 0.004$; Fig. 5B). No other differences were seen between treatment groups at PD8, PD12, PD16, or PD28 ($p > 0.2$; Fig. 5B).

3.6. Type of ELA contributed to sex differences in risk for expression of anxiety-like behavior

3.6.1. Light-dark box

To determine if the type of adversity experienced conferred risk for development of anxiety-like behavior, separate groups of mice were tested in the light-dark box task at three developmental time points: juvenile (PD22), adolescent (PD35), and adult (PD75). Analysis of effects of treatment were carried out separately at each developmental timepoint.

3.6.1.1. Juvenile. An overall main effect of treatment was found for time spent in the light-side of the box (2-way ANOVA, $F_{(2, 97)} = 5.921$, $p = 0.004$, $\eta^2 = 0.101$). In addition, an effect of sex ($F_{(1, 97)} = 4.967$, $p = 0.028$, $\eta^2 = 0.043$) but no treatment × sex interaction ($F_{(2, 97)} = 0.238$, $p = 0.788$, $\eta^2 = 0.004$; Fig. 6A) were found. Post-hoc analysis revealed that LB female mice spent less time in the light side compared to both controls ($p = 0.043$) and MS female mice ($p = 0.038$), while LB male mice did not ($p = 0.245$ and $p = 0.146$, respectively; Fig. 6A). In addition, MS and control mice (both males and females) spent an equivalent amount of time in the light ($p > 0.05$; Fig. 6A). Further, for the latency to enter the light side, no effect of treatment (2-way ANOVA, $F_{(2, 111)} = 1.366$, $p = 0.259$, $\eta^2 = 0.023$), sex ($F_{(1, 11)} = 0.6450$, $p = 0.4236$, $\eta^2 = 0.005$) or interaction ($F_{(2, 111)} = 1.222$, $p = 0.299$, $\eta^2 = 0.021$; Fig. 6B) were found.

3.6.1.2. Adolescent. Analysis of anxiety-like behavior in adolescent mice revealed a similar overall pattern to those observed in juvenile mice. A main effect of treatment was found (2-way ANOVA, $F_{(2, 110)} = 6.320$, $p = 0.002$, $\eta^2 = 0.102$) but no main effect of sex ($F_{(1, 110)} = 0.7247$, $p = 0.396$, $\eta^2 = 0.005$) or treatment × sex interaction ($F_{(2, 110)} = 0.431$, $p = 0.651$, $\eta^2 = 0.007$; Fig. 6C). Again, LB female

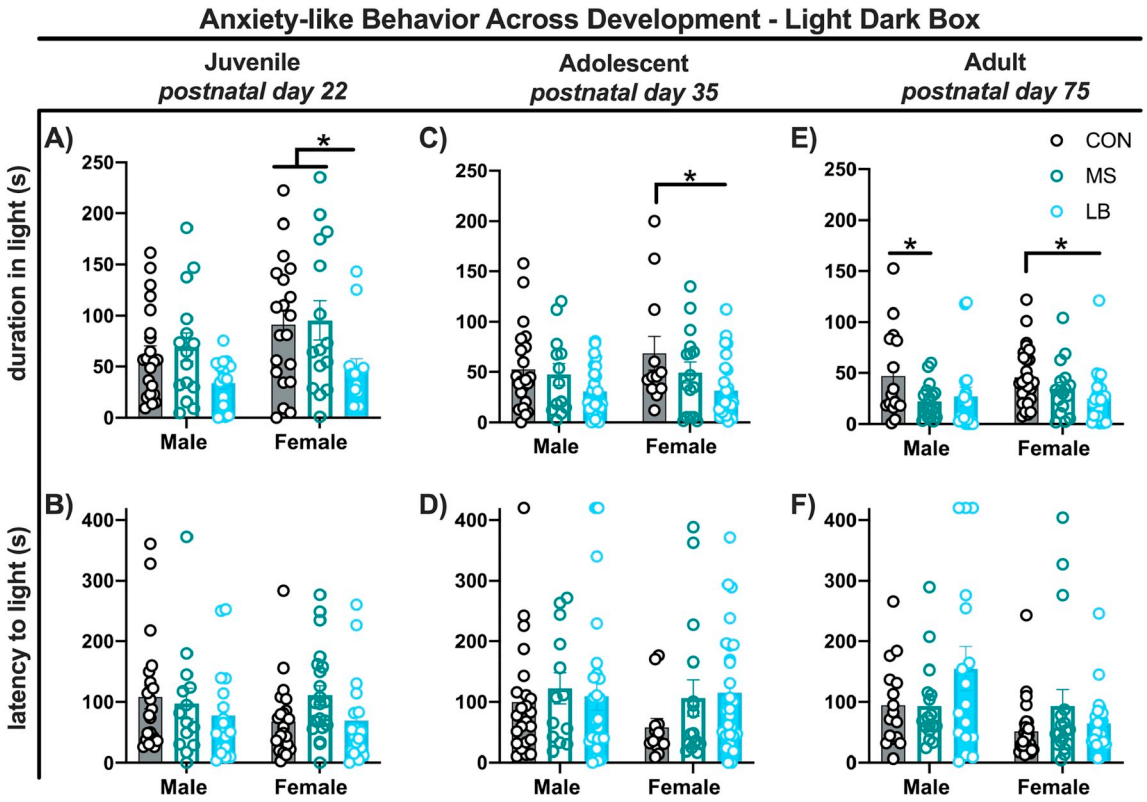

Fig. 6. ELA in the form of LB, but not MS, results in anxiety-like behaviors throughout development. Bar graph depicting mean duration and latency to the light side at PD22 (A–B), P35 (C–D) and PD75 (E–F) in male and female mice reared in control (gray-filled), MS (green-unfilled) or LB (blue-filled) conditions. All bars depict mean, standard error of the mean and individual data points. *Tukey's LSD $p < 0.05$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

mice, but not males ($p = 0.107$) spent decreased time in the light compared to control ($p = 0.015$; Fig. 6C). In addition, MS male ($p = 0.914$) and MS female ($p = 0.391$; Fig. 6C) mice spent an equivalent amount of time in the light side compared to their control counterparts. There were no treatment effects on the latency to enter the light-side of the box (2-way ANOVA, $F_{(2,\ 112)} = 1.370$, $p = 0.258$, $\eta^2 = 0.023$), no effects of sex ($F_{(1,\ 112)} = 0.779$, $p = 0.379$, $\eta^2 = 0.006$), and no treatment × sex interaction ($F_{(2,\ 112)} = 0568$, $p = 0.568$, $\eta^2 = 0.009$; Fig. 6D).

3.6.1.3. Adult. For duration of time spent on the light side of the box for adults, a main effect of treatment ($F_{(2,\ 112)} = 5.315$, $p = 0.006$, $\eta^2 = 0.085$) was found, but no effect of sex ($F_{(1,\ 112)} = 0.2666$, $p = 0.606$, $\eta^2 = 0.002$) or treatment × sex interaction ($F_{(2,\ 112)} = 0.565$, $p = 0.569$, $\eta^2 = 0.009$; Fig. 6E). Consistent with both juvenile and adolescent ages, LB female mice (but not male $p = 0.142$) spent less time in the light side compared to controls ($p = 0.048$; Fig. 6E). Interestingly, while MS male mice did not differ from controls in the duration spent in the light in either juvenile or adolescent ages, a difference emerged in adults, with MS males spending decreased time in the light compared to control males ($p = 0.055$; Fig. 6E). There were no treatment differences in the latency to enter the light-side of the box (2-way ANOVA, $F_{(2,\ 113)} = 1.813$, $p = 0.168$, $\eta^2 = 0.027$), or treatment × sex interaction ($F_{(2,\ 113)} = 2.535$, $p = 0.838$, $\eta^2 = 0.040$; Fig. 6F). For latency to enter the light side, there was a trending effect of sex ($F_{(1,\ 113)} = 7.700$, $p = 0.007$, $\eta^2 = 0.059$ Fig. 6F), with no observed significant post-hoc effects.

4. Discussion

Early experiences provide critical signals to support both neural and behavioral development, which serve as building blocks for continued maturation. ELA can come in many forms, including alterations in the quality, quantity, and reliability of care, loss of resources, and physiological stress signals provided by the parent or environment. To causally assess the effects of different forms of early life adversity on maturation, we used two common rodent models of ELA, limited bedding and maternal separation, to respectively model resource restriction and loss of care. Both models had unique effects on weight gain, eye opening, motor behavior maturation, neural maturation, and basal CORT levels, with several of these effects being sex and/or model dependent (see Table 1 for summary).

4.1. ELA in the form of LB, but not MS, affected physical growth

In the current report, LB and MS differed in their effects on physical development. LB resulted in a significant decrease in body weight across all ages measured and MS resulted in no change in early weight gain, and a modest increase in body weight relative to controls shortly following weaning in males. These results are consistent with prior work in the LB model, where LB rearing impacted physical growth (Bath et al., 2016; Manzano Nieves et al., 2019; Rice et al., 2008), and work from others where MS did not impact weight gain (George et al., 2010). However, effects of weight gain in MS are heterogeneous and may depend upon the strain and specific method of ELA implemented. Shorter protocols show no effect on weight (George et al., 2010; Roque et al., 2014) while longer protocols lead to reduced weight gain in MS pups (de Almeida Magalhães et al., 2018). Further, for MS in rats, reductions in weight during the separation period has been shown to recover shortly after weaning (Coley et al., 2019; Mesquita et al., 2007).

While both ELA models impact dam-pup interactions and the care of the offspring (Bailoo et al., 2014; Gallo et al., 2019; Ivy et al., 2008), differences in the design and execution of each manipulation could account for the disparate effects on weight gain in the present study.

Table 1
Summary of results. Type of ELA confers different developmental and behavioral outcomes in a sex-dependent manner.

	Maternal separation	Limited bedding
Weight gain	PD35-55 increased ♂	PD6-75 decreased ♂ ♀
Eye opening	PD14-16 decreased ♂	PD12-17 decreased ♂
	PD12-16 decreased♀	PD12-18 decreased♀
Righting reflex	PD6-7 decreased ♂	PD6-8 decreased ♂ ♀
	PD6-8 decreased ♀	
Motor cortex maturation (qPCR- *dcx*)	PD8 increased *DCX* ♂	PD8 increased *DCX* ♂ ♀
Basal corticosterone	PD16 decreased ♂	PD16 decreased ♂
		PD21 increased ♂ ♀
Light-dark box–Juvenile		Reduced duration in light ♀
Light-dark box–Adolescence		Reduced duration in light ♀
Light-dark box–Adult	Reduced duration in light ♂	Reduced duration in light ♀

First, the LB paradigm chronically restricts access to bedding throughout the full seven-day manipulation, while MS separates pups from mom for 3 h per day for 7 days. In this case, for MS, the effects of separation on access to care may be transient and compensated for during the 21 h per day of normal housing conditions (Bailoo et al., 2014), leading to less robust effects. The effects of LB on pup-dam interactions, however are chronic, occur during the full period of manipulation, and result in fragmented care (Gallo et al., 2019; Ivy et al., 2008). Alternatively, differences between the models may be the result of the impact of the form of adversity on maternal physiology, with the stress of LB housing impacting maternal health, milk production, and hormones associated with milk ejection, effects that may not occur in the MS model. Further study of the effects of the two forms of ELA on these processes may provide important insights into ELA effects on basic physiological processes over development, and additional challenges faced by pups in varied ELA conditions.

4.2. Both forms of ELA delay signs of visual development

Early sensory experience drives dramatic changes in synaptic organization of the cerebral cortex (Prusky et al., 2008; Sirevaag and Greenough, 1988; Wiesel and Hubel, 1963). For the visual system, a significant component of visual development occurs postnatally and relies, at least partially, upon input from the environment. For example, an accelerated development of the visual system has been reported both in pups reared in enriched environments who received increased levels of licking (Sale et al., 2004), and in pups who received artificial tactile stimulation in the absence of a dam (Smart et al., 1990). Understanding how ELA impacts the timing of sensory, and underlying neural development, can provide insights into possible mismatches in the unfolding of neurodevelopmental programs and timing of key experiences. Here, we found that both forms of ELA resulted in delayed timing of eye opening of males and females, with the degree of delay depending on the form of adversity. LB and MS rearing led to a significant delay in the initiation and the completion of eye opening compared to controls. Further, the effects of LB rearing on eye opening were more profound than the effects observed in MS mice. Based on these observations, it is possible that LB rearing is a more potent paradigm of adversity than MS rearing, due to the chronicity of effects on maternal care (Walker et al., 2017). Our current findings in MS reared mice contradict previous findings in other rodent models of MS rearing, where MS resulted in either no changes in eye opening date (Farkas et al., 2009) or accelerated eye opening after MS rearing (Mesquita et al., 2007). The divergence from prior reports may be due to inconsistency in the implementation of MS protocols, methods of data collection, and specific strains or species used. Prior reports using daily three-hour MS from PD1-14 in rats showed no differences in terms of final day of eye opening, however, the trajectory of eye opening was not measured (Farkas et al., 2009). Eye opening does not occur abruptly, and the trajectory of development provides useful information regarding the rate of maturation. Interestingly, a group utilizing a six-hour MS paradigm from PD2-PD15 in rats found an acceleration in eye opening, with eye opening onset occurring earlier in MS males and females compared to controls (Mesquita et al., 2007). In that report, the authors propose that glucocorticoid levels resulting from the prolonged separation may serve to stimulate growth and maturation. Alternatively, in some MS paradigms, there is an uptick in maternal care following daily reunion of pups with the dam (Macrí et al., 2004). The increased care upon reunion in some iterations of this model could contribute to the disparate outcomes. Notably, in a separate line of work, an enriched early rearing environment has been associated with precocious eye opening, which was associated with increased maternal licking (Sale et al., 2004) and precocious expression of brain derived neurotrophic factor (BDNF) in the visual cortex (Cancedda, 2004). Thus, the results from LB and MS rearing, show how subtle differences in the way that maternal care and resources that are provided can profoundly impact key milestones that are important for basic sensory and cortical development.

4.3. Both forms of ELA delay maturation of motor behavior

Motor experiences during postnatal life are crucial drivers of normative maturation and produce long-lasting changes in the brain (Kolb and Gibb, 2011). Righting reflex requires the recruitment of muscular and motor systems and is also dependent on successful coordination between the left and right side of the body (Feather-Schussler and Ferguson, 2016). This fine motor coordination is an important milestone for the rodent pup as it allows them to begin to engage with their environment in richer ways and changes their ability to interact with the dam. Rodents raised in enriched housing have been shown to engage in increased motor activity compared with those reared in standard caging. Enrichment reared animals have increased cortical thickness, dendritic branching and spine density in regions measured (Sirevaag and Greenough, 1988). Consistent with other work in MS mice (Farkas et al., 2009; Mesquita et al., 2007), ELA drove significant delays in motor development in both males and females. Thus, like visual development, motor development is highly sensitive to the early rearing environment. Interestingly, delays in motor function were apparent as early as two days after the initiation of ELA manipulations. Thus, motor development appears to be highly sensitive to these forms of ELA during this period of rapid development. Importantly, abnormalities in the development of motor milestones in infants have been linked to other neurodevelopmental disorders, including autism (Leonard et al., 2014), impaired social communication (Bhat et al., 2012), and delayed language development (Iverson, 2010). Thus, ELA effects on the timing of neurodevelopmental and physical development have the potential to compound effects of other genetic and environmental drivers of risk and potentially impact the expression or severity of symptom development in other conditions.

4.4. ELA alters maturation of the motor cortex

ELA has been associated with significant effects on the timing of neural maturation (Bath et al., 2016), as well as on morphometry of regional brain structure measured in adolescence and adulthood (Bagot et al., 2009; Brunson, 2005; Champagne et al., 2008; Chen et al., 2008; Chen and Baram, 2016; Gee et al., 2013; Joëls and Baram, 2009; Wang et al., 2011). To date, studies have principally focused on later maturing cortical regions, such as the prefrontal cortex (Pascual and Zamora-León, 2007), and subcortical structures, including hippocampus (Oomen et al., 2010) and amygdala (Danielewicz and Hess, 2014). Here, we have found early emerging and significant effects of ELA on the timing of motor development. The motor cortex is one of the earliest maturing cortical regions (Gao et al., 2015), and could provide additional and important insights into the effects of ELA on the timing of regional brain development. Here, *dcx* gene expression (a marker found in immature neurons) was measured as a proxy of neural maturation, with motor cortex showing adversity- and sex- dependent effects. When measured across development, there is a general decline in *dcx* expression, indicative of the process of maturation (more mature and fewer immature cells). At PD8, LB and MS reared males had increased *dcx* expression compared with controls (indicative of a later developmental decline in expression of this gene). However, for females, only LB rearing was associated with increased *dcx* expression at early time points. While we observed a general trend toward higher *dcx* levels in ELA conditions at early time points, the less robust effects in females may have been the result of sex differences in the timing of this developmental process. It is possible that motor cortex maturation in females occurs slightly earlier than males and that analysis of *dcx* at PD6 (two days earlier) might have revealed a more robust difference between MS females and controls, matching the results found in males. Despite this, the current results suggest that ELA leads to a delay in one marker of motor cortex maturation, which may drive, or be the result of, motor behavioral deficits observed in ELA reared animals. While *dcx* expression is a useful marker for immature neurons undergoing differentiation, it does not index the multitude of additional processes comprising cortical development. For a more complete picture of motor cortex development, other morphological, cellular and behavioral variables would need to be measured. Future studies will be needed to test for markers of proliferation, division, migration, death, etc. and for morphological changes in motor cortex.

Importantly, while these data suggest a delay in brain maturation after ELA, other research has shown accelerated neuronal development of other regions such as the hippocampus (Bath et al., 2016) and amygdala (Gee et al., 2013; Honeycutt et al., 2020), which were associated with accelerated development of fear learning, and altered emotional regulation. Thus, we propose a mixed model of adversity's effect on neurobehavioral maturation, where resources are being allocated to support precocious maturation of some brain regions (limbic and paralimbic structures) while other regions are delayed or possibly failing to develop completely (motor cortex). Alterations in the timing of maturation is likely an adaptive process and provides short-term advantages by promoting investment in processes and structures that will support successful survival within and adaptation to the rearing environment.

4.5. ELA alters developmental changes in basal corticosterone levels

In rodents, the adrenals continue to develop during the early postnatal period, resulting in the stress hyporesponsive period, indexed by low CORT levels and a diminished or absent CORT response to stress (Schmidt et al., 2003). Interestingly, despite low basal levels of CORT, the developing nervous system is sensitive to changes in circulating CORT levels. For example, repeated administration of exogenous CORT through drinking water or injection can change the maturational profile of the brain through effects on cell proliferation and cell death (Gould et al., 1991a; Gould et al., 1991b). Administration of drugs that suppress CORT synthesis have been shown to prevent normal dendritic spine turnover (Liston and Gan, 2011). Activation of CORT receptors, including mineralocorticoid and glucocorticoid receptors, have been associated with changes in transcription of genes involved in spine plasticity, growth factors (including BDNF) (Schaaf et al., 1997), and synaptic transmission (Datson et al., 2001). Thus, developmental changes in levels of CORT can play a major role in regulating postnatal development of the central nervous system. Here, we tested the effects of ELA on developmental changes in basal plasma CORT levels of pups.

The type of ELA experienced, and the sex of the mouse, led to distinct effects on basal CORT levels over early development. In males and females reared in control conditions, CORT levels were low at PD8 and PD12 and then rose by PD16, consistent with our previous work (Bath et al., 2016). Although, a mature hormonal response to a stressor has been shown to occur as early as PD12 (Schmidt et al., 2003) or even PD9 (Gilles et al., 1996), male mice reared in LB conditions showed what appeared to be a delayed maturational profile, with the developmental increase in CORT levels occurring at PD21, five days later than control males. Conversely, while female LB mice were not different from controls at PD16, LB females showed elevated levels of basal CORT compared to controls at PD21. The present results are consistent with previous reports in rats and indicate that male pups exposed to LB show reduced CORT levels early in development and a delayed rise in basal CORT levels, while females show elevated CORT levels at PD21 (Moussaoui et al., 2017, 2016). In contrast to LB, ELA in the form of MS resulted in no changes in females and lower basal CORT levels in males at PD16, with no subsequent elevation at PD21. These results are consistent with other work in mice, with groups showing no effects on basal CORT after repeated MS (Horii-Hayashi et al., 2013). Notably, this work showed elevated CORT levels after a 3 h separation at both PD14 and PD21, suggesting that repeated MS does not habituate nor sensitize to separation-induced elevations in CORT. Likewise, other studies in rats have shown that MS pups were able to mount a stress response to restraint during the stress hypo-responsive period (Dent et al., 2000; Vazquez et al., 2006). A recent study in rats compared basal CORT levels in male and female pups at PD21 who had experienced LB and MS rearing and found parallel ELA-specific results with LB rearing leading to the most robust changes in basal CORT levels (Moussaoui et al., 2017).

One theory is that the sex- and adversity- specific changes in basal CORT levels are likely the result of changes in the quality of maternal care (Francis et al., 1999). As a consequence of LB rearing, maternal care becomes fragmented, unpredictable, and, in some cases, abusive (Gallo et al., 2019; Ivy et al., 2008; Walker et al., 2017). Changes in maternal care have been proposed to alter normative development of the HPA axis and systems involved in stress regulation (Gunnar and Quevedo, 2007), and tactile stimulation with feeding of neonates reversing the effects of MS (van Oers et al., 1998). In contrast to LB, MS rearing leads to higher maternal care upon reunion and may act as a buffer to protect against HPA-axis dysregulation (Daskalakis et al., 2011; Gee et al., 2014; Sanchez et al., 2015). Alternatively, the timing of the HPA axis development may also be the consequence of exposure to exogenous CORT, delivered through the breastmilk of the stressed dam (Ivy et al., 2008). While, prior work has shown that LB rearing drives a robust CORT response in the dam during the light-cycle, others fail to show this increase during the dark-cycle (Opala et al., 2019). Divergent results may reflect circadian rhythm-dependent changes in CORT response in LB dams, disruptions in synchronization of circadian rhythms of CORT secretion, or true differences in basal levels. Exposure to exogenous CORT has been shown to stimulate regional brain development (Gould et al., 1991a, 1991b) and levels of CORT receptor expression in the brain (Thompson et al., 2004). More work will be needed to better understand whether altered timing of HPA development is either the result of the psychological and physical stress of LB rearing, or receipt of chemical signals of stress from the lactating dam.

Experiencing adversity early in life has lasting consequences for HPA axis function and likely mediates vulnerabilities to developing psychiatric disorders such as anxiety (van Bodegom et al., 2017). Females have been shown to be particularly vulnerable to stress-associated pathology due to HPA-axis reprogramming (Carpenter et al., 2017), are more likely to develop anxiety-like phenotypes after experiencing ELA (Kalinichev et al., 2002) and show increased rates of anxiety (Reynolds et al., 2015). Supporting these and other reports (Kanatsou et al., 2016), LB rearing led to anxiety-like behaviors in the light dark box in females, but not males, an effect that was present throughout development. It is possible that these effects may also be sensitive to the type of task used to assess anxiety-like behavior, as we had not previously observed an anxiety-like phenotype in LB reared animal on the elevated plus, elevated zero, or open field task (Goodwill et al., 2018a, 2018b). MS rearing resulted in a reversed pattern of effects, with males being affected only at adult time point, but not females. This sex-specific consequence of MS is in agreement with others and has been linked to changes in HPA axis programming (Papaioannou et al., 2002) and is potentially explained by sex-specific differences in maternal care during MS rearing (Moore and Morelli, 1979). Further characterization of the relationship between developmental changes in CORT and expression of behavioral markers of pathology may elucidate possible mechanisms of disease and stress-associated vulnerability and resilience.

5. Conclusions

In summary, a better understanding of the effects of ELA on early physical, behavioral, and neural developmental trajectories can provide important new insights into the way circuit development adapts to the early environment. Specifically, ELA supports a shift in the timing of physical weight gain, early visual maturation, motor development, and the maturation of a stress associated marker. Using two different forms of ELA, in both male and female mice, we find that the presence and degree of ELA effects depend upon the sex- and type of adversity experienced. These results highlight the complex nature of ELA as it relates to the developing organism and likely reflect adaptations of the system in response to the early caregiving environment. That said, additional research will be needed to further isolate the developmental mechanisms that are impacted by different adverse experiences and how males and females adapt differently to these early experiences. Such findings will not only reveal targets for earlier medical and therapeutic interventions but will also provide critical groundwork for individualized medicine.

Declaration of competing interest

The authors declare no competing interests.

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