Investigating Mechanisms Underlying Differential Effects of Early Life Adversity on Maternal Care, Neuronal Activation, and Social Behavior in Mice

A Thesis submitted to

Indian Institute of Science Education and Research Pune in partial fulfilment of the requirements for the BS-MS Dual Degree Programme

by

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Under the guidance of

Dr. Elizabeth Gould,

Princeton Neuroscience Institute, USA

Certificate

This is to certify that this dissertation entitled **'Investigating Mechanisms Underlying Differential Effects of Early Life Adversity on Maternal Care, Neuronal Activation, and Social Behavior in Mice'** towards the partial fulfilment of the BS-MS dual degree programme at the **Indian Institute of Science Education and Research, Pune** represents work carried out by **Vibha Ajit Bapat** at **Princeton Neuroscience Institute** under the supervision of **Dr. Elizabeth Gould**, **Dorman T. Warren Professor of Neuroscience,** during the academic year 2023-24.

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Declaration

I hereby declare that the matter embodied in the report entitled **'Investigating Mechanisms Underlying Differential Effects of Early Life Adversity on Maternal Care, Neuronal Activation, and Social Behavior in Mice'** are the results of the work carried out by me at the **Princeton Neuroscience Institute** under the supervision of **Dr. Elizabeth Gould**, and the same has not been submitted elsewhere for any other degree. Wherever others contribute, every effort is made to indicate this clearly, with due reference to the literature and acknowledgement of collaborative research and discussions.

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Abstract

Early life adversity (ELA) has been known to predispose individuals to developing neuropsychiatric diseases in later life. ELA is a broad term encompassing a multitude of negative childhood experiences. The history of different types of childhood adversities have been known to result in predispositions to specific neuropsychiatric conditions in later life. Maternal separation combined with early weaning (MSEW) and limited bedding and nesting (LBN) are two rodent models of ELA that are known to cause differential and sex-dependent long-term effects on structural hippocampal plasticity and social behavior in adult mice. Here, we did a comparative study of MSEW and LBN during the postnatal period to characterize model-specific rearing experiences and delineate the mechanisms linking early experiences to the differential outcomes of these models. We found that LBN leads to fragmented maternal care and lower pup body surface temperature, whereas MSEW leads to compensatory higher maternal care-giving. Both the models resulted in lower neuronal activation in the hippocampus. MSEW and LBN models have also been shown to result in impaired social recognition memory in adult males but not females. We investigated the emergence of these sex differences through a longitudinal assessment of social recognition memory in males and females over development and found that these sex differences emerge after puberty. Lastly, we identified increased senescent cells in the subgranular zone (SGZ) in MSEW adult males, as a potential mechanism facilitating the decreased adult neurogenesis after ELA. Further, we found that environment enrichment housing can rescue the increase in senescent cell population in the SGZ. Together, these results provide insights into the early experiences, developmental trajectory, and mechanisms underlying differential outcomes of ELA.

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Contributions

Contributor name	Contributor role
Elizabeth Gould	Conceptualization Ideas
Vibha Bapat, Elizabeth Gould, Renée Waters	Methodology
------	Software
Vibha Bapat	Validation
Vibha Bapat	Formal analysis
Vibha Bapat, Renée Waters	Investigation
Vibha Bapat, Elizabeth Gould, Renée Waters, Wang lab PNI	Resources
Vibha Bapat, Renée Waters	Data Curation
Vibha Bapat	Writing - original draft preparation
Vibha Bapat, Elizabeth Gould, Renée Waters	Writing - review and editing
Vibha Bapat	Visualization
Elizabeth Gould	Supervision
Elizabeth Gould	Project administration
Elizabeth Gould	Funding acquisition

Table 1 : List of contributors to the thesis work

Introduction

Neuropsychiatric Outcomes of Early Life Adversity (ELA)

Early life adversity (ELA) has been known to predispose individuals to developing neuropsychiatric diseases in later life (Li et al., 2016; Dunn et al., 2018). ELA is a broad term encompassing a multitude of negative experiences in early life, including parental neglect, poverty, physical, mental, and sexual abuse, witnessing violence, etc. Numerous medical conditions such as respiratory and inflammatory disorders, cardiovascular disease, metabolic syndrome, diabetes (Taylor et al., 2011), and shortened life expectancy due to multiple health conditions (Lippard & Nemeroff, 2020) have also been associated with a history of ELA**.** Individuals with a history of childhood adversities are known to be at higher risk of cognitive disorders, depression, mood and personality disorders, substance use disorder, and chronic pain (Widom et al., 1999; Bandelow et al., 2005; Scott et al 2010; Antoniou et al., 2023). Brain development during childhood is sensitive to experience, thus adverse experiences can facilitate long-term enduring adverse effects of ELA leading to predispositions to neuropsychiatric conditions. Different brain regions and circuits can be affected based on the type and time of adversities experienced during development (Luby et al., 2020). Through studies looking at the link between specific types of childhood adversities and later life outcomes, we now understand that the type, timing, duration, and intensity of ELA can predispose individuals to developing certain neuropsychiatric conditions (Waters and Gould 2022). For example, separation from parents in childhood has been associated with a higher risk of personality disorders (Lahti et al., 2012), sexual abuse has been associated with a predisposition to PTSD (Strathearn et al., 2020) and history of emotional neglect childhood has been identified as a risk factor for clinical depression in later life (Jacobson et al., 1975). Considering the prevalence of ELA and the neuropsychiatric outcomes associated with it, it is essential to develop therapeutic interventions. Hence, understanding the mechanisms underlying the connection between ELA and the associated vulnerability to neuropsychiatric diseases is crucial. To this end, several studies using animal models are being done to probe the underlying neurobiological mechanisms that facilitate the long-term outcomes of ELA.

Rodent Models of ELA

Studies using rodent models of ELA have provided insights into the neurobiological circuit- and cellular- level phenomena underlying the connection between the ELA and resulting mental and physical health outcomes. The usage of robust, translationally relevant rodent models in ELA research has been prevalent since the development of

the first ELA model was developed more than 50 years ago (Levine, 1957). Rodent models allow high resolution control over the type, duration, and intensity of stress manipulations during the postnatal period prior to weaning that may correspond to human childhood (Guzman et al., 2016; Murthy and Gould 2018; Murthy and Gould 2020). Rodent ELA models typically involve manipulations of maternal care, environment, and resources available during rearing thus allowing efficient modeling of specific types, duration, and intensity of adverse experiences. Since most mechanisms of neuronal plasticity and chemical modulation are well conserved across species from rodents to humans (Robbins 2021), the susceptibility of structural plasticity as well as behavior to ELA can be modeled well. Though complex human behaviors cannot be modeled exactly in rodents, ELA manipulations in rodents result in robust behavioral phenotypes such as increased anxiety-like behaviors (Walker et al., 2017; George et al 2010).

In the current study, we use two models; maternal separation combined with early weaning (MSEW) and limited bedding and nesting (LBN). MSEW is a model of parental neglect or deprivation of parental care in childhood (George et al., 2010). This involves repeated maternal separation (MS) across the postnatal period combined with early weaning (EW). EW alone has been known to cause increased anxiety- and depression- like behavior (Kikusui et al., 2004). The combined MSEW model has been shown to increase anxiety- and depression- like behaviors, as well as to cause hyperactivity and gene dysregulation (George et al., 2010). The limited bedding and nesting (LBN), on the other hand, is a model for poverty or scarce resources in childhood (Brunson et al., 2005; Cui et al., 2006; Ivy et al., 2008). The LBN model has also been shown to increase anxiety like behaviors (Walker et al., 2017; Prusator & Greenwood-Van Meerveld 2015)

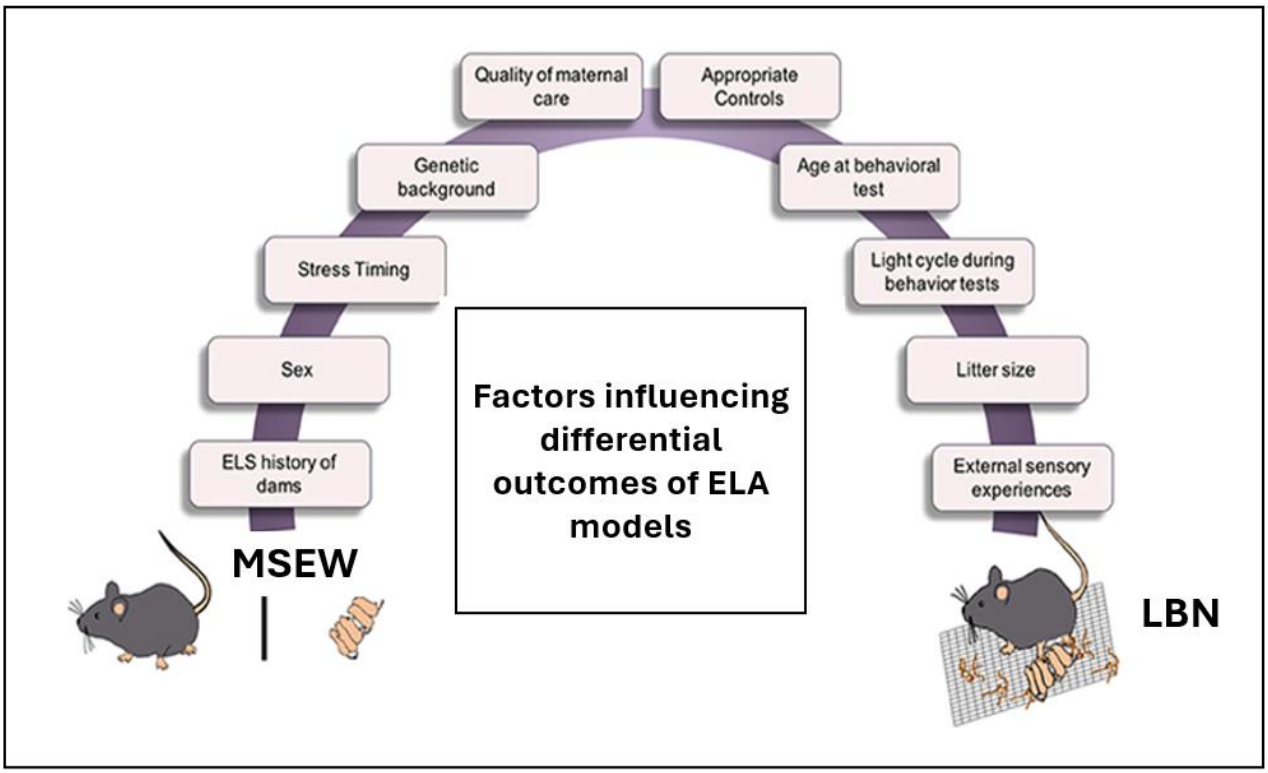

Figure 1. Factors influencing differential outcomes in rodent ELA models (Adapted from Murthy & Gould 2018)

Differential Effects of MSEW and LBN

As mentioned previously, the type of adversity as well as the design of the ELA manipulation in the rodent models can influence the outcomes. Studying the mechanisms underlying model specific outcomes of ELA is necessary. Additionally, very few studies have reported direct comparisons between multiple ELA models to study the differential effects (Demaestri et al., 2020; Johnson et al., 2018). Work in our lab has focused on investigating the long-term enduring effects of ELA in adults in MSEW and LBN. These two models have been found to have differential and sex dependent enduring effects on various structural hippocampal plasticity measures and hippocampus dependent social behavior (Waters et al., 2022). In the current project we addressed questions that arise from previous results regarding differential outcomes of the two ELA models in adult mice. We designed a comparative study of the MSEW and LBN models in the first two postnatal weeks, to characterize the differential rearing experience and gain insights into the differential experiences of ELA in the two models. Though we have a fair understanding of the long-term differential outcomes of MSEW and LBN in adults, we do not completely understand the differential experiences during the ELA manipulations in the postnatal period. Thus, we worked towards addressing this gap in our knowledge.

Postnatal Experience in MSEW and LBN Models

To better understand the long-term differential effects of MSEW and LBN, it is essential to do a comparative study investigating the differences between these models during the MSEW and LBN experience. Here, we characterize the differences in the rearing experiences in each model. Several studies have looked at the rearing experiences in terms of the stress levels of the mother and maternal caregiving behaviors and patterns (Orso et al., 2019). The focus of most studies in the field studying the postnatal ELA experience is centered on the disruption in maternal care, however, many studies have been done using just one ELA paradigm with often contradictory results (discussed below). Furthermore, a focus on maternal care does not permit a comprehensive understanding of the entire ELA experience. Thus, in addition to maternal care, we looked at thermoregulation in pups undergoing MSEW and LBN manipulations and we mapped the brain-wide differential neuronal activation during these manipulations to characterize how the pups encode the environment and experience of different types of ELA during the early postnatal period.

Maternal Care

Studies in rodents report that the quality of maternal care and attachment with pups in the postnatal period facilitates healthy neurodevelopment (Kaffman & Meaney 2007). Disrupted caregiving patterns have been associated with aberrant synaptic connectivity and maturation of cognitive and emotional circuits (Glynn & Baram 2019). In maternal separation models of ELA, studies have shown that the altered maternal caregiving patterns can contribute to the negative experience in addition to the lack of maternal presence during the separations (Boccia and Pedersen, 2001; Huot et al., 2004), however other studies report increased compensatory care including licking, and grooming, arched back nursing (Orso et al., 2019), which has also been speculated to potentially attenuate the effects of the separation (Millstein and Holmes, 2007). The LBN model on the other hand reports disrupted, fragmented, and unpredictable care, reduced licking, and grooming (Pardo et al., 2023, Orso et al., 2018) and abusive behaviors kicking behaviors (Gallo et al., 2019). However, the reports from different studies using either model are somewhat conflicting, and not all studies report decreased or altered caregiving and abusive behaviors. These inconsistencies in the reported studies could potentially be due to the variations in ELA models, experimental designs, and rodent species and strains used. Furthermore, none of the studies directly compare the two models of maternal separation and LBN within the same study. Here, we did a comparative study of the maternal caregiving behaviors and patterns in the MSEW and LBN models in C57BL/6 mice.

Differential Neuronal Activation During ELA

A comprehensive understanding of how MSEW and LBN affect the brain and behavior requires information about which brain circuits are activated during the different experiences. Investigating neuronal activation patterns through the expression of immediate early genes (IEGs) can provide the foundation for establishing a mechanistic link between the long-term enduring structural and behavioral outcomes after ELA. IEGs are a class of genes that are rapidly and transiently expressed in response to extracellular calcium influx during neuronal activation (Okuno 2011). The expression of IEGs is thus coupled with cellular activity linked to experiences and behaviors and plays a role in regulation of synaptic plasticity (Minatohara et al., 2016). Thus, neuronal activation can be indirectly quantified by immunolabeling IEG protein products. Studies investigating the IEG - cFos expression in ELA models have largely been focused on adult males and there aren't many studies reporting IEG expression in pups or ELA females. Studies in adults have shown brain-wide overall increase in cFos expression, in most of the regions commonly studied in ELA including hippocampus, amygdala, prefrontal cortex, paraventricular nucleus, VTA (Schuler et al., 2022). However, studies investigating ELA model specific experiences dependent IEG expression during postnatal period have not been done. Though IEGs are typically studied for certain behavioral tasks, the pattern of constitutive IEG expression has been known to occur in response to the cues associated with the home cage environment (Marrone et al., 2008). In our study, we investigate the brain-wide differential cFos activation in both male and female P7 mice undergoing MSEW and LBN. We aim to create a global brain-wide map of differentially activated brain regions in these two models. We are interested in looking at encoding of sensory, homeostatic, and emotional experiences. We determined our key regions of interest based on the pathways and circuits known to be susceptible to stress and based on the development of regions at P7. These include olfactory and tactile sensory regions, which are the primary sensory modalities developed in mice at P7 (Arakawa 2019). We were interested in exploring the activation patterns in the piriform cortex and somatosensory cortex. The pain and reward pathways including regions like the anterior cingulate cortex, ventral tegmental area, nucleus accumbens and prefrontal cortex that, are known to be affected in adults as well as during development post ELA (Hanson et al., 2021; Birnie et al., 2020). Studies also report anhedonia like behaviors and altered fear conditioning after ELA, suggesting impaired dopaminergic and reward pathways (Bolton et al., 2018; Birnie et al., 2023). We were also interested in investigating activation in hypothalamic regions involved in thermoregulation and homeostasis such as the paraventricular nucleus and preoptic nucleus. Lastly, we were interested in activation of sub regions of hippocampus, and emotional and limbic regions including the amygdala, BNST, anterior cingulate cortex and locus coeruleus, which are known to be affected in adults after ELA (Raineki et al., 2019; Kunzler et al., 2015)

Susceptibility of Hippocampus to ELA

The hippocampus has a protracted developmental period during the postnatal period in which it undergoes highly plastic developmental processes such as neurogenesis, dendritic remodeling, and synaptogenesis (Khazipov et al., 2001; Avishai-Eliner et al., 2002), which makes it sensitive to ELA. The susceptibility of the hippocampal structures and functions to stress has also been widely studied. In humans, smaller hippocampal volumes have been associated with the history of ELA (Rao et al., 2010, Carballedo et al., 2012). Similar results of reduced hippocampal volumes have been found in rodent ELA models (Aksic et al., 2013). Studies in humans have shown that ELA can lead to impaired hippocampus dependent functions including cognitive functioning (Molet et al., 2016), poor contextual memory (Lambert et al., 2017) and weak emotional learning (Krugers et al., 2017). Studies in adult rodents investigating effects of ELA on synaptic plasticity by investigating electrophysiological properties in the hippocampus suggest that ELA can lead to decreased hippocampal LTP (Sousa et al., 2014; Heydari et al., 2019). ELA also affects structural plasticity in the hippocampus. Studies investigating structural plasticity and architecture in the hippocampus have found decreased dendritic architecture complexity and spines in pyramidal and granule cells (Murthy & Gould 2020) as well as decreased adult neurogenesis (Boku et al., 2015; Korosi et al., 2012). ELA has been shown to increase perineuronal nets surrounding parvalbumin-positive interneurons in the ventral hippocampus of adult mice, and this structural alteration has been linked to altered avoidance behaviors and neuronal oscillations (Murthy et al., 2019). Structural plasticity in dorsal hippocampus and dorsal hippocampus dependent social behavior has also been shown to be susceptible to ELA in mice (Waters et al., 2022). Further, electrophysiological properties of the hippocampus have also been studied in rodents after ELA. Changes in structural plasticity and neuronal oscillations in the ventral hippocampus linked to increased anxiety- like behavior have been reported in mice (Murthy et al., 2019), and increased theta power in hippocampal oscillations during rapid eye movement (REM) sleep in adult mice have been linked to history of ELA (Sampath et al., 2014). In the current project we focus on studying the effects of ELA on hippocampus dependent social behaviors and the mechanism underlying decreased adult neurogenesis.

Hippocampus Dependent Social Behavior

The hippocampus plays a major role in regulating social behaviors (Rubin et al., 2014; Montagrin et al., 2018). The CA2 of the hippocampus is known as the ‘social hub’ and is particularly responsible for regulating social recognition behaviors (Hitti & Siegelbaum, 2014; Meira et al., 2018; Smith et al., 2016, Diethorn & Gould 2023). Additionally, structural hippocampal plasticity mechanisms susceptible to stress such as perineuronal nets and adult neurogenesis can influence social behaviors (Cope et al., 2022, Cope et al., 2020). In rodents, the function and connectivity of adult born

neurons in the existing hippocampal circuits plays a role in social behaviors and response to stress (Monteiro et al., 2014; Garrett et al., 2015; Pereira-Caixeta et al., 2017; Cope et al., 2020).

Studies in humans have shown that childhood neglect or abuse can lead to impaired social behavior in the form of altered facial cue recognition in crowds (Iffland & Neuner., 2020) or attentional bias towards facial threat cues (Masten et al., 2008). Social interactions, recognition and memory of conspecifics are crucial to survive in social groups. Healthy social interactions and memory are essential for distinguishing between threatening versus rewarding interactions with conspecific (Jones & Monfils, 2016; Montagrin et al., 2018) and for mating, caregiving (Ophir, 2017; Winslow et al., 1993), and establishment and maintenance of dominance hierarchies (Tibbetts et al., 2022). Social behavior is disrupted in neuropsychiatric disorders linked to ELA (Farrington, 2005; Opendak et al., 2017; Tzanoulinou & Sandi, 2017). Thus, studying the social behavior impairment outcomes of ELA and the underlying hippocampal circuits using rodent ELA models is important.

Previous work from our lab focusing on long term enduring effects of ELA in MSEW and LBN models in adults has shown that these models lead to sex dependent effects on social memory. In both the models, only the adult males, but not females show impaired long term social memory (Waters et al., 2022). In the current study we were interested in investigating the emergence of sex differences in this social behavior impairment over development, particularly with respect to puberty.

Emergence of Sex Differences in Social Behavior Impairment

Sex dependent vulnerability to ELA and the associated neuropsychiatric outcomes of ELA have been reported in human studies (Hill et al., 2010; Dragan et al., 2019). The likelihood of having a history of childhood adversity as well as the types of adversities experienced are known to be sex dependent, with females being more likely to have a history of childhood adversities along with experiencing more complex and varied patterns of ELA (Pederson et al., 2020). Studies in humans and rodents suggest that sex-differences in the effects of ELA might not be evident in childhood but can emerge in adult life post puberty and have been associated with the changes in circulating hormone levels (Hodes & Epperson., 2019). Changes in gonadal hormone levels during puberty have been known to affect neurodevelopment and social behavior development in rodents (Schulz & Sisk., 2016). Studies in rodents have shown that social recognition memory can be influenced by estrogens in adults (Ervin et al., 2015). In adult female mice, higher degree of social recognition memory has also been associated with the proestrus phase of the estrous cycle when estrogen levels are highest (Sánchez-Andrade and Kendrick, 2011). Improvement in social interaction and long-term social recognition memory in ovariectomized female mice has also been associated with chronic estrogen replacement (Tang et al., 2005). Further, a study in

female rats also found that ovariectomy in females prior to puberty prevented development of social recognition in adulthood, which could not be rescued by estradiol administration suggesting the potential role of ovarian hormones in development of circuitry and mechanisms underlying social recognition behavior in females (Yoest et al., 2023)

Studies from our lab using the MSEW and LBN adult mice, have shown sex differences in the social behavior impairment (Waters et al., 2022). In the current project we are interested in addressing the questions of whether the sex differences in the social recognition memory emerge over development in these ELA models, and if so, when they emerge. to address these questions, we designed a longitudinal behavioral study including males and females from both models and controls. We assessed social behavior at a juvenile timepoint (P21-immediately after weaning), followed by post puberty adolescent timepoint (P45).

Adult Neurogenesis in the Hippocampus

Decreased adult neurogenesis is one of the long-term effects of ELA on hippocampal plasticity mechanisms (Korosi et al., 2012). ELA has been shown to result in impaired adult neurogenesis through diminished differentiation and proliferation capacity of the progenitor cells (Boku et al., 2015; Brydges et al., 2018). New neurons are generated in the subgranular zone in the dentate gyrus of the hippocampus throughout adulthood. These newly born neurons get integrated into the existing circuitry in the granule cell layer of the dentate gyrus. The adult born neurons have been shown to actively participate in the functioning of these circuits (Cope & Gould, 2019). In rodents, the function and connectivity of these adult born neurons in the existing hippocampal circuits plays a role in social behaviors and response to stress (Monteiro et al., 2014; Garrett et al., 2015; Pereira-Caixeta et al., 2017, 2018; Cope et al., 2020).

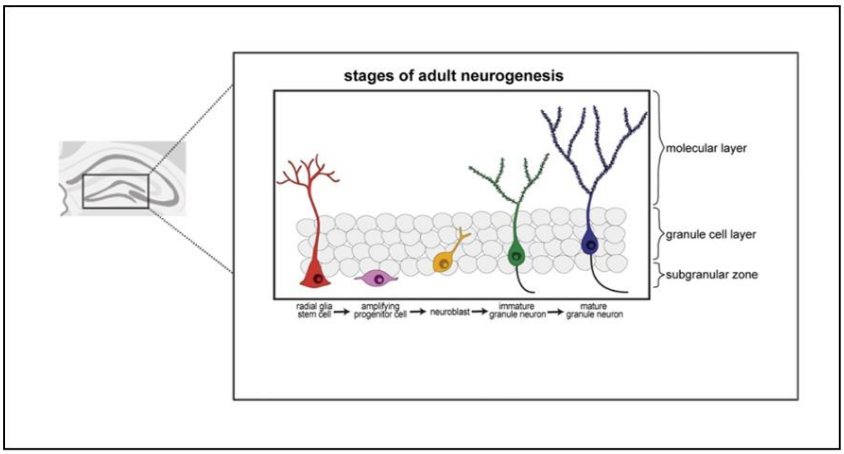

Figure 2. Adult neurogenesis in the subgranular zone of the dentate gyrus of hippocampus of rodents (Adapted from Cope & Gould 2019)

Work in our lab has shown that adult neurogenesis is decreased in the MSEW model in adult male mice. The decrease in neurogenesis was shown through decreased newly born adult born granule cells marked by 3R-tau and decreased SOX2 positive pluripotent progenitor cells (Waters et al., 2022). ELA has also been associated with higher vulnerability to severe medical conditions which result in shortened life expectancy (Lippard and Nemeroff, 2020), accelerated aging through epigenetic (Drury et al., 2012; Choudhari et al., 2022) and cellular mechanisms such as mitochondrial dysfunction and oxidative stress (Hoffmann & Spengler., 2018) Individuals with neuropsychiatric disorders and history of childhood adverse experience have been found to have shorter telomere lengths (Kananen et al., 2010; Tyrka et al., 2010), which is a cellular senescence marker used as a measure of biological aging. Furthermore, cellular senescence in the SGZ has been associated with declined adult neurogenesis in aging brains and the ablation of senescent cells in the neurogenesis-niche area in the SGZ has been shown to enhance proliferation and survival of adult born neurons and partially restore neurogenesis and associated cognitive function. This suggests that senescent cells in the SGZ can negatively influence the neural progenitor cells thereby affecting neurogenesis. (Fatt et al., 2022).

In this project we aimed to probe the mechanism causing the decrease in adult neurogenesis after MSEW in adult males by investigating the senescent cell population of the dorsal SGZ of adult male MSEW mice. Further, we were also interested in investigating whether housing the adult MSEW males in environment enriched (EE) housing could rescue the potential increase in senescent cells. EE has been known to enhance adult neurogenesis (Nilsson et al 1999; Van Praag, et al., 2000).

The goal of this thesis was to address 3 questions that arise from previous results regarding differential outcomes of the two ELA models in adult mice. First, we did a comparative study of the MSEW and LBN models, to characterize the differential postnatal rearing experience and gain insights into the differences in experience during the ELA manipulations of the two models. Second, based on the sex dependent outcomes of ELA seen in adult life, we asked whether and when sex differences in the social behavior impairment emerged during development into adulthood. Lastly, we investigated the effect of ELA on hippocampal plasticity specific to the MSEW model, by probing the mechanism of decreased adult neurogenesis observed in adult MSEW males.

Materials and Methods

1. Animals

C57BL/6J mice were obtained from Jackson Laboratories (strain #000664) and bred in-house for experiments. Mice were housed in Optimice cages with bedding and nesting material (unless mentioned otherwise) on a 12 h reverse dark-light cycle, with unlimited access to food and water. Pups were weaned into same-sex groups on P21 (unless mentioned otherwise). All animal procedures were approved by Princeton University's Institutional Animal Care and Use Committee and were in accordance with the guidelines of the National Research Council's Guide for the Care and Use of Laboratory Animals.

2. Early Life Adversity Models

Maternal Separation & Early Weaning (MSEW)

Daily maternal separations were done from P3 to P16. The pups are separated from the dam for 4 hours per day from P3–P6 and for 8 hours from P7–P16. The dam is removed from the home cage, and each cage containing the pups is placed on a thermal heating blanket and maintained at 34℃. Pups remained with their littermates, in a separate room from the dam, for the entire period of separation after which the dam was returned to the home cage. These pups are weaned early at P17. After weaning, the pups were housed in groups of same sex littermates (Murthy et al., 2019).

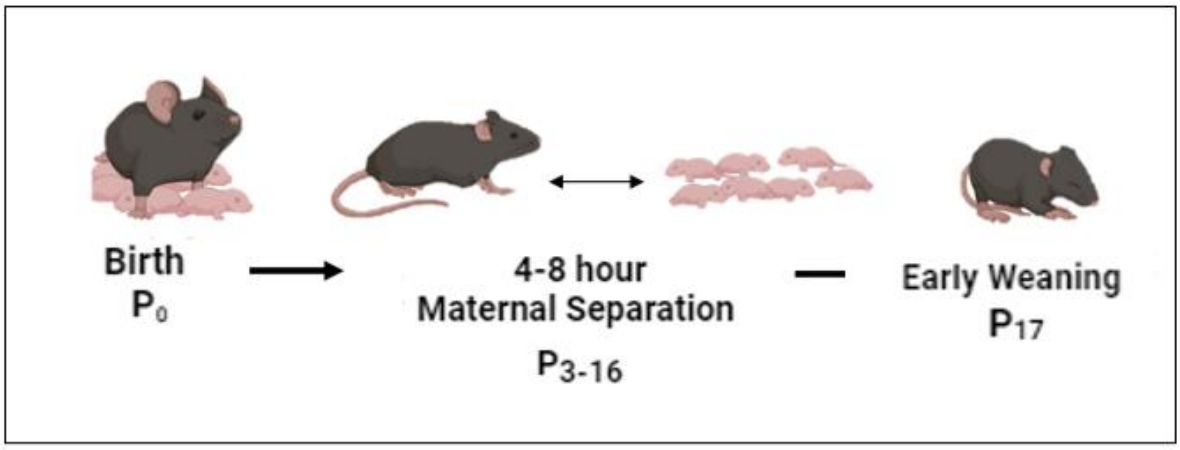

Figure 3. Schematic showing design of maternal separation combined with early weaning (MSEW) rodent model of ELA.

Limited Bedding and Nesting (LBN)

LBN treatment is done from postnatal day 4 (P4) to P11. The dam and her pups were transferred to a new cage on P4 with a perforated metal floor and were provided with no bedding accessible and minimal nesting material. On P11, the dam and her pups were returned to standard housing, with regular bedding and nesting and remained in the regular cage until weaning at P21. After weaning, the pups were housed in groups of same sex littermates (Waters et al., 2022).

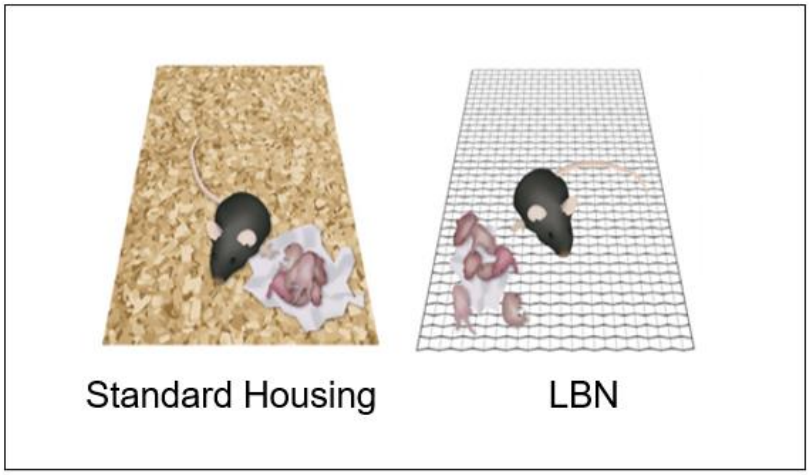

Figure 4. Schematic showing design of limited bedding and nesting (LBN) rodent model of ELA.

Both MSEW and LBN pups were compared to mice that were reared under standard laboratory cage conditions.

3. Environment Enrichment Housing

The MSEW and control adult male mice were housed in an enriched environment in satellite housing for a duration of 6 weeks, starting at P90. The EE housing involved satellite housing in Sterilite containers (34”x18”x12”), which were larger in size compared to the standard Optimice cages (13.5” X 11.5” X 6.1”) used in the standard vivarium housing. The MSEW and control colony mice were housed in the standard cages The EE containers provided adequate ventilation, food, a water bottle, and clean bedding. In addition to the regular nesting material, enriching objects including plastic washable pet-quality rodent toys and climbing structures. All objects were removed from the container once a week and cleaned. New objects were rotated into the environment every week. 6 age- and sex- matched mice were placed per enrichment container.

Figure 5. A. Standard mouse housing, cage dimensions - 13.5” X 11.5” X 6.1” B. Environment enriched (EE) housing design, cage dimensions 34”x18”x12”

4. Maternal Care Behavior

Pregnant female mice were housed in a satellite housing room in the same Optimice cages and with unlimited food and water supply. Three rearing groups – MSEW, LBN and control rearing (N = 6) were used for the study. The LBN and MSEW manipulations were done in the satellite housing, following the same protocols listed above. The control cages were left undisturbed. Home cage behaviors of the dams with their pups were videotaped. Video recordings of one hour were done at 3 equally spaced timepoints across the 24 h day; 2 dark phase recordings (9:00 – 10:00 am, 6:00 – 7:00 pm) and one light cycle recording (2:00 am – 3:00 am) (The MSEW, dams were present in the cages with the pups at all recording times) . The recordings

were done on 5 postnatal days – P3, P4, P6, P10 and P13. Three 5-minute-long videos were analyzed from equally spaced intervals of time across each 1-hour recording clip. Behavioral analyses of the videos were done using the Behavioral Observation Research Interactive Software (BORIS v. 8.24.1). The caregiving behaviors of the dam including presence on nest, entries and exits from the nest, arched back nursing, and nest maintenance were analyzed. The time spent on the nest was defined as the dam being in direct physical contact with the pups on the nest or within the region of the home cage where the pups were huddled together (in case on LBN), the entries and exits from nest were counted as entries and exits from these defined areas. Time spent on each behavior, number of occurrences, frequency, and mean bouts of each of these behaviors were calculated using the time budget function in BORIS. The transition coefficients were also calculated for each of the analyzed videos as a measure of transitions between each of these unique behaviors. All the behaviors were compared across different time points of the day and across postnatal days in the control, MSEW and LBN groups.

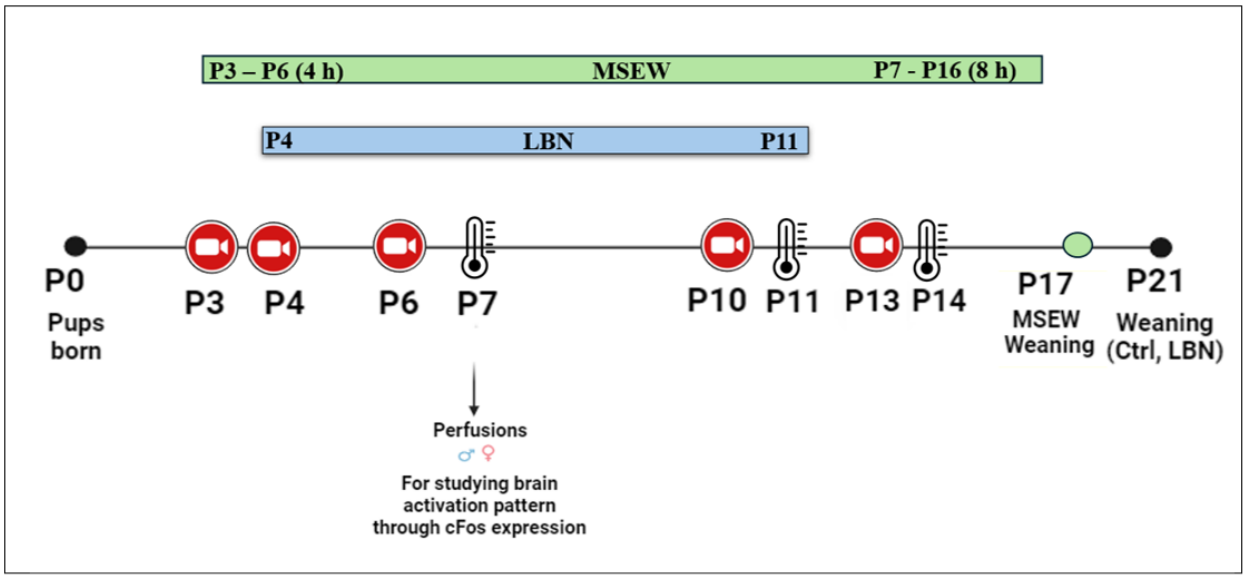

Figure 6. Experimental design and timeline for comparative study of postnatal experience in MSEW and LBN models.

5. Assessment of Body Surface Temperature in Postnatal Mice

Abdominal surface temperatures of male and female pups were recorded at P7, P11 and P14 during the dark cycle using an IR temperature gun. The dam was removed from the home cage and placed in a clean cage while taking temperature readings of the pups. Each mouse pup was taken out of the cage and taped to a clean table surface on its back with the abdomen facing up, temperature readings were taken immediately within few seconds of the pup being removed from its cage. The IR

temperature gun was pointed at the abdominal surface, close to the body, approximately within 5-10mm. The pups and dams were returned to their home cage after the readings were taken.

6. Direct Social Interaction Test (DSIT)

The direct social interaction test (DSIT) was done on both males and females from the MSEW, LBN and control rearing groups, to test social recognition memory (Diethorn and Gould 2022). The test was conducted on P21 (M = 10, F = 10 per group) and P45 (M = 10, F = 10 per group). Age- and sex- matched mice from a different litter were used as stimulus mice. A 12” x12” square acrylic arena with black walls and a clear base was used to conduct the DSIT. The test was conducted under low light conditions. The behavior was conducted between 9 am and 6 pm during the dark phase of the light cycle. The mice were habituated to the testing room for 15 mins prior to behavior and each mouse was habituated to the testing arena for 5 minutes before the actual test on the same day as testing. The DSIT consisted of two 5-minute trials, separated by a 1 hour inter trial interval. In trial 1 or ‘novel’ trial, the test mouse was paired with a novel stimulus age- and sex- matched conspecific and allowed to interact freely in the arena for 5 minutes. In the inter trial interval, the mice were returned to their home cages. Following the 1-hour inter-trial interval, in trial 2 or ‘familiar’ trial, the same two mice were paired again and were allowed to interact freely for 5 minutes. The DIST trials were recorded using a video camera placed above the arena. The time spent by the test mouse actively interacting and investigating the stimulus conspecific in both the trials was manually scored with the experimenter blinded to the trial and experimental group of the mouse. Active social interaction and investigation by the test mouse included behaviors like anogenital sniffing and allogrooming. The events counted as social interactions were strictly restricted to interactions initiated by the test mouse and were defined as the events were test mouse oriented its nose and touched the stimulus mouse to initiate social interaction. The DSIT arena was cleaned thoroughly by 70% ethanol between each trial and animal. The time spent in active social investigation in trial 1 (novel) was compared to trial 2 (familiar) for each mouse. A discrimination index was calculated for each mouse that assessed the novelty preference. A positive discrimination index, indicating higher time spent investing a novel mouse compared to the familiar mouse is the expected response in controls (Diethorn and Gould 2022) that represents an intact novelty preference.

Calculation of discrimination index:

N = Time spent actively investigating Novel mouse in trial 1

F = Time spent actively investigating familiar mouse in trial 2
Total investigation time = N + F

Discrimination Index (D.I) = (N-F) / T

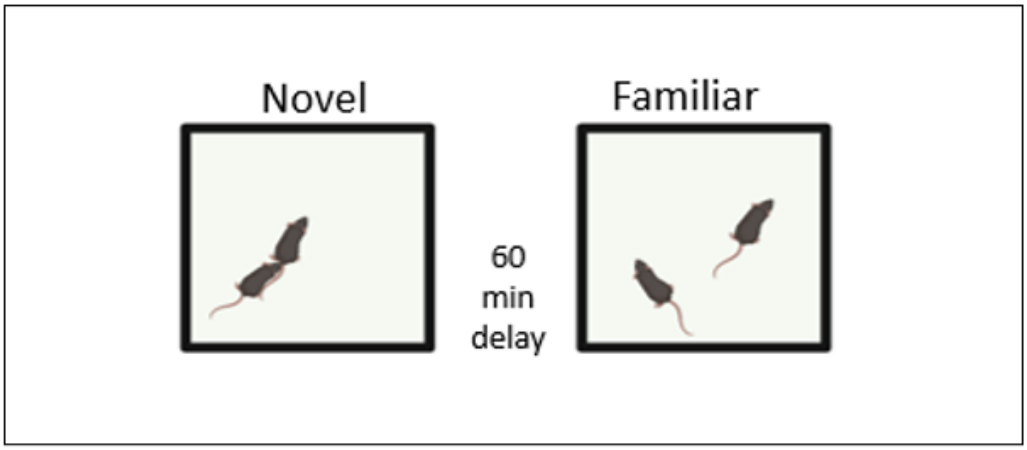

Figure 7. Schematic showing design of the direct social interaction test (DSIT), depicting the 'novel' trial1, and 'familiar' trial 2, separated by a 60 min (1h) inter-trial-interval.

7. Assessment of Puberty Onset

External markers of onset of puberty, including preputial separation in males and vaginal opening in females, were monitored every other day, in males and females (N = 10 M, F from each group) from all three groups – MSEW, LBN and controls, starting from P21. The protocol for this was followed from - Hoffmann 2018**.**

8. Histology

8.1 cFos Immunolabeling

Pups from each litter from all three rearing groups; MSEW, LBN and controls (N = 18 males and N = 18 females) were perfused on P7. Two pups (1 male and 1 female, were taken from each litter). The pups were deeply anesthetized with Euthasol and perfused with cold 4% paraformaldehyde (12mL). Whole brains were extracted and postfixed in 4% paraformaldehyde for 48 h. Following this, the right halves of the brains

were stored in 0.1 M PBS azide at 4℃ until tissue clearing for iDISCO. The left halves of the brains were cryoprotected in 0.1 M PBS with 10% sucrose solution and were frozen and stored at -80℃ until sectioning for conventional immunohistochemistry. cFos immunolabeling was done using both the conventional immunohistochemistry as well as iDISCO tissue clearing.

Brain clearing and cFos immunolabelling was done on the right halves of the P7 brains using the iDISCO+ protocol (Pisano et al., 2022). The brains were rinsed in 0.1M PBS at room temperature for three 30 min sessions. Immediately brains were dehydrated 30 min at each ascending concentration of methanol (20, 40, 60, 80, 100,100%) and placed overnight in 5% hydrogen peroxide and methanol at room temperature. The following day, brains were rehydrated for 30 min at each descending concentration of methanol (100, 80, 60, 40, 20%) and lastly in 0.1M PBS. Samples were then placed in 0.2% Triton X-100 in 0.1M PBS for 30 min and then placed in 20% Dimethyl sulfoxide (DMSO), 0.3M glycine, 0.2% Triton X-100 in 0.1M PBS for 2 days at 37 °C. Brains were blocked in 10% DMSO, 6% donkey serum, 0.2% Triton X-100 in PBS at 37 °C for 5 days. Once at room temperature, samples were washed twice in PTwh (0.2% Tween-20,10 µg/ml heparin in PBS) for 1 h each and then transferred to the primary antibody (rabbit anti-Fos (226 003, Synaptic Systems, Goettingen,Germany, 1:1000) in a solution in 5% DMSO and 3% donkey serum in PTwh solution for 3 days at 37°C. Brains were then washed in PTwH five times in increasing amounts of time (10,15, 30, 60, 120 min) and then placed in secondary donkey anti-Rabbit Alexa Fluor 647-conjugated secondary antibody (A-21449; Thermo Fisher Scientific, MA, USA, Invitrogen; 1:200) in 3% donkey serum in PTwh solution for 5 days at 37 °C. Brains were then washed in PTwH five times in increasing amounts of time (10, 15, 30, 60, 120 min) and then dehydrated 1 h at each ascending concentration of methanol (20, 40, 60, 80, 100, 100%) until being placed in 66% dichloromethane (DCM)/ 33% methanol for 1 h at room temperature. Brains were then cleared with 100% DCM for two 15 min steps and then placed in 100% benzyl ether (DBE). Brains were kept in fresh DBE prior to imaging on a light sheet microscope and after for long-term storage.

For free floating conventional immunohistochemistry, the left half of the brains were cut on a Leica CM3050S cryostat in 40 µm-thick sections and collected into 0.1 M PBS with 0.1% sodium azide. Sections were first washed in 0.1M PBS for 15 min on a shaker at room temperature and then transferred to a blocking solution of 3% normal donkey serum in 0.1M PBS with 0.3% Triton X-100 for 1.5 h on a shaker at room temperature. Sections were then incubated in the primary antibody (rabbit anti-Fos (226 003, Synaptic Systems, Goettingen,Germany, 1:250) solution in 0.3% Triton X-100 in 0.1 M PBS overnight on a shaker at 4℃. Following this, sections were washed in 0.1M PBS for 15 min on a shaker at room temperature and then incubated in the secondary antibody (A-21449; Thermo Fisher Scientific, MA, USA, Invitrogen; 1:250) solution in 0.3% Triton X-100 in 0.1 M PBS for 2h on a shaker at room temperature. Following this, sections were incubated in the counterstain Hoechst 33342 (1:5000 µl) for 10 min on the shaker at room temperature and then transferred to 0.1 M PBS.

Sections were then mounted on Superfrost Plus slides (Fisher Scientific) and were dried and then coverslipped with Vectashield antifade mounting media (Vector Labs) before imaging.

8.2 Senescence-Associated Beta Galactosidase (SA-β-gal) Staining.

4-5-month-old male MSEW and control mice were deeply anesthetized with Euthasol and perfused with cold 4% paraformaldehyde (40 mL for adult mice). Whole brains were extracted and postfixed in 4% paraformaldehyde for 48 h. following this, the brains were cryoprotected in 0.1 M PBS with 30% sucrose solution and were frozen and stored at -80℃ until sectioning. Brains were cut on a Leica CM3050S cryostat in 40 µm-thick sections and collected into 0.1 M PBS with 0.1% sodium azide. 6 to 8 unilateral sections spanning the entire dorsal hippocampus were sampled from each brain in a 1 in 4 series. Neuroanatomically matched sections between experimental groups were used. Free-floating staining was performed using the Senescence β-Galactosidase staining kit - 9860, Cell Signaling Technology, Danvers MA, USA, to visualize the senescent cells in the subgranular zone of the dentate gyrus. The standard staining protocol from the manual of the kit was followed which involved first washing the sections in 0.1M PBS for 15 min on a shaker at room temperature, followed by the fixing in 1X fixative solution. The sections were then washed twice in 0.1M PBS for 15 min on a shaker at room temperature. Sections were then incubated in the beta-gal staining solution at 37℃ in the dark for 48h. Sections were mounted wet in the beta-gal staining solution on Superfrost Plus slides (Fisher Scientific) and cover slipped wet with glycerol as mounting medium.

9. Imaging and analysis

9.1 Light Sheet Microscopy

To obtain a brain wide map of differential cFos activation, the iDISCO+ cleared cFos immunolabelled P7 brain samples were imaged using a LaVision Ultramicroscope II - light sheet microscope. The cleared brains were glued (Loctite, 234796) to a 3D printed holder and were imaged in DBE. cFos images were acquired using 640 nm excitation and 680 nm emission (×1 magnification, ×1.3 objective, 0.1 numerical aperture, 9.0mm working distance, 12.0 × 12.0 mm field of view, LVMI-Fluor 1.3x, LaVision Biotech) with a 10 µm step-size using a 0.016 excitation NA. Cells with positive immunolabeling were counted using ClearMap (Renier et al., 2016), on high-performance computing clusters. The tissue image processing and registration were performed using - https://github.com/PrincetonUniversity/BrainPipe, a custom Python

code. The DevCCF developmental mouse brain atlas (https://kimlab.io/brain-map/DevCCF/ - Kronman et al., 2023) was used to identify the activated regions in the P7 brains and Neuroglancer - a Google WebGL-based was used to create 3-D representations of volumetric data for each brain to analyze region specific and brain wide cFos immunolabelled regions.

9.2 Confocal Microscopy

Images of conventional immunohistochemistry were collected using a Leica SP8 confocal microscope with LASX imaging software. Images were taken with 10x or 40x objectives, and images used for quantitative analyses were collected as z-stacks with a 1 µm interval between optical sections. Confocal images were analyzed using Fiji (Image J) (NIH).

9.3 Brightfield microscopy

The SA-*β*-Gal-stained sections were imaged on a Olympus BX60 brightfield microscope using the MBF bioscience Stereo investigator software. Quantification of the SA-*β*-Gal positive cells in the SGZ of DG of the dorsal hippocampus was done using the particle analysis plugin in the Fiji (ImageJ-NIH) software. Area measurements of the SGZ were taken on the MBF bioscience Stereo investigator.

10. Statistical Analysis

Statistical analyses were conducted using GraphPad prism 10.1.0 and lme4 package (Bates et al., 2015). All graphs were prepared using GraphPad Prism 10.1.0. All data are presented as the mean ± standard error of mean (SEM), unless mentioned otherwise. The p value being less than 0.05 was set as the criterion of statistical significance in all tests. The maternal care behavior data sets were analyzed using the linear mixed effects model (lme4 package). The cFos immunolabeling data were analyzed using a non-parametric Kruskal-Wallis test with Dunn's multiple comparisons or one-way ANOVAs in GraphPad prism. All the other behavioral analyses and temperature, puberty data and SA-*β*-Gal histology involving more than two groups were analyzed using mixed effects model, one-way or two- way ANOVAs in GraphPad prism as appropriate. Tukey's, Sídák's (puberty onset data) or uncorrected Fisher's LSD (SA-*β*-Gal histology) post hoc multiple comparisons were used to follow up any main effects or significant interactions in the ANOVAs.

Results

1. Differential Experience of MSEW and LBN Rearing in the Early Postnatal Period

1.1 Maternal Care

Through a comparative study of maternal care in MSEW and LBN models in the first two postnatal weeks, we characterized differential maternal care-giving behavior patterns of the two models ELA models and compared them to control rearing patterns. We assessed care-giving behaviors of the dam including presence on nest, entries to nest, exits from nest, arched back nursing, and nest maintenance. Home cages were recorded at 3 equally spaced timepoints across the 24 h day, for 1h each, including 2 dark phase and 1 light phase recording on P3, P4, P6, P10 and P13. Behavioral analyses were done by sampling three 5-minute-long videos from equally spaced intervals of time across each 1-hour recording clip. First, we analyzed the time spent by the dam on the nest, or the time spent by the dam in direct physical contact with the pups within the region of the home cage where the pups were huddled together, in case of LBN, where there is no defined nest. After running a linear mixed effects analysis on the overall time spent (represented as % of total time) on the nest, we found that the MSEW dams spend significantly higher time on the nest ($p = 0.0313$). No difference was seen between time spent by LBN moms compared to controls ($p = 0.6819$). However, the mean bout of time on nest was significantly decreased in the LBN group ($p < 0.0001$).

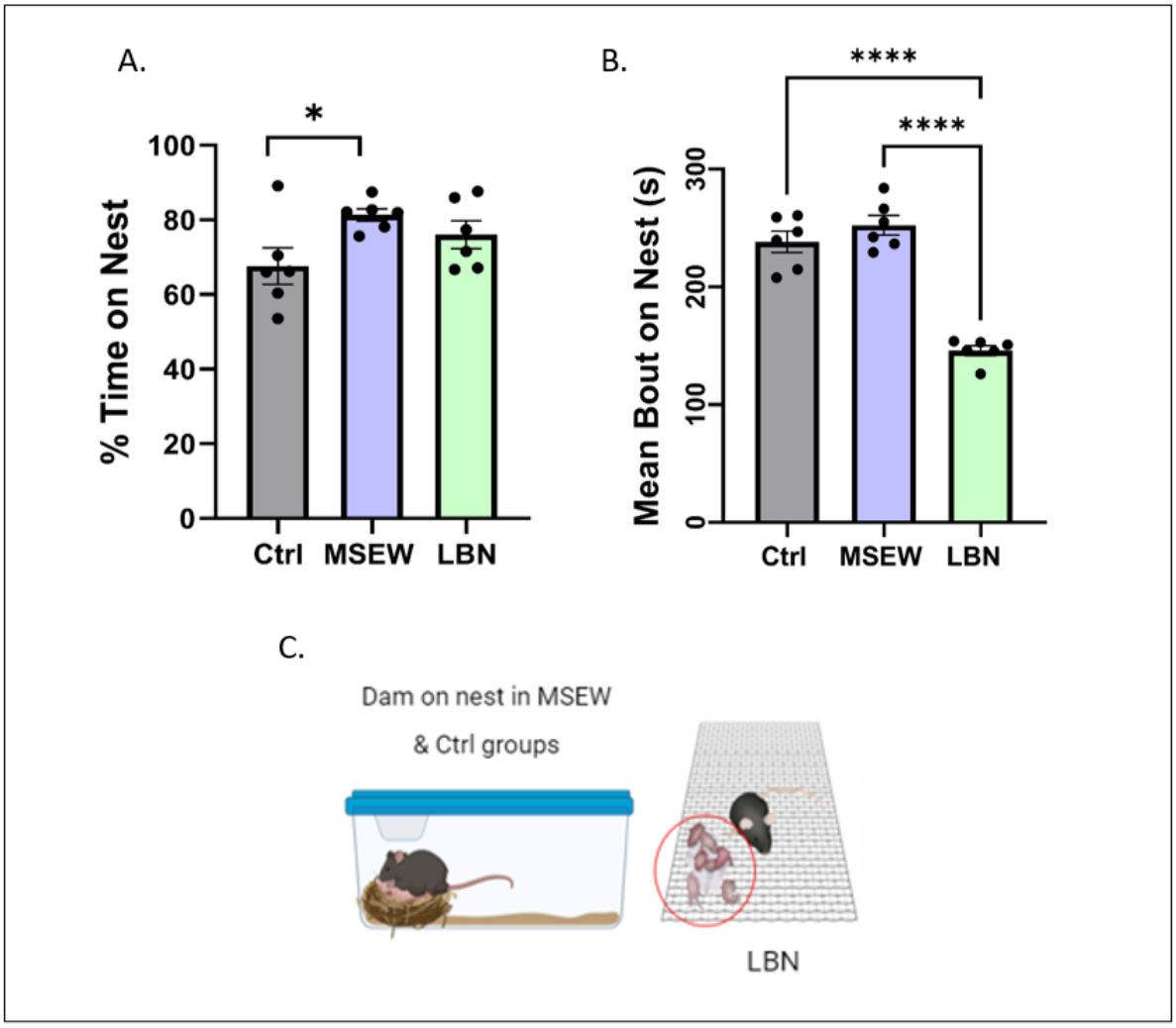

Figure 8. Analysis of time spent on nest by dam. A. MSEW dams show increased time on nest compared to controls (p = 0.0313). B. LBN dams show significantly lower duration of mean bout spent on nest (p < 0.0001) *C. Schematic showing the behavior defined as presence dam on nest; left- standard home cage for MSEW and controls, and right, LBN cage – presence of mom in contact with pups within the red circled region was defined as presence of the dam on nest.* (*Control N = 6, MSEW N = 6, LBN N = 6) *p < 0.05, **** p ≤ 0.0001*

In addition to the overall time spent and bouts of time on nest, in order to assess the continuity in care-giving we quantified the transitions of the dam to- and from- the nest by counting the average number of entries to the nest and exits from nest per video session. The LBN dams showed greater number of entries to the nest compared to the control ($p<0.0001$), as well as MSEW dams ($p<0.0001$), and larger number of exits from the nest compared to the control ($p<0.0001$), as well as MSEW dams ($p<0.0001$) suggesting larger number of discontinuous caregiving bouts on the nest. These findings correspond to the lower mean bout time on nest shown by LBN dams.

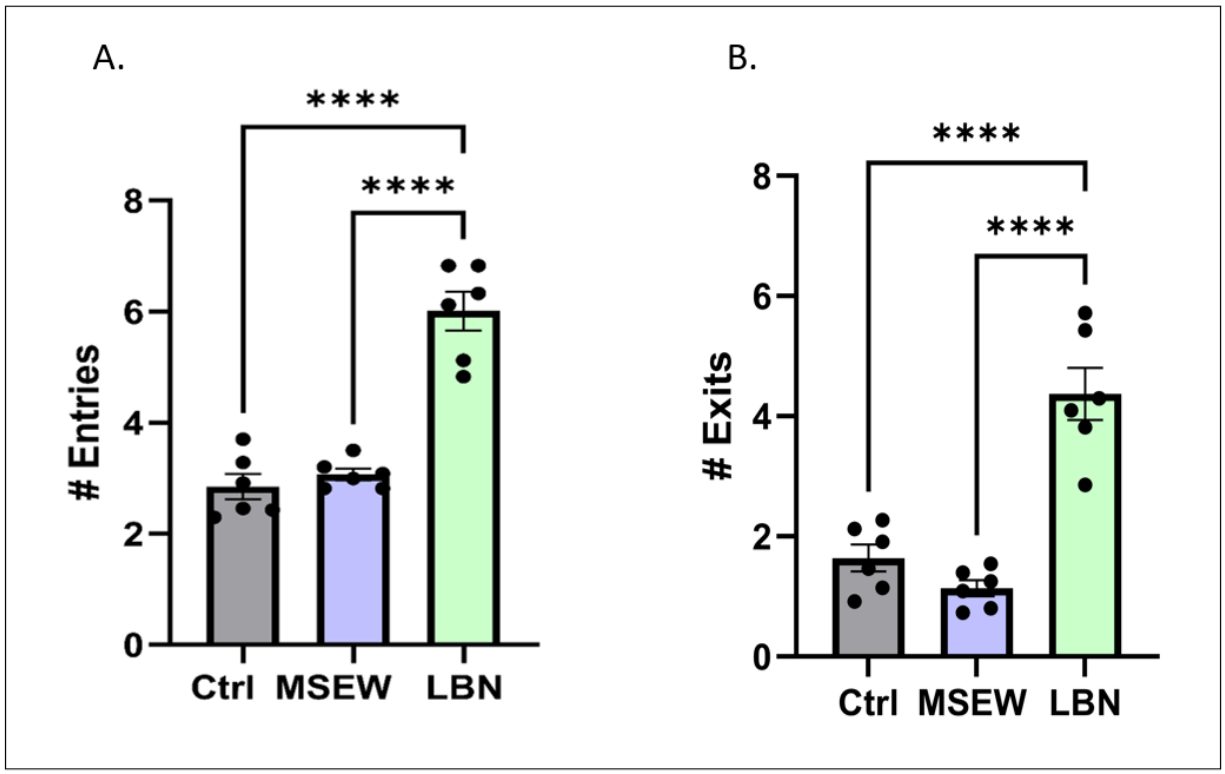

*Figure 9. Average number of entries to nest by dam. A. LBN dams show increased number of entries to the nest compared to controls ($p <0.0001$) and MSEWs ($p <0.0001$) B. LBN dams show increased exits from nest compared to controls ($p <0.0001$) and MSEWs ($p <0.0001$). (Control N = 6, MSEW N = 6, LBN N = 6), **** $p \leq 0.0001$*

Next, we analyzed the time spent by dams in active arched back nursing, only the time spent in the characteristic arched back position while nursing (depicted in Fig 10C) was counted, passive nursing was not considered. Based on the linear mixed effects model, no difference was seen in time spent in arched back nursing across the groups (Ctrl vs MSEW, p = 0.2775, Ctrl vs LBN, p = 0.2412), the mean time of each bout of nursing was also not different across groups (Ctrl vs MSEW, p = 0.2727, Ctrl vs LBN, p =0.2775).

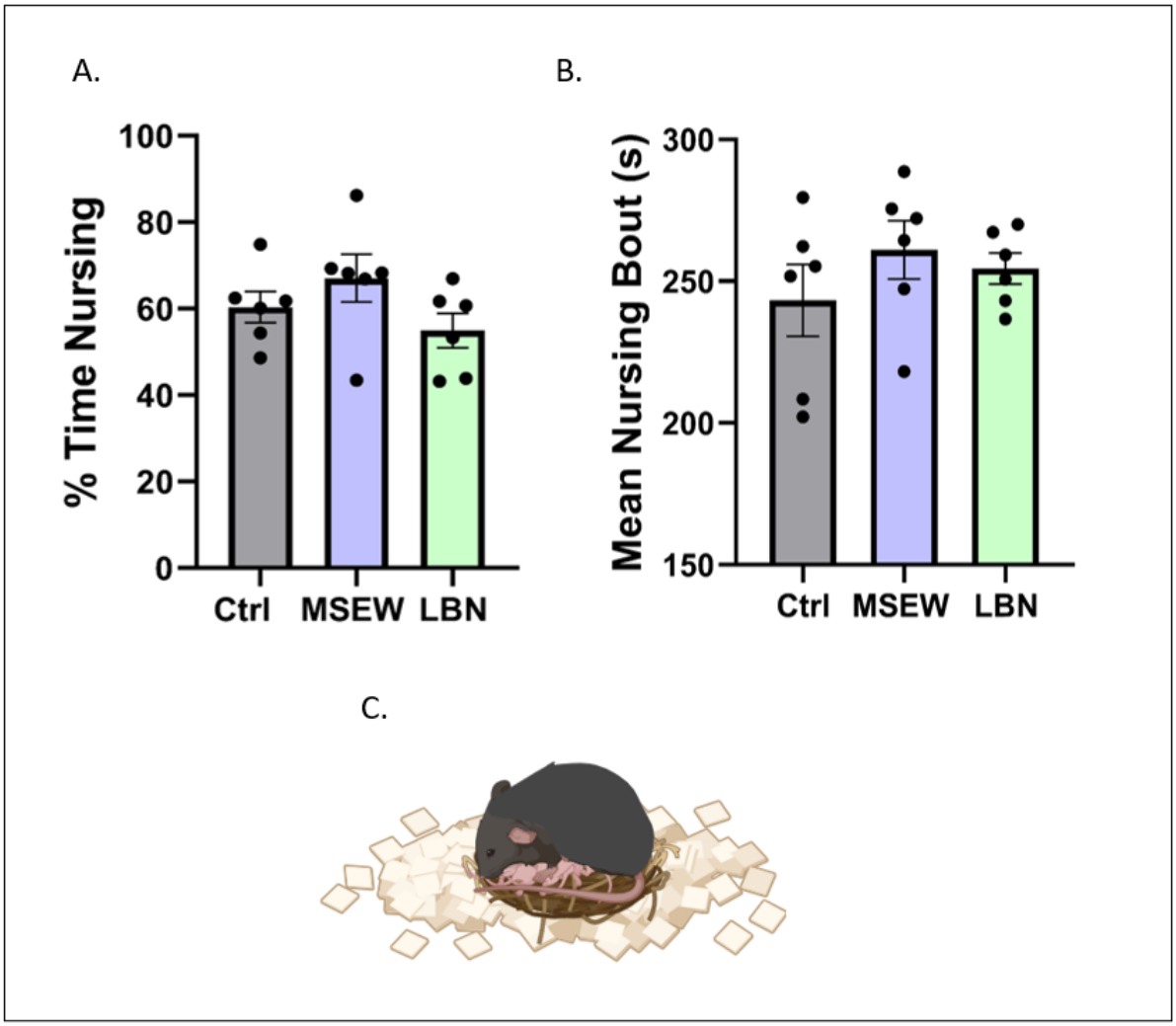

Figure 10. Time spent in arched back nursing by dam. A. No difference in time spent in arched back nursing by dams across groups. (Ctrl vs MSEW, p = 0.1389, Ctrl vs LBN, p = 0.2775), B. No difference seen in average time nursing per bout (Ctrl vs MSEW, p = 0.2727, Ctrl vs LBN, p =0.2775) C. Schematic showing the characteristic arched back nursing position of dam. (Control N = 6, MSEW N = 6, LBN N = 6)

Lastly, we looked at the nest maintenance behaviors of dams in all the three groups. Nest maintenance behaviors included the dam using her forelimbs and mouth to compose the nest, fraying, chewing of nesting material strands and reorganizing them to cover the pups and maintain integrity of nest, and organizing bedding to support nest. A linear mixed effects analysis showed no differences were found in this behavior across groups (Ctrl vs MSEW, p = 0.6183, Ctrl vs LBN, p = 0.6656).

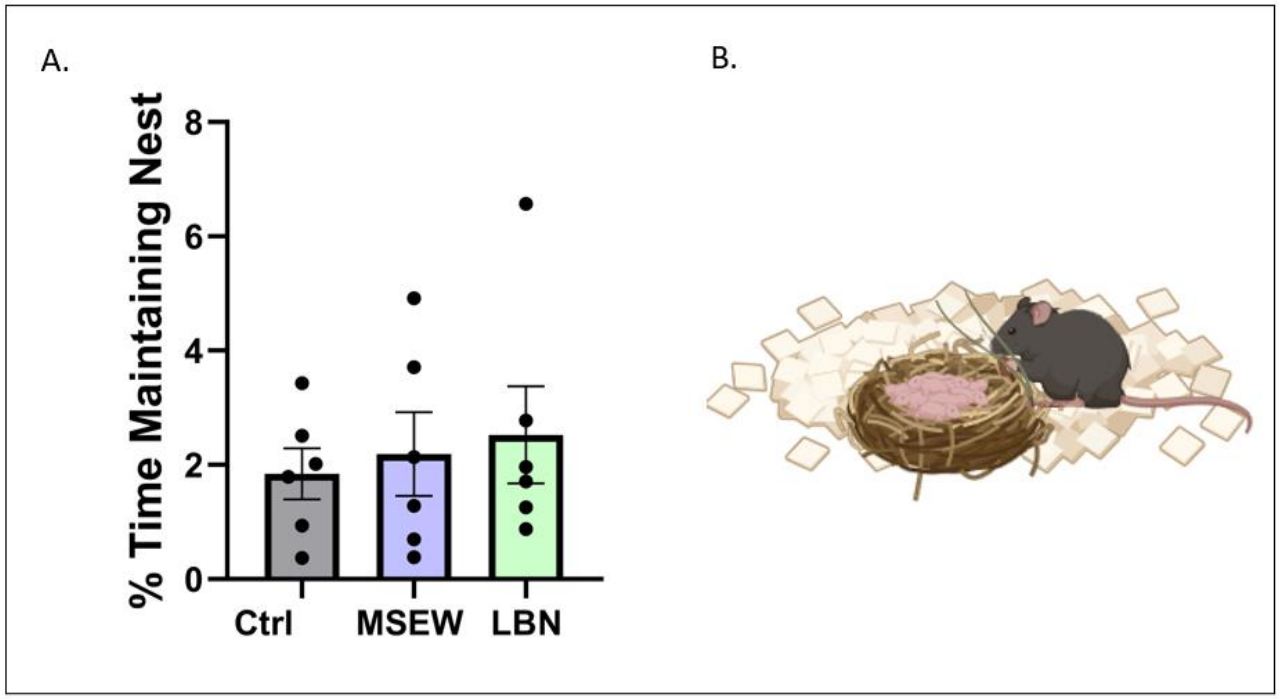

Figure 11. Time spent in nest maintenance behaviors by dam. A. No difference in time spent in nest maintenance behaviors by dams across groups. (Ctrl vs MSEW, p = 0.2775, Ctrl vs LBN, p = 0.2412), (Control N = 6, MSEW N = 6, LBN N = 6), B. Schematic showing the nest maintenance behaviors involving use of forelimbs and mouth.

1.2 Body Surface Temperature

To assess the effects of the unique home cage environments and rearing experience of the MSEW and LBN manipulations on thermoregulation in pups, we measured abdominal surface temperatures of male and female pups from each litter from all 3 rearing groups on postnatal days (PND); P7, P11 and P14. A mixed-effects model revealed a significant interaction between PND and group ($p < 0.0001$). Significantly lower abdominal surface temperatures were observed in the LBN group on P7 and P11 compared to the other two groups (Fig 12). However, on P14 the control group showed lower temperatures compared to MSEW and LBN. No effect of sex nor significant interaction between group and sex was seen on abdominal surface temperatures on any of the postnatal days (Fig 13).

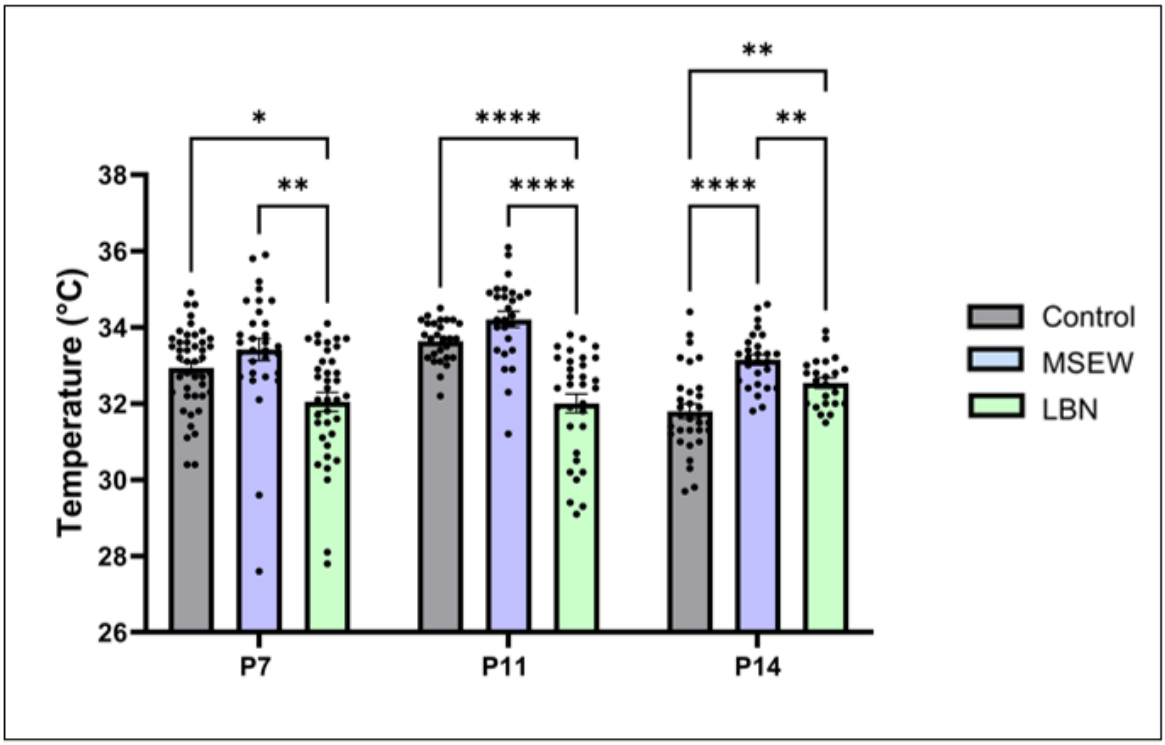

*Figure 12. Abdominal surface temperature of postnatal mice undergoing ELA manipulations on P7, P11, P14. LBN pups show decreased abdominal surface temperature on P7 (Ctrl v. LBN: p = 0.0103, MSEW v. LBN: p = 0.0018, Control N = 44, MSEW N = 31, LBN N = 37) and P11 (Ctrl v. LBN, p <0.0001, MSEW v. LBN, p<0.0001, Control N = 27, MSEW N = 27, LBN N = 32), on P14, MSEW and LBN show higher temperature compared to the controls (MSEW v. Ctrl, p < 0.0001, LBN v. Ctrl, p = 0.0074, Control N = 32, MSEW N = 27, LBN N = 23), MSEW show higher temperature than LBN (MSEW v. LBN, p =0.0087). *p < 0.05, **p ≤ 0.01, **** p ≤ 0.0001.*

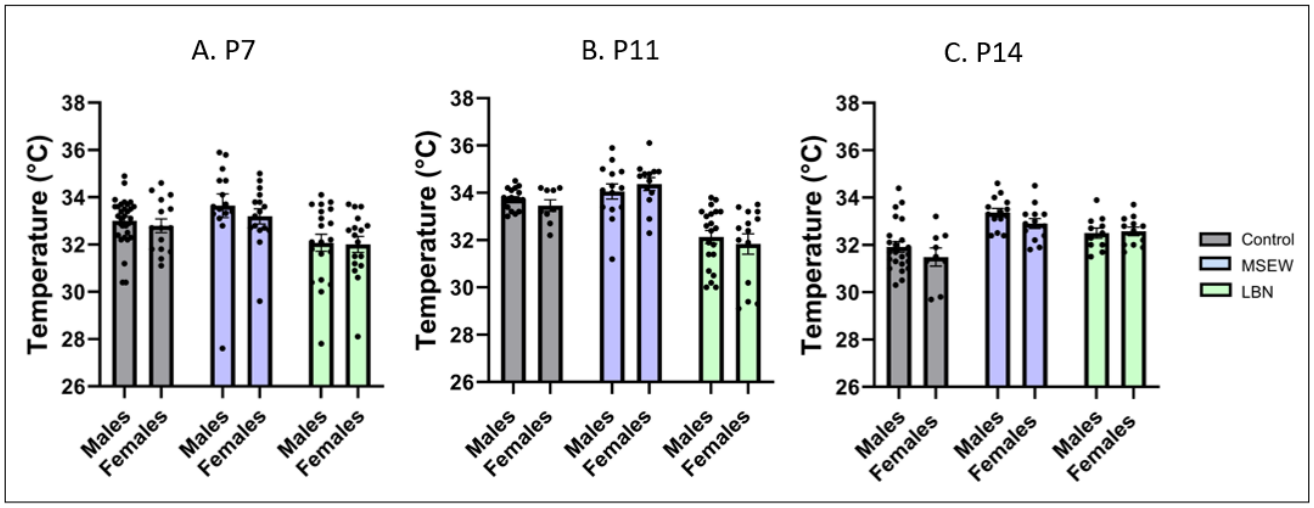

Figure 13. Sex separated, PND wise abdominal surface temperature. No sex differences seen on any of the days. A. P7 (p = 0.1418, Control M = 29, F = 15 , MSEW M = 15, F= 16, LBN M = 20, F = 17), B. P11 (p = 0.7267, Control M = 18, F = 9 , MSEW M = 14, F= 13, LBN M = 20, F = 12), C. P14 (p = 0.1348, Control M = 22, F = 10 , MSEW M = 14, F= 13, LBN M = 11, F = 12)

1.3 Differential Neuronal Activation

To develop a comprehensive understanding of how the differential maternal caregiving and environmental experiences are encoded and affect different brain regions in MSEW and LBN, we are mapping differential activation of brain regions during ELA experiences by looking at cFos expression in P7 pups using the iDISCO method for brain clearing and immunolabeling. Based on some preliminary analyses of the cFos expression in the overall hippocampus, CA2 and CA3, a one-way ANOVA with Tukey's post hoc comparisons showed that LBN resulted in significantly lower cFos positive cells in the overall hippocampus compared to controls ($p = 0.0288$), no significant difference was found between controls and MSEW ($p = 0.1081$), or between MSEW and LBN ($p = 0.7958$). In the CA2 and CA3 subregions of the hippocampus, both MSEW and LBN resulted in lower activation compared to controls (CA2 - Ctrl vs MSEW p = 0.0079, Ctrl vs LBN, 0.0084), (CA3 - Ctrl vs. MSEW, p = 0.0188, Ctrl vs LBN, 0.0047). We are currently working on further analyses of other brain regions.

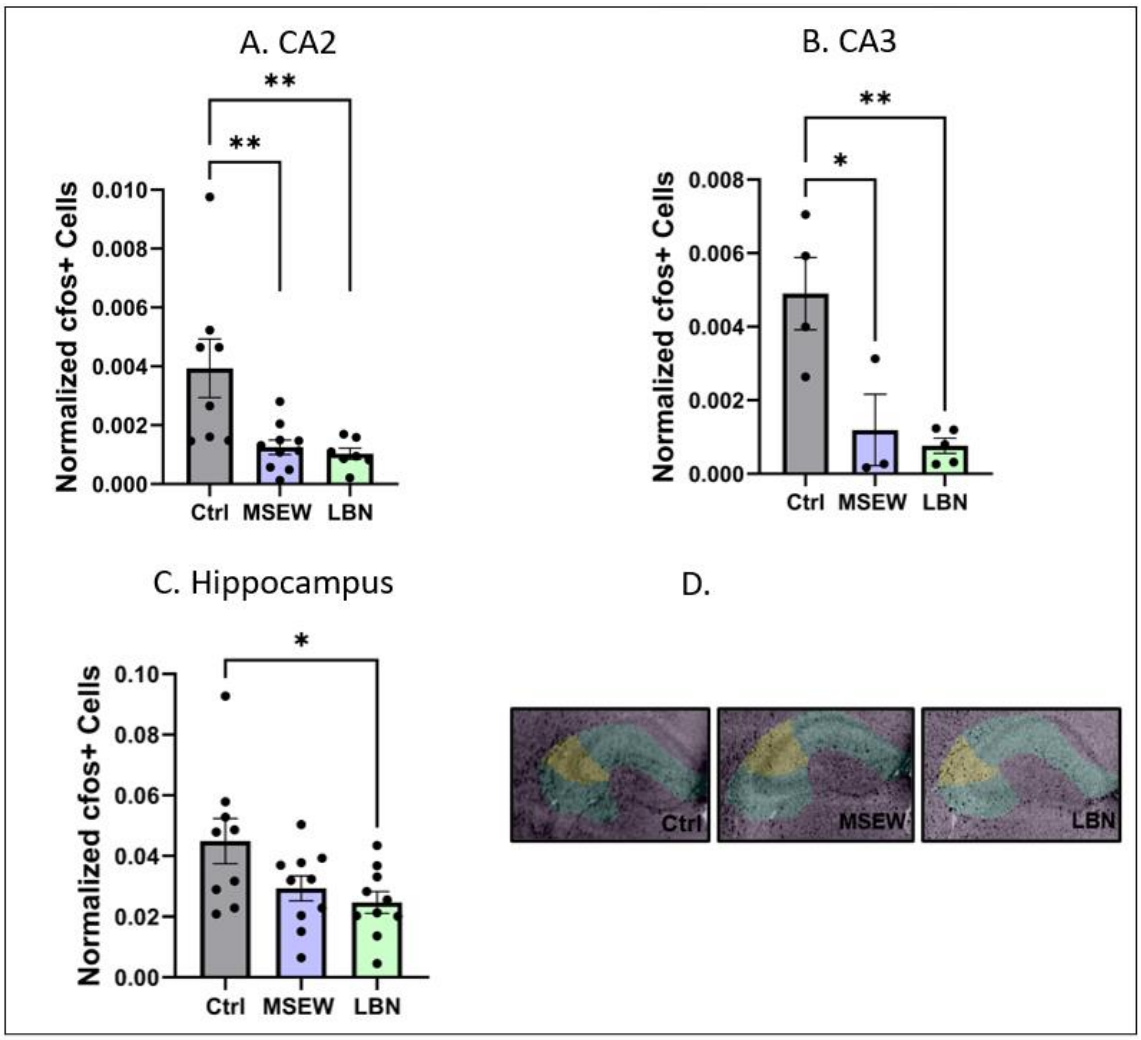

Figure 14. Normalized cFos counts in overall hippocampus, CA2, and CA3 at P7. A. MSEW and LBN show lower number of cFos+ cells in CA2 compared to controls (Ctrl

*vs. MSEW, p = 0.0079, Ctrl vs LBN, 0.0084, N = 8 Controls, 10 MSEW, 7 LBN) B. MSEW and LBN show lower number of cFos+ cells in CA3 compared to controls (Ctrl vs. MSEW, p = 0.0188, Ctrl vs LBN, 0.0047, N = 4 Controls, 3 MSEW, 5 LBN). C. LBN shows lower number of cFos+ cells compared to the controls in the hippocampus (Ctrl v. LBN, p = 0.0288), no difference between control and MSEW (Ctrl vs MSEW, p = 0.1081, N = 9 Controls, 10 MSEW, 10 LBN). D. Representative images of cFos+ cells detected in the hippocampus. *p<0.05, **p < 0.001*

2. Emergence of Sex Differences in Social Behavior Impairments

Previous work from our lab showed that both MSEW and LBN lead to impaired social recognition memory in adult males but not females (Waters et al., 2022). In the current study we were interested in investigating the emergence of this sex difference in social behavior impairment over development, particularly with respect to puberty. For that, we did a longitudinal study assessing social recognition memory using a direct social interaction test for males and females at P21; a juvenile, pre-pubertal timepoint, followed by a second test at P45 at a post puberty, adolescent timepoint. A two-way ANOVA with repeated measures revealed that at P21, the control group showed a significant difference between the novel and familiar investigation times in both the males ($p = 0.0227$) and females ($p = 0.0278$), 100% males, and 90% females showed a positive discrimination index indicative of a clear novelty preference. In the MSEW group, no difference between the novel and familiar investigation times was seen in the males ($p = 0.3579$) or females ($p = 0.0809$), and only 40% males, and 50% females showed a positive discrimination index. In the LBN group, no difference between the novel and familiar investigation times was seen in the males ($p = 0.3733$) or females ($p = 0.4007$), and only 40% males, and 60% females showed a positive discrimination index. A two-way ANOVA of the discrimination indices showed a main effect of group ($p = 0.0002$) but did not show a main effect of sex ($p = 0.4422$), nor a significant interaction between group and sex ($p = 0.5623$), thus showing that there are no sex differences in social recognition memory at P21.

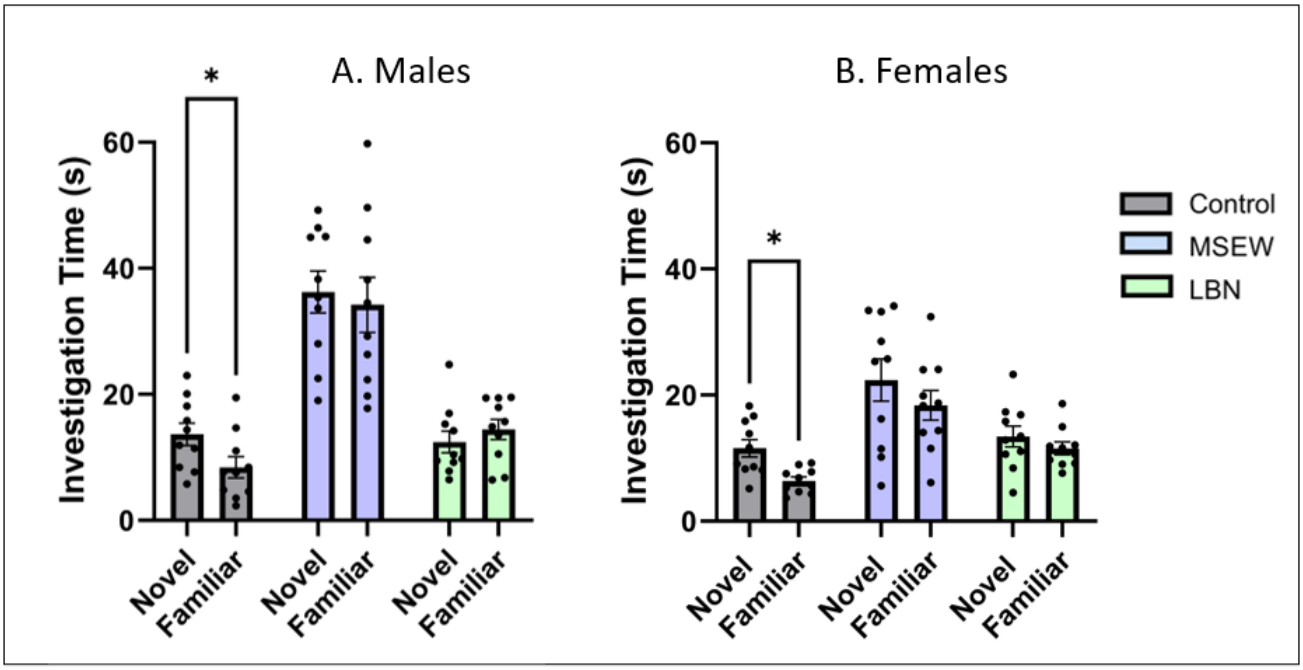

*Figure 15. Investigation time during DSIT at P21. A. Control males show significant reduction in social investigation time from novel to familiar trial (p = 0.0227), this reduction in social investigation time is not seen in MSEW (p = 0.3579) and LBN (p = 0.3733) males. B. Control females show significant reduction in social investigation time from novel to familiar trial (p = 0.0278), this reduction in social investigation time is not seen in MSEW (p = 0.0809) and LBN (p = 0.4007) females. (Control N = 10, MSEW N = 10, LBN N = 10) *p < 0.05*

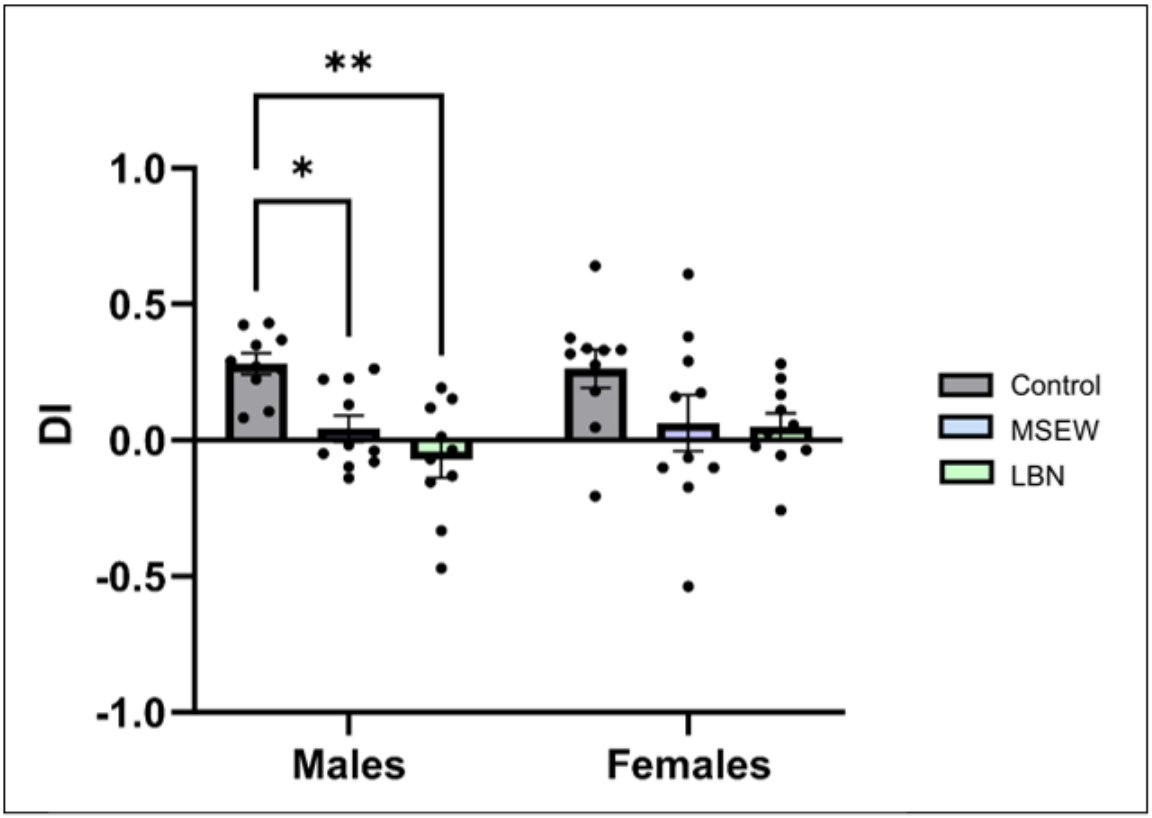

*Figure 16. Social discrimination indices (DI) at P21 of males and females from control, MSEW and LBN. No interaction between sex and group seen (p = 0.5623, Control N = 10, MSEW N = 10, LBN N = 10). *p < 0.05, **p ≤ 0.01*

At P45, a two-way ANOVA with repeated measures revealed that at P45, the controls continued to show a significant reduction in investigation time from the novel to familiar trial. This reduction was shown by both the males ($p<0.0001$) and females ($p < 0.0001$) and 100% males, and 100% females showed a positive DI indicative of a clear novelty preference (Fig 18). In the MSEW group, though a difference between the novel and familiar investigation times was seen in the males ($p = 0.02$) only 70% males showed a positive DI, and the mean DI was significantly lower than control males ($p = 0.0052$), whereas 100% MSEW females showed reduced investigation time from the novel to familiar trial ($p < 0.0001$) and showed a positive DI which was comparable to control females ($p =0.5990$). In the LBN group, no difference between the novel and familiar investigation times was seen in the males ($p =0.2291$), though 80% males showed a positive DI, the mean DI significantly lower than controls ($p = 0.0071$). In contrast, 100% LBN females showed a positive discrimination index and reduced investigation time from novel to familiar trial ($p < 0.0001$) and had DIs comparable to controls ($p = 0.7717$. A two-way ANOVA of the discrimination indices showed a main effect of group ($p =0.0067$) and sex ($p< 0.0001$), thus suggesting that the sex differences in social recognition memory emerge by P45.

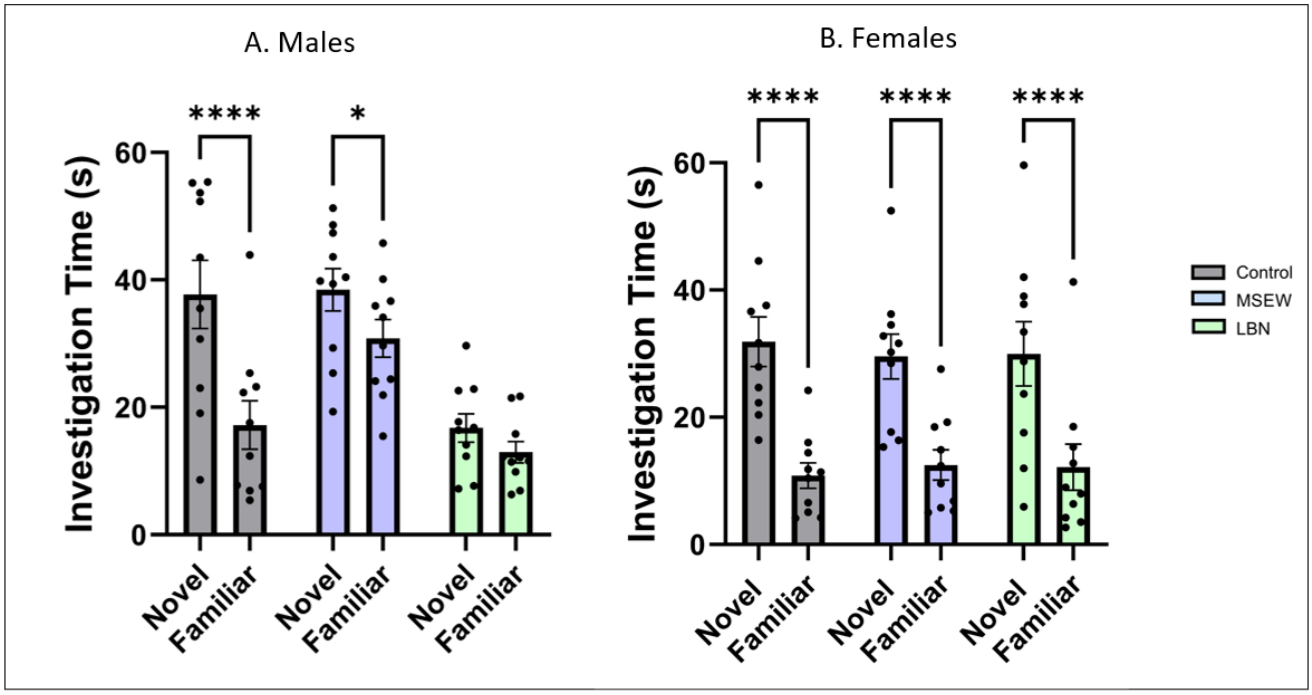

*Figure 17. Investigation time during DSIT at P45. A. Significant reduction in social investigation time from novel to familiar trial in males seen in control (p< 0.0001) and MSEW (p = 0.02), this reduction in social investigation time is not seen in LBN (p = 0.2291). B. Significant reduction seen in social investigation time from novel to familiar trial in all groups of females, control (p< 0.0001), MSEW (p< 0.0001), LBN (p< 0.0001). (Control N = 10 M, F, MSEW N = 10 M,F, LBN N = 10 M,F). *p < 0.05, **** p ≤ 0.0001*

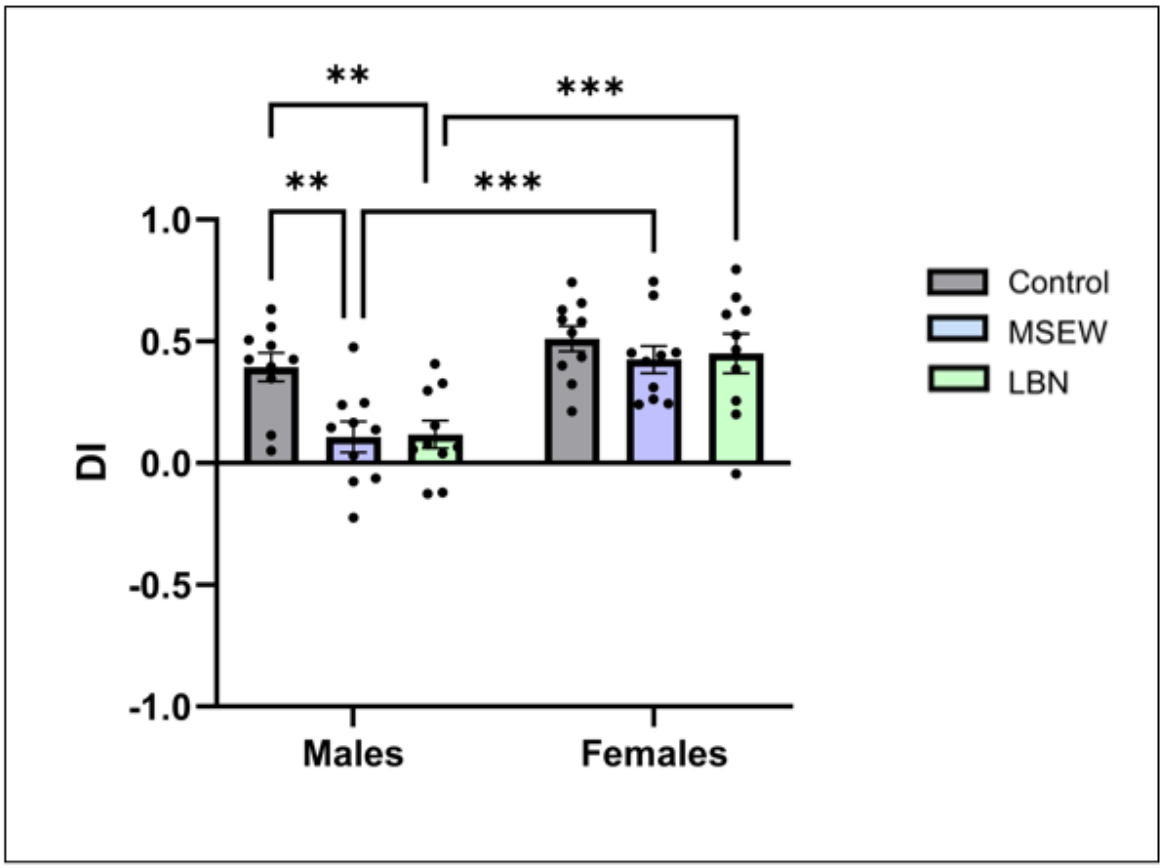

*Figure 18. Social discrimination indices (DI) at P45 of males and females from control, MSEW (0.0052) and LBN (0.0071). MSEW and LBN males have significantly lower DI compared to controls. Sex differences in DI seen in MSEW males vs. females (p = 0.0006) and LBN (p = 0.0004). (Control N = 10 M, F, MSEW N = 10 M, F, LBN N = 10 M, F). **p ≤ 0.01, *** p ≤ 0.001.*

To evaluate the social investigation memory over development, we compared the DIs across P21 and P45. A two-way ANOVA with repeated measures showed that the DI improves in females form P21 to P45 in the controls (p = 0.0227), MSEW (p = 0.0015) and LBN (p = 0.0005) groups. In contrast, only the LBN males show an improved DI (p =0.0218).

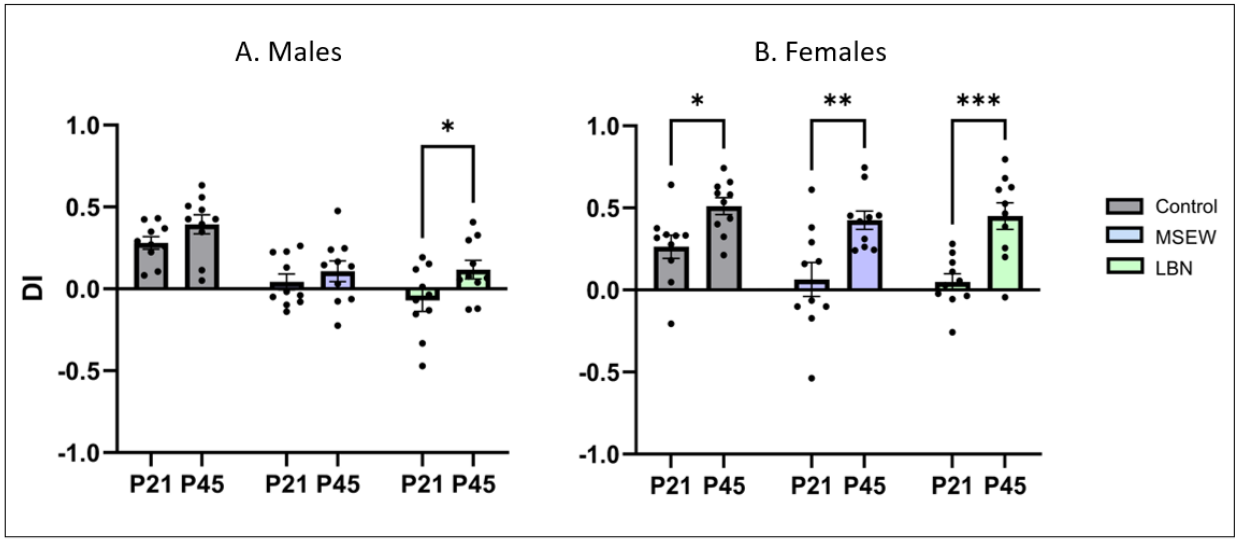

*Figure 19. Longitudinal analysis of social discrimination indices (DI) of ELA males and females. A. Social DI of P21 and P45 MSEW, LBN and control males. LBNs show improved DI from P21 to P45 (p =0.0218). B. Social DI of P21 and P45 MSEW, LBN and control females. All groups show improved DI from P21 to P45 (Ctrl - p =0.0227, MSEW, p = 0.0015, LBN, p = 0.0005). (Control N = 10 M, F, MSEW N = 10 M, F, LBN N = 10 M, F) *p < 0.05, **p ≤ 0.01, *** p ≤ 0.001*

Since the emergence of sex differences in social behavior impairment are linked to onset of puberty, we were interested in assessing the effect of ELA on puberty onset timepoint in MSEW and LBN. A one-way ANOVA with Sídák's comparisons revealed that the LBN results in delayed onset of puberty in the males (p =0.0139) as well as females (p = 0.0216). However, no difference was observed between the timing of onset of puberty in the MSEW males (p = 0.9732) or females (p = 0.1333).

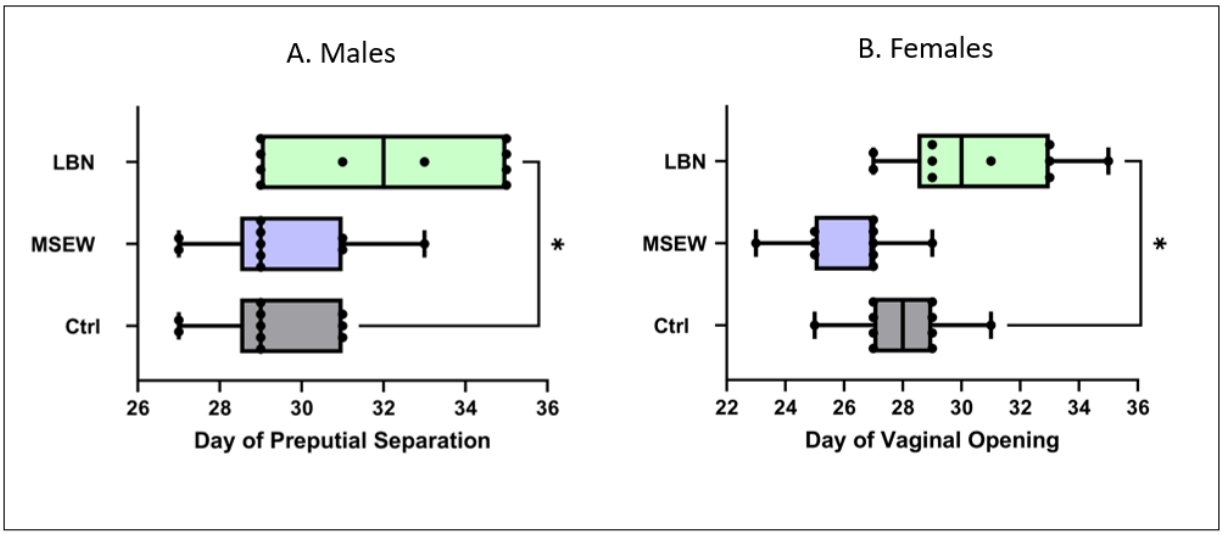

*Figure 20. Timing of onset of Puberty. Data represented as median ± IQR. A. LBN males show delay in onset of puberty, marked by preputial separation (p = 0.0139), no difference between puberty onset in MSEW and Ctrl males (p = 0.9732) B. LBN females show delay in onset of puberty, marked by vaginal opening (p = 0.0216), no difference between puberty onset in MSEW and Ctrl females (p = 0.1333). (Control N = 10 M, F, MSEW N = 10 M, F, LBN N = 10 M, F) *p < 0.05.*

3. Senescent Cells in Dorsal Dentate Gyrus

To probe increase in senescent cells in the dorsal subgranular zone as the potential mechanism driving the decreased adult neurogenesis observed in MSEW adult male mice, quantified, and compared the SA-β-gal positive cell in the subgranular zone (SGZ) of the dorsal hippocampus. Further, we investigated whether housing the control and MSEW adult males in environment enrichment (EE) following ELA affected the SA-β-gal positive cell population in the dorsal SGZ, compared to regular colony housing. A two-way ANOVA revealed a significant interaction between housing and group ($p = 0.0119$) and showed that the MSEW group had significantly higher SA-β-gal positive cell density in the dorsal hippocampus ($p = 0.0114$). Further, we also saw that the EE housing results in a significant decrease in SA-β-gal positive cells in the control ($p = 0.0164$) as well as MSEW group($p<0.0001$) as well as nullified the difference between the two groups ($p = 0.028$).

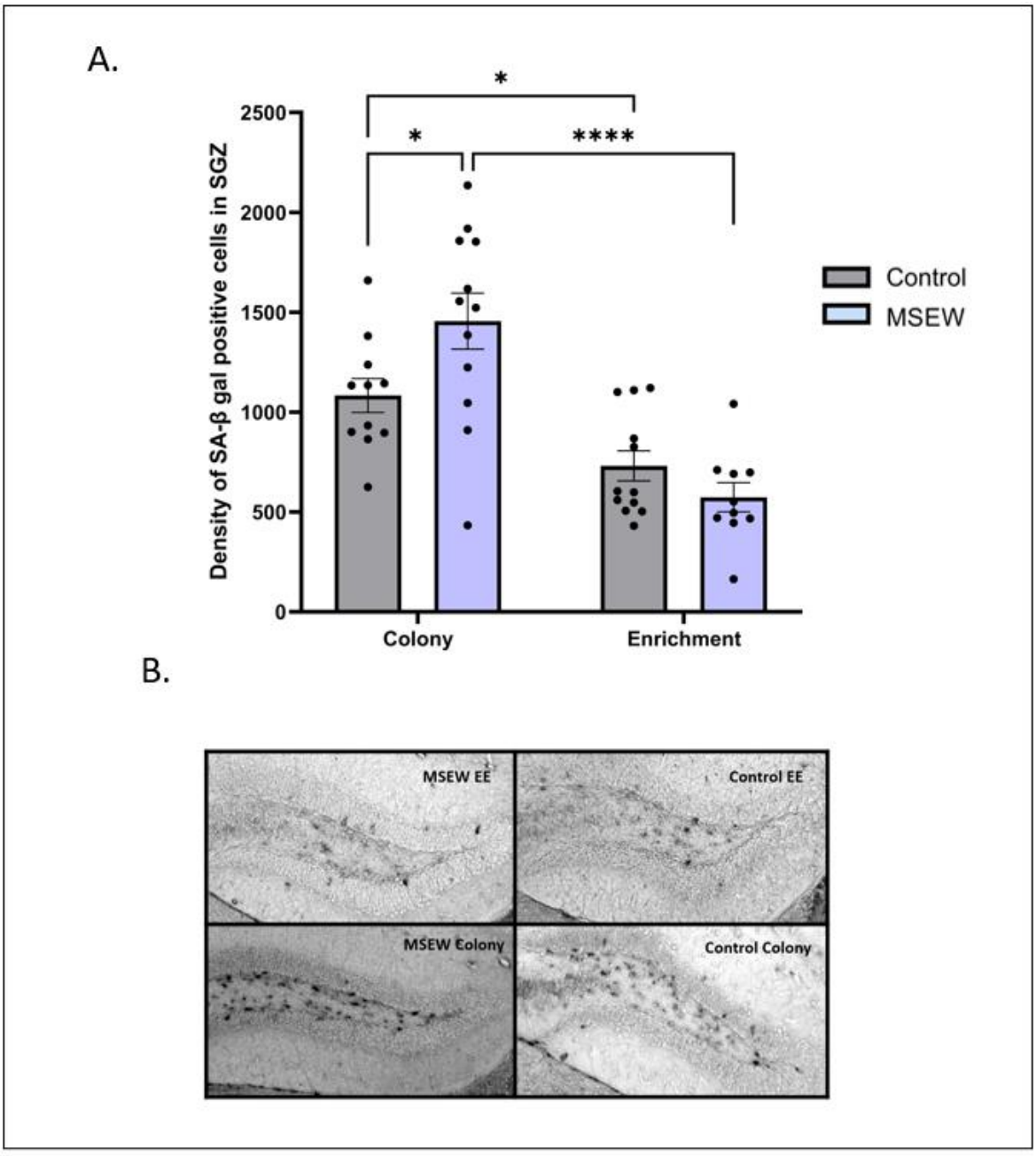

*Figure 21. SA-β-gal positive cells in the SGZ of DG in dorsal hippocampus. A. Environment enriched housed MSEW mice show a decrease in SA-β-gal positive cell counts compared to colony housed MSEW groups (p = 0.0012), (N = MSEW Colony = 12, Ctrl Colony =12, MSEW EE = 11, Ctrl EE = 12), *p <0.05, **** p ≤ 0.0001. B. Representative bright field microscopy images of SA-β-gal positive cells in dorsal DG.*

Table 2 : Statistical Analysis

Data Set	Statistical Test	Main effects	Post hoc comparisons
Fig 8A. - Time on nest	Linear mixed effects model	Ctrl v. MSEW $t_{(15.76)} = 2.364$ p=0.03126 Ctrl v. LBN $t_{(17.681)} = 0.417$ p=0.6819	
Fig 8B. - Mean presence on nest bout time	Linear mixed effects model	Ctrl v. MSEW $t_{(181)} = 0.898$ p = 0.3702 Ctrl v. LBN $t_{(181)} = -5.776$ p<0.0001	
Fig 9A. #Entries to nest	Linear mixed effects model	Ctrl v. LBN $t_{(182)} = 6.785$ p<0.0001 Ctrl v. MSEW $t_{(182)} = 0.787$ p = 0.4324	
Fig 9B. #Exits from nest	Linear mixed effects model	Ctrl v. LBN $t_{(177.00)} = 7.357$ p < 0.0001 Ctrl v. MSEW $t_{(177.00)} = -1.130$ p = 0.2599	
Fig 10A. Time arched back nursing	Linear mixed effects model	Ctrl v. LBN $t_{(15.099)} = -1.220$ p = 0.2412 Ctrl v. MSEW $t_{(13.7750)} = 1.131$	

		p = 0.2775	
Fig 10B. Mean arched back nursing bout time	Linear mixed effects model	Ctrl v. LBN $t_{(16.041)} = 1.136$ p = 0.2727 Ctrl v. MSEW $t_{(13.382)} = 1.574$ p = 0.1389	
Fig 11. Time maintaining nest	Linear mixed effects model	Ctrl v. LBN $t_{(164)} = 0.499$ p = 0.6183 Ctrl v. MSEW $t_{(164)} = 0.433$ p = 0.6656	
Fig 12. Body surface temperature across PND	Mixed effects model Tukey's multiple comparisons	**Post natal day (PND)**, $F_{(1.899, 153.8)} = 9.884$ p=0.0001 **Group**, $F_{(2,109)} = 30.18$ P<0.0001 **PND × Group**, $F_{(4,162)} = 8.251$ P<0.0001	**P7** Ctrl vs MSEW, p = 0.3224 Ctrl vs LBN, p = 0.0103 MSEW vs LBN, p = 0.0018 **P11** Ctrl vs MSEW, p = 0.0502 Ctrl vs LBN, p < 0.0001 MSEW vs LBN, p < 0.0001 **P14** Ctrl vs MSEW, p < 0.0001 Ctrl vs LBN, p < 0.0074 MSEW vs LBN, p = 0.0087

Fig 13. Sex separated, PND wise body surface temperature	Mixed effects model Tukey's multiple comparisons	**P7** **Group**, $F_{(2,62)} = 6.215$ p = 0.0035 **Sex** $F_{(1,44)} = 2.238$ p = 0.1415 **Group × Sex** $F_{(2,44)} = 0.2311$ p = 0.7946 **P11** **Group**, $F_{(2,83)} = 32.46$ p <0.0001 **Sex** $F_{(1,83)} = 0.1230$ p = 0.7267 **Group × Sex** $F_{(2,83)} = 0.6630$ p = 0.5180 **P14** **Group**, $F_{(2,46)} = 17.51$ P<0.0001 **Sex** $F_{(1,30)} = 2.362$ p = 0.1348 **Group × Sex** $F_{(2,30)} = 0.8905$ p = 0.4210	**P7** Ctrl M v. F, p = 0.3592 MSEW M v. F, p = 0.2131 LBN Mv. F, p = 0.7129 **P11** Ctrl M v. F, p = 0.5517 MSEW M v. F, p = 0.4697 LBN Mv. F, p = 0.4416 **P14** Ctrl M v. F, p = 0.1385 MSEW M v. F, p = 0.1667 LBN Mv. F, p = 0.8329

Fig 14 A. Normalized cFos counts in hippocampus	One-way ANOVA Tukey's multiple comparison	F (2, 26) = 4.049 p=0.0294	Ctrl vs MSEW p = 0.1081 Ctrl vs LBN p = 0.0288 MSEW vs LBN p = 0.7958
Fig 14 B. Normalized cFos counts in CA2	One-way ANOVA Tukey's multiple comparison	F (2, 22) = 7.357 p=0.0036	Ctrl vs MSEW p = 0.0079 Ctrl vs LBN p = 0.0084 MSEW vs LBN p = 0.9624
Fig 14 C. Normalized cFos counts in CA3	One-way ANOVA Tukey's multiple comparison	F (2, 9) = 10.57 p=0.0043	Ctrl vs MSEW p = 0.0188 Ctrl vs LBN p = 0.0047 MSEW vs LBN p = 0.9104
Fig 15A. Trial wise investigation times in DSIT – males	Two-way ANOVA, Repeated measures Tukey's multiple comparisons	**Group × Trial** $F_{(2,27)}$ = 2.769 p = 0.0806 **Group**, $F_{(2,27)}$ = 30.81 P<0.0001 **Trial** $F_{(1,27)}$ = 1.994 p = 0.1693	Novel vs Familiar Ctrl, p = 0.0227 MSEW, p = 0.3579 LBN, p = 0.3733
Fig 15B. Trial wise investigation times in DSIT – females	Two-way ANOVA, Repeated measures Tukey's multiple comparisons	**Group × Trial** $F_{(2,27)}$ = 0.5581 p = 0.5788 **Group**, $F_{(2,27)}$ = 13.12	Novel vs familiar Ctrl, p = 0.0278 MSEW, p = 0.0809

		p=0.0001 **Trial** $F_{(1,27)}$ = 8.309 p = 0.0076	LBN, p = 0.4007
Fig 16. Discrimination indices of P21 social recognition behavior	Two – way ANOVA Tukey's multiple comparisons	**Group**, $F_{(2,54)}$ = 10.03 p=0.0002 **Sex** $F_{(1,54)}$ = 0.5994 p=0.4422 **Group × Sex** $F_{(2,54)}$ = 0.5820 p = 0.5623	Males vs Females Ctrl, p=0.8548 MSEW, p=0.8190 LBN, p=0.2009 Ctrl Vs MSEW, M, p = 0.0364 F, p = 0.0934 Ctrl vs LBN, M, p = 0.0012 F, p = 0.0671 MSEW vs LBN, M, p=0.4485 F, p=0.9874
Fig 17A. Trial wise investigation times in DSIT – males at P45	Two-way ANOVA, Repeated measures Tukey's multiple comparisons	**Group × Sex** $F_{(2,27)}$ = 8.013 p = 0.0019 **Group**, $F_{(2,27)}$ = 10.69 p=0.0004 **Trial** $F_{(1,27)}$ = 35.62 p <0.0001	Novel vs familiar Ctrl, p < 0.0001 MSEW, p = 0.02 LBN, p = 0.2291
Fig 17B. Trial wise investigation times in DSIT – females at P45	Two-way ANOVA, Repeated measures Tukey's multiple comparisons	**Group × Sex** $F_{(2,27)}$ = 0.4036 p = 0.6719 **Group**, $F_{(2,27)}$ = 0.0032 p=0.9968 **Trial**	Novel vs familiar Ctrl, p < 0.0001 MSEW, p < 0.0001 LBN, p < 0.0001

		$F_{(1,27)} = 94.02$ $p < 0.0001$	
Fig 18. Discrimination indices of P45 social recognition behavior	Two – way ANOVA Tukey's multiple comparisons	**Group × Sex** $F_{(2,54)} = 1.910$ $p = 0.1580$ **Group** $F_{(2,54)} = 5.496$ $p=0.0067$ **Sex** $F_{(1,54)} = 25.67$ $p<0.0001$	Males vs Females Ctrl, p=0.1883 MSEW, p=0.0006 LBN, p=0.0004 Ctrl Vs MSEW, M, p = 0.0052 F, p = 0.5990 Ctrl vs LBN, M, p = 0.0071 F, p = 0.7717 MSEW vs LBN, M, p=0.9931 F, p=0.9571
Fig 19A. Longitudinal comparison of DI across ages males	Two-way ANOVA, Repeated measures Tukey's multiple comparisons	**Age x Group** $F_{(2,27)} = 0.6453$ $p = 0.5324$ **Age** $F_{(1,27)} = 7.474$ $p = 0.0109$ **Group** $F_{(2,27)} = 17.38$ $p < 0.0001$	Ctrl P21 vs P45 p = 0.1562 MSEW P21 vs P45 p = 0.4074 LBN P21 vs P45 p = 0.0218
Fig 19B. Longitudinal comparison of DI across ages females	Two-way ANOVA, Repeated measures Tukey's multiple comparisons	**Age x Group** $F_{(2,27)} = 0.6104$ $p = 0.5504$ **Age** $F_{(1,27)} = 32.51$ $p < 0.0001$ **Group** $F_{(2,27)} = 2.645$ $p = 0.0893$	Ctrl P21 vs P45 p = 0.0227 MSEW P21 vs P45 p = 0.0015 LBN P21 vs P45 p = 0.0005

Fig 20A. Puberty onset – males	One – way ANOVA, Sídák's multiple comparison test	$F_{(2,27)} = 5.313$ p=0.0113	Ctrl vs MSEW, p = 0.9732 Ctrl vs LBN, p = 0.0139
Fig 20B. Puberty females	One –way ANOVA, Sídák's multiple comparison test	$F_{(2,27)} = 10.83$ p=0.0004	Ctrl vs MSEW, p = 0.1333 Ctrl vs LBN, p = 0.0216
Fig 21 Senescence-associated beta-gal positive cells in SGZ	Two-way ANOVA Uncorrected Fisher's LSD	**Housing x Group** F (1, 41) = 6.937 p = 0.0119 **Housing** F (1, 41) = 37.57 p = <0.0001 **Group** F (1, 41) = 1.136 p = 0.2927	Colony housed Ctrl vs MSEW p = 0.0114 EE Ctrl vs MSEW, p = 0.2800 Control Group Colony vs EE p = 0.0164 MSEW Colony vs EE p <0.0001

Discussion

Differential Experience of MSEW and LBN Rearing in the Early Postnatal Period

To understand the link between the rearing experience and ELA-type specific differential outcomes of MSEW and LBN, we characterized the differential rearing experience of MSEW and LBN during the first two postnatal weeks, by assessing multiple parameters. Through analysis of maternal care-giving behaviors and patterns, we saw that aside from the time they spent physically separated from their pups, MSEW dams spent a greater amount of time on the nest in direct contact with the pups. In LBN, although the overall time spent on the nest by the dams (in direct physical contact with the pups) did not differ from the controls, the average bout time spent on nest was significantly lower, which indicates an interrupted and possibly unpredictable care-giving pattern. This was also reflected through the higher entries and exits to- and from- the nest. No differences were seen in the overall time spent arched back nursing or the average bouts of nursing across days between all groups, and no difference was seen in the overall time spent in nest maintenance behaviors across groups. Previous studies have reported mixed results on maternal care-giving patterns in ELA models. Altered behavioral patterns observed in our results in MSEW and LBN, concur with some of the results from previous studies, including overcompensation in caregiving in the maternal separation models (Orso et al., 2019) and fragmented caregiving in the LBN model (Pardo et al., 2023, Orso et al., 2018). The maternal caregiving quality and patterns in the postnatal period are known to facilitate healthy neurodevelopment (Kaffman & Meaney 2007) and thus ELA model specific altered maternal care-giving patterns are an important aspect to characterize, while studying the link between the rearing experience and type specific outcomes. Unpredictable and disrupted maternal care has been linked to altered neurodevelopment and later life psychopathologies (Glynn & Baram 2019), thus suggesting fragmented maternal care as a mechanism facilitating adverse outcomes in LBN. Though compensatory care is observed in MSEW, which has also been speculated to potentially attenuate the effects of the separation (Millstein and Holmes, 2007), it is important to note that the disruption in maternal care during the repeated daily long (4-8 h) maternal separations are a major part of the adverse rearing experience which can contribute to the adverse outcomes. Our current experimental design limited the inclusion of analysis of maternal caregiving behaviors such as licking and grooming, as well as analysis of self-directed or non-caregiving behaviors of the dam and investigation of maternal stress. Future studies including these factors can characterize the postnatal rearing experience to gain further insights.

Next, to investigate the effect of the ELA manipulations on thermoregulation of pups, we assessed abdominal surface temperatures of male and female pups on P7, P11 and P14. We found lower abdominal surface temperatures in the LBN group compared to the MSEW and control mice on P7 and P11. No sex differences were seen in the body surface temperatures on any of the days. These findings are in line with previously reported studies showing lowered body temperatures in LBN pups (Shupe & Clinton 2021). On P7 and P11, the LBN dam and pups are housed in cages with a metal floor plate with no access to bedding and minimal nesting material, thus the lack of proper nest which is known to facilitate adaptive behavioral thermoregulation strategies in pups such as huddling (Lapp et al 2020), and the pups being in direct contact with metal plate, could be resulting in lower body temperatures. On P14, LBN and MSEW groups showed higher temperature than controls. By P14, the LBN group is transferred back to standard housing cages, whereas the maternal separations continue which involve keeping the separated pups on a heating pad. Additionally, potential higher mobility of control pups and less huddling in the cage at P14 could also lead to these observed differences.

We are currently mapping the brain-wide differential neuronal activation in MSEW and LBN compared to controls at P7, to compare and characterize how the pups encode the environment and experience of different types of ELA. Through some preliminary analysis from this ongoing study, we saw reduced neuronal activation, through decreased cFos expression in LBN pups compared to controls, however though the MSEW group also showed a trend towards decreased activation in the in the overall hippocampus, there was no significant difference between control and MSEW. When looking specifically at the CA2 and CA3 subregion of the hippocampus at P7, we found a decrease in activation in both the MSEW and LBN groups compared to controls. The susceptibility of the hippocampus to ELA due to its protracted development in the early postnatal period is known (Khazipov et al., 2001; Avishai-Eliner et al., 2002) and has been discussed at length in previous sections. Neuronal activation in the hippocampus during the postnatal period is also crucial for activity-dependent circuit and plasticity development (Cline et al., 2023), thus the decreased activity in the hippocampus of ELA groups could be a link between ELA and the circuit- and plasticity-based outcomes of ELA seen in the hippocampus. CA2 is a subregion of hippocampus which is known to play a role in the emergence of social recognition behavior (Diethorn and Gould 2023). Although the CA2 is partially developed prior to birth, it undergoes development during the early postnatal period involving forming connections with other brain regions and expression of molecular markers that are involved in regulation of plasticity (Diethorn and Gould 2023). Thus, decreased activation of CA2 in the ELA groups could be linked to the social behavior impairments as well as other hippocampal plasticity related outcomes seen in later life. The entorhinal cortex is another region that has high connectivity with the hippocampus and is known to undergo developmental changes in the postnatal period (Piguet et al., 2020.). These activity dependent effects on development could be major players in facilitating the ELA experience dependent outcomes. However, we need further investigation and analyses of the brain wide neuronal activation maps to provide more

insights into the encoding of differential type specific ELA experiences. Additionally, future studies involving manipulations such as activation or inhibition of certain brain regions of interest during ELA rearing experience can provide causal insights into the encoding of ELA experiences in specific brain regions and its link to the adverse outcomes.

Emergence of Sex Differences in Social Behavior Impairment

Previous studies from our lab have shown that MSEW and LBN impairs social recognition memory in adult male mice, but not adult female mice (Waters et al., 2022). In the current project we investigated whether these sex differences in the social recognition memory observed in adults are present throughout development or emerge later in development after puberty. For this, we did a longitudinal study assessing social recognition memory in MSEW and LBN males and females at P21 and P45. We found that the sex differences in social recognition memory observed in adult mice are not present at a juvenile age (P21) and that they emerge in adolescence after puberty (P45). We saw that normal social recognition memory is well established in the control males as well as females at P21, which is reflected through clear social novelty preference assessed indicated by a decrease in investigation time from the novel to familiar trial and a positive social discrimination index (DI). This has also been shown previously by our lab for P21 control mice (Diethorn and Gould 2022). The control males and females also showed similar normal social recognition memory at P45 as well. However, in MSEW and LBN, we observed that both the males and females showed impaired social recognition memory at P21, as they did not show a decrease in investigation time in the familiar trial, and their DIs were significantly lower than the corresponding controls. However, at P45, the MSEW and LBN females had developed normal social recognition memory similar to controls. By contrast, the MSEW and LBN males did not show social recognition memory comparable to controls. The LBN males did not show a decrease in investigation time from the novel to familiar trial, and their Dis were significantly lower than controls. Whereas in the MSEW males, although the investigation time decreased from the novel to familiar trial, their discrimination indices were significantly lower than the controls, thus indicating impaired social recognition memory. Additionally, the DIs of males and females were significantly different in both the MSEW and LBN groups at P45. Together these results provide evidence for the divergence of social recognition memory in males and females over development and suggest that the onset of puberty and changes in hormone levels post puberty could play a role in this divergence. Our results are consistent with a study done in LBN rats, examining early versus later adolescent timepoints. Similar to our results, Holman and colleagues (2021) found that both male and female LBN rats were impaired on a social novelty test at the early adolescent time point, but by late adolescence, females showed evidence for restored function. Additionally, the behavior we observed at P45, in the males and females corresponds to behavior previously reported in adult P60 mice (Waters et al.,2022), which supports the hypothesis that sex differences emerge after puberty and persist through adulthood. This suggests that the higher levels of estrogen as well as changes

in ovarian hormones in females post puberty could play a role in protecting them against the social behavior impairment. Previous studies have suggested the role of estrogen in the development of social behavior and neurodevelopment (Schulz & Sisk, 2016). Further investigation into the neurodevelopmental trajectory and divergence in sexes following ELA is required to develop a better understanding of the neurobiological mechanisms underlying the emergence of sex differences in the outcomes of ELA.

Since onset of puberty is an important developmental milestone and is relevant to the emergence of sex differences, we were interested in assessing the effects of MSEW and LBN on the timing of puberty onset. We looked at external markers of onset of puberty, including preputial separation in males and vaginal opening in females, to assess the time point of onset of puberty in the MSEW and LBN models with respect to controls. We found that the LBN results in delayed onset of puberty in the males as well as females. Previous studies report puberty onset be affected by ELA (Cowan & Richardson 2019; Manzano et al., 2019), including delay in puberty onset as well as somatic milestones such as eye opening and weight gain (Manzano et al., 2019; Pardo et al 2023) in the LBN model. Other studies have also reported precocious onset of puberty in females in the maternal separation model (Oliveira et al., 2016; Cowan & Richardson., 2019). However, we did not find any significant effects on the time point of onset of puberty relative to controls in the MSEW males or females.

Senescent Cells in Dorsal Dentate Gyrus

We hypothesized that increase in senescent cells in the subgranular zone of MSEW males would be a potential mechanism driving the decreased adult neurogenesis. Further we hypothesized that housing mice in environment enrichment (EE) as adults following ELA, could reduce the senescent cells in the SGZ, and rescue the potential increase in senescent cells due to MSEW. After quantifying and comparing the SA-β-gal positive cell in the subgranular zone (SGZ) of the dorsal hippocampus the adult male MSEW and controls, our data confirmed our hypotheses. We saw that the MSEW males have higher senescent cells in the dorsal SGZ compared to controls in the colony housed group. Further, we also see that the EE housing results in a significant decrease in SA-β-gal positive cells overall as well as compared to the MSEW colony housed group. Thus, we have evidence suggesting increased senescent cells in the MSEW colony housed group as a potential mechanism for facilitating the decrease of adult neurogenesis in MSEW adult males. Cellular senescence in the SGZ has been previously shown to be associated with declined adult neurogenesis, and ablation of senescent cells in the SGZ have been shown to restore neurogenesis as well as cognitive functions associated with adult born neurons (Fatt et al., 2022). Based on our current research, future studies exploring whether ablating the senescent cells in the SGZ of the adult MSEW mice to restore neurogenesis and the impairment seen in the social behavior involving adult born neurons (Garrett et al., 2015; Cope & Gould, 2019) can provide insights into potential therapeutics to restore neurogenesis after

MSEW. Furthermore, EE has been known to enhance adult neurogenesis (Nilsson et al 1999; Van Praag, et al., 2000). Thus, future studies investigating the effects of ablation of senescent cells as well as EE as an intervention, on neurogenesis and hippocampus dependent social behavior impairment shown by MSEW males can be done to investigate the mechanistic link between senescence and neurogenesis and explore this as a potential intervention for the outcomes of MSEW.

Taken together, through this thesis involving three different questions, we have characterized the differential experience of ELA during the postnatal period, provided evidence for the emergence of sex differences in social behavior impairment in ELA and we have identified senescence in the neurogenesis niche as one of the potential mechanisms underlying decreased adult neurogenesis in MSEW adult males. Our studies provide insights to understand the link between types of adverse experience and predisposition to certain neuropsychiatric conditions in later life. Future correlational and causal studies to dissect out the relationships between early adversity type specific experiences and later life differential outcomes, as well longitudinal studies across the developmental trajectory following ELA to understand the emergence of outcomes of ELA and mechanisms underlying sex differences are required. These studies will lead toward developing interventions and therapeutics specific types of childhood adverse experience and additionally, better understanding of the developmental trajectory following ELA can also help develop early interventions and preventive therapies for the ELA linked long term risks.

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