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EARLY LIFE ADVERSITY AND HIPPOCAMPAL PLASTICITY: EXPLORING GENETIC, SEX, AND EXPERIENCE-DEPENDENT VARIATION

Waters, Renee Chanel

Princeton, NJ : Princeton University

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EARLY LIFE ADVERSITY AND HIPPOCAMPAL PLASTICITY: EXPLORING GENETIC, SEX, AND EXPERIENCE-DEPENDENT VARIATION

Renée Chanel Waters

A DISSERTATION PRESENTED TO THE FACULTY

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Advisor: Elizabeth Gould

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Abstract

Early life adversity (ELA) is a predisposing factor for several neuropsychiatric diseases. Specific types of ELA are known to produce differing vulnerabilities to certain conditions. This thesis focuses on ELA-induced behavioral dysfunction in avoidance behavior and social discrimination function, both of which are key characteristics in some neuropsychiatric diseases. The goal of this thesis is to identify and target underlying mechanisms for therapeutic intervention in adulthood. Chapter 1 takes a broad look at the human literature surrounding early life adversity and its connection to adult neuropsychiatric diseases to understand the variation that exists in neuropsychiatric diagnoses. In subsequent chapters, mouse models that likely reflect two broad types of ELA in humans—neglect and resource scarcity—were used to investigate the effects of different types of ELA. Avoidance behavior has been shown to characteristically increase after ELA experiences in humans and rodents. Chapter 2 examines avoidance behavior after neglect using MSEW (Maternal Separation with Early Weaning) and the role of adult neurogenesis, the addition of new neurons to the adult brain, in the hippocampus. Contrary to previous studies, the neglect model (MSEW) did not produce any behavioral changes in male mice. However, reducing adult-born granule cells (abGCs) produced a decrease in avoidance behavior, indicating a potential role of abGCs in avoidance behavior. In Chapter 3, the two ELA models were directly compared in a social behavioral task and neural mechanisms were investigated. The neglect model produced a deficit in social recognition that lasted into adulthood in males, but not females. Consistent with the hypothesis that abGCs are required for social memory, male mice who experienced MSEW produced fewer stem cells, fewer abGCs, and abGCs had fewer connections within the hippocampus. Using a double transgenic approach to increase the activation of the remaining new neurons in ELA-neglect mice was sufficient to restore social recognition abilities. Additionally, increasing the number of abGCs using environmental enrichment was also sufficient to restore social recognition abilities. Lastly, for the neglect model (MSEW), in vivo recordings of the hippocampus established electrophysiological signatures for ELA-induced impairment and restoration of function. The model of scare resources (Limited Bedding and Nesting; LBN) produced a deficit in social novelty preference in adulthood in males, but not females. Unlike the model of neglect, the model of scarce resources did not alter adult neurogenesis but instead increased the expression of specialized extracellular matrix structures known to inhibit plasticity, called perineuronal nets (PNNs), in the hippocampus. Targeted disruption of hippocampal PNNs in LBN mice successfully restored social novelty preference and social discrimination function. Future studies will investigate electrophysiological network differences between control and LBN mice and whether these differences are restored by PNN degradation. Taken together, these findings suggest that ELA-induced outcomes are dependent zzon both genetic and experiential factors.

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Chapter 1: Early Life Adversity and Neuropsychiatric Disease: Differential Outcomes and Translational Relevance of Rodent Models

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Abstract

It is now well-established that early life adversity (ELA) predisposes people to develop several neuropsychiatric conditions, including anxiety disorders and major depressive disorder. However, ELA is a very broad term, encompassing multiple types of negative childhood experiences, including physical, emotional, and sexual abuse, neglect, as well as trauma associated with chronic illness, natural disasters, accidents, and witnessing a violent crime. Emerging literature suggests that, in humans, different types of early adverse experiences are more or less likely to produce susceptibilities to certain conditions including affective dysfunction. To investigate the driving mechanisms underlying the connection between experience and subsequent disease, neuroscientists have developed many different rodent models of ELA including maternal deprivation, limited resources, and chronic stress exposure. These studies have also shown different types of ELA paradigms producing different behavioral phenotypes. In this chapter, components of ELA that may be driving different neuropsychiatric outcomes in humans are described. The neuropsychiatric sequelae arising from different types of ELA that can be successfully translated into rodent models are also discussed.

Chapter Introduction

Early life adversity (ELA) has long been recognized as a predisposing factor to the emergence of mental illness. While most ELA research has focused on childhood maltreatment, including abuse (physical, sexual, and emotional) and neglect (physical and emotional), observations that other types of childhood experiences can have deleterious effects on mental health have led to the recognition that ELA encompasses a broad range of negative experiences. Among these include exposure to natural disasters, war, chronic illness, death or incarceration of a caregiver, family separation, witnessing a violent crime, racism, and poverty. In this broad sense, ELA has been associated with an increased likelihood of developing several neuropsychiatric disorders, including, but not limited to, mood and anxiety disorders, posttraumatic stress disorder (PTSD), substance use disorders, and personality disorders (Scott et al., 2010; Widom, 1999; Dagnino et al., 2020; Norman et al., 2012; Lahti et al., 2012; Bandelow et al., 2005; Strathearn, 2020).

ELA also produces vulnerability to numerous physical conditions that are known to contribute to cognitive and emotional dysfunction, including excessive weight gain, diabetes, cardiovascular disease, autoimmune disorders, and certain types of cancer (Lippard and Nemeroff, 2020). Not surprisingly, given this long list of serious medical conditions, ELA has also been associated with shortened lifespan (Lippard and Nemeroff, 2020). Considerable research that investigates the connection between ELA and later life outcomes has considered ELA as a general umbrella term under which multiple conditions that produce chronic stress reside with the assumption that inherent differences in susceptibility, perhaps due to genetics, account for specific differences in health outcomes. However, a growing number of studies report evidence that certain types of ELA may produce specific vulnerabilities that lead to some, but not other, ELA-linked outcomes. This chapter considers a brief history of the study of the effects of ELA on negative health

outcomes (with a focus on neuropsychiatric conditions). It then considers the degree to which variability in diagnostic outcomes can be explained by differential ELA experiences. In addition, the impact of different types of ELA on specific neural circuits and whether these changes can be effectively modeled in experimental animals are discussed.

The link between ELA and negative health outcomes: historical considerations

Although the impact of early childhood experience on the development of healthy mental processes in adulthood was recognized in Western society as early as the 18th century (Gianoutsos, 2006), it was not until the late 19th century that child abuse was recognized as a criminal offense in the US and resources were put into protecting children (Crosson-Tower, 2021). Although physical maltreatment (abuse and neglect) during childhood was acknowledged to be inhumane and potentially harmful to long-term health outcomes, the link to mental health was not reported in the scientific literature until the 1960s when "battered child syndrome", which includes signs of what would now be referred to as major depressive disorder (MDD) and PTSD, was characterized (Kempe et al., 1962; Galdston, 1965), and shown to have lasting impacts on psychiatric health into adulthood (Isaacs, 1968). It was then recognized that childhood sexual abuse increases the likelihood of anxiety disorders, MDD, suicidality, and substance use disorder (Tsai and Wagner, 1978; Rosenfeld, 1979). Subsequently, scientific reports showed that increased incidence of psychiatric disorders has been linked to verbal abuse (Zanarini et al., 1989; Kjelsberg et al., 1994).

Around the same time that reports of physical and sexual abuse increasing susceptibility to mental illness emerged, studies showed that emotional neglect in childhood is also a risk factor for clinical depression (Jacobson et al., 1975), with additional studies reporting that loss of the

primary caregiver, typically the mother, which often leads to emotional neglect, has a similar effect (Goldney, 1981; Harris et al., 1986; Patten, 1991).

Although many of the earlier studies presented compelling evidence for links between childhood adversity and mental illness, most included relatively small samples and used basic metrics for determining whether abuse or neglect occurred. To address these issues and to determine the overall health outcomes of individuals subjected to childhood adversity, a landmark study was done by the Center for Diseases Control (CDC) and Kaiser-Permanente beginning in 1995 (Fellitti et al., 1998). This study used family health history and health appraisal questionnaires that collected a large amount of information from over 13,000 adults living in the US about childhood experiences and health (both mental and physical). The questionnaire included three ELA categories, each with distinct subtypes: abuse (physical, emotional, sexual), neglect (physical, emotional), and household dysfunction (mental illness, incarcerated relatives, mother treated violently, substance abuse, and parental divorce). The study presented strong evidence that a high degree of childhood adversity increased the risk of clinical depression, suicidality, and substance use disorder, as well as of physical conditions that are often comorbid with psychiatric diagnoses, including obesity, cardiovascular disease, diabetes, and cancer (Fellitti et al., 1998). This study also presented evidence for a "dose-response" relationship between childhood adversity and health outcomes whereby a greater number of adversities was statistically correlated with a higher risk for certain illnesses. Since the publication of this report, numerous studies have used similar questionnaires. These studies have firmly established the relationship between ELA and mental illness and allowed for estimates by the World Health Organization (WHO) that ELA accounts for about thirty percent of all psychiatric disorders (Kessler et al., 2010). However, even with the broad range of experiences considered to be ELA in these

studies, they do not capture the diversity of adversity experienced by the global population or even within the United States. More recent studies have been added to the list of adverse childhood experiences that increase the risk of health problems, such as bullying by peers (Biedermann et al., 2021; Iob et al., 2021), being involved in an environmental disaster (Adebäck et al., 2018), being treated for a chronic illness (Bergmans and Smith, 2021), being subjected to family separation due to parental incarceration within countries (Murray and Farrington, 2008; Swisher and Shaw-Smith, 2015) and at the US-Mexico border (Teicher, 2018; de la Peña et al., 2019; MacLean et al., 2020; Sidamon-Eristoff et al., 2022), economic hardship (Mawson and Gaysina, 2021), neighborhood violence (van Rooij et al., 2020), and racism (Thomas Tobin and Moody, 2021).

Variation in deleterious effects of ELA

Although the link between ELA and increased vulnerability to illness has been well-established through earlier studies, the high variability in individual health outcomes is notable. A sizable proportion of individuals exposed to ELA do not seem to differ from the non-ELA exposure population in terms of risk for developing mental health problems. This resilience has been attributed to possible genetic or mitigating environmental factors (Ensink et al., 2021). Even within the affected population of ELA-exposed individuals, considerable variability in the type of psychiatric illness, as well as the types of symptoms exhibited within the diagnostic criteria, exists in the outcome. Studies have considered how different onset, severity, and duration of ELA experiences contribute to much of the variability. Many findings align with the idea that earlier occurrences of maltreatment and poverty are associated with greater stress-related brain changes (Andersen and Teicher, 2008) and mental health issues in adulthood (Fitzsimons et al., 2017). Theorists who prioritize timing in models of the impact of ELA often base their predictions on

"experience-expectant" mechanisms of neurodevelopment, where adversity during sensitive or critical periods is more likely to have profound effects on neural function in adulthood (Nelson and Gabard-Durnam, 2020).

It is also relevant to mention that gender is a significant mediator between ELA and later life neuropsychiatric outcomes (Meng et al., 2016; Thompson et al., 2004; Fisher et al., 2009; Fletcher et al., 2009). These gender differences may be attributed to differences in the adverse experience itself, in terms of frequency and/or intensity (Giano et al.,2020; Gauthier-Duchesne et al., 2017; Maikovich-Fong and Jaffee, 2010; Thomspson et al., 2004; Meng et al., 2016; Khan et al., 2021). It is also probable that the differences between these two populations are reflective of differences in reporting rates between sexes. For example, males are less likely to report sexual abuse and interpersonal violence, while they're more likely to report physical and emotional abuse (Meng et al., 2016). Gender differences in the relationship between ELA and neuropsychiatric outcomes can also be attributed to reduced sample sizes in male participants due to fewer seeking professional help for mental health issues (Pattyn et al., 2015). Lastly, and most relevant to the current review, during development there are likely significant differences in the developmental trajectories of stress-sensitive circuits between sexes. Consequently, ELA may impact male and female youth differently, ultimately contributing to differences in neuropsychiatric outcomes (Bath 2020; Dragan et al., 2019; Hill et al., 2010).

Studies show that less than five percent of childhood adversity occurs as a single event. Not only do people often experience multiple types of ELA but the cumulative effects of these different types of ELA have proven to be deleterious (Dich et al., 2015). In the CDC/Kaiser-Permanente study discussed above, researchers found that nearly one in five children had experienced two or more different types of ELA experiences. Researchers have found that the combination and

accumulation of ELA increase the severity of neuropsychiatric diseases across multiple psychiatric diagnoses, including MDD and anxiety disorders (Anda et al., 2006). Taken together, studies that have considered whether timing or severity determines the qualitative outcome have concluded that although these factors play some role, they do not account for all the variability.

The unexplained variability raises the possibility that specific types of childhood experiences change the brain in ways that lead to disparate negative health outcomes. Although some of the earliest reports linking childhood experiences with mental health outcomes have attempted to draw connections between specific experiences and specific symptoms (Bornstein, 2006), the field has moved increasingly toward considering ELA experiences as having common stress consequences that produce different outcomes because of factors inherent to the individual. Recently, however, a growing literature has investigated whether specific adverse experiences are major determinants of symptom variability. In the next section, different categories of ELA are considered in the context of neuropsychiatric correlates, as well as what is known about related neural changes. As mentioned previously, childhood maltreatment rarely occurs in an isolated form (Felitti et al., 1998), however, it is important to consider evidence suggesting that there may be specific contributions of each subtype to the etiology of some neuropsychiatric illnesses. It should be noted at the outset of this discussion that there is considerable overlap in conditions that have been associated with specific categories of ELA and that some studies have reported that only nonspecific relationships exist, including that there are no major differences in brain effects (Haidl et al., 2021). Because the majority of research on this topic has considered forms of ELA that involve childhood maltreatment, the following discussion will focus on physical abuse, sexual abuse, and emotional maltreatment.

Physical abuse

Despite numerous societal efforts to reduce its occurrence, childhood physical abuse remains common (CDC, 2021). The field seems to generally conclude that individuals who experience physical abuse are at an increased risk to develop anxiety disorders, PTSD, and certain personality disorders, including antisocial and borderline (Schorr et al., 2020; Bornoalova et al., 2006; Fergusson et al., 2008; Springer et al., 2007; Kiser et al., 1991). Perhaps not surprisingly, the mental health risks for children who experience both physical and sexual abuse are potentiated (Roth et al., 1997). When the field has attempted to dissect these findings, such as to determine which abuse subtype is the strongest predictor, the evidence seems to differ considerably, possibly because most studies have not considered other factors, such as duration, severity, or onset. A recent study attempted to fill this gap by examining 1,200 emerging adults and found that physical abuse beginning between 6-12 years old is associated with several types of psychopathologies, including anxiety disorders, but that physical abuse at age 13 or older was a significant predictor of mainly PTSD (Adams et al., 2018).

These findings raise questions about how physical abuse might change the brain to increase the likelihood of anxiety disorders and PTSD. Studies have shown that compared to healthy controls, physically abused children have smaller amygdala, prefrontal cortex, and hippocampal volumes (Gold et al., 2016; Lambert et al., 2017; Nogovitsyn et al., 2020), as well as altered activation in brain regions involved in emotional regulation and fear processing, including greater activation of the amygdala, hippocampus, and medial prefrontal cortex when presented with emotional faces (Table 1). These studies suggest that the lasting effects of physical abuse may be the result of changes in emotion and fear processing circuits brought about by threat-induced activation of these brain regions (Figure 1).

Table 1: Brain changes and neuropsychiatric disorders associated with different types of ELA in humans

Type of ELA	Reported brain changes	References	Psychiatric vulnerability
Physical abuse	↓ volume of AMY, HIP, PFC ◑▽ ↑ activation in response to emotional and threat stimuli of AMY, HIP, PFC ◆	◑ Nogovitsyn et al., 2020 ▽ Lambert et al., 2017 ▽ Gold et al., 2016 ◆ Garrett et al., 2012	PTSD, anxiety disorders
Sexual abuse	↓ volume of AMY, ACC, HIP ◆◑■ Thinning of visual and somatosensory cortex ■ Altered activation of HIP, PFC to stress and sexual abuse memories ▽ ↓ connectivity AMY-PFC ◆	◑ Nogovitsyn et al., 2020 ■ Cohen et al., 2006 ◆ Rinne-Albers et al.,2017 ◆ Weniger et al., 2008 ■ Tomoda et al., 2009 ■ Heim et al., 2013 ▽ Purcell et al., 2021 ◆ Duque-Alarcón et al., 2019	PTSD, BPD
Verbal abuse	↑ size of auditory cortex ▽ ↓ fiber tracts connecting language and emotional processing regions ◑ ↓ activation of striatum ▽	▽ Tomoda et al., 2011 ◑ Choi et al., 2009 ▽ Kopala-Sibley et al., 2020	MDD, increased PTSD severity
Emotional neglect	↑ volume of AMY ◆ ↓ activation of striatum, ACC, PFC to reward ◑▽	◆ Roth et al., 2018 ◑ Hanson et al., 2015 ▽ Morgan et al., 2015	MDD

◆: Brain changes associated with clinical diagnoses; ◑: Brain changes linked to symptoms, but not formal diagnosis; ▽: Not specified whether brain changes are linked to diagnoses; ■: Brain changes that occur in mentally healthy as well as mentally ill individuals

ACC, anterior cingulate cortex; AMY, amygdala; HIP, hippocampus; PFC, prefrontal cortex; PTSD, post-traumatic stress disorder; BPD, borderline personality disorder; MDD, major depressive disorder

Sexual abuse

There is considerable overlap between the most common mental health outcomes associated with sexual abuse and those associated with physical abuse, although the relationship between sexual abuse and increased susceptibility to PTSD and borderline personality disorder (BPD) seems especially strong (de Aquino Ferreira et al., 2018; Hailes et al., 2019; Breuer et al., 2020; Cancilliere et al., 2021). In addition, studies suggest that sexual abuse leads to increased dissociative symptoms (Tschoeke et al., 2021) and non-suicidal self-injury and suicidal ideation, but not completion (Mullen et al., 1994; de Aquino Ferreira et al., 2018). The latter effects seem to be magnified when sexual abuse was combined with physical abuse, as well as when the abuse was repeated and occurred over a long time (de Aquino Ferreira et al., 2018). Aside from diagnostic outcomes, sexual abuse has been linked to interpreting neutral facial expressions as negative (Pfaltz et al., 2019) and to deficits in social cognition (Duque-Alarcón et al., 2019) and other cognitive functions (Navalta et al., 2006) as well as impaired parenting skills (Burkett, 1991; Cole et al., 1992; Ethier et al., 1995).

Several studies suggest that childhood sexual abuse changes the brain in ways that are not necessarily specific to the type of ELA or the presence or absence of psychopathology. Childhood sexual and physical abuse have been linked to reduced volume of the amygdala regardless of clinical diagnosis (Nogovitsyn et al., 2020) suggesting that early exposure to trauma alters the structural development of this region, perhaps contributing to vulnerability to subsequent stress. Childhood sexual, but not physical, abuse has been associated with diminished stress-induced

activation of the dorsolateral frontal cortex and hippocampus (Purcell et al., 2021). Childhood sexual abuse produces thinning of the visual cortex and somatosensory cortex associated with genital representations in a way that may not be related to the development of psychopathology as the effects can be observed even in individuals without diagnoses (Tomoda et al., 2009; Heim et al., 2013). Additional brain changes have been observed in individuals with a history of childhood sexual abuse presenting with a psychiatric diagnosis. For example, people with a history of childhood sexual abuse and a PTSD diagnosis exhibit greater activation of the prefrontal cortex in response to childhood sexual abuse memories (Bremner, 1999), a shift in activation from the frontal cortex to the hippocampus and amygdala when viewing emotional faces (Duque-Alarcón et al., 2019), as well as diminished volume of the anterior cingulate (Cohen et al., 2006; Rinne-Albers et al., 2017). Childhood sexual abuse victims with a BPD diagnosis have diminished connectivity between the amygdala and the frontal cortex (Duque-Alarcón et al., 2019) (Table 1). These results suggest that sexual abuse likely has general as well as specific effects on the brain, some of which may be protective and others that contribute to vulnerability, perhaps in cases where the individual experiences additional adversities during development or in adulthood. Differences in the size of key brain regions as well as in the recruitment of specific circuitry in response to subsequent stress may explain the increased likelihood for childhood sexual abuse victims to develop certain symptoms, particularly those related to emotional processing (Figure 1).

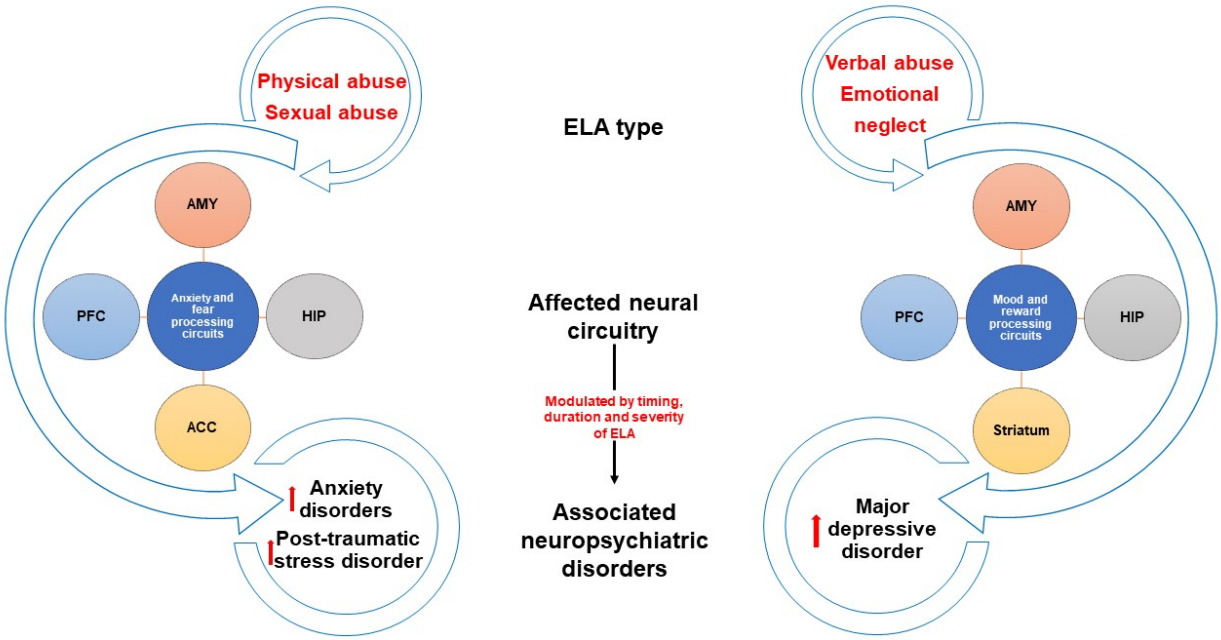


Figure 1. Brain changes and neuropsychiatric outcomes associated with different ELA types. Diagram depicting brain regions that are altered by different types of childhood maltreatment. Studies show that physical and sexual abuse can alter regions involved in anxiety/fear processing circuits while verbal abuse and emotional neglect affect regions related to mood/reward processing circuits. These brain changes likelihood contribute to differential predisposition to certain neuropsychiatric disorders, such as anxiety disorders, post-traumatic stress disorder, and major depressive disorder. ACC, anterior cingulate cortex; AMY, amygdala, HIP, hippocampus, PFC, prefrontal cortex.

Emotional maltreatment

While physical and sexual abuse both evoke threat of bodily harm, emotional maltreatment involves threat to self-image. In addition, emotional maltreatment often involves either direct or indirect emotional deprivation, although studies have shown that even in cases where emotional abuse does not involve net emotional deprivation, the effects are negative. For example, emotional abuse has been strongly linked to MDD, with studies showing that its effects are not mitigated by emotional affection by the abusive or other parent (Polcari et al., 2014). It should be

noted that some studies have shown that childhood physical and sexual abuse have been linked to MDD (Maniglio., 2009; Lansford et al., 2002), but the relationship between emotional abuse and neglect appears to be stronger (Infurna et al., 2016; Martins et al., 2014; Gibb et al., 2001; Kim and Cicchetti, 2006), and, of course, it is important to consider that physical and sexual abuse are often accompanied by verbal abuse, making it difficult to study these types of ELA separately. Related to the impact of parental emotional abuse on the later emergence of psychopathology, many recent studies have shown a link between bullying by peers and the development of MDD in adulthood (Valera-Pozo et al., 2021; Biedermann et al., 2021; Iob et al., 2021), raising the possibility that similar mechanisms are invoked by emotional abuse from caregivers as from peers. In addition to increasing the likelihood of MDD, emotional abuse during childhood has been linked to the severity of PTSD (Rameckers et al., 2021), which is often comorbid with MDD.

As mentioned earlier, emotional abuse, particularly when delivered by a parent, is often accompanied by emotional neglect, so it is particularly difficult to study the latter alone. However, studies of children raised by mothers with postpartum depression, who are therefore less interactive, as well as those who lose their primary caregiver as a result of death or incarceration may provide some insight. These studies have shown an increased incidence of MDD (Berg et al., 2016; Netsi et al., 2018; Heard-Garris et al., 2019) with the timing of parental loss as a mediating factor (Berg et al., 2016).

There are several aspects of emotional abuse and neglect that may contribute to its strong ties to depressive disorders. A long line of developmental research supports the view that attachment figures are critical for the development of healthy social schemas of the world (Amos et al., 2011; Szepsenwol and Simpson, 2019). Emotional neglect deprives the child of necessary prosocial

experiences, while emotional abuse shifts social interactions in a negative direction, increasing the likelihood of developing a "negative self-model", which often leads to depressive illness (Infurna et al., 2016). Emotional abuse in childhood also has a significant impact on social function in adulthood by increasing the likelihood of fear and avoidance of social situations. Since positive social relationships can buffer against subsequent stress and social avoidance is often a feature of MDD, it is reasonable to assume that these changes are related.

Given the long-term impact of emotional maltreatment, studies have sought to determine how the brain is changed by these experiences. Emotional maltreatment in general has been shown to increase activation of the amygdala (Puetz et al., 2020). Verbal abuse in particular, has been shown to increase the size of parts of the auditory cortex (Tomoda et al., 2011), as well as diminish fiber tracts connecting language and emotional processing regions (Choi et al., 2009; Tomoda et al., 2011) (Table 1). These findings suggest that persistent activation of auditory and language processing regions, particularly under conditions of stress, alter the developmental trajectory of these circuits, likely affecting how subsequent verbal information is processed. Emotional neglect has been shown to produce not only a larger amygdala volume (Roth et al., 2018) but reduced volume and altered function in a frontoparietal network that has been associated with complex social and cognitive processing (McLaughlin et al., 2019: Puetz et al., 2020) (Table 1).

These findings suggest that emotional maltreatment may alter development of circuits that are critical for processing salient socioemotional information in adulthood. Evidence also suggests that emotional maltreatment produces changes in reward circuitry, including activation of the prefrontal cortex and striatum in response to reward (Hanson et al., 2015; Morgan et al., 2014; Kopala-Sibley et al., 2020). This dysregulation may contribute to the emergence of mood

disorders, of which reward processing deficits can be defining symptoms. It should be noted that some research has shown that sex differences exist in the extent to which brain regions are impacted by emotional maltreatment, findings that may be related to sex differences in symptoms, as well as in rates of MDD and anxiety disorders (Altemus et al., 2014).

Remaining questions about specific impacts of ELA subtypes in humans

Given that physical abuse, sexual abuse, verbal abuse, and emotional deprivation have been shown to predispose to different, albeit overlapping, neuropsychiatric conditions, it seems reasonable to consider not only whether psychiatric patients have histories of ELA, but the individual specific life history when developing treatment plans. Despite this somewhat obvious conclusion, it remains unknown whether a psychiatric condition, e.g., MDD, that arises from one type of ELA, e.g., physical abuse, has the same or different underlying neural dysfunction as that which arises from another type of ELA, e.g., parental loss. While talk therapy can address childhood experiences directly, biomedical interventions may benefit from information about specific experiential etiologies, particularly if it is found that they involve unique brain changes. It seems relevant to note that although multiple types of antidepressant treatments exist, it remains unclear which treatments will be most effective for individual patients, and 10-30% of patients with MDD either show only a partial improvement or appear to be resistant to all treatments (Al-Harbi, 2012). Similar issues exist regarding the inability of health care professionals to alleviate suffering in many people with PTSD and BPD, conditions that are typically treated with drugs developed for other conditions, like MDD and anxiety disorders. A more informed approach toward treating such conditions in a way that is tailored to the individual patient can be envisioned to arise from a better understanding of how specific forms of ELA impact the brain differentially. Even though the field has progressed considerably by recognizing that different

subtypes of ELA predispose more heavily to certain psychiatric conditions than others, and that ELA subtype-specific brain changes are known to occur, there remain considerable unanswered questions about how ELA impacts neural mechanisms that might serve as potential therapeutic targets.

Modeling ELA in experimental animals

To study the neurobiological consequences of ELA, scientists have developed models using experimental animals to test causal mechanisms and investigate levels of analyses that are not possible to probe in humans. While all of the types of ELA experienced by humans cannot be captured in these models, the expectation has been that this approach will provide some insight into how ELA impacts molecular and cellular processes in specific neural circuits, so that the information can be used to build a preclinical framework that would be useful for treating humans suffering from ELA-related conditions. Although some nonhuman primate models of ELA exist, practical and ethical issues have limited their usefulness, and most of the research on this topic has used rodents (Murthy and Gould, 2018). For rodent experiments to effectively model ELA-induced predispositions to psychopathology seen in humans, they should produce behavioral phenotypes that mimic some aspects of the relevant disorders. This is a difficult problem to solve since neuropsychiatric diseases linked to ELA in humans, including MDD, anxiety disorders, PTSD, and personality disorders, cannot be replicated in rodents without resorting to debatable anthropomorphic interpretations of animal behavior (LeDoux and Pine, 2016; Murthy and Gould, 2020). Despite this important caveat in interpreting behavioral data from these studies, several ELA rodent models have emerged that produced interesting behavioral results pointing to the potential for modeling differential effects in humans. They are described below grouped into the types of human ELA they might represent, with the

recognition that rodent experiences are likely very different from human ones, even when they share some common surface features.

A comprehensive understanding how ELA affects the brain and behavior requires consideration of potential sex differences. In humans, there are gender differences in the prevalence of exposure to adversity (e.g., girls are more likely than boys to be exposed to sexual abuse; boys are more likely than girls to be exposed to nonsexual physical abuse, Tourigny et al., 2008; Lippus et al., 2020), as well as in diagnoses of neuropsychiatric disorders linked to ELA (e.g., women are more likely than men to be diagnosed with anxiety disorders or major depressive disorder; men are more likely to be diagnosed with substance use disorder, Altemus et al., 2014; NIDA, 2021), and societal influences are difficult to rule out. ELA studies in experimental animals have traditionally included only male rodents, but a growing number of studies have considered both sexes with a view toward identifying potential sex differences (Bath et al., 2020. In the next section, rodent sex difference findings are incorporated into our discussion of the effects of specific rodent ELA paradigms.

It should be noted that prenatal stress has also been shown to impact behavior of offspring in ways that may reflect aspects of neuropsychiatric disease in humans (reviewed in Weinstock, 2016), but since this chapter is focused on modeling adversity during infancy, childhood, and adolescence, our discussion will consider only studies that include manipulations during postnatal life.

Physical abuse

Some researchers have investigated the impact of painful experiences during development on rodent behavioral outcomes in adulthood. Since these manipulations impact somatosensory pain pathways and directly signal threats to safety, they may share common outcomes with physical abuse in children. Studies have used unpredictable electric shock in neonates and adolescents, formalin injection, or paw surgery to examine behavioral outcomes and found increased avoidance behavior (Negrigo et al., 2011; Sarro et al., 2014; Li et al., 2015; Tao et al., 2017; Lovelock and Deak, 2019; Ririe et al., 2021; Zhao et al., 2022) and enhanced fear conditioning (Quinn et al., 2014) in rats. Increased avoidance behavior has been observed in both male and female rats treated with formalin injection, with greater effects observed in females (Negrigo et al., 2011). Foot shock during adolescence has been shown to increase avoidance behavior in male but not female rats (Lovelock and Deak, 2019), however another study showed increased avoidance behavior in female rats (Tao et al., 2017). The reasons for these sex difference discrepancies remain unknown but could be due to different timing and duration of stress exposure.

Increased avoidance and enhanced fear conditioning with models of early life physical abuse appear to be consistent with the most common neuropsychiatric disorders stemming from physical abuse in humans, namely, PTSD and anxiety disorders. Likewise, brain data obtained from these models is also consistent with what has been observed in humans subjected to childhood and adolescent physical abuse, such as changes in the amygdala (Sarro et al., 2014), including increased activation (Lovelock and Deak, 2019) (compare Table 2 with Table 1).

Table 2: Brain changes and behaviors resulting from different types of ELA in rodents

Type of ELA	Affected brain regions	Behavioral change	References
Physical pain (electric shock, paw injection or surgery)	◆AMY	"Anxiety-like behavior"	◆Lovelock and Deak, 2019 ◆Sarro et al., 2014
Sexual conspecific aggression	◆ HIP	Impaired cognition, maternal behavior	◆ Shors et al., 2016
Deprivation (MS, MSEW)	◆◑ HIP ◆Striatum ◆PFC ◆AMY	"Anxiety-like" and "depressive-like" behavior	◆Murthy et al., 2019 ◑ Hill et al, 2019 ◆Pena et al., 2019 ◆Mejía-Chávez et al., 2021
Mixed – scarcity and abuse (LBN)	◆◑ AMY ◆Striatum ◑ ACC	"Depressive-like behavior"	◆Raineki et al., 2012 ◆Wendel et al., 2021 ◑ Holschneider et al., 2016

◆ Brain changes associated with behavioral changes; ◑: Brain changes without behavioral analyses. ACC, anterior cingulate cortex; AMY, amygdala; HIP, hippocampus; PFC, prefrontal cortex. "Anxiety-like behavior" refers to increased avoidance on behavioral inhibition tasks (EPM, NSF, OF); "depressive-like behavior" refers to decreased reward-seeking (SP) and stress-coping (FST, TST) behavior. It should be noted that these behaviors cannot be used to identify rodents with anxiety disorders or clinical depression, but they may reflect basic responses that reflect some aspects of these disorders in humans.

Sexual abuse

It is particularly difficult to model childhood sexual abuse in laboratory rodents because mating activity depends on hormonal as well as behavioral sexual receptivity, which only emerges after puberty. Shors et al (2016) developed a novel model of repeated sexual abuse whereby adolescent

female rats were repeatedly paired with large sexually experienced male breeders, which produced chasing, fighting, and mating attempts. Because the females were not through puberty, and as such, were not behaviorally sexually receptive, there were no completed intromissions, but these experiences were stressful for the females in that they produced activation of the HPA axis (Shors et al., 2016). This paradigm was not examined for effects on avoidance behavior, but the researchers found that female rats exposed to sexually aggressive males exhibited diminished cognitive ability, as well as reductions in the maternal care of unrelated pups (Shors et al., 2016). The extent to which these rodent behavioral changes reflect those observed in victims of early sexual abuse in humans remains unknown, but it may be relevant that childhood sexual abuse has been associated with reduced cognitive function (Navalta et al., 2006) as well as impaired parenting skills (Burkett, 1991; Cole et al., 1992; Ethier et al., 1995). Very limited evidence exists regarding the effects of this paradigm on the rodent brain, but available data suggest a reduction in neuronal growth in the hippocampus (Shors et al., 2016), which is consistent with work in humans showing that hippocampal volume and activation is reduced after childhood sexual abuse (Weniger et al., 2008; Purcell et al., 2021; Yuan et al., 2020) (compare Table 2 with Table 1). Clearly, this approach presents an innovative experimental animal paradigm that should be explored further for potential translational relevance. To date, there are no available animal models of sexual abuse in male rodents, so examination of sex differences is not possible.

Emotional and physical deprivation

The first ELA model to be developed for rodents, a model of deprivation called maternal separation (Ader et al., 1960), remains the most used to this day. This paradigm involves separating pups from their dam for varying amounts of time (usually for 1-8 hours daily) during the first 2 postnatal weeks. Despite some attempts to standardize the separation times, the

behavioral results of this manipulation vary considerably. Several studies have discussed how maternal separation has differential outcomes regarding behaviors that have been considered "anxiety-like" and "depressive-like" (Millstein and Holmes, 2007; Pryce and Feldon 2003). Systematic reviews and meta-analyses of the literature regarding maternal separation show that there are no consistent increases in avoidance behavior following maternal separation (Wang et al., 2020; Tractenberg et al., 2016; Murthy and Gould, 2020). This is not surprising given the variability in response to ELA due to sex (Bath et al., 2020), genotype (Holmes et al., 2005; Mehta and Schmauss, 2011; Millstein and Holmes, 2007), and maternal separation procedure (Wang et al., 2020; Lehmann and Feldon, 2000). While there is no consensus on how maternal separation impacts avoidance behavior, there is a stronger case for the connection between maternal separation and "depressive-like" behavior in mice (Tractenberg et al., 2016). Despite differences in strain, species, and protocol, many studies agree that maternal separation leads to an increase in "depressive-like behavior", defined as reduced stress coping in the forced swim test (FST) and tail suspension test (TST), as well as reduced reward seeking in a sucrose preference test in mice and rats (e.g., Tchenio et al., 2017; He et al., 2020; Thornton et al., 2021; Wei et al., 2021). As with the physical stress/pain studies, maternal separation studies have shown varied differences with sex; some studies show effects on males with no effects on females (He et al., 2020; Demaestri et al., 2020), others show effects in females and not males (Honeycutt et al., 2020), while still others show comparable effects on both sexes (Thornton et al., 2021). The reasons for these discrepant results remain unknown but could be due to differences in species (mouse – He et al., 2020; rat – Honeycutt et al., 2020; Thornton et al., 2021), in durations of maternal separation per day (6 hours per day -Thornton et al., 2021; 4 hours per day - He et al., 2020, Honeycutt et al., 2020), or different durations of maternal separation overall (P2-P12 - He et al., 2020; P2-P20 -Honeycutt et al., 2020; P1-P21 - Thornton et al., 2021).

A newer model than maternal separation alone, maternal separation and early weaning, which may be a more cumulative model of ELA, has produced results that seem to show increased "depressive-like" behavior (George et al., 2010; Montalvo-Ortiz et al., 2016; Sun et al., 2021) as well as increased behavioral avoidance (George et al., 2010; Carlyle et al., 2012; Murthy et al., 2019; Sun et al., 2021), perhaps reflecting the high comorbidity between MDD and anxiety disorders, but there are still reports showing inconsistent results (Tan et al., 2017; Waters et al., 2022). These findings are consistent with human studies on emotional and physical neglect, which most commonly increase the likelihood of MDD. Maternal separation and early weaning studies have generally shown effects in male mice and rats (George et al., 2010; Montalvo-Ortiz et al., 2016; Murthy et al., 2019; Sun et al., 2021), with studies either not examining females or, if so, finding no effects (Murthy et al., 2019; Sun et al., 2021). The lack of effects in females may be due to the protective action of certain stages of the estrous cycle in that maternal separation and early weaning mice only shows increased avoidance behavior during diestrus, a time when the ratio of progesterone to estrogen is relatively high (Laham et al., 2022). These findings raise possible connections with human studies showing that childhood maltreatment increases the likelihood of emotional dysfunction linked to fluctuations in ovarian hormones (Azoulay et al., 2020).

Aside from depriving preweaning pups of maternal contact, additional studies have examined deprivation in adolescence by using models of social isolation housing during development. These studies have shown that social isolation reduces stress coping, social interaction, and reward seeking, behaviors that may reflect "depressive-like" and "anxiety-like" phenotypes (Chang et al., 2015; Matsumoto et al., 2017; Potrebić et al., 2021; Novoa et al., 2021).

Studies involving maternal separation during the preweaning period or social isolation during adolescence have revealed brain changes in areas that have been shown to be affected in humans subjected to early life emotional neglect, such as the medial prefrontal cortex, hippocampus, amygdala, and striatum (Hill et al., 2018; Morgan et al., 2014; Edmiston et al., 2011; Matsumoto et al., 2017; Murthy et al., 2019; Pena et al., 2019; Mejía-Chávez et al., 2021; Novoa et al., 2021; Laham et al., 2022). The extent to which the reported brain changes after maternal separation are similar to those in humans subjected to emotional neglect (compare Table 2 with Table 1), beyond the commonalities in the affected brain regions remains unknown.

Mixed and multiple hit models

The limited bedding and nesting (LBN) model is now widely used as an ELA paradigm. LBN involves reducing or eliminating bedding material and reducing nesting material available to the dam. LBN is thought to model scarce resources in childhood that can lead to erratic and potentially abusive maternal care (Gallo et al., 2019), so this model may reflect a combination of environmentally induced neglect and abuse in humans. However, it should be noted that LBN, although reported to increase aggressive behavior in some dams toward their pups (Gallo et al., 2019), has also been shown to increase nursing and attentive care, perhaps as a compensatory response (Eck et al., 2020), so its impact on offspring may vary dramatically depending on individual differences in maternal response. Similar to maternal separation studies, experiments using LBN have resulted in varied behavioral outcomes related to "anxiety-like" and "depressive-like" behavior in adulthood with some studies showing increases (Dalle Molle et al., 2012; Raineki et al., 2012; Wendel et al., 2021) and others showing decreases or no change (van der Kooji et al., 2015; Johnson et al., 2018). Regarding sex differences, seemingly discrepant results from LBN rearing have been reported. For example, one study showed LBN-induced increased

avoidance behavior in males but not females (Dalle Molle et al., 2012) while another showed no changes in avoidance behavior in either sex but reduced stress coping and reward seeking in females (Goodwill et al., 2019). The reasons for these differences could be related to the species (rat – Dalle Molle et al., 2012; mouse – Goodwill et al., 2019) or timing of LBN (P2-P9 – Dalle Molle et al., 2012; P4-P11 – Goodwill et al., 2019). In studies where increases in "anxiety-like" and "depressive-like" behaviors have been observed, associated brain changes point to alterations in regions linked to avoidance and fear processing circuitry, including the amygdala (Raineki et al., 2012) and anterior cingulate (Holschneider et al., 2016), as well as reward circuitry, including the ventral tegmental area and nucleus accumbens (Wendel et al., 2021) (Table 2).

Rodent models have also been developed to simulate the unpredictable, cumulative effects of ELA that often occur throughout childhood and adolescence in humans. By exposing rodents to repeated exposure to the same stressor and/or by combining exposure to different types of stressors over the developmental period, recovery and/or adaptation to adversity may be prevented. These models may be more naturalistic and reflective of the human experience, where adversity is often diverse and repeated, and have been generally successful at inducing behavioral changes (Molet et al., 2014). "Multiple-hit" models during development have been shown to have a significantly greater impact than a single adversity alone (Hollis et al., 2013).

A relevant animal model of the impact of repeated emotional and physical abuse may be found with studies investigating repeated social defeat during adolescence. This paradigm typically involves a brief episode of physical contact with an aggressive conspecific, after which the aggressor and test animal are separated by a physical barrier that permits repeated visual, auditory and olfactory contact without the possibility of physical injury. Studies using this

approach have yielded findings that are generally consistent with the human literature with repeated social defeat producing increased "depressive-like" symptoms in both mice and rats (Iñiguez et al., 2016; Burke et al., 2017; Alves-Dos-Santos et al., 2020; Shimizu et al., 2020; Mancha-Gutiérrez et al., 2021). These behavioral effects also emerge under circumstances where adolescent mice witness, but are not directly involved in, social defeat, a paradigm called vicarious social defeat (VSD) (Garcia-Carachure et al., 2020; Warren et al., 2020; Finnell et al., 2018) and can be further exacerbated by additional environmental adversities, such as eating an unhealthy diet (Sial et al., 2021). Although the majority of studies using chronic social defeat in adolescence have examined males, due to the ease with which aggressive mice and rats will attack smaller males, studies using the VSD procedure in adolescent male and female rodents have shown roughly similar effects across sexes (Warren et al., 2020; Finnell et al., 2018), Social defeat studies have identified changes in the same brain regions shown to be altered after the LBN approach, including the hippocampus, prefrontal cortex, ventral tegmental area and nucleus accumbens (Rodríguez-Arias et al., 2018; Blanco-Gandia et al., 2019).

Additional paradigms have modeled exposure to multiple types of adversity beginning before weaning and extending into adolescence by either combining a more traditional ELA paradigm, like maternal separation, with subsequent exposure to additional stressors in mice and rats (Huang et al., 2021; Knox et al., 2021; Saavedra et al., 2021), or by subjecting developing mice and rats to different types of stressors (Meyer et al., 2021; Page and Coutellier, 2018; Yohn and Blendy, 2017). In general, these studies have shown that multiple adversity exposure throughout development increases avoidance behavior as well as impairs stress coping and reward seeking (Knox et al., 2021; Saavedra et al., 2021; Yohn and Blendy, 2017; Page and Coutellier, 2018; Huang et al., 2021; Meyer et al., 2021; Chaby et al., 2015), findings that are generally consistent

with the human literature demonstrating a link between multiple ELA exposures not only increasing the risk and severity of neuropsychiatric disease (Dich et al., 2015; Anda et al., 2006) but also increasing the likelihood of anxiety/depression comorbidity (Espejo et al., 2007). Multiple hit developmental models have successfully produced behavioral phenotypes consistent with "anxiety-like" and depressive-like" behavior in both rats and mice, and the overall picture that has emerged suggests similar general behavioral findings in males and females (Knox et al., 2021; Saveedra et al., 2021; Yohn and Blendy, 2017; Page and Coutellier, 2018). Developmental multiple hit rodent studies have also reported changes in brain regions that have been associated with both depression and anxiety in humans, including the amygdala, hippocampus, and prefrontal cortex (Yohn and Blendy, 2017; Page and Coutellier, 2018; Huang et al., 2021; Saavedra et al., 2021; Igsor et al., 2004; Oztan et al., 2011). It should be noted that, as with most ELA experimental animal studies, these experiments used a region-of-interest approach, so it cannot be ruled out that other brain regions are affected as well.

Cellular effects of ELA: similarities and differences across paradigms

The wide range of behavioral outcomes observed in response to ELA in rodents has been generally interpreted in the context of individual differences in stress susceptibility related to inherent differences in the experimental organism, i.e., strain, sex, maternal care, but when viewed against the overall body of literature on ELA in humans, an important possibility to consider is that specific aspects of the ELA paradigm itself, e.g., whether it is primarily a model of neglect or abuse, may be responsible for some of the differences across studies. Future studies that compare outcomes of different rodent ELA paradigms regarding differential brain changes are likely to be informative. Since most studies understandably use one model (for an exception see

Demaestri et al., 2020), comparisons across models must be made across studies where dependent measures often differ.

Some evidence suggests that maternal separation and LBN models exert similar effects on the brain in that they generally result in decreased complexity, connectivity, and activation of neurons in the amygdala, hippocampus, and prefrontal cortex (Molet et al., 2014). However, some studies have reported differences in the outcomes of exposure between these models. For example, Walker et al. (2017) showed that LBN attenuates the development of dendritic layers II, III, and V in PFC, resulting in decreased cortical function, while Farrell et al (2016) showed that maternal separation results in enhanced dendritic branch number and dendritic length in PFC.

Additionally, while it is possible that some of the neural outcomes of these two models may be similar, the underlying processes by which they arise may be different. For example, it has been shown that in the hippocampus, both maternal separation and LBN result in decreased adult neurogenesis in the hippocampus (Zhang, et al., 2021; Naninck et al., 2015). Maternal separation studies suggest that altered proliferative of neural stem cells results in decreased adult neurogenesis in adulthood (Zhang, et al., 2021). LBN studies, on the other hand, report increases in the microglia phagocytic marker CD68, suggesting that more local, cell non-autonomous changes may be contributing to the reduction of cells (Hoeijmakers et al., 2017.

Along these lines, studies have shown that microglia are also more broadly impacted in rodent models of ELA. Maternal separation during the early postnatal period, as well as social isolation during adolescence, alters microglia morphology, activation, and number in the hippocampus and prefrontal cortex (Delpech et al., 2016; Bachiller et al., 2020; Reshetnikov et al., 2020;

Gildawie et al., 2020; Usui et al., 2021), and some evidence suggests that ELA-induced increases in microglial activation may be responsible for some of its effects on stress vulnerability, including reduced reward seeking and stress coping after subsequent stress exposure. Given the important role that microglia play in synaptogenesis, synapse elimination, and synaptic plasticity (Wu et al., 2015; Wilton et al., 2019), it seems likely that microglial activation might be causally linked to ELA-induced behavioral phenotypes. Indeed, treatment with minocycline, a drug that inhibits microglial activation, prevents many ELA-induced changes (Wang et al., 2018; Han et al., 2019). The translational significance of ELA-induced changes in microglia and psychiatric vulnerability remains relatively unexplored, although several studies have shown that childhood maltreatment results in increases in inflammatory biomarkers, some of which have been associated with microglial activation (D'Elia et al., 2018; Gill et al., 2020; Lacey et al., 2020).

Regarding potential molecular and cellular effects of specific types of ELA that may predispose individuals to certain neuropsychiatric diseases, the field might benefit from descriptive studies involving multiple brain regions in addition to more commonly used region-of-interest approaches. For example, several studies using different rodent models of ELA have reported changes in perineuronal nets (PNNs), extracellular matrix structures that are known to play important roles in suppressing plasticity (Santiago et al., 2018; Murthy et al., 2019; Gildawie et al., 2020; 2021). These results are difficult to interpret together given that the ELA paradigms and examined brain regions differ across studies. A recent report examining human postmortem brain tissue reported that MDD adults with a previous history of childhood maltreatment have higher numbers of PNN+ neurons in the prefrontal cortex than those without such a history (Tanti et al., 2021). Collectively, the rodent and human findings raise intriguing possibilities regarding potential effects of ELA as well as therapeutic targets, however, the available evidence

is very limited. Comparative characterization of effects of different ELA types on PNNs in multiple brain regions in both humans and rodents would be a useful first step toward a better understanding of whether and how PNNs are causally linked to ELA-induced vulnerabilities to neuropsychiatric disease.

Genetic influences on ELA effects across species

Any attempt to understand how different types of ELA influence mental health in humans must consider the likelihood that a significant amount of the variability across individuals may be related to genetic differences. While environmental variables could drive some of these individual differences, it seems likely that at least some of the variability arises from genetic differences. Along these lines, researchers have identified the val66met BdNF polymorphism as a genetic vulnerability factor to early life stressors in humans (Kim et al., 2007; Bukh et al., 2009; Gatt et al., 2009; Youssef et al.2018; Zhao et al., 2018). Furthermore, transgenic val66met BdNF mice also show a greater likelihood of stress-induced behavioral change (Chen et al., 2006; Notaras et al., 2017). These and related findings have led to suggestions for treatment plans related to the genetic composition of individual patients.

Early life experiences also have the ability to alter gene expression independent of inherent genomic differences between individuals. Indeed, ELA has been shown to induce transcriptional changes increasing or decreasing expression of different genes, and some of these changes have been associated with ELA-induced neuropsychiatric disorders (Heim and Binder, 2012; Nugent et al., 2011; Caspi et al., 2003; Karg et al., 2011). Examining epigenetic changes in rodent models of ELA has been a fruitful pursuit in understanding the physiological mechanisms of

ELA. Studies focusing on SLC6A4 (serotonin transporter), NR3C1 and FKBP5 (involved in glucocorticoid activity) and BDNF gene expression have revealed promising targets for potential intervention (Parel and Peña et al., 2022). Klopack and colleagues have investigated accelerated epigenetic aging as a link between ELA and adult neuropsychiatric disease (2022). While a comprehensive evaluation of genetic and epigenetic variables mediating ELA-induced neuropsychiatric disease is far beyond the scope of this review, considering these factors in the context of vulnerabilities induced by specific types of ELA should be the focus of future research.

Conclusion

Considerable research efforts have been made into understanding the variability in outcomes that result from ELA in humans. Numerous studies have explored how timing, duration, intensity, and genetic vulnerabilities are important for understanding the varied impact of ELA. A growing literature shows how different types of ELA may predispose individuals to different neuropsychiatric outcomes. Available evidence suggests that physical and sexual abuse are most often associated with anxiety disorders, PTSD, and personality disorders, and emotional maltreatment is most often associated with MDD and suicidal ideation. Attempts to model types of ELA in rodents have produced mixed results with regard to consistency with the human literature. Models involving physical and sexual abuse in rodents have produced behavioral changes that may be consistent with some relevant human neuropsychiatric outcomes, including anxiety disorders and PTSD, while available brain data, although limited, highlight changes in the amygdala and anterior cingulate with physical abuse and changes in the hippocampus with sexual abuse in both rodents and humans. Models involving deficient maternal care in rodents as well as social isolation during adolescence, which may be reasonable models of childhood emotional maltreatment and deprivation, have been more widely used and despite varied results,

have revealed behavioral changes that may be relevant to MDD, while available brain data points to changes in reward and mood processing circuits in both rodents and humans. Taken together, adversity type seems to play an important role in determining mental health outcomes, as is the case with timing, duration, and intensity or ELA. More research using rodent models that directly compare brain changes resulting from different ELA models is likely to be informative in understanding how specific subtypes of ELA influence neural circuitry, which could result in potential targets for therapeutic intervention.

Therefore, this thesis aims to understand the mechanisms through which early life adversity contributes variation in behavioral outcomes. With a particular focus on hippocampal plasticity, due to its heightened vulnerability to early life experiences, we investigated two different hippocampal dependent behaviors including anxiety-like behavior and social memory.

Chapter 2: Inhibition of adult neurogenesis reduces avoidance behavior in male, but not female, mice subjected to early life adversity

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Abstract

Early life adversity (ELA) increases the risk of developing neuropsychiatric illnesses such as anxiety disorders. However, the mechanisms connecting these negative early life experiences to illness later in life remain unclear. In rodents, plasticity mechanisms, specifically adult neurogenesis in the ventral hippocampus, have been shown to be altered by ELA and important for buffering against detrimental stress-induced outcomes. The current study sought to explore whether adult neurogenesis contributes to ELA-induced changes in avoidance behavior. Using the TK-GFAP transgenic model, which allows for the inhibition of adult neurogenesis, and CD1 littermate controls, mice were subjected to an ELA paradigm of maternal separation and early weaning (MSEW) or control rearing. Mice with intact adult neurogenesis were found to show no behavioral changes in response to MSEW. After reducing adult neurogenesis, however, male mice previously subjected to MSEW had an unexpected decrease in avoidance behavior. This finding was not observed in female mice, suggesting that a sex difference exists in the role of adult-born neurons in buffering against ELA-induced changes in behavior. Taken together with the existing literature on ELA and avoidance behavior, this work suggests that strain differences exist in susceptibility to ELA and that adult-born neurons may play a role in regulating adaptive behavior.

Chapter Introduction

The striking relationship between early life adversity (ELA) and a wide range of neuropsychiatric disorders has led researchers to search for mechanisms underlying this connection. ELA encompasses a variety of negative experiences including abuse (physical, emotional, and sexual), neglect, chronic illness, witnessing violence, and natural disasters, and more (CDC, 2020; NIMH, 2018). It has been shown that negative early life experiences can significantly diminish cognitive, emotional, social, and physical health (Lupien et al., 2009; Chen et al., 2016). More specifically, ELA has been strongly associated with an increased prevalence of anxiety disorders (Fonzo et al., 2016; Gallo et al., 2018; Heim et al., 2010) and it has been estimated that over fifty percent of anxiety disorder diagnoses can be linked to self-reported childhood maltreatment (Li et al., 2016).

To explore mechanisms underlying the connection between ELA and adult psychopathology, researchers have developed a variety of animal models. Among the most common are maternal separation, a putative model of neglect (Murthy et al., 2018), and limited bedding/nesting, a putative model of scarcity and abuse (Walker et al., 2017; Gallo et al., 2019). Using these approaches, several studies have demonstrated that ELA increases behaviors that have been characterized as avoidance, in that they involve avoidance of potentially threatening environments (Janus, 1987; Huot et al., 2002; Kalinichev et al., 2002; Romeo et al., 2003; Daniels et al., 2004; Colorado et al., 2006; Lee et al., 2007; Wei et al., 2010; Aya-Ramos et al.,2017; Masrour et al., 2018; Cui et al., 2006; Raineki et al., 2012; Dalle Molle et al., 2012; Wang et al., 2012). Although controversy exists over the interpretation of these behaviors (LeDoux and Pine, 2016), it is generally accepted that their increase may reflect an "anxiety-like"

state in rodents that shares some features with less complex symptoms of anxiety disorders in humans (LeDoux and Pine, 2016; Murthy et al., 2020).

Despite successful attempts to model ELA in rodents and observe increased avoidance behavior, several studies using these same approaches have failed to report such effects (Lehmann et al., 1999; Slotten et al., 2006; Eklund and Arborelius, 2006; Millstein and Holmes, 2007; Savignac et al., 2011; Candemir et al., 2019; Rice et al., 2008; Ivy et al., 2010; van der Kooij et al., 2015; Johnson et al., 2018). This overall collection of contradictory findings is difficult to comprehend, but it may be reflective of the variability of responses to ELA observed in humans. Indeed, many people who experience ELA do not develop neuropsychiatric diseases (Li et al., 2016; Dunn et al., 2018) and some even show improved coping to subsequent stressors (Shapero et al., 2015). Factors including genetic differences, as well as the type and timing of ELA, likely contribute to the varied vulnerability of neuropsychiatric outcomes. In this latter regard, it is relevant to note that repeated bouts of trauma during sensitive periods of development may be most likely to produce long-term dysfunction in humans (Dunn et al., 2018; Schalinski et al., 2019). To potentially model this more closely in rodents and to avoid compensatory responses of rodent mothers to maternal separation (Orso et al., 2019; Murthy et al., 2018), researchers developed the maternal separation and early weaning model (MSEW) which has been shown to increase avoidance behavior in adult mice (George et al., 2010; Carlyle et al., 2012; Murthy et al., 2019; Laham et al., 2020).

In adult rodents, the ventral hippocampus has been implicated in the regulation of avoidance behavior through lesion studies (Weeden et al., 2015; Bannerman et al., 2003), optogenetics (Padilla-Coreano et al., 2019), and electrophysiological recordings (Murthy et al., 2019). The hippocampus undergoes significant neurogenesis, synaptogenesis, dendritic remodeling, and glial

growth during the postnatal period (Avishai-Eliner et al., 2002; Khazipov et al., 2001) making it a possible target for ELA-induced structural impairment. The hippocampus continues to exhibit these plastic processes well into adulthood, including adult neurogenesis, the addition of new dentate gyrus granule cells, which become integrated into established neuronal circuits (Cope and Gould, 2019). These new neurons develop throughout the entire dorsoventral axis and are important for avoidance behavior, stress regulation, and cognitive function, including social memory (Snyder et al., 2011; Revest et al., 2009; Hill et al., 2015; Anacker et al., 2018; Cope et al., 2020). Some studies report that ELA decreases the number of adult-born granule cells (abGCs) (Mirescu et al., 2004; Oomen et al., 2010; Leslie et al., 2011; Loi et al., 2014; Naninck et al., 2015; Ruiz et al., 2018; Youssef et al., 2019), as well as diminishes dendritic morphology (Oomen et al., 2010; Leslie et al., 2011), function and connectivity (Huot et al., 2002) of newborn cells. abGCs have also been shown to play a role in social behavior (Cope et al., 2020; Monteiro et al., 2014; Garrett et al., 2015; Pereira-Caixeta et al., 2017, 2018), which is disrupted in many neuropsychiatric disorders that have been linked to ELA (Farrington et al., 2005; Opendak et al., 2017; Tzanoulinou and Sandi, 2016).

Given that abGCs have been linked to avoidance behavior, the current study sought to investigate whether adult neurogenesis contributes to changes in avoidance behavior following ELA. The hypothesis that reducing abGCs following MSEW would potentiate already increased avoidance behavior compared to control reared animals was tested, and given the role of abGCs in social memory, whether this would also impair social recognition. A transgenic mouse model that expresses herpes simplex virus thymidine kinase (TK) under the control of the GFAP promoter on a CD1 background was used. Adult neurogenesis is inhibited in TK mice by treatment with the antiviral drug valganciclovir (VGCV) (Snyder et al., 2011). Male and female

TK and CD1 wild-type littermate pups were subjected to MSEW or control rearing. Following maturation and VGCV treatment, mice were tested on the elevated plus maze (EPM), an avoidance behavior task, and the direct social interaction test, a social memory task.

Materials and methods

Animals

All animal procedures were performed in accordance with Princeton University Institutional Animal Care and Use Committee and followed the guidelines of the National Research Council's Guide for the Care and Use of Laboratory Animals (2011). Male and female transgenic mice expressing herpes simplex virus thymidine kinase (TK) under the GFAP promoter were bred in the Princeton Neuroscience Institute animal colony with founders provided by Dr. Heather Cameron at the National Institute of Mental Health. GFAP-TK mice were generated by crossbreeding CD1 male mice with heterozygous GFAP-TK female mice. Both male and female CD1 and GFAP-TK mice were used for this study. To control for potential litter-specific genetic and prenatal factors, pups were cross-fostered with those of age-matched litters on postnatal day (P) 2. On P15, mice were genotyped by taking ear punch samples that were processed by Transnetyx.

Maternal Separation and Early Weaning

Pups were randomly assigned to either a control-rearing or MSEW group as previously described (George et al., 2010; Murthy et al., 2019) (Figure 1). Control-reared litters were left undisturbed until weaning on P21. MSEW mice were separated on P3-P6 from their dam for 4 hours per day. On P6-P16, separations increased to 8 hours. For separations, dams were removed from the

home cage, and each cage containing the pups was placed on top of a thermal heating blanket maintained at 34°C. Pups remained with their littermates for the entire period of separation after which the dam was returned to the home cage. Maternally separated pups were then weaned four days earlier than controls, at P17. Body weights of MSEW pups were measured daily, while those of control pups were taken on the day of weaning to minimize disruption. At weaning, all mice were housed by genotype and sex with 4-5 mice per cage in Optimice cages on a reverse 12-hour light/dark cycle.

Valganciclovir treatment

Beginning at P60, valganciclovir (VGCV), an antiviral drug that selectively reduces adult neurogenesis in TK-GFAP animals (Figure 6), was administered to half of the TK and CD1 male and female mice in the study. These mice received VGCV (VGCV+) mixed in powdered rodent chow (227 mg of VGCV per kg chow) for 5 days/ week and standard pellet chow for 2 days/week as previously described (Cope et al., 2020). VGCV treatment continued until the time of perfusions. Standard chow without VGCV (VGCV-) was administered to the other half of the TK and CD1 mice. Thus, for each sex and MSEW/control-reared group, there were 4 groups: CD1 VGCV+, CD1 VGCV-, TK-GFAP VGCV+, TK-GFAP VGCV-. Group sizes were as follows: males n =15-17/group, females n= 8-10/group.

Estrous cycle monitoring

To determine the stage of estrous before behavioral testing, C57 female mice were lavaged daily. Stage classification used vaginal cytology as previously described (McLean et al., 2012). Stages of the estrous cycle were determined based on observation of leukocytes, cornified epithelial cells,

and nucleated epithelial cells. Females were tested during sexually receptive (proestrus/estrus) and sexually non-receptive (diestrus/metestrus) periods.

Elevated plus maze test

After 6 weeks of VGCV treatment, male and female mice were tested on the elevated plus-maze (EPM) usually during the active cycle (dark phase) between 08:00-12:00. The 6-week time point was selected for behavioral testing because abGCs are known to be functionally integrated into the hippocampal circuitry by this time (Denny et al., 2012; Kee et al., 2007). The EPM measures 44 x 44 x 20 inches) with two closed arms each 20 inches long and with high walls (13 inches). The two open arms, also 20 inches in length, and the central intersection had no walls. The open arms were illuminated to 200 lux. Mice were placed on the EPM, facing the open arm and exploratory behavior was recorded for 5 mins. After each mouse was tested, the EPM was cleaned with 70% ethanol. The behavior test was videotaped by a camera suspended over the EPM. Videos were scored by an experimenter blind to the genotype, treatment, and group. The number of entries and time spent in each of the arms were collected. Since open arms are less protected and more exposed to light, more time spent in the open arms is considered to reflect less avoidance behavior. Total entries into the arms are a measure of overall activity levels.

Direct social interaction test

The direct social interaction test was conducted in an open-field box (23 × 25 × 25 cm) in low light (10–20 lux) and during the active cycle for mice (dark phase). Mice were first habituated to the testing room for a minimum of 30 mins and then to the testing box for 5 mins. For the test, each animal has 2 trials. In trial 1, the subject mouse was allowed to freely interact with a novel mouse stimulus mouse for 5 minutes. Following a 60-minute delay, the subject mouse was then

placed back with the same, now familiar mouse for a second 5-minute trial. Because mice prefer exploring novelty, they typically decrease their investigation time when exposed to a familiar mouse. More specifically, social memory is operationalized as a mouse's decrease in investigation time from trial 1 to trial 2.

Histology

To verify that VGCV treatment successfully reduced abGCs only in TK mice, mice were perfused, and brain tissue was immunolabeled for PSA-NCAM (CD56), a marker of immature granule cells. Mice were anesthetized with Euthasol and transcardially perfused with 4% paraformaldehyde (PFA) in PBS. A random, representative subset (n=5-6/group) of brains from each experimental group was chosen for analysis. Brains were post-fixed for 48 hours in 4% PFA in PBS followed by 48 hours in PBS with 30% sucrose for cryoprotection. Unilateral coronal sections were collected at 40 μm thickness using a Leica CM3050S cryostat. Sections were washed in PBS then pre-blocked in 0.3% Triton X-100 and 3% normal donkey serum for 1.5 hours at room temperature. Sections were then incubated in a solution containing a primary antibody against PSA-NCAM (rat anti-mouse CD56, 1:500; BD Pharminogen), a marker of immature neurons, for 24 hours at 4°C. Rinsed sections were next transferred to 0.3% Triton X-100 in PBS containing secondary antibody goat anti-rat Alexa Fluor 568 (1:250; Invitrogen). Rinsed sections were then counterstained with Hoechst 33342 (1:5000, Invitrogen). Finally, sections were mounted onto slides and cover slipped with Vectashield (Thermo-Fisher Scientific). Slides were coded and analyzed with the investigator blind to the treatment group. Each brain was checked to determine whether abGCs were present in the dentate gyrus at the time of perfusion using a Leica TCS SP8 confocal microscope.

Statistical analysis

Statistical tests were performed using GraphPad Prism 9.0 (GraphPad Software). Data were first analyzed using linear regression to assess the ability of sex or estrous cycle stage in females to predict each metric of interest (percent time and entries to open arms, percent time and entries to closed arms, and overall entries to both arms). Sex was a significant predictor of some measures of the EPM; hence data were analyzed separately by sex. Estrous cycle was not a significant predictor of any measure, therefore, the values for females tested on the EPM during diestrus and estrus were averaged together for each mouse. For the direct social interaction test, a linear regression model revealed no significant interactions between genotype and treatment so data these groups were collapsed and analyzed using a 3-way ANOVA model. The normality test was used to test sample normality to determine whether parametric or non-parametric tests were appropriate and then the data were analyzed using 3-way ANOVA or if a data set did not pass tests of normality, it was log-transformed and then analyzed using parametric statistics. Data are presented as mean ± standard error of the mean.

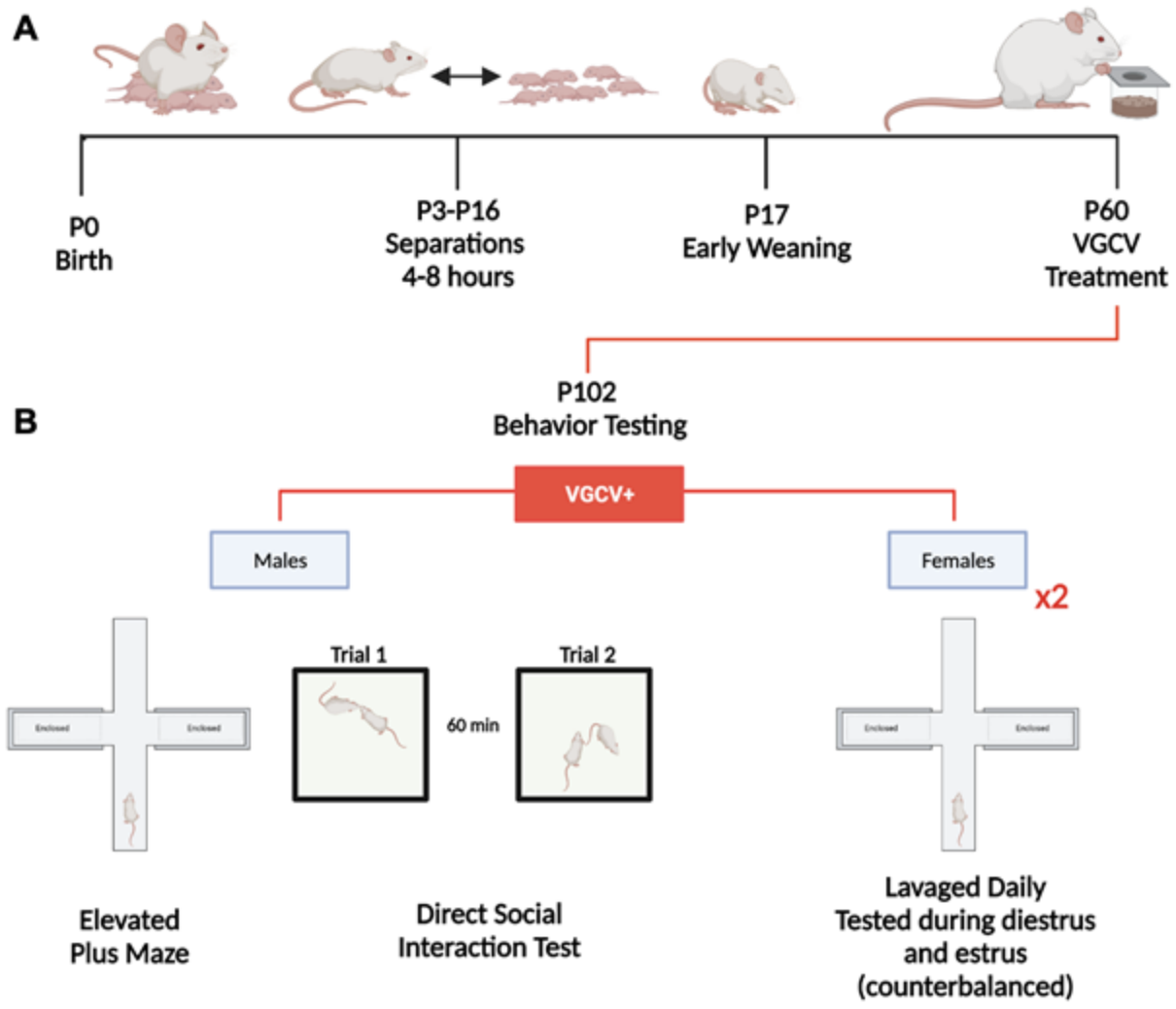


Figure 1. Timeline and experimental design. Litters were subjected to either control rearing, where they were undisturbed until weaning on P21, or A) MSEW rearing. B) Half of each genotype and rearing condition was then fed VGCV for 6 weeks. Male mice were then tested on the EPM for 5 mins, followed by the direct social interaction test using a 60-minute intertrial delay. Female mice were tested twice on the EPM, once during estrus and diestrus as determined by a daily lavage.

Results

Sex differences in avoidance behavior are evident in mice with and without MSEW and males are more active than females

Hyperactivity is often comorbid with anxiety disorders in humans (Mao et al., 2014) and previous studies have shown that MSEW increases activity levels in C57 male mice (George et al., 2010; Murthy et al., 2019). The total entries to the open and closed arms were summed to determine activity levels on the EPM. A significant regression equation was found ($F_{(1,195)}$ =557.71, p=<0.0001, with R^2 =0.222) with male mice making more total entries into the arms than female mice (Figure 2). Multiple linear regression revealed sex differences in the percent of time and entries made to the open arm. A significant regression equation was observed with analysis of time spent in the open arms ($F_{(1,180)}$ =11.54, p=0.0008, with R^2= 0.060). Sex was a significant predictor of percent time in the open arms with males spending more time than females. It was found that open arm entries differed according to sex with males showing more entries than females. Given our observed sex differences on these measures and the interrelatedness of all other measures of the EPM, sexes were analyzed separately.

MSEW does not alter activity levels on the EPM, nor does reducing adult neurogenesis in male or female mice.

Male MSEW CD1 VGCV-/+ did not differ from control CD1 VGCV-/+ in the total number of entries to the arms of the EPM (Figure 2A). Additionally, reducing abGCs in control and MSEW TK VGCV+ groups did not alter the total number of entries on the EPM (group: $F_{(1,115)}$=4.277, p=0.0509; genotype: $F_{(1,115)}$=0.2193, p=0.6404; treatment: $F_{(1,115)}$=0.1486, p=0.7006; group x genotype x treatment: $F_{(1,115)}$=0.2836, p=0.5954), suggesting that there were no changes to activity levels across groups. Similarly, female mice showed no changes in activity

levels across any of the groups examined (group: $F_{(1,65)}$ =0.4723, p=0.4944; genotype: $F_{(1,65)}$ =0.0147, p=0.9036; treatment: $F_{(1,65)}$ =0.7667, p=0.3845; group x genotype x treatment: $F_{(1,65)}$ =0.2527, p=0.5954).

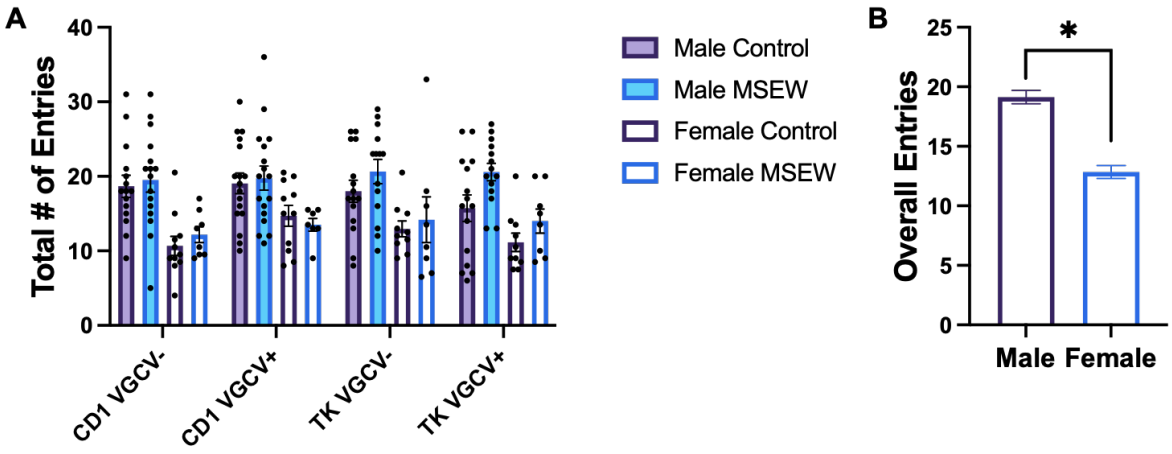


Figure 2. Sex differences in behavior on the EPM with males showing greater activity than females. A) Total entries to open and closed arms, a measure of activity levels, was greater in males than females. Within each sex, there were no significant differences in total arm entries between control and MSEW. B) A linear regression revealed that sex was a significant predictor of overall entries to both arms.

In male mice, reducing adult neurogenesis after MSEW decreases avoidance behavior

To assess the influence of abGCs on avoidance behavior after MSEW, the transgenic GFAP-TK mice with inhibited adult neurogenesis following administration of VGCV (VGCV+) was used and tested mice on the EPM. Unexpectedly, in contrast to previous studies (Murthy et al., 2019; George e t al., 2010), MSEW did not increase avoidance behavior compared to control reared CD1 mice. MSEW CD1 mice, either treated with VGCV or not, did not show differences in the percentage of time spent in the open arms (Figure 3A). However, reducing adult neurogenesis had differential effects in mice that experienced MSEW. MSEW TK VGCV+ spent a

significantly greater percentage of time on the open arms of the EPM compared to control TK VGCV+ suggesting reduced avoidance behavior (Figure 3A; group: $F_{(1,112)}$ =5.086, p=0.0261; genotype: $F_{(1,112)}$ =0.6698, p=0.4148; treatment: $F_{(1,112)}$ =0.6665, p=0.4160; group x genotype: $F_{(1,112)}$ =6.994, p=0.0094).

MSEW CD1 mice, either treated with VGCV or not, did not show differences in the percent of time spent in the closed arms (Figure 3B), compared to control reared mice with or without VGCV. In TK GFAP control-reared mice treated with VGCV, there was also no change in avoidance behavior. However, MSEW TK VGCV+ spend a lower percentage of time in the closed arms compared to control TK VGCV-, animals with normal levels of abGCs (group: $F_{(1,116)}$ =3.500, p=0.0639; genotype: $F_{(1,116)}$ =0.0018, p=0.9655; treatment: $F_{(1,116)}$ =1.880, p=0.1730; group x genotype: $F_{(1,116)}$ =7.387, p=0.0076).

Preference for closed or open arms by the percentage of total entries made to each set of arms on the EPM was also measured. Control and MSEW CD1 mice with normal levels of adult neurogenesis (VGCV-/+) did not differ in the percentage of entries made to the open arms (Figure 3C). Reducing adult neurogenesis in control mice (TK VGCV+) caused a significant reduction in the percentage of time spent in the open arms relative to MSEW TK VGCV+, MSEW TK VGCV-, and MSEW CD1 VGCV-. MSEW mice with reduced adult neurogenesis entered the open arms significantly more than control TK VGCV+, mice with similar reductions to abGCs (group: $F_{(1,114)}$ =14.05, p=0.0003; genotype: $F_{(1,114)}$ =0.0204, p=0.8865; treatment: $F_{(1,114)}$ =0.0784, p=0.7799; group x genotype x treatment: $F_{(1,114)}$ =6.218, p=0.0141).

A similar relationship was true for the percentage of entries into the closed arms and the center. Control CD1 VGCV-, control and MSEW CD1 VGCV+, and control TK VGCV- entered

closed arms comparably. Reducing adult neurogenesis in control mice (TK VGCV+) caused a significant increase in the percentage of time spent in the closed arms compared to MSEW TK VGCV+, MSEW TK VGCV-, and MSEW CD1 VGCV-. MSEW mice with reduced adult neurogenesis entered the closed arms significantly less than control TK VGCV+, mice with similar reductions to abGCs (group: $F_{(1,113)}$ =13.36, p=0.0004; genotype: $F_{(1,113)}$ =0.0060, p=0.9382; treatment: $F_{(1,113)}$ =0.2484, p=0.6192; group x genotype x treatment: $F_{(1,113)}$ =5.795, p=0.0177). Altogether, increased time and entries into the open arms and decreased time and entries in the closed arms suggest decreased avoidance behavior in male MSEW mice with reduced numbers of abGCs (MSEW TK VGCV+).

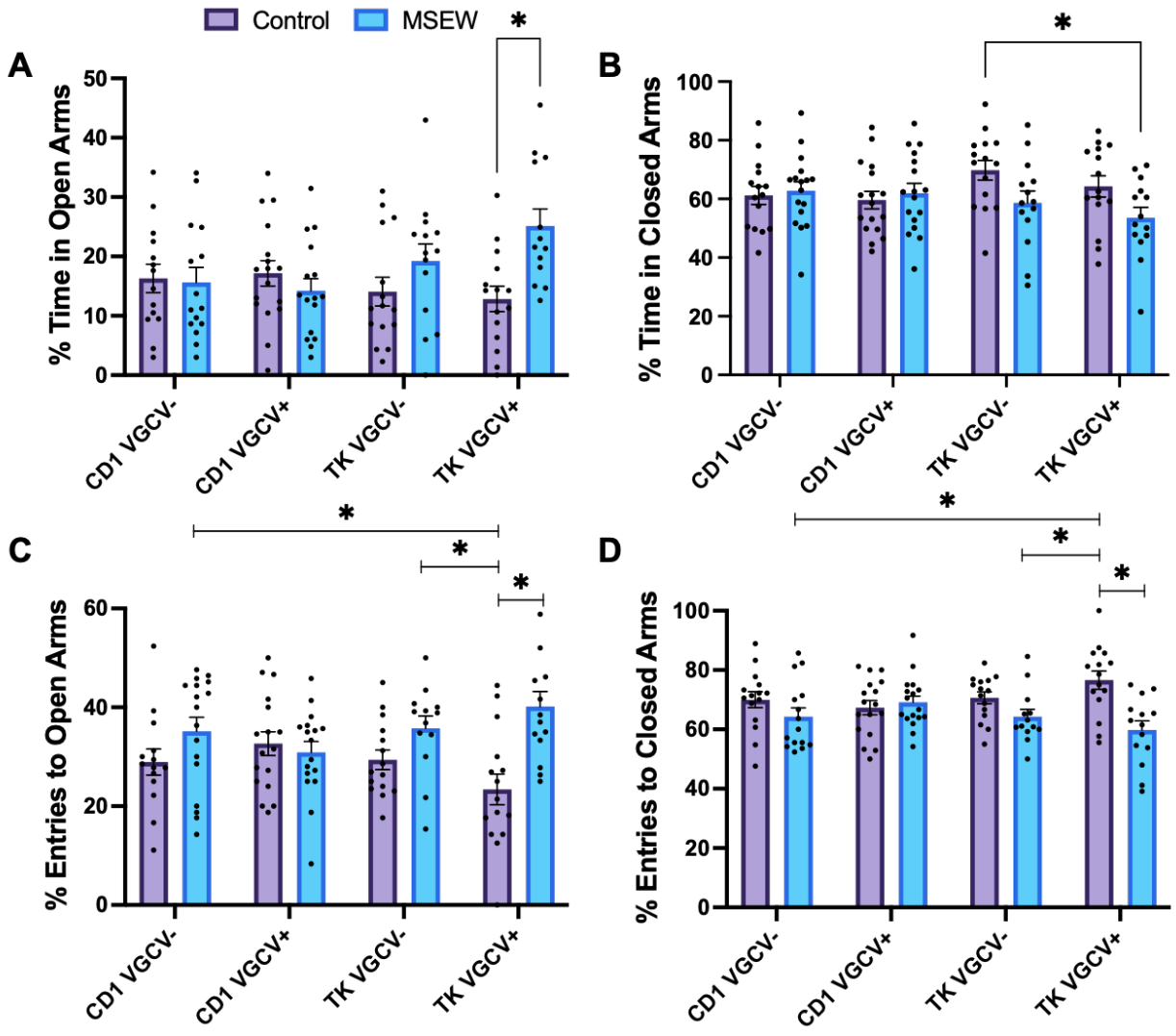


Figure 3. In males, reduction of adult-born neurons after ELA decreases avoidance behavior. A) Percent time on the open arms of EPM, a measure of avoidance behavior was higher in MSEW TK VGCV+ compared to control TK VGCV+ (group x genotype: $F_{(1,112)}$ =6.994, p=0.0094; Tukey posthoc test: MSEW TK VGCV+ vs control TK VGCV+, p=0.0304). B) Percent time spent in the closed arms of EPM. MSEW TK VGCV+ animals spend less time in the closed arms compared to control TK VGCV- mice (effect of group x genotype: $F_{(1,116)}$ =7.387, p=0.0076; Tukey posthoc test: MSEW TK VGCV+ vs control TK VGCV-, p=0.0258). C) Percent entries to open arms of EPM. Control TK VGCV+ mice entered the open arms significantly less than MSEW TK VGCV+, MSEW TK VGCV-, and MSEW CD1 VGCV- (effect of group x genotype x treatment: $F_{(1,114)}$=6.218, p=0.0141; Tukey posthoc test: MSEW

TK VGCV+ vs control TK VGCV+, p=0.0005; Control TK VGCV+ vs MSEW CD1 VGCV-, p=0.0293, Control TK VGCV+ vs MSEW TK VGCV-, p=0.0351) D) Number of entries to closed arms of EPM. Control TK VGCV+ enter the closed arms significantly more than MSEW TK VGCV+, MSEW TK VGCV-, and MSEW CD1 VGCV- (effect of group x genotype x treatment: $F_{(1,113)}$ =5.795, p=0.0177; Tukey posthoc test: MSEW TK VGCV+ vs control TK VGCV+, p=0.0005; Control TK VGCV+ vs MSEW CD1 VGCV-, p=0.0260, Control TK VGCV+ vs MSEW TK VGCV-, p=0.0360). Data were analyzed by 3-way ANOVA. Bars represent mean +SEM.

In female mice, reducing adult neurogenesis had no effect on avoidance behavior after MSEW or control-rearing

The stage of estrous and its corresponding fluctuations in hormone levels have been shown to significantly influence female mouse behavior (Pentkowski et al., 2018, Luine et al., 2013). Additionally, studies have shown that avoidance behavior in female C57 mice following MSEW is increased only during diestrus (Laham et al., 2022). As a result, female mice were lavaged daily and tested them on the EPM during estrus and diestrus using a counterbalanced design.

First, a multiple linear regression was used to predict the percent time mice spent in the open and closed arms as well as entries to the open arms and closed arms based on the estrous stage. Significant regression equations were found however, estrous stage was not a significant predictors of any of these measures (percent time in open arms: $F_{(1,131)}$=1.639, p=0.2027; percent time in closed arms: $F_{(1,139)}$=0.1297, p=0.7193; percent entries to open arms: $F_{(1,139)}$=0.0381, p=0.8454; percent entries to closed arms: $F_{(1,139)}$=0.0406, p=0.8405). Therefore, data from each estrous stage were averaged within each mouse and the resulting dataset was analyzed using 3-way ANOVA. Control CD1 VGCV +/- female mice did not differ significantly from MSEW mice in the percent of time spent in the open arms (Figure 4A) Additionally, reducing abGCs in

TK VGCV+ mice also had no apparent effect on percent time in the open arms in the MSEW or control mice (group: $F_{(1,54)}$=0.1559, p=0.6945; genotype: $F_{(1,54)}$=0.0466, p=0.8298; treatment: $F_{(1,54)}$=2.505, p=0.1194). The same was the case for the percent of time spent in the closed arm (Figure 4B; group: $F_{(1,63)}$ =2.076, p=0.1546; genotype: $F_{(1,63)}$ =0.0145, p=0.9044; treatment: $F_{(1,63)}$ =4.294, p=0.0423).

Additionally, MSEW, genotype, or treatment did not associate with the percent of entries made to the open arms (Figure 4C; group: $F_{(1,65)}$=0.1411, p=0.7084; genotype: $F_{(1,65)}$=0.0117, p=0.9142; treatment: $F_{(1,65)}$=4.119, p=0.0465) or closed arms (Figure 4D; group: $F_{(1,62)}$=0.0003, p=0.9855; genotype: $F_{(1,62)}$=0.2603, p=0.6117; treatment: $F_{(1,61)}$=5.166, p=0.0265). In sum, these data show that there are no changes to avoidance behavior in female mice following MSEW with or without reduced numbers of abGCs.

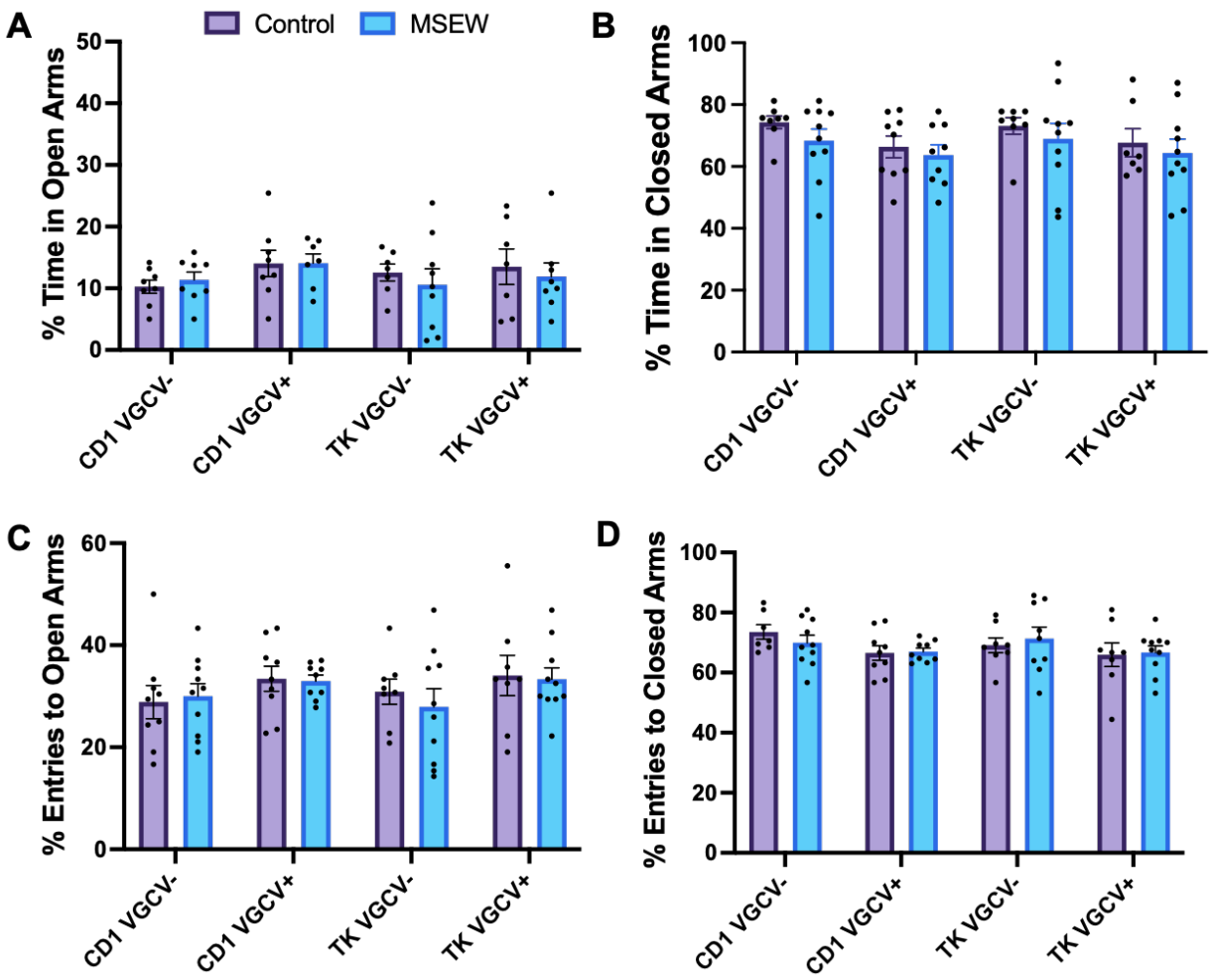


Figure 4. In females, reducing abGCs does not alter avoidance behavior in MSEW or control-reared mice. A) Percent time spent in the open arm of EPM. There were no significant main effects or interactions between group, genotype, or treatment. B) Percent of time spent in the closed arm of EPM. There was a main effect of treatment however the post hoc did not reveal any significant comparisons. (effect of treatment: $F_{(1,63)} = 4.294$, $p=0.0423$). C) Number of entries to open arm of EPM. There were no significant main effects, or interactions between group, genotype, or treatment. D) Number of entries to the closed arm of EPM. There were no significant main effects, or interactions between group, genotype, or treatment. Data were analyzed by 3-way ANOVA. Bars represent mean + SEM.

MSEW does not alter social memory in CD1 male mice, but reducing abGCs in TK mice diminishes social memory

Given the stress-induced changes to abGCs that have been reported in the literature (Oomen et al., 2011; Mirescu et al., 2004; Huot et al., 2002) and the role of abGCs in social memory (Cope et al., 2020), whether MSEW impaired social memory function was investigated as well as, whether reducing abGCs would impact any effects. As described above, social memory function is defined as a significant decrease in investigation time when the stimulus mouse is novel versus when it is familiar. A multiple linear regression was calculated to predict mice's trial 2 times based upon trial 1 time, group, genotype and treatment. A significant regression equation was found ($F_{(14,75)}$ =4.486, p=0. <0.0001, with R^2= 0.4557). Group, genotype or treatment were not significant predictors; therefore, genotype and treatment were collapsed, and the entire data set was analyzed using a 3-way ANOVA. MSEW and control CD1 VGCV-/+ males exhibit normal social memory function. These groups of mice significantly decrease their investigation time from trial 1 to trial 2 (Figure 5A). However, TK VGCV+ mice from both MSEW and control-rearing groups showed impaired social memory function in that they did not exhibit differences in the amount of time spent investigating a stimulus animal between trials (Figure 5B). These results are further illustrated by a difference score, where the trial 2 investigation time is subtracted from trial 1. Given the complete lack of MSEW effect on social memory in male mice, female mice were not tested on the direct social interaction test.

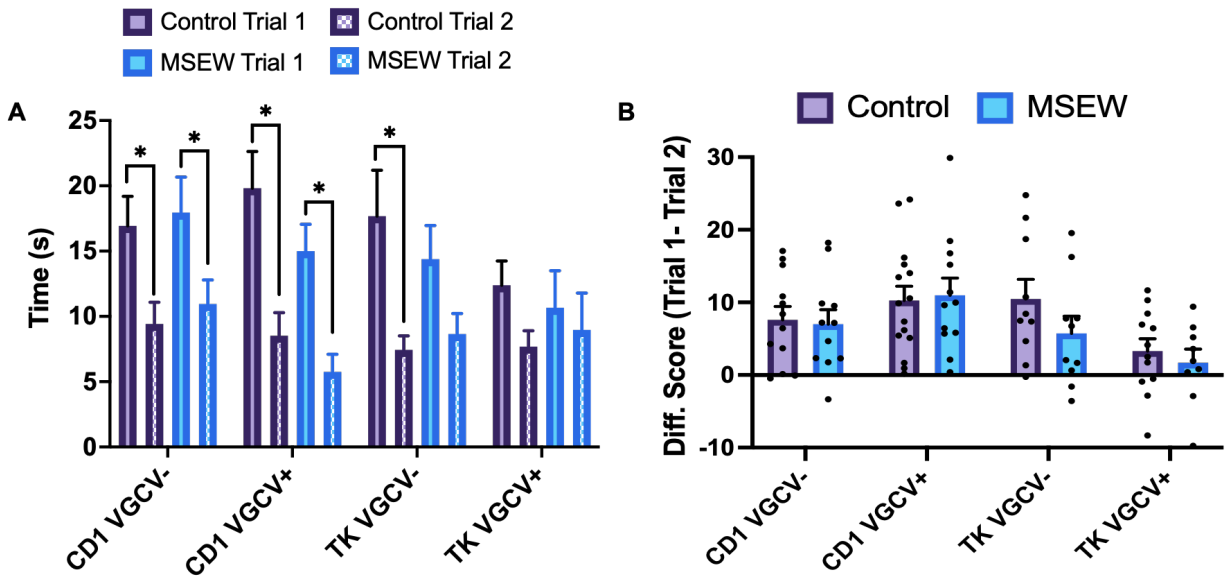


Figure 5. MSEW does not alter social memory in male CD1 mice but reducing adult neurogenesis in TK mice causes significant impairment. A) Control CD1 VGCV -/+ animals display a significant reduction in time investigating the novel animal in trial 1 to the familiar animal in trial 2. MSEW CD1 -/+ animals also significantly reduced the time spent investigating the novel stimulus animal in trial 1 to the familiar stimulus animal in trial 2. TK VGCV+ MSEW as well as control-reared mice showed similar investigation times in trial 1 and trial 2. (effect of genotype/treatment x trial: $F_{(3,73)}$=4.263, p=0.0074; Tukey posthoc test: Control CD1 VGCV- Trial 1 vs. Control CD1 VGCV- Trial 2, p=0.0378; MSEW CD1 VGCV- Trial 1 vs. MSEW CD1 VGCV- Trial 2, p= 0.0477; Control CD1 VGCV+ Trial 1 vs. CD1 Control VGCV+ Trial 2, p= <0.0001; MSEW CD1 VGCV+ Trial 1 vs. MSEW CD1 VGCV+ Trial 2, p=0.0011; Control TK VGCV- Trial 1 vs Control TK VGCV- Trial 2, p=0.0030) B) TK VGCV+ mice in both MSEW and control-reared groups had lower difference scores (trial 1-trial 2) during the direct social interaction test. (effect of genotype x treatment: $F_{(1,83)}$ =8.791, p=0.0040)

VGCV reduces abGCs in the dentate gyrus of TK mice

In the dentate gyrus, abGCs were almost completely eliminated in male and female MSEW and control-reared TK mice administered VGCV. A randomly selected subset of experimental mice

was immunolabeled for PSA-NCAM CD56, a marker of immature granule cells. Both control and MSEW CD1 male and female mice VGCV-/+ as well as control and MSEW TK VGCV- mice maintained substantial numbers of immature abGCs in the dentate gyrus. Control and MSEW TK VGCV+ male and female mice had almost no immature abGCs in the dentate gyrus (Fig. 6). This observation suggests that VGCV treatment significantly reduced the number of abGCs in the dentate gyrus of the hippocampus in both MSEW and control-reared TK VGCV+ groups.

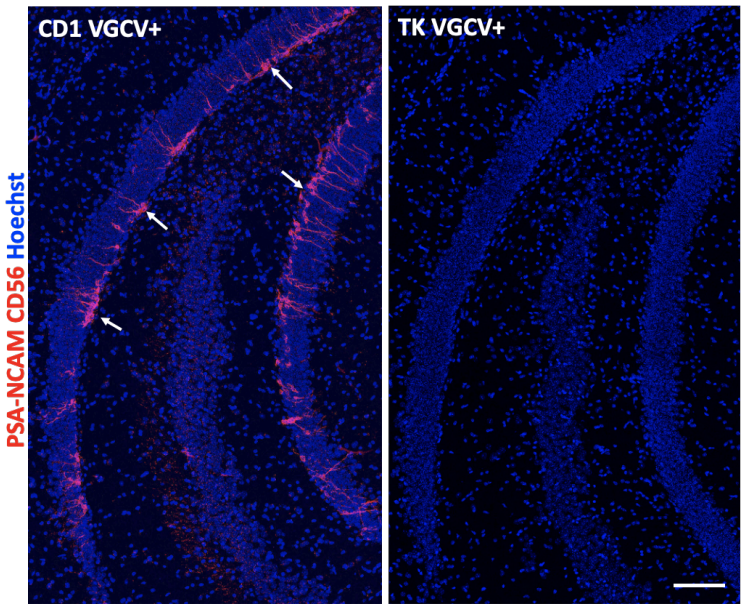


Figure 6. Administration of VGCV reduced adult neurogenesis in the ventral dentate gyrus. Confocal images of PSA-NCAM labeled cells (red) the ventral dentate gyrus from CD1 and TK mice both treated with VGCV. CD1 VGCV+ mice had a typical distribution of immature neurons, while TK VGCV+ mice showed no immature neurons. Scale bar= 100μm.

Discussion

This study was designed to explore whether abGCs buffer against MSEW-induced increases in avoidance behavior. Based on our previous findings, it was hypothesized that MSEW would

increase avoidance behavior in both male and diestrus female mice (Murthy et al., 2019; Laham et al., 2022). Given the literature on adult neurogenesis and avoidance behavior (Revest et al., 2009; Hill et al., 2015; Anacker et al., 2018), it was further hypothesized that eliminating abGCs would potentiate the negative effects of MSEW. Our results were not consistent with either of these hypotheses. First, neither male nor female mice in either stage of estrous tested (estrus or diestrus) as well as of either genotype tested (TK or CD1 littermates) showed an increase in avoidance behavior after MSEW. Second, reducing abGCs had no effect on control or MSEW females but produced an unexpected reduction in avoidance behavior in MSEW males. MSEW TK VGCV+ male mice spent more time in the open arms and made more entries to the open arms than control TK VGCV+ males. These unexpected results suggest that abGCs may normalize avoidance behavior in MSEW males. Since behavioral inhibition is likely an adaptive response in potentially threatening circumstances, these findings suggest that in males, abGCs may serve to stabilize the system after MSEW, dampening the expression of potentially high-risk behavior.

The findings that MSEW did not increase avoidance behavior in either genotype or sex were surprising given results showing such an effect in males and diestrus females (George et al., 2010; Carlyle et al., 2012; Murthy et al., 2019; Laham et al., 2022). Similarly, no MSEW effect on social memory in CD1 males was found, which was unexpected given previous results (Franklin et al., 2011; Emmons et al., 2019) showing that ELA impairs social discrimination. The most obvious difference between the previous studies and the current one is the strain of mice used – previous studies used C57 mice while the current study used TK mice on a CD1 background as well as their CD1 wildtype littermates. Strain differences in stress effects have been reported (Kundakovic et al., 2013; Daskalakis et al., 2014), and although previous studies have not

examined MSEW effects on avoidance or social behavior in CD1 mice, it seems plausible that genetic differences play a role in these discrepant results. In this regard, it may be relevant that several studies investigating behavior on the EPM of CD1 and/or C57 mice have shown that CD1 mice engage in more avoidance behavior, i.e., less time on the open arms, than C57 mice (Tambour et al., 2005; Livneh et al., 2010; Dori et al., 2011; compare the present study with Murthy et al., 2019). Additionally, in the present study, regardless of genotype, group, and treatment, female mice make fewer overall entries to the arms than male mice. This finding may be indicative of even higher baseline avoidance behavior, which is consistent with human literature suggesting that females experience higher rates of anxiety disorders than males (McLean et al., 2011; Altemus et al., 2014). However, this is the opposite of rodent studies investigating avoidance behaviors that find that avoidance behavior is unchanged or decreased in females following ELA (Botterill et al., 2021; Scholl et al., 2019). Thus, this sex difference may be specific to CD1 mice and potentially contribute to obscuring MSEW effects on avoidance behavior in CD1 mice. Since avoidance behavior is already high in CD1 mice, further increases after ELA would likely produce a maladaptive state, with almost complete behavioral inhibition in a novel environment. This interpretation is consistent with studies examining prenatal and adult stress effects in rodents rated as high or low on avoidance behavior in which mice with high avoidance behavior at baseline do not show further stress-induced increases perhaps because they are already close to the ceiling on those measures (Füchsl et al., 2014; Bosch et al., 2006).

Another potential explanation for the differential effects of MSEW on CD1 versus C57 mice may be strain differences in maternal care. Previous studies have shown that maternal separation can lead to an increase in maternal care (licking and grooming, nest building, and nursing) when dams are reunited with their pups (Orso et al., 2019; Berman et al., 2014) and that this increase

in maternal care may minimize or alter behavioral phenotypes in some cases. Furthermore, it has been suggested that enrichment has the potential to completely reverse the physiological and behavioral impact of maternal separation (Hegde et al., 2020). Although to our knowledge no studies have compared maternal behavior of C57 to CD1 mice under control or ELA conditions, it is known that CD1 mice typically have much larger litters than C57 mice (average number of pups is 10 for CD1 dams versus 5 for C57 dams) and that pup survival is higher for CD1 than C57 mice (Bramanti, 1999; Lambert, 2007). These differences raise the possibility that CD1 dams may be better suited at providing maternal care under challenging circumstances and may be better able to compensate for periods of separation, a feature that may contribute to the lack of behavioral effects in MSEW CD1 offspring. MSEW studies that involve fostering pups from C57 to CD1 mice would help to determine whether this was the case.

Mouse strain differences in effects of ELA raise the interesting possibility of parallels to human genetic influences in susceptibility to anxiety disorders. Although ELA greatly increases the likelihood of developing an anxiety disorder, a relatively large percentage of people with anxiety disorder diagnoses report no history of childhood adversity, and still others experience ELA without developing anxiety disorders (Li et al., 2016). Taken together, these findings suggest potentially complex interactions between genes and the environment, which are supported by numerous studies identifying genetic risk factors for the development of anxiety disorders (Gottschalk et al., 2017). Thus, CD1 mice seem to have a genetic predisposition to increased avoidance behavior even without ELA, and strain-related maternal care may serve to protect against maladaptive exacerbations of their already high baseline.

Given previous studies linking abGCs to the regulation of stress responses and avoidance behavior (Snyder et al., 2010; Revest et al., 2009; Hill et al., 2015; Anacker et al., 2018), as well

as evidence that abGCs participate in recovery from deleterious consequences of stress (Schoenfeld et al., 2019; Alves et al., 2017), a major goal of this study was to test whether abGC reduction would exacerbate MSEW-induced increases in avoidance behavior. In this study, elimination of abGCs reduced avoidance of the open arms, but only in the MSEW male mice. These findings suggest that MSEW impacted circuits involved in avoidance behavior but that abGCs buffered against these changes, a possibility that is consistent with views of adult neurogenesis serving to promote adaptive brain function (Opendak and Gould, 2015; Raichlen and Alexander, 2017).

Since ELA is also known to increase risk-taking behavior in humans (Duffy et al, 2018; Lee et al., 2019; Slavich et al., 2019), the current findings suggest that plastic processes, such as those associated with abGCs, may protect against the behavioral manifestation of these changes after ELA. Along these lines, studies have shown that ELA has similar effects on neural circuitry in humans with and without neuropsychiatric diagnoses (Teicher et al., 2016), which suggests that as yet unidentified mechanisms may protect against functional consequences in some individuals but not others. These results suggest that at least for TK mice, MSEW only alters avoidance behavior in the absence of abGCs. The extent to which these effects are adaptive remains unknown, but it is worth noting that the role of abGCs in preventing MSEW-induced decreases in avoidance behavior was only observed in male, not female, mice. This sex difference may be similar to that observed in humans where men are more likely to engage in risky behavior, which is known to be higher after ELA compared to for women (Staton et al., 1999; Crouch et al., 2018; Brookmeyer et al., 2019).

In the hippocampus, neuronal oscillations in the theta frequency range have been linked to self-reported threat/anxiety in humans (Khemka et al., 2017) and increased avoidance of the open

arms in the EPM in mice (Padilla-Coreano et al., 2019). ELA-induced increases in avoidance behavior on the EPM have been associated with increased ventral hippocampal theta power in C57 male and female mice (Murthy et al., 2019; Laham et al., 2020) and ELA has been shown to increase theta power in the hippocampus of adult mice during rapid eye movement (REM) sleep (Sampath et al., 2014). As further evidence that increased theta power is linked to avoidance behavior in both humans and rodents, anxiolytic drugs have been shown to reduce hippocampal theta (McNaughton et al., 2007; Yeung et al., 2012). AbGCs in the hippocampus contribute to the regulation of neuronal oscillations in that they increase theta power (Nokia et al., 2012; Park et al., 2015) and may prevent gamma oscillations from increasing above an optimal level (Lacefield et al., 2012; Murthy and Gould, 2020). It is possible that abGCs stabilize the hippocampal network in a way that facilitates rhythmic firing of neurons supporting behavioral inhibition. Since removing these cells from the network decreases hippocampal theta power, it is perhaps not surprising that avoidance behavior would be diminished as well. Despite observing no MSEW effect on social memory, an expected impairment in social memory in both control and TK mice with reduced abGCs was found. These findings are consistent with previous work (Cope et al., 2020; Monteiro et al., 2014; Garrett et al., 2015) and raise questions about whether neuronal oscillations supporting social memory, such as theta-coupled sharp-wave ripples (Tao et al., 2021) may be influenced by abGCs, findings that have been supported by a recent study (Laham et al., 2024).

Conclusions

Although study described in this chapter produced unexpected results, these findings are less surprising when viewed in the context of the overall rodent and human literature on ELA, which provides numerous examples of individual differences in vulnerability, resistance, and resilience.

These results show that for the strains of mice that were examined, ELA did not affect our behavioral measures, but this does not mean that the circuits underlying these behaviors were unchanged. Indeed, behavioral changes emerged when abGCs were reduced but only in MSEW males, suggesting that this form of plasticity plays an important role in normalizing behavior after ELA. The extent to which a similar process may underlie resilience to ELA in humans remains unknown.

Chapter 3: Different types of early adverse experience engage distinct mechanisms to alter social behavior

Abstract

Specific types of ELA have been shown to increase the likelihood of different neuropsychiatric outcomes, which include social dysfunction, in humans. Maternal separation and early weaning (MSEW), a rodent model of neglect, and limited bedding and nesting (LBN), a rodent model of scarcity and abuse, was used to investigate effects on social recognition, a common impaired function in neuropsychiatric disorders. MSEW and LBN male mice both showed social memory impairments that differed, with MSEW males showing a social recognition deficit and LBN males showing reduced social novelty preference. By contrast, both MSEW and LBN female mice showed enhanced social novelty preference, indicating possible sex-specific protective mechanisms. Then, the neural substrates of these hippocampus-dependent impairments, adult born granule cells (abGCs) and perineuronal nets (PNNs) in the CA2, were explored. MSEW, but not LBN, mice had diminished abGC numbers as well as a lower density of abGC axons in the target region CA2 compared to controls. MSEW males also show reduced sharp wave ripples (SWR) in the CA2. Environmental enrichment restored the number of abGCs, while chemogenetic activation in a double transgenic model restored abGC activity levels and CA2 network activity patterns. In LBN males, reducing CA2 PNNs using a degradative enzyme restored social recognition function. These findings suggest that distinct ELA forms impact social memory through different neural mechanisms. Understanding these mechanisms provides insights into the neurodevelopmental consequences of early life stress and potential therapeutic targets.

Introduction

Early life adversity (ELA) is known to increase susceptibility to several neuropsychiatric diseases in adulthood (Felitti et al., 1998). Studies show that distinct types of early life adversity, predispose people to different, sometimes overlapping, neuropsychiatric conditions (Waters and Gould, 2022). For example, children subjected to physical abuse have an increased incidence of posttraumatic stress disorder (PTSD), while those subjected to parental loss have an increased incidence of major depressive disorder (reviewed in Waters and Gould, 2022). Among the neuropsychiatric diseases linked to ELA, several, including PTSD and depression, present with deficits in social cognition (DeGangi et al.,2017; Beck et al., 2009; Ladegaard et al., 2014). Social cognition also seems to be distinctly affected by certain types of early adverse experiences. For example, children subjected to emotional abuse have a low threshold for assuming negative emotions on facial expressions, while those subjected to emotional neglect have deficits in facial recognition (Iffland and Neuner, 2020). Importantly, many neuropsychiatric diseases affect regions such as the amygdala, prefrontal cortex, and hippocampus, regions that are also involved in sociocognitive processes, particularly social recognition memory (Tanimizu et al., 2017). Given that social cognition impairments are a common symptom of several neuropsychiatric conditions and their negative effects on quality of life (reviewed in Schaffer and Schiller, 2019), identifying neural mechanisms connecting specific types of ELA to social processing is an important goal. Toward this end, numerous studies have used animal models of ELA to explore neural and behavioral outcomes, with the majority focused on nonsocial behavioral outcomes, such as avoidance behavior, stress coping, and nonsocial cognition (Laham et al., 2022; Ishakawa, et al., 2015; Bolton et al., 2018; Yajima et al., 2018).

Two studies showed that the ELA paradigm of limited bedding and nesting (LBN) reduced investigation time of novel versus familiar conspecifics (Holman et al., 2021, Kohl et al., 2015) although another study did not find any significant differences (Shupe and Clinton, 2021). Additional studies showed that maternal separation diminishes social discrimination in mice (Franklin et al., 2011) and rats (Lukas et al., 2011). Even fewer studies have compared effects of more than one ELA paradigm on behavioral outcomes and of these (Demaestri et al., 2020; Johnson et al., 2018), none have investigated differential social behavior outcomes.

The hippocampus plays an important role in social memory (Trinkler et al., 2009; Viskontas et al., 2009). It is well-established that the hippocampus undergoes considerable postnatal development, making it potentially vulnerable to the effects of early life adversity (Dick et al., 2022). During hippocampal development there are critical periods, that are significantly dependent on experience and sensory inputs to facilitate important short-range and long-range synaptic connections, making the experiences during this time particularly critical for normal brain development (Andersen et al, 2014; Hodel et al;2015). Indeed, studies have shown that ELA reduces hippocampal volume in humans (Dannlowski et al., 2012; Paquola et al., 2016), and diminishes neurogenesis, dendritic arborization, and synaptogenesis in rodents (Mirescu et al., 2004; Leslie et al., 2011; Monroy et al., 2010). The CA2 region of the hippocampus has been referred to as a "social hub" because it integrates social information from many afferents in support of social recognition (Hitti and Siegalbaum, 2014; Diethorn and Gould, 2023). Afferents to this region, particularly from the dentate gyrus and supramammillary nucleus, undergo considerable development during the postnatal period (Diethorn and Gould, 2023). In adulthood, the CA2 receives input from adult-generated cells (abGCs) of the dentate gyrus, which have been causally linked to social recognition (Cope et al. 2020; Laham et al., in press).

The CA2 also contains a large concentration of perineuronal nets (PNNs), extracellular matrix structure implicated in plasticity inhibition, which have also been linked to social recognition both during development and in adulthood (Cope et al., 2022; Huang et al., 2023). Both abGCs and PNNs contribute to neuronal oscillations in the hippocampus, including sharp wave ripples and phase amplitude coupling (Laham et al., 2024; Sun et al., 2018), which have been linked to social recognition function. Furthermore, abGCs and PNNs are sensitive to early life stress (Catale et al., 2022; Jakovljevic et al., 2022), raising the possibility that social recognition might be altered through actions on these plastic processes and their effects on CA2 network activity. Despite these intriguing connections, no previous studies have compared the effects of more than one ELA paradigm on social recognition with a view toward identifying whether distinct mechanisms are engaged by different types of experiences.

This chapter describes studies that show that exposure to ELA paradigms of deprivation (maternal separation and early weaning, MSEW) or scarcity (limited bedding and nesting, LBN) impairs social recognition function in adult male, but not female, mice. Despite similarities of behavioral outcomes between these paradigms, each type of ELA was found to affect different hippocampal plasticity mechanisms to impair social recognition. MSEW males exhibit reduced numbers of progenitor cells and abGCs in the dentate gyrus, as well as reduced abGC axons in the CA2 region. These differences are accompanied by changes in CA2 network activity. LBN males show no difference in these measures but an increase in PNN intensity in the CA2. Normalizing levels of abGCs in MSEW mice by enriched environment living or chemogenetic enhancement restores social recognition function, while normalizing PNN intensity in LBN mice by enzymatic degradation followed by timed restoration, is similarly beneficial. These findings

show that targeted intervention directed at ELA-specific mechanisms can restore social recognition function in adulthood.

Methods

Animals

All animal procedures were approved by the Princeton University Institutional Animal Care and Use Committee and were in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals. C57BL/6 mice were obtained from Jackson laboratories and were used for most studies. Nestin-CreERT2 and R26-LSL-hM3Dq-DREADD single transgenic mice were obtained from Jackson labs and bred in the Princeton Neuroscience Institute as single or double transgenic mice for behavioral and electrophysiology experiments. Initial studies used mixed-sex groups; however, some investigations were male-only due to behavioral impairments that were discovered because they were follow-up studies based on ELA-induced behavioral impairments. Transgenic mice were genotyped by Transnetyx from ear punches at P15 using real-time PCR. Control mice were weaned from their dams at P21 and housed in same sex groups of 4-5 per cage. Single and double transgenic animals were group housed with mixed genotypes. Animals were housed in Optimice cages on a reverse 12/12 h light/dark cycle.

Maternal separation and early weaning (MSEW)

C57, single transgenic Nestin-cre, and double transgenic Nestin-cre: Gq pups were randomly assigned to either a control-rearing or MSEW group as previously described (Murthy et al., 2019; Laham et al., 2022). MSEW mice were separated on P3-P6 from their dam for 4 hours per day. On P7-P16, separations increased to 8 hours. For separations, dams were removed from the home cage, and each cage containing the pups was placed on top of a thermal heating blanket maintained at 34°C. Pups remained with their littermates for the entire period of separation after which the dam was returned to the home cage. Maternally separated pups were then weaned four days earlier than controls, at P17. Body weights of MSEW pups were measured daily, while those of control pups were taken on the day of weaning to minimize disruption. For transgenic experiments, all litters contained both genotypes.

Limited bedding and nesting

C57, Nestin-cre, and Nestin-cre: Gq pups were randomly assigned to control-rearing or LBN protocol adapted from previous studies (Gallo et al., 2019). Following cross-fostering on P2, control litters were left undisturbed until weaning on P21. Control litter cages contained standard bedding and a normal amount of nesting material. LBN pups and their dam were moved from their home cages and placed into a cage with a perforated metal floor, no bedding, and very little nesting material from P4-P11. Following P11, LBN mice were returned to cages with standard bedding and nesting until weaning on P21.

Environment Enrichment Housing

C57 control and MSEW adult male mice were housed in an enriched environment (EE) outside of the vivarium for 6 weeks, starting at P60. EE housing took place in large Sterilite containers (34"x18"x12"). Food and water were provided ad libitum. In addition to the standard nesting material and bedding, pet-quality toys made of wood and plastic, shelter structures, and running wheels were provided. All objects were removed from the container once a week and cleaned. Additionally, new objects were rotated into the environment once a week to maintain novelty.

Behavioral analysis

The direct social interaction test was used (Laham et al., 2021; Chapter 2) to determine whether adult mice were able to discriminate between a novel and familiar mouse of the same sex and age. Adult C57, single transgenic Nestin-cre, and double transgenic, Nestin-cre, and Nestin-cre: Gq underwent the direct social interaction test. Following habituations to the room and the testing arena, the mice were tested in a 2-trial test. In trial 1 (novel), the test mouse was placed in an open field box and a novel stimulus mouse was introduced. The mice were allowed to interact for 5 minutes. After a 1 hour (Figure 1 only) or 24-hour delay in their respective home cages, trial 2 (familiar) began in which the test mouse was placed back in the open field with the same mouse for 5 minutes. Social exposures were videotaped and the time the test mouse spent investigating (sniffing, close following, allogrooming with a distance of ≤2 cm) the familiar or novel mouse was measured. As mice prefer novelty, social memory is measured as the mouse's decrease in investigation time from trial 1 with the "novel" mouse to trial 2 with the "familiar" mouse (Moy et al., 2004).

Drug treatments

On P60, Nestin-cre and Nestin-cre: Gq mice were administered intraperitoneal (i.p.) injections of tamoxifen (75 mg/kg) dissolved in corn oil to induce recombination. Tamoxifen was administered every other day, for 9 days (5 total injections). 5 weeks post-tamoxifen administration, Nestin-cre and Nestin-cre: Gq mice underwent social discrimination testing (with novel and familiar stimulus mice) twice, once after vehicle systemic i.p. injection and once after CNO (5 mg/kg) i.p. injections 30 minutes before the trial. The order of drug administration (vehicle or CNO) was counterbalanced across groups. Electrode-implanted mice underwent direct social interaction testing after receiving systemic vehicle or CNO administration following the same experimental design. Mice were given a 48-hour minimum rest period between vehicle and CNO tests.

Surgical manipulations

Mice were deeply anesthetized with isoflurane (2-3%) and placed in a stereotaxic apparatus (Kopf) under a temperature-controlled thermal blanket for all surgeries. The head was leveled using bregma, lambda, and medial-lateral reference points before virus injection or implantation electrode. MSEW and control-reared double transgenic male mice were implanted unilaterally with a custom-made 4-wire electrode array (Microprobes) into the right hemisphere targeting dorsal CA2 (AP: -1.82, ML: ±2.15, DV: -1.67). 4 bone screws were implanted into the skull with 1 screw positioned on the contralateral hemisphere serving as ground. The ground wire was wrapped around the ground screw and covered with metallic paint to ensure maximum contact. Electrode implants were kept in place using metabond followed by dental cement (Bosworth Trim).

Two weeks after surgery, electrode mice received systemic injections of vehicle or CNO 30 minutes prior to electrophysiological recordings (see Electrophysiology recordings).

For the retrovirus experiment, a replication-deficient retroviral vector derived from the Moloney leukemia virus, designed to express GFP under the CAG promoter, was employed to selectively transduce abGCs, following the method outlined by Zhao, Teng, Summers, Ming, and Gage (2006). To deliver the retrovirus, adult male mice (P60) underwent identical surgical preparation and leveling as described above. A microsyringe was used to deliver CAG-GFP retrovirus bilaterally to the dorsal DG (1.0 µl per injection at 0.15 µl/min; AP: −2.0, ML: ±1.3, DV (suprapyramidal blade): −2.0, DV (infrapyramidal blade): −2.3). Mice were perfused 6 weeks post-injection to examine histologically.

For the PNN manipulation experiment, a microsyringe was used to deliver bilateral infusions of penicillinase (Pnase) or chondroitinase ABC (chABC) to the dorsal CA2 of control and LBN C57 adult males (15 nanoliter per injection at 3 nanoliters/min; AP: −1.82, ML: ±2.15, DV: −1.67). Mice were tested 10 days post infusion in the direct social interaction test.

Electrophysiology

Local field potentials (LFPs) were recorded from control-reared and MSEW Nestin-cre: Gq mice with CA2 electrodes, using a tethered head stage (HST/16D Gen2, Plexon). After 5 minutes of habituation, the behavioral testing paradigm consisted of a 3-minute baseline followed by a 5-minute social interaction period. Animals underwent this

recording paradigm for both novel and familiar conspecifics. Vehicle or CNO (5mg/kg) was i.p. administered 30 minutes before testing. LFPs were recorded continuously throughout baseline and stimulus trials. The data were transmitted to a receiver (OmniPlex Hydrid eChassis, Plexon) and recorded using PlexControl software (Plexon).

Sharp wave-ripple analysis

Electrophysiology recordings were processed using Neuroexplorer software (Nex Technologies) and custom Python scripts (Laham and Zahn, 2023). For SWR analyses, continuous LFP data were first notched at 60 Hz and band-pass filtered between 140 and 220 Hz. Signal underwent Hilbert transform before being z-scored. Using custom Python script, events qualified as SWRs when they exceeded 3 standard deviations for a minimum of 15 milliseconds. SWRs occurring within 15 ms of one another were grouped into a single SWR event. SWR frequency, peak amplitude, integral, duration, and raw number were normalized to each animal's baseline trial. SWR measures were investigated for baseline, novel, and familiar conspecific trials for the entirety of each testing period.

Phase-amplitude coupling analysis and Power Analyses

Using a custom Python script, theta (4-12 Hz), slow gamma (25-50 Hz), and mid-gamma (50-100 Hz), and fast gamma (100-130) oscillations were extracted from the LFP signal obtained during the baseline, novel, and familiar conspecific during periods immobility.

All theta and gamma recordings underwent a Hilbert transform, and theta phase and gamma analytic signal were stored. PAC was quantified using a Modulation Index (Tort et al., 2010).

Power was calculated by filtering for the desired frequency range. For each trial and baseline, the proportion of power in that frequency range to the overall power spectrum was then calculated. Lastly, the trial proportion was then divided by the baseline proportion of the same frequency range.

Histology

Mice were first anesthetized with Euthasol (Virbac) and were transcardially perfused with cold 4% paraformaldehyde (PFA). Extracted brains were postfixed for 48 h in 4% PFA at 4◦C followed by an additional 48 h in 30% sucrose at 4◦C for cryoprotection before being frozen in tissue freezing medium at -80◦C. Hippocampal coronal sections (40 µm) were collected using a cryostat (Leica). Sections were pre-blocked for 1.5 hours at room temperature in a PBS solution that contained 0.3% Triton X-100 and 3% normal donkey serum. Sections were then incubated overnight while shaking at 4◦C in the blocking solution that contained combinations of the following primary antibodies: mouse anti-3 microtubule-binding domain tau protein (3RTau, 1:500, Millipore, Cat# 05-803), rabbit anti-Purkinje cell protein 4 (PCP4, 1:500, Sigma-Aldrich, Cat# HPA005792), rabbit anti-zinc transporter 3 (ZnT3, 1:500, Alomone labs, Cat# AZT-013), mouse anti SRY-related HMG-box 2 (Sox2, 1:100, Millipore, Cat#AB5603), rabbit anti-green fluorescent protein (GFP, 1:500, Molecular Probes, Cat#G10362), or Wisteria floribunda agglutinin lectin (WFA, 1:1000, Sigma Aldrich, Cat# L1516. For 3R-Tau immunohistochemistry, sections

were subjected to an antigen retrieval protocol where sections were first incubated in sodium citrate and citric acid buffer for 30 minutes at 80◦C before blocking solution. Washed sections were then incubated for 1.5 hours at room temperature in secondary antibody solutions that contained combinations of the following secondaries: donkey anti-rabbit Alexa Fluor 568 (1:500, Abcam), donkey anti-mouse Alexa Fluor 488, donkey anti-rabbit Alexa Fluor 488, donkey anti-rabbit Alexa Fluor 488, or Streptavidin 488. Washed sections were then counterstained with Hoechst 33342 for 10 minutes (1:5,000 in PBS, Molecular Probes), mounted onto slides, and cover slipped with Vectashield (Vector labs). Slides were coded until completion of the data analysis. Sections through the dorsal hippocampus from electrophysiology studies were stained for Hoechst 33342 to verify accurate cannula and electrode placement.

Optical intensity measurements

Z-stack images of the DG and CA2 were taken using a 40x objective with a 0.5m step size through the entire 40um stack on a Leica SP8 confocal with LAS X software (version 35.6). The CA2 was defined by PCP4 labeling, which is specific to the CA2 in the hippocampal CA fields (San Antonio et al., 2014). Collected z-stack images were analyzed for optical intensity in Fiji (NIH).

For overall intensity values of 3R-Tau to assess abGC innervation of CA2, the ROI included the pyramidal cell layer and stratum radiatum. The area, mean gray value, and integrated density of the ROI was calculated for each z-slice and the maximum integrated

density value for each z-stack was taken from 3 sections per brain. Averages were obtained for each brain and statistical comparisons were made between groups.

Identical intensity methods were used for intensity values of WFA in the CA2; however, the ROI was restricted to the pyramidal cell layer.

Cell density/Varicosity Density and percentage measurements

The number of 3R-Tau+ and SOX2+ cells was manually counted in the dorsal dentate gyrus of the hippocampus on 3 neuroanatomically matched sections using Fiji (ImageJ). The area measurements were collected using Fiji. The density of 3R-Tau and SOX2 was determined by dividing the total number of positively labeled cells by the volume of the subregion (ROI area multiplied by 40 μm section thickness).

For the GFP+ bouton analysis, 6 sections per brain were preliminarily scanned for GFP+ axon segments in the stratum radiatum of the CA2 and CA3. GFP+ varicosities were identified and quantified as circular shaped GFP+ staining that was 0.5–2.2 μm in length and 0.5–1.5 μm in width. Proportions of GFP+ varicosities in CA2 compared to CA3 were calculated.

Statistical Analyses

For histological analyses, data sets were analyzed using an unpaired 2-tailed Student's t-test or Mann-Whitney U tests. For behavioral analyses involving 2 group comparisons, data sets were analyzed using either unpaired two-tailed Student's t-tests or a repeated measures 2-way

ANOVA, as appropriate. For behavioral analyses involving experimental manipulations in adulthood, data sets were analyzed using either a 2-way ANOVA, a mixed effects model, or a repeated measures 3-way ANOVA. Tukey or Holm-Sidak post hoc comparisons were used to follow up any significant main effects or interactions of the ANOVAs. Because electrophysiological measurements were taken from multiple electrodes within each mouse, these data were analyzed with linear mixed-effects ANOVAs using the lme4 package (Bates et al., 2015). Tukey post hoc comparisons were used to follow up any significant main effects or interactions using the emmeans package (Lenth, 2022). All data sets are expressed as the mean ± SEM on the graphs and statistical significance was set at $p < 0.05$ with 95% confidence. GraphPad Prism 9.2.0 (GraphPad Software), Excel (Microsoft), or R studio were used for statistical analyses. All graphs were prepared using GraphPad Prism 9.2.0 (GraphPad Software).

Results

MSEW and LBN both impair social recognition in male mice but enhance social novelty preference in female mice

The effects of two different models of ELA on social recognition in adult C57 male and female mice were investigated first (Figure 1A). Using the two-trial direct social interaction test with a 24 intertrial delay we tested social recognition in control-reared, MSEW, and LBN adult mice. Mice were first exposed to novel sex- and age-matched mice for five minutes (Novel trial), and 24 hours later, exposed to the same mouse in a second trial (Familiar trial) for an additional five minutes. Because control-reared mice tend to prefer novel mice over familiar mice, social recognition was defined as a significant decrease in investigation time between Novel and Familiar trials. Male control-reared mice had significantly lower investigation times for the

familiar mouse than the novel mouse, while MSEW and LBN male mice did not have significant differences in interaction time across trials (Figure 1B). LBN and MSEW male mice were similar in total social recognition times except LBN, but not MSEW, mice exhibited significantly lower investigation times of novel mice compared to control-reared mice (Figure 1B). Social discrimination ratios were calculated by subtracting the familiar investigation time from the novel investigation time and then dividing it by the total investigation time and found that MSEW and LBN males had significantly lower discrimination ratios than control-reared males (Figure 1C).

Next, female ELA mice were examined for social discrimination function in adulthood using the direct social interaction test. Control-reared, MSEW, and LBN females all had a significant decline in investigation time between novel and familiar trials (Figure 1D). In contrast to what was observed with male mice, both MSEW and LBN females showed significantly higher investigation of novel mice compared to control-reared females (Figure 1D). Both MSEW and LBN females had comparable discrimination ratios to control-reared females (Figure 1E).

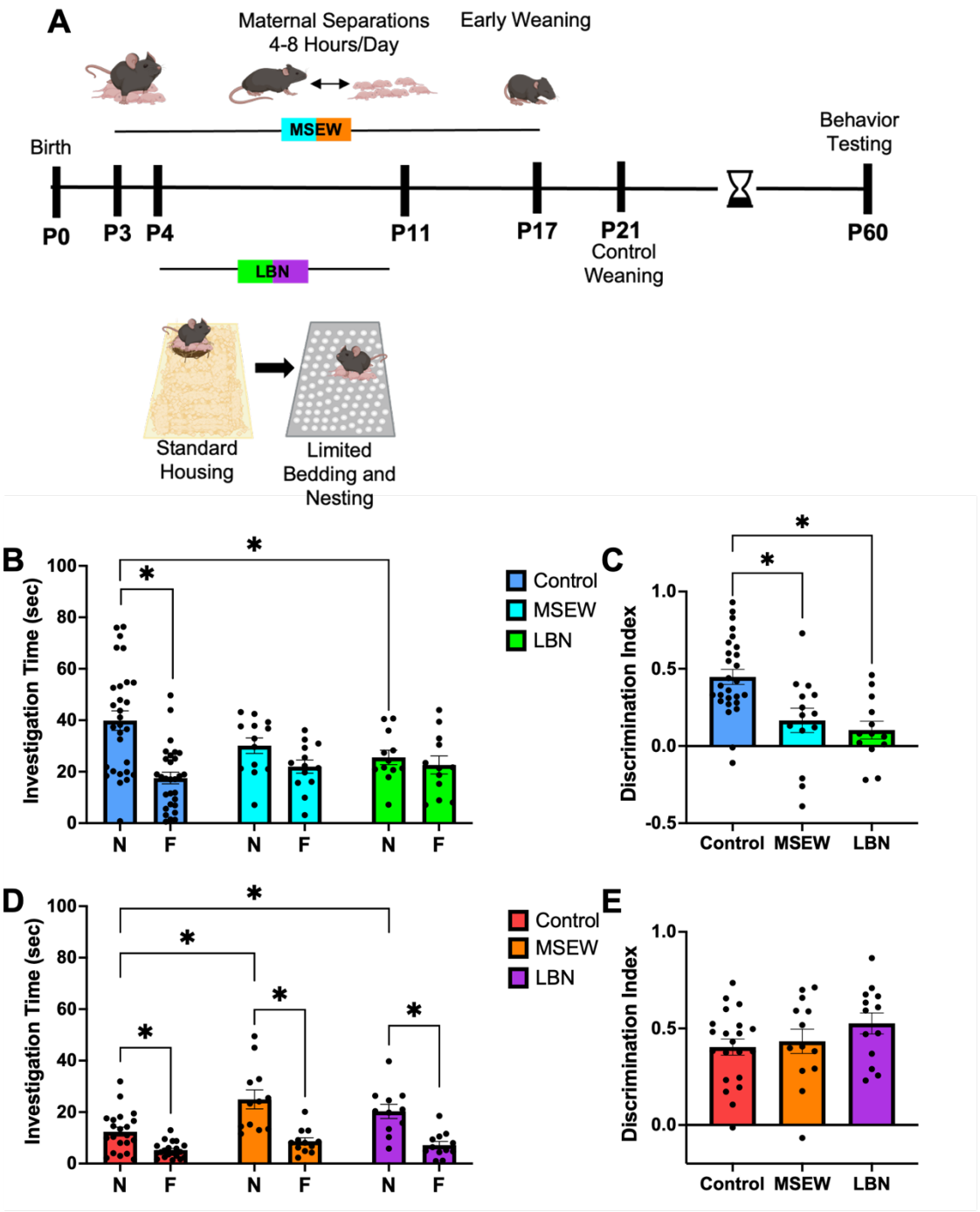


Figure 1. MSEW and LBN impair social discrimination in males, but not females. **A)** Experimental timeline and schematic of MSEW and LBN. **B)** Control-reared males display a significant reduction in time investigating the novel animal in trial 1 (N) to the familiar animal in trial 2 (F). MSEW and LBN animals showed similar investigation times in trial 1 and trial 2. Two-way ANOVA: effect of group x trial: $F_{(2,51)} = 8.548$, $p=0.0006$; Holm-Sidak posthoc test:

Control: N vs F, p= <0.001; Novel: Control vs LBN, p=0.0134; n= Control: 29, MSEW: 14, LBN: 13. **C)** MSEW and LBN males both had lower discrimination indices (N- F/N+F). One-way ANOVA: Group: $F_{(2,51)} = 0.4684$, p= 0.0002; Holm-Sidak posthoc test: Control vs MSEW, p=0.0029; Control vs LBN, p=0.0006. **D)** Control-reared, MSEW, and LBN females display a significant reduction in time investigating the novel animal in trial 1 to the familiar animal in trial 2. MSEW and LBN females show an increase in novel investigation. Two-way ANOVA: effect of group x trial: $F_{(2,38)}=6.512$, p=0.0037; Holm-Sidak posthoc test: Control: N vs F, p= <0.0001; MSEW: N vs F, p=<0.0001; LBN: N vs F, p=<0.0001; Novel: Control vs MSEW, p=<0.0001, Novel: Control vs LBN, p=0.0159; n=Control: 19, MSEW: 12, LBN: 10. **E)** Control, MSEW, and LBN all show similar discrimination indices.

Male MSEW, but not LBN, mice exhibit decreased progenitor cells and adult neurogenesis in the dorsal dentate gyrus

abGCs are critical for social recognition in healthy animals (Cope et al., 2020). abGC afferents to the CA2 have been shown to facilitate social memory retrieval and to play an important role in overall CA2 network activity (Laham et al., 2024). Whether MSEW or LBN affect adult-born granule cells, their progenitor cells, and their projections to CA2 in males and females was investigated. Using 3R-Tau to label immature granule cells in dorsal dentate gyrus (Llorens-Martin et al., 2015), MSEW, but not LBN, males had significantly fewer 3R-Tau+ cells than their respective control-reared males (Figure 2A, B). Neither MSEW nor LBN females showed any difference in the number of 3R-Tau+ abGCs (Figure 2C, D). Next, the possibility that the decrease in abGCs in MSEW males is associated with diminished abGC progenitor cells in the dentate gyrus was investigated Progenitor cells were labeled using Sox2 (Graham et al., 2003; Gage, 2010). MSEW males had a significant decrease in Sox2+ cells in the dorsal dentate gyrus when compared to their respective control-reared males (Supplementary Figure 1A, B). Because a reduction in number of abGCs would affect projections to target regions, hippocampal CA2

and CA3, 3R-Tau axon staining was examined. MSEW, but not LBN, males had less intense 3R-Tau staining in the stratum radiatum of the CA2 and in the stratum lucidum of CA3 suggesting reduced projections to both target regions compared to control-reared males (Supplementary Figure 1C-F). Moreover, to assess whether the proportion of abGC projections projecting to CA2 and CA3 differed after MSEW, a GFP-retrovirus was injected into the dentate gyrus of control-reared and MSEW males at P60. After 6 weeks, GFP was specially transduced in abGCs, including their axons. Control-reared males had roughly similar GFP+ axonal varicosities in CA2 and CA3, but MSEW males had fewer GFP+ axonal varicosities in CA2 than CA3, suggesting greater CA2 than CA3 attenuation of synaptic sites (Supplementary Figure 1G, H) after MSEW. Lastly, to assess whether this MSEW-induced change in dentate gyrus projections to the CA2 was specific to immature abGC axons or whether a similar reduction would exist in mature granule cell axons, which include developmentally born cells, the zinc transporter ZnT3 was used to label mature granule cell axons. Control-reared control and MSEW males had similar projection intensities of ZnT3 axons in the stratum radiatum of the CA2 (Supplementary Figure 1I, J). These results suggest that MSEW and LBN females, which have no deficit in social recognition, exhibit no difference in number of abGCs or their CA2 afferents. By contrast, MSEW and LBN males, both of which have social recognition deficits, exhibit differential effects on abGCs. MSEW males have significantly fewer abGCs and their CA2 afferents while LBN males do not show these effects.

Stimulating adult neurogenesis with enriched environment living restores social recognition in MSEW male mice

Previous studies have shown that environmental enrichment stimulates adult neurogenesis in the dentate gyrus in mice and rats (Olson et al., 2006; Monteiro et al., 2013; Speisman et al., 2013).

Given that MSEW males exhibit lower numbers of abGCs and impaired social recognition, living in an enriched environment might boost adult neurogenesis and thereby improve social recognition in ELA adult males. In a new cohort of animals, male mice that underwent MSEW were placed into enriched environments in young adulthood (P60) (Figure 1E). Six weeks later, male mice were tested in the direct social interaction test. MSEW mice housed in the colony room (CH MSEW) again showed impaired social recognition when investigating a sex- and age-matched conspecific, while colony-housed controls investigated a novel mouse significantly more. However, this impairment was rescued in the MSEW males by housing enriched environments (Figure 1F) with no effect on control-reared mice housed in enriched environments (Figure 1F). MSEW males living in an enriched environment were able to discriminate between novel and familiar mice by significantly reducing their investigation time during the familiar trial (Figure 1F). Analyses of discrimination ratios also followed this pattern; colony-housed MSEW males showed low discrimination ratios compared to colony-housed control-reared males while enriched environment living corrected this measure in MSEW males with no effect on control-reared males (Figure 1G). Enriched environment living increased the number of abGCs and number of Sox2+ progenitor cells in the dentate gyrus of MSEW male mice so that they were not significantly different from control-reared mice (Figure 1H, I). These findings suggest that an experience-dependent increase in the number of abGCs may be sufficient to restore social recognition memory in MSEW males.

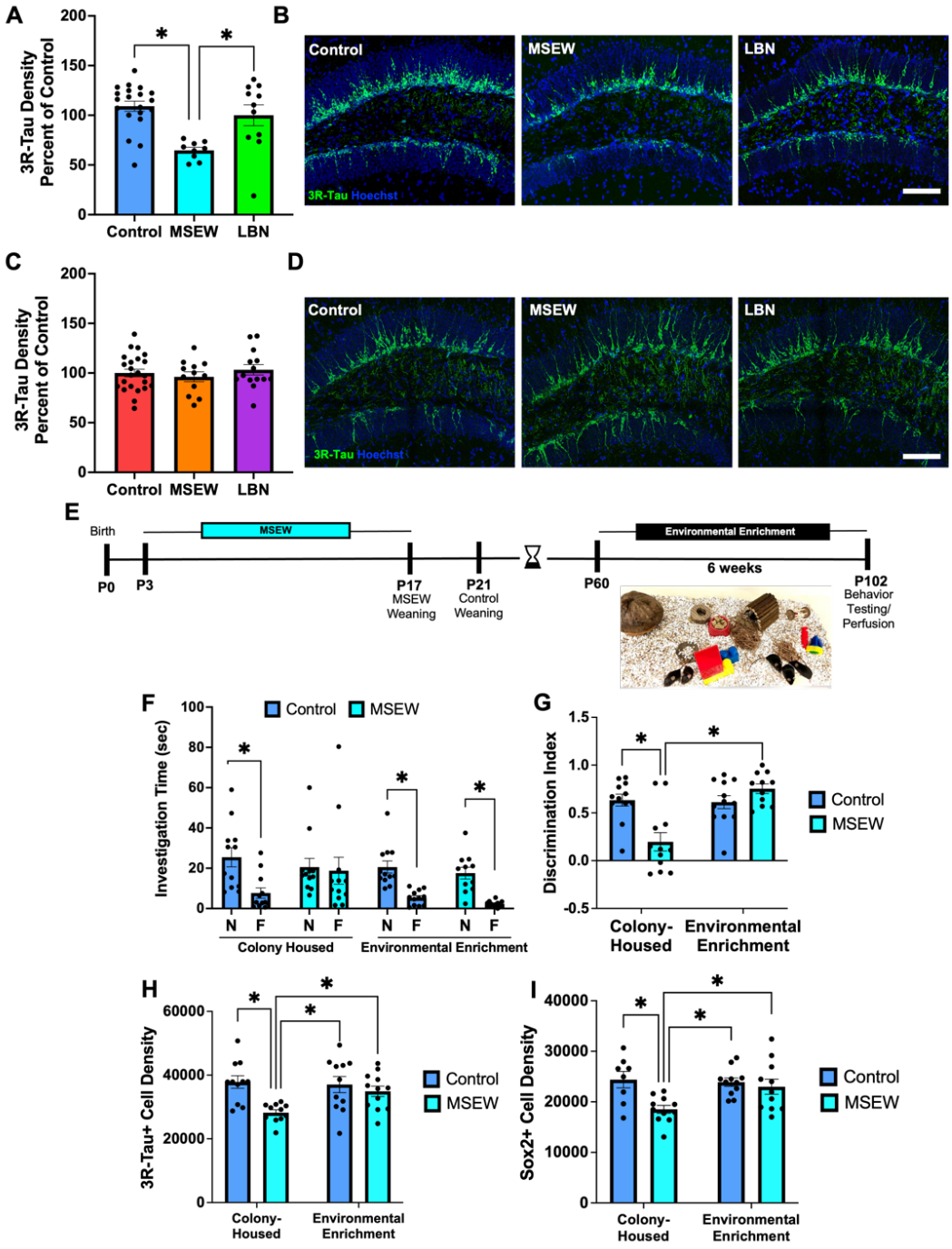
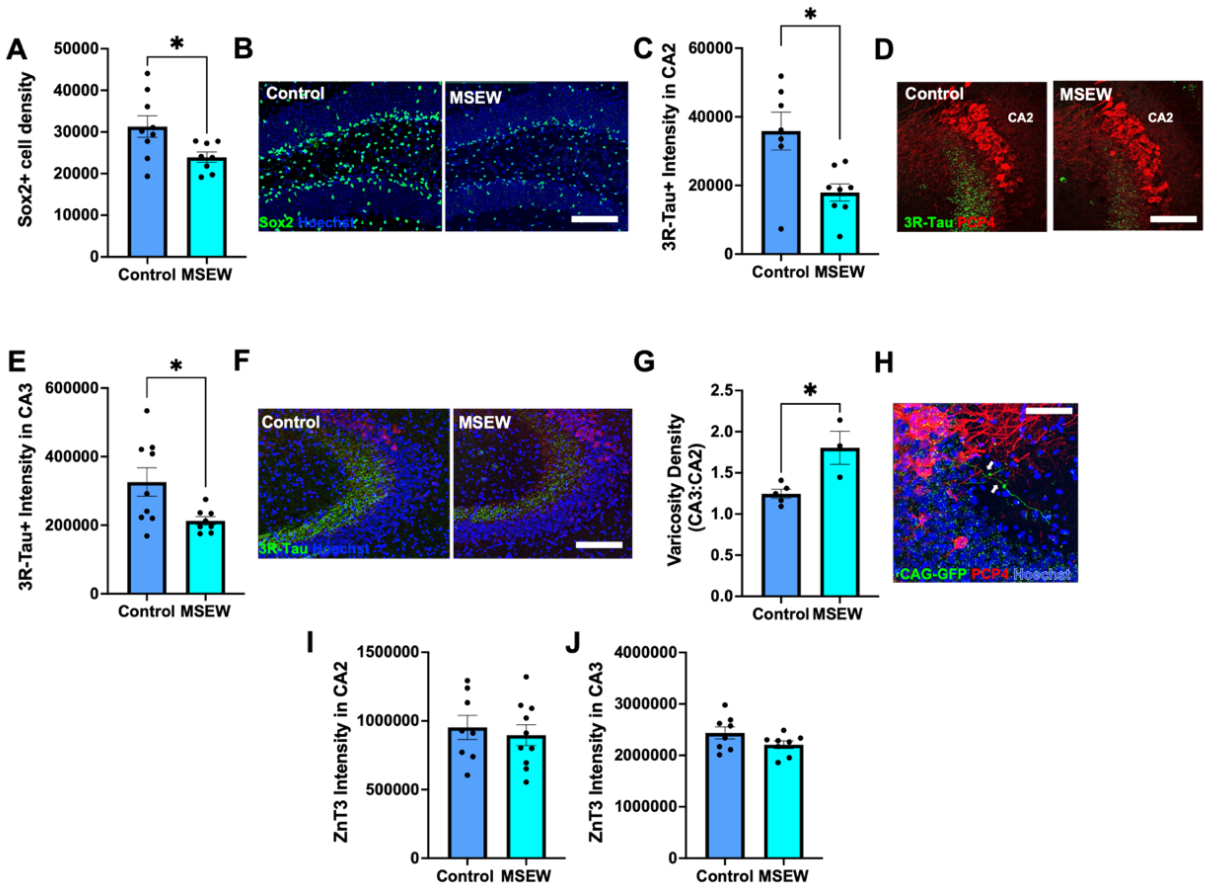


Figure 2. Adult neurogenesis is reduced in male MSEW mice, restored by environmental enrichment, along with social recognition abilities. **A)** MSEW males show reduced 3R-Tau+ cells in dorsal dentate gyrus compared to Control-reared and LBN males. One-way ANOVA: Group, $F_{(2,37)} = 2.662$, p=0.0003; Holm-Sidak posthoc test: Control vs MSEW, p=0.0002; LBN vs MSEW, p=0.0063; n= Control: 20, MSEW:9, LBN:11. **B)** Confocal images of 3R-Tau+ cells

(green) in dorsal dentate gyrus of Control-reared, MSEW, and LBN male mice. **C)** Control-reared, MSEW, and LBN female mice show comparable distributions of 3R-Tau+ cells in dorsal dentate gyrus. **D)** Confocal images of 3R-Tau+ cells (green) in dorsal dentate gyrus of Control-reared, MSEW, and LBN female mice. **E)** Timeline of enriched environment experiment including image of enriched environment housing. **F)** Colony-Housed Control-reared (CH Control-reared) males display a significant reduction in time investigating the novel animal in trial 1 (N) to the familiar animal in trial 2 (F). Colony-housed MSEW (CH MSEW) males showed similar investigation times in trial 1 and trial 2. Control-reared and MSEW males housed in enriched environment (EE Control-reared; EE MSEW) display a significant reduction in time investigating the novel animal in trial 1 (N) to the familiar animal in trial 2 (F). Three-way ANOVA: effect of group x housing x trial: $F_{(1,43)}$=7.307, p=0.0098; Holm-Sidak posthoc test: CH Control-reared: N vs F, p= <0.001; EE Control-reared: N vs F, p=<0.0001; EE MSEW: N vs F, p=0.0001; n= CH Control-reared : 12, CH MSEW: 12, EE Control-reared: 12, EE MSEW: 11. **G)** CH MSEW males had a significantly lower discrimination index compared to CH Control-reared males and EE MSEW males. Two-way ANOVA: Group x Housing, $F_{(1,43)}$ = 16.21, p=-0.0002; Fishers LSD posthoc test: CH: Control vs MSEW, p=<0.0001; MSEW: CH vs EE, p=<0.0001. **H)** CH MSEW males showed a significant reduction in 3R-Tau+ cells in dorsal dentate gyrus compared to CH Control-reared, EE Control-reared and EE MSEW males. Two-way ANOVA: Group x Housing, $F_{(1,41)}$=4.081, p=0.499; Fishers LSD posthoc test: CH: Control vs MSEW, p=0.0008; CH MSEW vs EE Control, p=0.0019; MSEW: CH vs EE, p=0.0135; n= CH Control: 11, CH MSEW: 11, EE Control: 11, EE MSEW: 12. **I)** CH MSEW males showed a significant reduction in Sox2+ cell density in dorsal dentate gyrus compared to CH Control-reared, EE Control-reared and EE MSEW males. Two-way ANOVA: Group x Housing, $F_{(1,37)}$ =4.303, p=0.451; Fishers LSD posthoc test: CH: Control vs MSEW, p=0.0020; CH MSEW vs EE Control, p=0.0020; MSEW: CH vs EE, p=0.0089; n= CH Control: 8, CH MSEW: 11, EE Control: 11, EE MSEW: 12. Scale bar = 12.5 um.



Supplementary Figure 1. MSEW males have abnormal progenitor cell population and abGC projections. **A)** MSEW males had a significant reduction in Sox2+ cells in dorsal dentate gyrus compared to Control-reared animals. Unpaired t-test: $F_{(8,7)}$ =13.30, p=0.0265; n= Control: 9; MSEW: 8. **B)** Confocal images of Sox2+ cells (green) in dorsal dentate gyrus of Control-reared and MSEW males. **C)** MSEW males showed a significant reduction in 3R-Tau+ staining in CA2 compared to Control-reared animals. Unpaired t-test: $F_{(6,7)}$ =4.301, p=0.0086; n= Control: 7; MSEW: 8. **D)** Confocal images of 3R-Tau+ staining (green) in dorsal CA2 labeled by PCP4 (red) of Control-reared and MSEW males. **E)** MSEW males showed a significant reduction in 3R-Tau+ staining in CA3 compared to Control-reared animals. Unpaired t-test: $F_{(8,7)}$ =13.30, p=0.0254; n= Control: 9; MSEW: 8. **F)** Confocal images of 3R-Tau+ staining (green) in dorsal CA3 of Control-reared and MSEW males. **G)** MSEW males had significantly lower proportion of CA3:CA2 GFP+ varicosities than Control-reared males. Unpaired t-test: $F_{(2,4)}$ =7.962, p=0.0144; n= Control: 5; MSEW: 3. **H)** Confocal image of GFP+ axons (green) and varicosities (white arrows) in the CA2 labeled by PCP4 (red). **I)** Control-reared and MSEW males had

similar intensities of ZnT3+ labeling in dorsal CA2. **J)** Control-reared and MSEW males had similar intensities of ZnT3+ labeling in dorsal CA3. B, Scale bar= 12.5; D, F, scale bar = 25 um; H, 5 um.

Chemogenetic activation of abGCs restores social recognition and CA2 network activity in MSEW male mice

To more specifically investigate the role of abGC enhancement in social recognition after MSEW, tamoxifen-inducible Nestin-cre double transgenic mice where only abGCs express excitatory DREADDs were generated allowing the temporary activation of these cells with the ligand CNO. Previous studies have shown that abGCs exert an important influence over hippocampal microcircuitry between 4-6 weeks after their generation, with their influence diminishing after this time (Laham et al., 2024). To test whether activating abGCs of different ages mitigates social recognition dysfunction after MSEW, adult Control-reared and MSEW male single and double transgenic mice were treated with tamoxifen and tested on the direct social interaction task at both 4-6 weeks and 8-12 weeks post-injection (Figure 3A). Thirty minutes before each trial, mice were injected with either CNO (5mg/kg) or vehicle. Each mouse was tested twice, once with CNO and once with vehicle, with injections counterbalanced across tests with a 48-hour washout period between tests. At the 4–6 week post-tamoxifen injection time point, single transgenic mice showed the expected difference between control-reared and MSEW groups when treated with either CNO or vehicle; control reared single transgenics showed a robust decrease in investigation times between novel and familiar mice, while MSEW single transgenics showed no measurable discrimination between stimulus mice (Figure 3B, C). Double transgenic mice showed the same pattern of results when treated with vehicle. That is, control-reared double transgenic mice showed a substantial decrease in investigation time

between novel and familiar mice, while MSEW double transgenic mice showed no ability to discriminate between novel and familiar mice (Figure 3B). When double transgenic mice were treated with CNO, however, this pattern of results changed and both groups (Control-reared and MSEW) showed a significant decrease in investigation times (Figure 3C). Discrimination index analysis confirmed this pattern of results with single and double transgenic control-reared mice showing high discrimination indices regardless of whether they were treated with vehicle or CNO, and single transgenic MSEW mice showing low discrimination indices regardless of whether they were treated with vehicle or CNO (Figure 3D). Double transgenic MSEW mice showed low discrimination indices when treated with vehicle and significantly higher values after treatment with CNO (Figure 3D). These data suggest that temporarily activating 4-6 week old abGCs is sufficient to restore social discrimination function in MSEW male mice, while having no measurable effect on social recognition in control-reared male mice. The beneficial effects of activating abGCs on social recognition in male MSEW mice were lost when abGCs were activated 8-12 weeks after their generation (Supplementary Figure 2A-C).

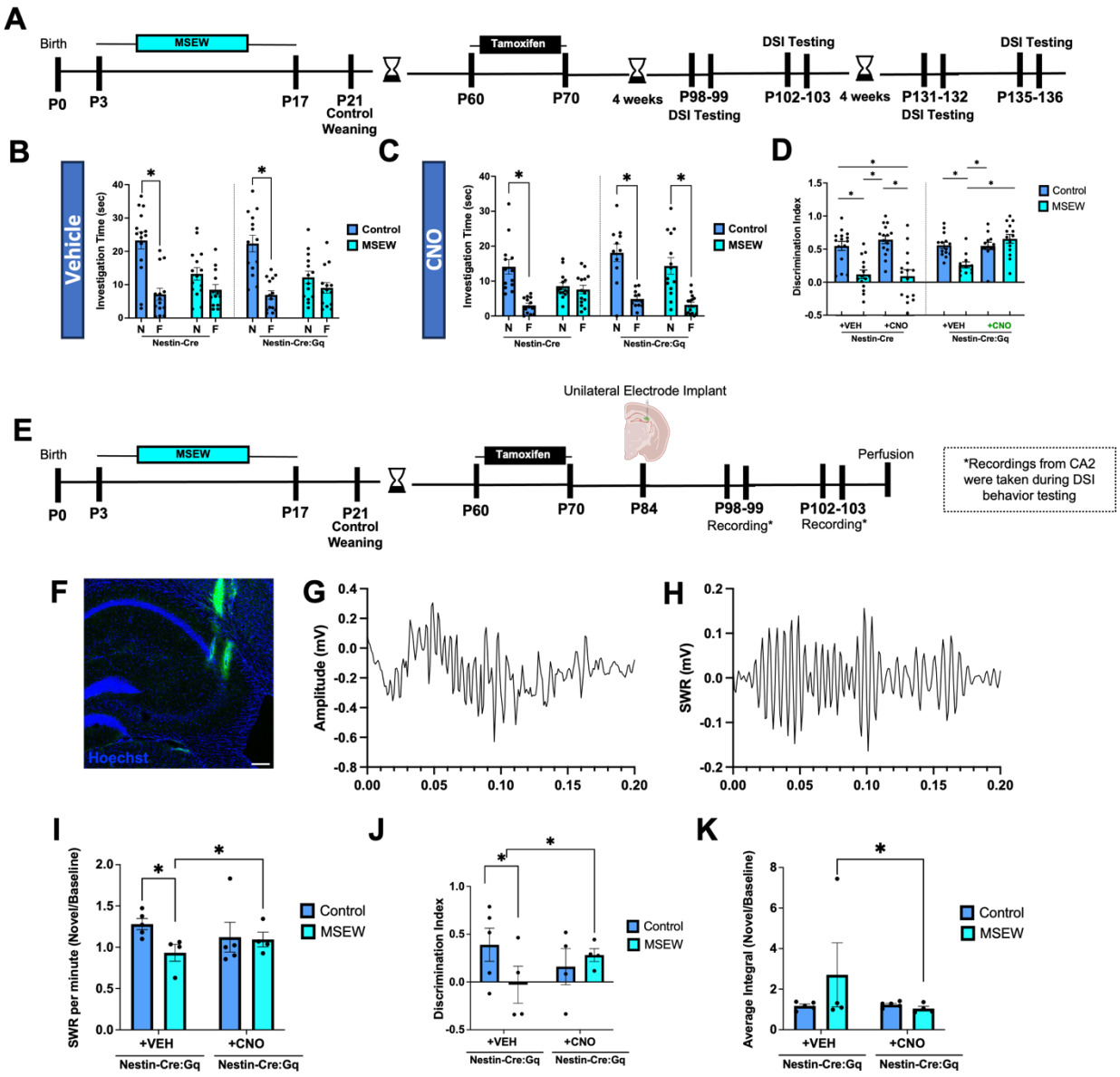
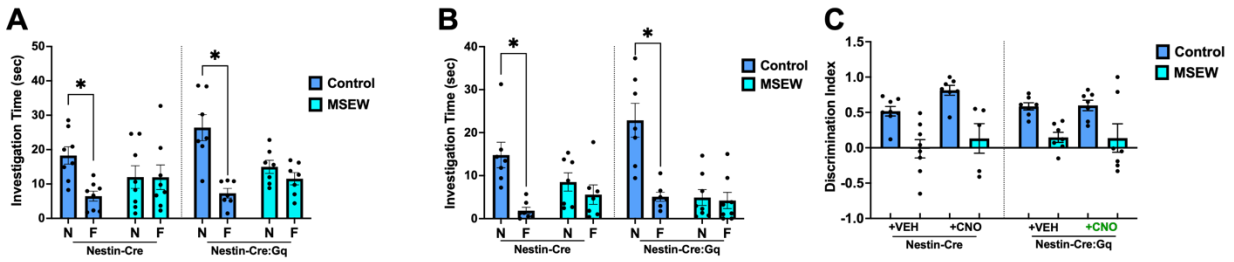


Figure 3. Activation of abGCs restores social discrimination function and CA2 network activity. **A)** Timeline of double transgenic experiment. **B)** Control-reared Nestin-cre and Nestin-cre: Gq with a vehicle injection both showed a significant reduction in time investigating the novel animal in trial 1 (N) to the familiar animal in trial 2 (F). MSEW Nestin-cre and Nestin-cre: Gq both showed similar investigation times in trial 1 and trial 2, with vehicle injection. Two-way ANOVA: effect of Group x Trial: $F_{(3,51)}=14.56$, p=<0.0001; Holm-Sidak posthoc test: Control Nestin-cre: N vs F, p= <0.001; Control Nestin-cre:Gq: N vs F, p=<0.0001; n= Control Nestin-cre: 14, MSEW Nestin-cre: 13, Control Nestin-cre:Gq: 14, MSEW Nestin-cre:Gq: 13. **C)** After CNO injection, Control-reared Nestin-cre, Control-reared Nestin-cre:Gq, and MSEW Nestin-

cre:Gq with a CNO injection all showed a significant reduction in time investigating the novel animal in trial 1 (N) to the familiar animal in trial 2 (F). MSEW Nestin-cre continued to show similar investigation times in trial 1 and trial 2, with CNO injection. Two-way ANOVA: effect of group x trial: $F_{(3,48)}$ =8.268, p=0.0002; Holm-Sidak posthoc test: Control Nestin-cre: N vs F, p= <0.001; Control Nestin-cre: Gq: N vs F, p=<0.0001; MSEW Nestin-cre: Gq: N vs F, p=<0.0001. **D)** With a vehicle injection, MSEW Nestin-cre and Nestin-cre: Gq both had significantly lower discrimination indices compared to Control Nestin-cre and Nestin-cre: Gq. However, after CNO injections, only MSEW Nestin-cre mice had a significantly lower discrimination index than Control Nestin-cre, Control Nestin-cre: Gq, and MSEW Nestin-cre: Gq mice. Three-way ANOVA: trial x group x genotype: $F_{(1,45)}$=5.431, p=0.0243; Holm-Sidak posthoc test: Vehicle: Control Nestin-cre vs MSEW Nestin-cre, p=<0.0001; VEH:Control Nestin-cre vs VEH: MSEW Nestin-cre:Gq, p=0.0010; VEH: Control Nestin-cre vs CNO: MSEW Nestin-cre, p=<0.0001; VEH:Control Nestin-cre:Gq vs CNO: MSEW Nestin-cre, p=<0.0001; Vehicle: Control Nestin-cre:Gq vs MSEW Nestin-cre:Gq, p=0.0213; VEH: Control Nestin-cre:Gq vs CNO: MSEW Nestin-cre, p=<0.0001; VEH:MSEW Nestin-cre vs CNO: Control Nestin-cre, p=<0.0001; VEH: MSEW Nestin-cre vs CNO:Control Nestin-cre:Gq, p=<0.0001; VEH: MSEW Nestin-cre vs CNO: MSEW Nestin-cre:Gq, p=<0.0001; VEH: MSEW Nestin-cre:Gq vs CNO: Control Nestin-cre:Gq, p=0.0249; VEH: MSEW Nestin-cre:Gq vs CNO: MSEW Nestin-cre:Gq, p=0.0008; CNO: Control Nestin-cre vs MSEW Nestin-cre, p=<0.0001; CNO: Control Nestin-cre:Gq vs MSEW Nestin-cre, p=<0.0001; CNO: MSEW Nestin-cre vs MSEW Nestin-cre:Gq, p=<0.0001. **E)** Timeline of double transgenic electrophysiology experiment. **F)** Electrode implant in CA2, electrode track in green. **G)** Raw LFP trace. **H)** Filtered SWR example trace from one animal. **I)** With vehicle injection, MSEW Nestin-cre: Gq animals had reduced SWR frequency during exposure to the novel animal (Trial 1). This reduction was restored after CNO injection. Linear mixed effect model: Group x Drug, $F_{(1,61)}$=17.03, p= 0.000114; Tukey posthoc test: VEH: Control Nestin-cre:Gq vs MSEW Nestin-cre:Gq, p=0.0487; MSEW: VEH vs CNO, p=0.0070, n= Control Nestin-cre:Gq: 5; MSEW Nestin-cre:Gq: 4. **J)** During immobility, with a vehicle injection, MSEW Nestin-cre:Gq males had a significantly lower SWR index than VEH: Control Nestin-cre:Gq and CNO: MSEW Nestin-cre:Gq. Linear mixed effect model: Group x Drug, $F_{(1,58)}$=4.30, p= 0.0425; Tukey posthoc test: VEH: Control Nestin-cre:Gq vs MSEW Nestin-cre:Gq, p=0.0478; MSEW: VEH vs

CNO, p=0.0007, n= Control Nestin-cre:Gq: 5; MSEW Nestin-cre:Gq: 4. **K)** During immobility, with a vehicle injection, MSEW Nestin-cre:Gq had a significantly lower average integral compared to MSEW Nestin-cre:Gq with vehicle injection. Linear mixed effect model: Group x Drug, $F_{(1,58)}$ =4.370, p= p=0.0188; Tukey posthoc test: MSEW: VEH vs CNO, p= 0.0029. Scale bar = 150 um.

In this experiment, the difference in social recognition between Control-reared and MSEW male mice remained the same regardless of whether abGCs were activated by CNO or not. Taken together, these findings suggest that activation of abGCs at a defined time window after their generation (4-6 weeks) but not later (8-12 weeks) is sufficient to restore social discrimination deficits in MSEW male mice.

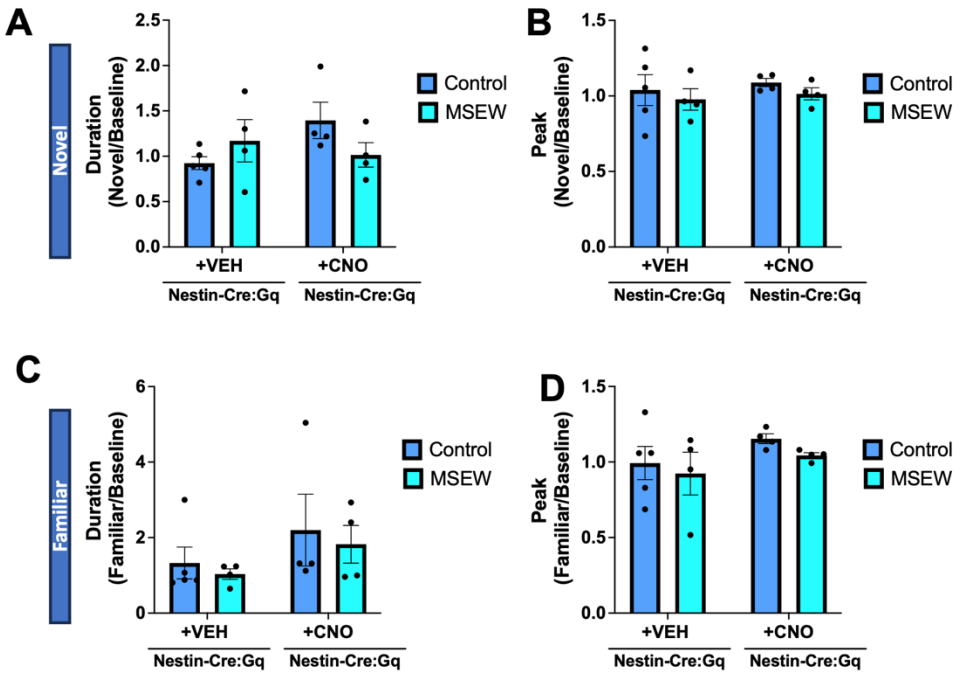


Supplementary Figure 2. Activating 8-12 week old abGCs does not rescue social discrimination function. **A)** Control-reared Nestin-cre and Nestin-cre: Gq with a vehicle injection both showed a significant reduction in time investigating the novel animal in trial 1 (N) to the familiar animal in trial 2 (F). MSEW Nestin-cre and Nestin-cre: Gq both showed similar investigation times in trial 1 and trial 2, with vehicle injection. Three-way ANOVA: effect of Group x Trial: $F_{(1,26)}$=35.42, p=<0.0001; Trial x Group: $F_{(1,26)}$=5.353, p=0.0289 ; Holm-Sidak posthoc test: Control Nestin-cre: N vs F, p= <0.0001; Control Nestin-cre:Gq: N vs F, p=<0.0001; n= Control Nestin-cre: 8, MSEW Nestin-cre: 8, Control Nestin-cre:Gq: 7, MSEW Nestin-cre:Gq: 7. **B)** Control-reared Nestin-cre and Nestin-cre:Gq with a CNO injection both showed a significant reduction in time investigating the novel animal in trial 1 (N) to the familiar animal in trial 2 (F). MSEW Nestin-cre and Nestin-cre: Gq both showed similar investigation times in trial 1 and trial 2, with vehicle injection. Three-way ANOVA: effect of Group x Trial:

$F_{(1,25)}$=27.47, p=<0.0001; Group x Genotype: $F_{(1,25)}$=4.659, p=0.0407 ; Holm-Sidak posthoc test: Control Nestin-cre: N vs F, p= 0.0001; Control Nestin-cre:Gq: N vs F, p=<0.0001; n= Control Nestin-cre: 7, MSEW Nestin-cre: 7, Control Nestin-cre:Gq: 7, MSEW Nestin-cre:Gq: 8. **C)** With a vehicle and CNO injection, MSEW Nestin-cre and Nestin-cre:Gq both had significantly lower discrimination indices compared to Control Nestin-cre and Nestin-cre:Gq. Three-way ANOVA: group: $F_{(1,41)}$ =36.77, p=<0.0001.

How reduced abGCs and chemogenetic activation of abGCs affect CA2 network activity was investigated next. For this experiment, Control-reared and MSEW double transgenic males implanted with electrodes in the unilateral CA2 were examined under conditions of CNO or vehicle 4-6 weeks after tamoxifen injection to express excitatory DREADDs in abGCs. It was first investigated whether CA2 SWRs differed between Control-reared and MSEW male mice during baseline, when exposed to novel versus familiar mice, or during a home cage recording (Figure 3E). Baseline recordings were used to normalize data from trial 1, 2, and home cage. During trial 1, but not trial 2, SWR frequency (SWRs per minute) was lower in MSEW males treated with vehicle compared to controls, and rescued by administration of CNO (i.e., by activation of abGCs) (Figure 3I). Similar to previously reported findings (Laham et al., 2024), when treated with vehicle, control-reared mice had higher CA2 SWR frequency while investigating novel mice compared to familiar mice. This difference did not exist with MSEW male mice treated with vehicle but was restored with CNO treatment. Thus, MSEW prevented the differential SWR frequency between novel and familiar stimulus exposure, an effect that was reversed by activation of abGCs by treatment with CNO. How SWRs change during different behaviors is also of interest, given the literature on SWRs during restful wakefulness. SWRs during immobility periods of each trial were also investigated. MSEW male mice treated with a vehicle showed a significantly lower SWR discrimination index, that was restored by activating new neurons with CNO (Figure 3J).

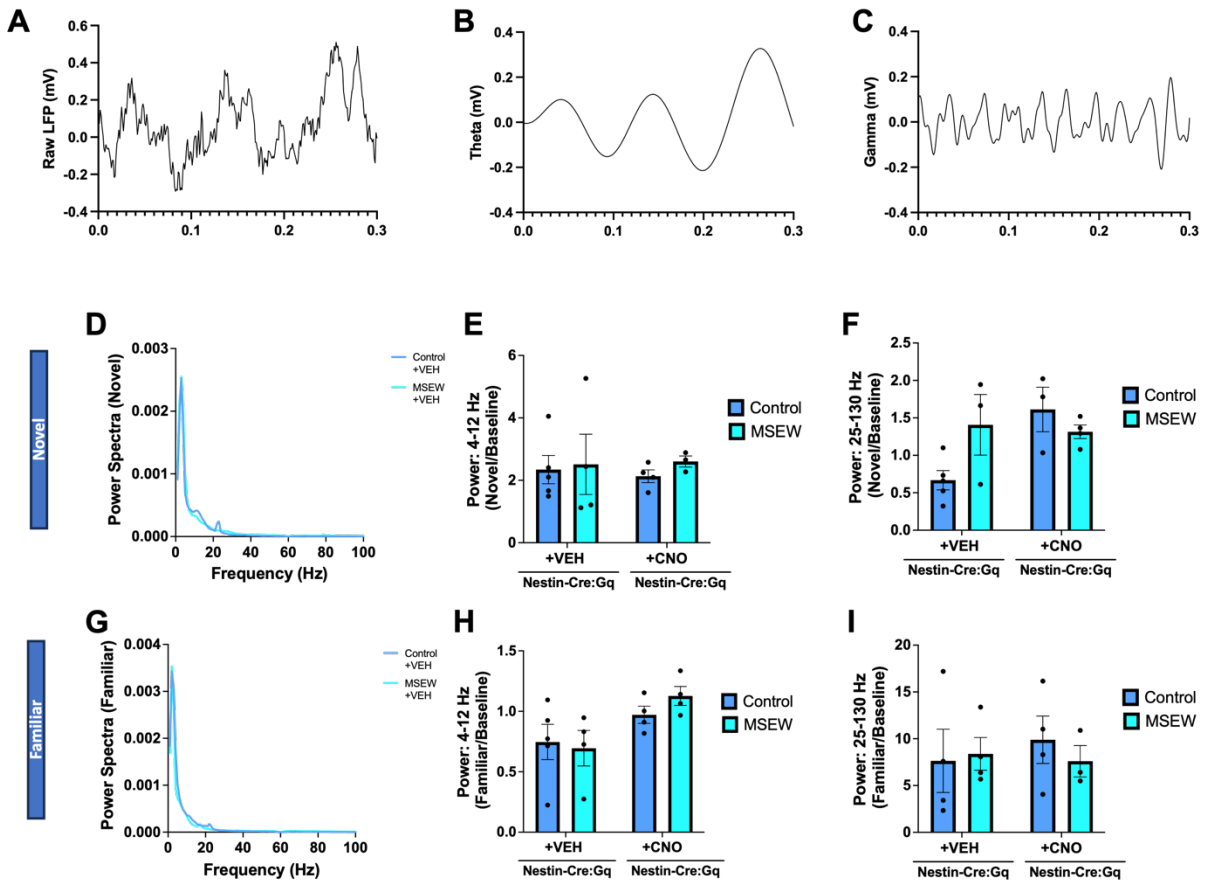
Other properties of SWR across groups were also examined, including average integral, peak, and duration. There were no significant differences between MSEW and control-reared mice on these measures (Supplementary Figure 3A, B). However, SWR integral did significantly decreased in MSEW mice after CNO injections, during Trial 1 when the animal was immobile (Figure 3K). There were no significant effects on SWR frequency, duration, integral (not shown), or peak amplitude in the home cage or during Trial 2 (Supplementary Figure 3C-D) or home cage (not shown).



Supplementary Figure 3. MSEW had no effect on other SWR characteristics. During the novel trial, activating abGCs has no significant effects on the average duration **(A)** or average peak of SWRs (**B).** During the familiar recording, activating abGCs had no effects on the **(C)**, average duration **(D)**, nor average peak. Homecage not shown.

Phase-amplitude coupling between theta (4-12) and gamma oscillations in the CA2 was investigated next, as well as theta and gamma power. Phase amplitude coupling has been shown to be higher when mice encounter a familiar mouse (their mother) compared to a novel mouse (Laham et al., 2024). No significant differences were found in PAC, when considering slow (25-50Hz), mid (50-100Hz) gamma, fast (100-130 Hz) gamma (not shown), and the full gamma frequency rage (25-130) .

Changes in theta and gamma power were examined considering low (4-7 Hz) and high (8-12 Hz) theta, as well as slow (25-50Hz), mid (50-100Hz), and fast (100-130 Hz) gamma because of previously noted associations between oscillations in these frequency ranges and different behavioral states (Zhu et al., 2023). Although no significant differences between MSEW and control-reared mice with vehicle treatment were found in power at any of the assessed frequency ranges (Supplementary Figure 4A-I), CNO treatment of MSEW mice increased theta (8-12 Hz and overall) during trial 1 (novel mouse exposure). These findings raise the possibility that the restoration of social recognition in MSEW double transgenic mice after activation of abGCs results from a net increase in oscillations at these frequencies rather than a recovery of normal network activity.



Supplementary Figure 4. Activating abGCs had no effects on Phase Amplitude Coupling or Power in the CA2. **A)** Example Raw LFP trace from CA2. **B)** Filtered theta oscillation trace, 4 - 12 Hz. **C)** Filtered gamma oscillation trace, 25-130 Hz. **D, F)** During the novel trial, activating abGCs had no effect on theta (4-12 Hz) or Gamma (25-130 Hz) in CA2. **E, G)** During the familiar trial, activating abGCs had no effect on theta (4-12 Hz) or Gamma (25-130 Hz) in CA2.

LBN, but not MSEW, male mice exhibit increased CA2 PNN intensity

Because LBN males show impaired social recognition abilities, but no differences in abGC number compared to control-reared males, the possibility that a different mechanism underlies LBN impairment was considered. Studies have shown that CA2 PNNs are important for the development and maintenance of social recognition abilities in mice (Diethorn et al., 2023; Cope et al., 2023) so the possibility that CA2 PNNs differed among control reared, LBN, or MSEW

male mice was investigated. Labeling of the plant lectin Wisteria floribunda agglutinin (WFA), which binds to glycosaminoglycan chains on PNNs, differed across groups. Confocal intensity analyses revealed that LBN, but not MSEW, male mice had greater CA2 WFA intensity than control-reared male mice (Figure 4A, B). By contrast, CA2 WFA intensity in LBN female mice did not significantly differ from control-reared mice (Figure 4C, D).

Reducing CA2 PNNs in LBN males restores social discrimination

To establish whether increased CA2 PNN intensity is causally linked to social recognition deficits, CA2 PNNs were lowered by direct infusion of the ECM degradative enzyme chondroitinase ABC (+chABC) in the bilateral CA2 of Control-reared and LBN C57 males (Figure 4E). Mice were tested on the direct social interaction task 10 days post injection, a timepoint when PNNs have been shown to have regrown to control-reared baseline levels. Additional groups of Control-reared and LBN males received direct infusions of the control enzyme penicillinase (+Pnase) into the bilateral CA2 and tested them at the same time point. It was found that control-reared males with penicillinase and chABC infusions had normal social recognition abilities in that they investigate novel mice significantly more than familiar mice (Figure 4F). By contrast, LBN males with penicillinase injections (+Pnase) showed impaired social recognition, investigating novel and familiar mice at similar levels (Figure 4F). However, normalizing CA2 PNNs in LBN males restored social discrimination abilities to control levels (Figure 4F). Likewise, LBN mice +Pnase had significantly lower discrimination ratios compared to all other groups (Figure 4G).

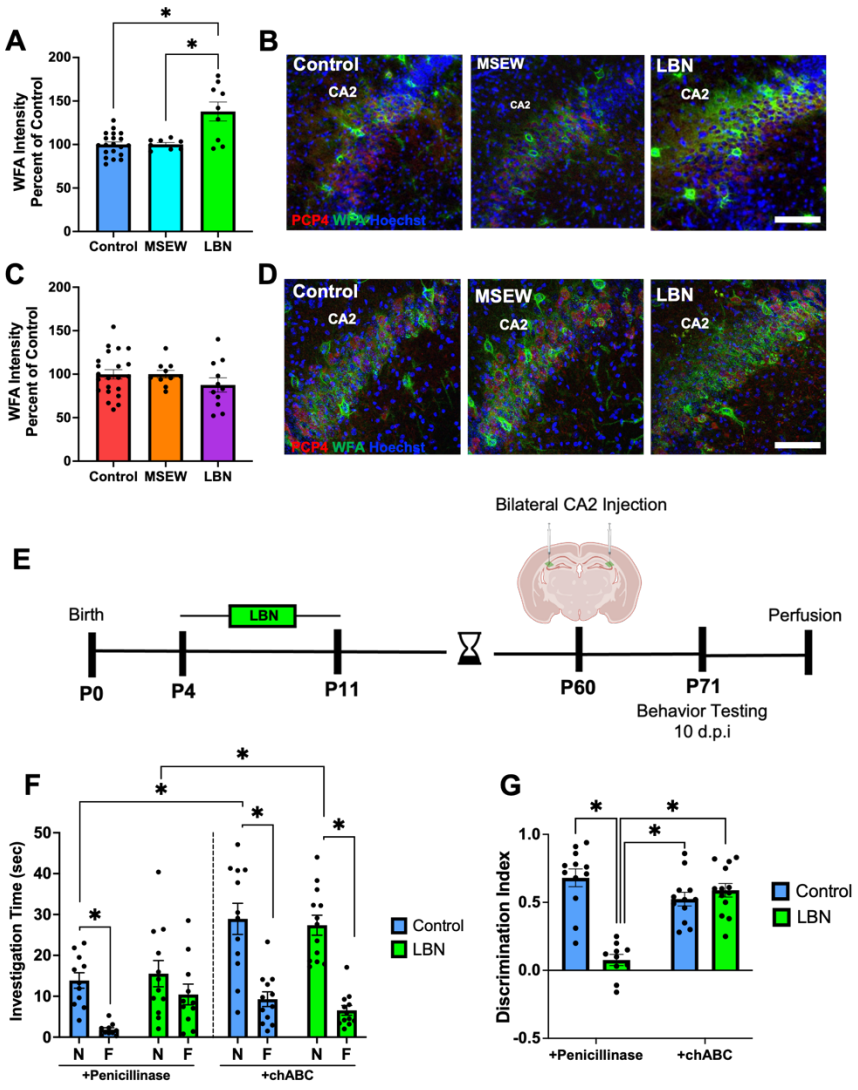


Figure 4. LBN, but not MSEW males, showed increased PNNs in CA2, social discrimination function restored by PNN degradation. **A)** LBN males were found to significantly lower intensity of WFA+ staining in the pyramidal layer of the CA2 compared to Control and MSEW. One-way ANOVA: effect of group: $F_{(2,34)} = 13.63$, p=<0.001; Holm-Sidak posthoc test: Control vs LBN, p=<0.0001; MSEW vs LBN, p=0.0005. **B)** Confocal images of WFA+ staining (green) in dorsal CA2 labeled by PCP4 (red) of Control-reared, MSEW and LBN males. **C)** Control, LBN and MSEW females had comparable intensities of WFA+ staining in the pyramidal layer of the CA2. **D)** Confocal images of WFA+ staining (green) in dorsal CA2 labeled by PCP4 (red) of Control-reared, MSEW and LBN females. **E)** Timeline of LBN chABC behavioral experiment. **F)** Reducing PNNs to Control levels restored social discrimination function in LBN males. Control +Pnase, Control +chABC, and LBN +chABC males display a significant reduction in

time investigating the novel animal in trial 1 (N) to the familiar animal in trial 2 (F). LBN +Pnase males showed similar investigation times in trial 1 and trial 2. Three-way ANOVA: group x trial x treatment: $F_{(1,41)}$=4.160, p=0.0479; Holm-Sidak posthoc test: Control +Pnase: N vs F, p=0.0003; Control +chABC: N vs F, p=<0.0001; LBN +chABC: N vs F, p=<0.0001; N: Control: +Pnase vs +chABC, p=0.0002; N: LBN: +Pnase vs +chABC, p=0.0007; n= Control +Pnase: 12, LBN +Pnase: 10, Control +chABC: 12, LBN +chABC:12. **G)** LBN +Pnase males had a significantly lower discrimination index compared to Control +Pnase, Control +chABC, and LBN +chABC males. Additionally, Control +chABC males had a significantly lower discrimination index compared to Control +Pnase males. Two-way ANOVA: group x treatment: $F_{(1,43)}$ =38.85, p=<0.0001; Holm-Sidak posthoc test: +Pnase: Control vs LBN, p=<0.0001; LBN +Pnase vs Control +chABC, p=<0.0001; LBN: +Pnase vs +chABC, p=<0.0001. Scale bar = 25 um.

Discussion

We compared the behavioral effects of two different ELA paradigms in adulthood. We found that both ELA paradigms impaired social recognition in males but not females. Closer examination of the behavioral data, however, revealed subtle differences in behavioral outcomes between MSEW and LBN. For males, social recognition deficits were accompanied by diminished social novelty investigation in LBN, but not MSEW, mice. For females, who did not show social recognition deficits, both ELA paradigms led to increased social novelty investigation. Furthermore, in adult males, we found differential effects on two measures of hippocampal plasticity depending on the ELA paradigm. Males previously subjected to MSEW, but not LBN, had reduced numbers of immature neurons in the dentate gyrus, whereas males subjected to LBN, but not MSEW, had elevated intensity of PNNs in the CA2 region. In subsequent experiments, we found that either increasing the number of new neurons or increasing the activation of existing new neurons restored social recognition in MSEW males.

Restoration of social recognition was accompanied by a normalization of CA2 SWRs in MSEW males following increased activation of new neurons. In LBN males, normalization of CA2 PNNs restored social recognition. Taken together, these findings suggest that different hippocampal plasticity mechanisms are affected by MSEW compared to LBN in males, to impair social recognition, and that adult interventions to normalize these abnormalities are sufficient to restore behavioral and electrophysiological function.

MSEW impairs social recognition through suppressed adult neurogenesis in the dentate gyrus

Previous studies have shown that abGCs in the dentate gyrus are important for social recognition in mice. The ability of adult mice to recognize a mouse as familiar 24 hours after the first encounter is impaired in mice lacking abGCs (Cope et al., 2020). Similar impairments in the ability to discriminate between a familiar mouse encountered during development (i.e., the mother) and a novel mouse have been observed in mice lacking abGCs (Laham et al., 2024). These findings are consistent with our observations that MSEW male mice have both deficient social recognition and reduced numbers of abGCs. In addition, our results showing that enriched environment living restores numbers of abGCs in MSEW mice to control levels along with recovery of social recognition function support the hypothesis that reduced numbers of abGCs are causally linked to MSEW-induced social dysfunction. In addition to restoring the density of new neurons, we also considered the functional consequences of reduced abGCs on network activity levels. Therefore, we asked whether activating abGCs, without altering the density, using double transgenic mouse model, would also restore social dysfunction after MSEW Similar to our behavioral results with enriched environment housing, activating abGCs in

MSEW males restored social recognition. Taken together, these findings strongly suggest that MSEW deficits in social recognition are causally linked to reduced abGCs, effects that can be reversed in adulthood by restoring the number, or increasing the activation, of existing abGCs. Studies have shown that in addition to their well-established connections to the hilus and the CA3 region, abGCs project to the CA2 region, an area known to be important for social recognition (Llorens-Martin et al., 2015). A previous study investigating this projection using an inhibitory DREADDs model, including precise CNO delivery to CA2, showed the importance of this circuit in discriminating mothers from novel mothers by adult male and female mice (Laham et al., 2024). The current study enhanced abGC activity generally, by giving CNO systemically, so we cannot rule out the possibility that abGC projections to the hilus and CA3 are involved in the restoration of MSEW-induced deficits in social recognition however, we found that MSEW mice had diminished abGC axons in the CA2, suggesting that this circuit is a likely candidate. We also found MSEW had diminished abGC axons in the CA3 region, although using retrovirus we discovered that the proportion of abGC boutons in CA3 compared to CA2 was increased in MSEW mice, suggesting that the impact of MSEW on abGC afferents may be greater in CA2 than CA3. It is also possible that the combined effect of abGCs on social recognition involves both projections to CA2 and CA3, as well as communication between these regions.

Our data further suggest that the reduction in number of abGCs and their axons in target regions in MSEW males is likely due to a deficit in cell proliferation, as opposed to cell survival, as MSEW males have fewer sox2+ progenitor cells in the dentate gyrus than control-reared mice, an effect that is normalized by enriched environment living. The restoration of Sox2+ cell numbers with enriched environment living likely caused by increased proliferation of progenitor cells, whose expansion increases their number, and ultimately the number of abGCs. These

findings are partially consistent with previous reports showing that enriched environment living stimulates cell proliferation and the number of Sox2+ cells in the dentate gyrus, although these findings were observed in control mice (Chandler et al., 2020; Veena et al., 2009; Tanti et al., 2012), and we only observed the increase in MSEW mice.

Since MSEW is a developmental experience, completed long before adulthood, our findings raise questions about how the hippocampus might be affected during the adverse experience to ultimately have fewer progenitor cells available to generate new neurons in adulthood. One possibility is that MSEW accelerates cell proliferation and neurogenesis such that the progenitor pool is depleted sooner, findings that are consistent with some developmental stress postnatal neurogenesis studies (Nanick et al., 2015). Accelerated cell proliferation may reflect an advanced developmental aging process that has been reported for some early life adversity models (Bath et al., 2016; Ruiz et al., 2018). For example, studies have shown that age-related decline in adult neurogenesis is causally linked to the expression of senescence molecules by progenitor cells in the hippocampus (Fatt et al., 2022). Taken together with the early life stress literature on accelerated aging, it is possible that the MSEW male dentate gyrus in young adulthood is more like a control-reared male at an older age. Importantly, MSEW effects can be reversed by housing mice in enriched environments, which has also been observed with other age-related phenomenon in control-reared mice (Birch and Kelly, 2019; Singhal et al., 2019; Schmidt et al., 2022). It has been hypothesized that ELA-induced accelerated aging could be adaptive if it hastens maturity and independence, although most studies have shown that if anything, ELA slows the achievement of developmental milestones, including puberty (Manzano Nieves et al., 2019; Knop et al., 2019).

Our findings of significantly decreased abGCs in the dorsal dentate gyrus of males after MSEW are in contrast to the lack of effect we observed with LBN. The difference in abGC effects between MSEW and LBN may be related to the different durations of the ELA paradigms (P2-P17 for MSEW versus P4-P11 for LBN), disparate influence on circuits results from experiential differences, or both. It should be noted that although some studies have shown no differences in adult neurogenesis between LBN and control-reared mice in cell proliferation or number of immature granule cells in the dorsal dentate gyrus (Hoeijthalers et al., 2018; Nanick et al., 2015; Dixon et al., 2024), others have found decreased long-term survival of abGCs (Nanick et al., 2015), a measure we did not make in our study. It is also worth mentioning that this latter study used an LBN protocol that is earlier than the one we used (P2-P9 compared to P4-P11).

LBN impairs social recognition through excessive CA2 PNNs

Previous studies have shown that the CA2 region plays important roles in social recognition both during development (Laham et al., 2022; Diethorn and Gould, 2023) and in adulthood (Hitti and Siegelbaum, 2014; Laham et al., 2022; 2024). The CA2 region has an unusually high concentration of PNNs, which in addition to surrounding inhibitory interneurons as is typical for other hippocampal subregions, surround pyramidal cells (Carstens et al., 2016; Cope et al., 2022). The emergence of PNNs in the CA2 appears to coincide with social recognition function during development (Diethorn and Gould, 2023). In adulthood, CA2 PNNs are important for the ability to distinguish between novel and familiar mice; degradation of PNNs impairs this function in otherwise healthy mice (Cope et al., 2022). Conversely, inbred and genetic mouse models of social dysfunction have been associated with excessive PNNs in the CA2; some

functional deficits can be restored by lowering levels of PNNs to control values (Carstens et al., 2021; Cope et al., 2022). These findings are consistent with our data showing a restoration of social recognition function in LBN males after normalization of PNNs after local infusion of the degradative enzyme chABC into the CA2 region. These findings, like those of MSEW males after abGC stimulation, suggest that the influence of LBN on circuitry underlying social recognition is not permanent and instead, can be repaired (at least temporarily) by adult intervention. The duration of these improvements, as well as whether it is possible to permanently restore function remains unknown.

Studies suggest that PNNs work to restrict plasticity by limiting postsynaptic sites available for synaptogenesis by incoming axons and/or by concentrating neurotransmitter receptors so that inhibition to the PNN+ cell is more robust (Wingert and Sorg, 2021). Previous work in other systems has shown that PNNs are sensitive to the environment (Laham and Gould, 2022) as well as to neuronal activity, with a complex relationship depending on the cell population. In the CA2, PNN formation appears to be negatively correlated with neuronal activity (Carstens et al., 2021) with chemogenetic inhibition increasing PNN intensity and chemogenetic excitation decreasing PNN intensity (Carstens et al., 2016). Thus, it is possible that the male LBN CA2 has higher PNNs because neuronal activity is inhibited in the region. There are many ways this could occur, including by LBN affecting intrinsic properties of CA2 excitatory or inhibitory neurons, which have been shown to be sensitive to PNN degradation (Carstens et al., 2021). Another possibility is that LBN might alter developmental innervation from afferents outside of the region, the majority of which are excitatory (Diethorn and Gould, 2023). If LBN diminishes excitatory afferents to the CA2, the corresponding decrease in activity in the postsynaptic region might contribute to excessive PNN formation. While the mechanism by

which increased PNN intensity in the CA2 of LBN males impairs social recognition remains unknown, the overall literature suggests that an optimal level is necessary for healthy social function, and our data suggest that the effect may be reparable with interventions designed to correct PNN levels. Taken together the MSEW and LBN findings, these data highlight the complex and heterogenous response of the hippocampus's response to different types of early life adversity.

CA2 network activity and social recognition

A growing number of studies have shown that CA2 network activity is associated with healthy social recognition capabilities. SWRs and PAC in the CA2 have been linked to social experience, social memory consolidation and retrieval (Oliva et al., 2020; Laham et al., in press; Zhu et al., 2023). CA2 SWRs are known to be causally linked with social memory consolidation through their coordination with the replay of neuronal ensembles (Oliva et al., 2020). In the CA2, SWRs are more likely to occur during quiet wakefulness than during sleep compared to other hippocampal subregions and are elevated during exposure to a novel versus familiar social stimulus (Laham et al., in press). Conversely, PAC is higher during exposure to a familiar compared to a novel social stimulus (Zhu et al., 2023; Laham et al., in press).

In control reared Nestin-cre: Gq males treated with vehicle we found similar patterns with SWRs and PAC with differential responses depending on whether the social stimulus was novel or familiar. Nestin-cre: Gq males previously subjected to MSEW and treated with vehicle lacked the differential SWR response to novel compared to familiar social stimuli, but had no significant differences from control-reared males in terms of CA2 PAC. When Nestin-cre: Gq MSEW mice

were treated with CNO to activate abGCs, the novel/familiar difference in SWR was evident, such that no differences were observed between Nestin-cre: Gq control-reared and MSEW males in terms of SWR. abGCs play a significant role in social cognition, including facilitating sparse encoding and participating in the generation of SWRs in the CA2 (Laham et al., in press; McHugh et al., 2022). Previous study reports that inhibiting the abGCs-CA2 pathway leads to decreased CA2 SWR diversification (Laham et al., in press). Taken together, MSEW-induced decreases in abGCs may lead to impair social memory through homogenous SWRs during encoding and retrieval of social memories. Although we found no significant differences in CA2 PAC between Nestin-cre: Gq control-reared or MSEW males treated with vehicle, we observed a significant increase in overall theta power in the CA2, which appeared to be mostly because of CNO-induced changes in low theta (4-7Hz). Although there were no differences between Nestin-cre: Gq control-reared and Nestin-cre: Gq MSEW mice treated with vehicle in terms of CA2 theta power, the further increase with CNO treatment might contribute to improved social recognition function. Although this result would not constitute a restoration of healthy network activity in MSEW males, it may suggest an alternative neurophysiological pathway through which social recognition could be improved. Along these lines, it is worth noting that afferents from the supramammillary nucleus (SUM) of the hypothalamus have been shown to modulate CA2 theta power (Farrell et al., 2021). SUM projections to the CA2 have been linked to social novelty responses and mimicking effects of SUM activation might contribute to improved function in MSEW males (Chen et al., 2020). Similar improvements in social recognition associated with increased theta power have been observed in a genetic model of social dysfunction, but in the ventral CA1, the main output of the CA2 (Cope et al., 2023).

Future studies will explore if/how Control reared and LBN male mice CA2 network activity differ at baseline. If so, determining whether restoring PNNs to control levels is sufficient to restore network activity is of interest.

Sex differences in ELA effects

Our results demonstrate divergent behavioral and cellular effects of different types of early life experience in males, as well as sex differences in behavioral response to ELA. In adulthood, both MSEW and LBN males have impaired, albeit slightly differently, social recognition. In striking contrast, adult female MSEW and LBN mice exhibit robust social recognition abilities, with females in both ELA types showing higher social novelty responses compared to control-reared females. These findings raise questions about whether the sex differences arise from differential developmental experience or whether they emerge over time. Regarding the former possibility, a few studies have shown that rodent mothers display differential care toward male and female pups, perhaps due to a greater need for licking and grooming in order to stimulate micturition in males (Sakamoto et al., 2021; Maggi et al., 1986). Thus, both MSEW and LBN, which are known to alter maternal behavior (Gallo et al., 2019; Shupe and Clinton, 2022; Baracz et al., 2020; Bailoo et al., 2015) may do so differently toward male and female pups, with greater long-term effects observed in males. However, it remains possible that the effects of MSEW and LBN are largely similar on male and female pups, but that the females recover as time passes, possibly due to influences that occur during puberty. Along these lines, one study has shown that after ELA, females exhibit social deficits during late development, with dysfunction disappearing as mice approach young adulthood (Holman et al., 2021). If this is the case, then understanding how changes in signaling molecules that occur during the peripubertal period, or differential

expression of stress-related genes, in females interact with circuitry underlying social recognition might provide some clues about how the adult sex difference we observed occurs. We did test the females during sexually receptive and sexually non-receptive stages of the estrus cycle and found no differences in social behaviors using the DSI test.

Translational relevance

Our findings suggest that behavioral outcomes after ELA are determined by the type of experience, as well as by sex. We also found that two different types of ELA affect behavior by affecting separate plasticity mechanisms. These findings are consistent with some human literature showing that different types of childhood maltreatment predispose individuals to develop certain types of neuropsychiatric illnesses over others, and that different neural circuits are altered as well (Waters and Gould, 2022). While childhood experience in humans is more complex than ELA models in rodents, our findings suggest that early life stress is not a general experience that results in similar outcomes regardless of individual experience. Our findings further suggest that different early life experiences alter different underlying mechanisms to affect behavior. Understanding how different ELA paradigms alter the brain during development using rodent models may yield potential biomarkers, and therapeutic targets for reversing dysfunction prior to adulthood and may help in the development of precision medicine approaches for assisting individuals with neuropsychiatric disease after childhood adversity.

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Chapter 1

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Chapter 2

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