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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 GFAP-TK transgenic model, which allows for the inhibition of adult neurogenesis, and CD1 littermate controls, we subjected mice to an ELA paradigm of maternal separation and early weaning (MSEW) or control rearing. We found that mice with intact adult neurogenesis showed 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.

1. 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 (physical and emotional), chronic illness, witnessing violence, experiencing natural disasters, and more (CDC, 2020). It has been shown that negative early life experiences can significantly diminish cognitive, emotional, social, and physical health (Lupien et al., 2009; Chen and Baram, 2016). More specifically, ELA has been strongly associated with an increased prevalence of anxiety disorders (Heim et al., 2010; Fonzo et al., 2016; Gallo et al., 2018). 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 and Gould, 2018), and limited bedding/-nesting, a putative model of scarcity and abuse due to altered maternal care (Walker et al., 2017; Gallo et al., 2019). Using these approaches, several studies have demonstrated that ELA increases behaviors that have been characterized as defensive, 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; Cui et al., 2006; Lee et al., 2007; Wei et al., 2010; Raineki et al., 2012; Dalle Molle et al., 2012; Wang et al., 2012; Aya-Ramos et al., 2017; Masrour et al., 2018; Demaestri et al., 2020). 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 and Gould, 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; Rice et al., 2008; Ivy et al., 2010; Savignac et al., 2011; Candemir et al., 2019; van der Kooij et al., 2015; Johnson et al., 2018). This overall collection of contradictory findings may be reflective of the variability of

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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 (McEwen, 2010; Karatsoreos and McEwen, 2013; McEwen et al., 2015; Shapero et al., 2015). Factors including genetic differences, as well as the type and timing of ELA, likely contribute to the varied vulnerability to 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 (Murthy and Gould, 2018; Orso et al., 2019), researchers developed the maternal separation and early weaning model (MSEW) which has been shown to increase defensive behavior in adult mice (George et al., 2010; Carlyle et al., 2012; Murthy et al., 2019; Laham et al., 2020). This model had the potential to increase the translational validity of mouse ELA studies while more consistently modeling negative health outcomes.

In adult rodents, the ventral hippocampus has been implicated in the regulation of defensive behavior through lesion studies (Bannerman et al., 2003; Weeden et al., 2015), 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 (Khazipov et al., 2001; Avishai-Eliner et al., 2002) 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). In mice, these new neurons develop throughout the entire dorsoventral axis and have been linked to avoidance behavior, stress regulation, and cognitive function (Revest et al., 2009; Snyder et al., 2011; Hill et al., 2015; Anacker et al., 2018; Huckleberry et al., 2018). Some studies report that developmental and adolescent stress decreases the number of adult-born granule cells (abGCs) (Mirescu et al., 2004; Oomen et al., 2011; Leslie et al., 2011; Barha 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., 2011; 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 (Monteiro et al., 2014; Garrett et al., 2015; Pereira-Caixeta et al., 2017, 2018; Cope et al., 2020), which is disrupted in many neuropsychiatric disorders that have been linked to ELA (Farrington, 2005; Opendak et al., 2017; Tzanoulinou and Sandi, 2017).

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. We tested the hypothesis that reducing abGCs following MSEW would potentiate previously reported increased avoidance behavior compared to control reared animals. Additionally, given the role of abGCs in social memory, and the potential for abGCs to be altered by ELA, we predicted that MSEW mice would also have impaired social recognition. We used a transgenic mouse model that expresses herpes simplex virus thymidine kinase (TK) under the control of the GFAP promoter on a CD1 background. Adult neurogenesis is inhibited in TK mice by treatment with the antiviral drug valganciclovir (VGCV) (Snyder et al., 2011). We first subjected male and female TK and CD1 wild-type littermate pups to MSEW or control rearing. Following maturation and VGCV treatment, we tested mice on the elevated plus maze (EPM), an avoidance behavior task, and the direct social interaction test, a social discrimination task.

2. Materials and methods

2.1. 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. 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 offspring 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.

2.2. Maternal separation and early weaning

Pups were randomly assigned to either control-rearing or MSEW as previously described (George et al., 2010; Murthy et al., 2019) (Fig. 1A). Aside from genotyping on P15, control-reared litters were left undisturbed until weaning on P21. MSEW mice were separated on P3–P6 from their dam for 4 h per day. On P7–P16, separations increased to 8 h. 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, in a separate room from the dam, 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. At weaning, all mice were housed by genotype and sex with 4–5 mice per cage in Optimice cages on a reverse 12-h light/dark cycle.

2.3. Valganciclovir treatment

Beginning at P60, valganciclovir (VGCV), an antiviral drug that selectively reduces adult neurogenesis in GFAP-TK mice, 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 cohort, there were 4 groups: CD1 VGCV+, CD1 VGCV-, GFAP-TK VGCV+, GFAP-TK VGCV-. Group sizes were as follows: males n = 15–17/group, females n = 8–10/group.

2.4. Estrous cycle monitoring

To determine the stage of estrous before behavioral testing, 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.

2.5. Elevated plus maze test

After 6 weeks of VGCV treatment, male and female mice were tested on the EPM (Fig. 1B) during the 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 × 44 × 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 min. After each mouse was tested, the EPM was cleaned with 70% ethanol. The behavior test was

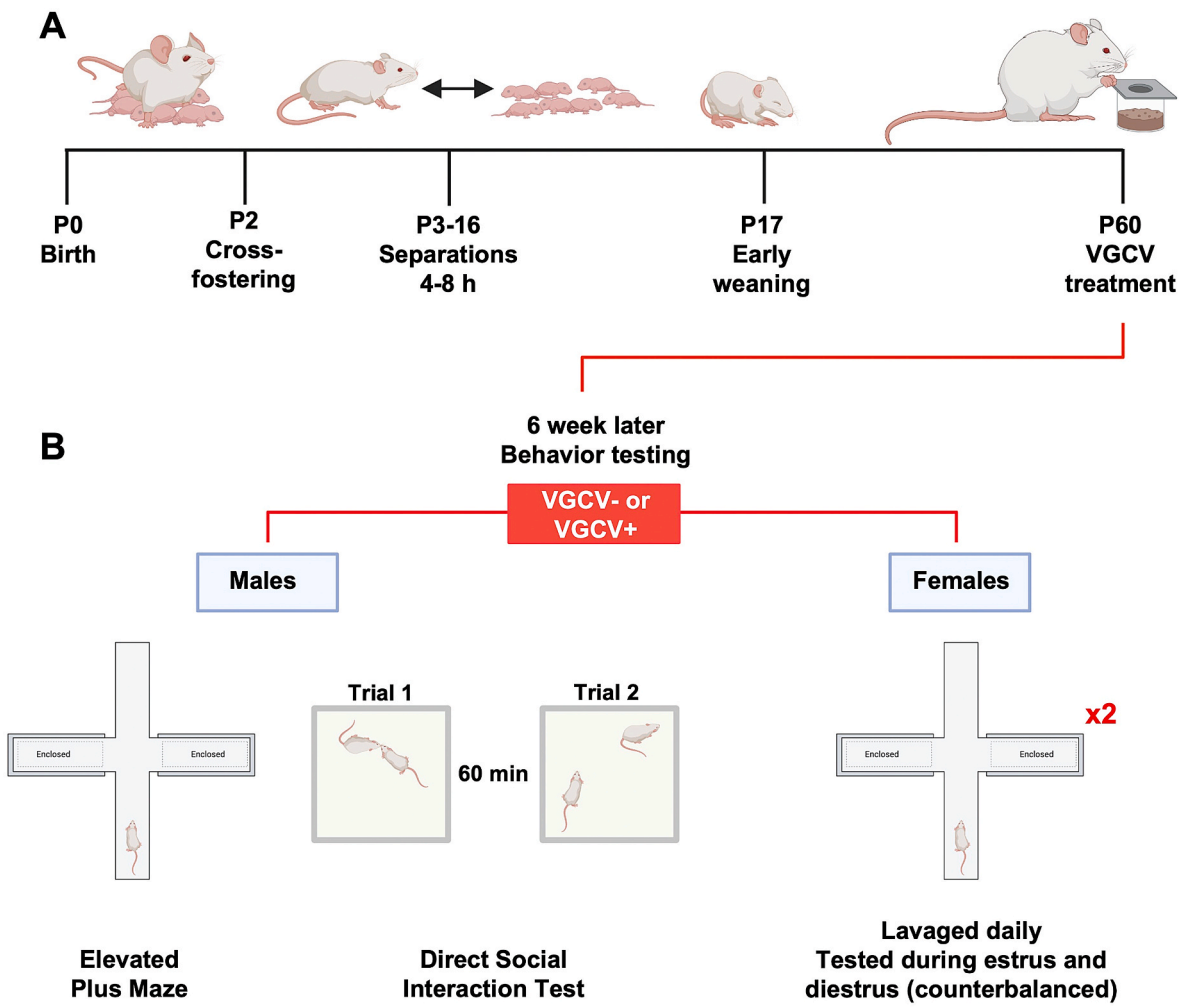


Fig. 1. Timeline and experimental design. Litters were subjected to either control rearing, where they were undisturbed until weaning on P21, or **A)** MSEW rearing. **B)** Half of each genotype and rearing condition was fed VGCV for 6 weeks. Male mice were then tested on the EPM for 5 min, followed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

videotaped by a camera suspended over the EPM. Videos were scored by an experimenter blind to the genotype, rearing condition, and VGCV treatment. The numbers of entries and time spent in each of the arms were collected. Criteria for arm entries was front two paws and snout inside of the arm. Since open arms are less protected and more exposed to light, more time spent in the open arms is considered to reflect less avoidance or defensive behavior. Total entries into the arms are a measure of overall activity levels.

2.6. 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 min and then to the testing box for 5 min. For the test, each animal had 2 trials. In trial 1, the subject mouse was allowed to freely interact with a novel stimulus mouse for 5 min. Following a 60-min delay, the subject mouse was then placed back with the same, now "familiar", mouse for a second 5-min trial. The amount of time spent investigating the stimulus mouse was quantified. The criteria for investigation were: anogenital sniffing, allogrooming, close following, and sniffing (snout pointed toward stimulus mouse $\leq$2 cm). Because mice prefer exploring novelty, they typically investigate familiar mice less than novel mice. More specifically, social discrimination memory is operationalized as a mouse's decrease in investigation time from trial 1 to trial 2. The direct social interaction test was conducted only on male mice. We did not test female mice on this task because no changes were observed with MSEW in males, nor with MSEW in females on the EPM.

2.7. 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 h in 4% PFA in PBS followed by 48 h 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 h 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 h 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 coverslipped with Vectashield (Thermo-Fisher Scientific). Slides were coded and analyzed with the investigator blind to the experimental group. Each brain was checked to determine whether immature granule cells were present in the dentate gyrus using a Leica TCS SP8 confocal microscope.

2.8. Statistical analysis

Statistical tests were performed using GraphPad Prism 9.0 (GraphPad Software). The normality test was used to determine whether parametric or non-parametric tests were appropriate. Statistical outliers were excluded from statistical analyses. Data are presented as mean ± standard error of the mean. For each rearing group (control and MSEW) data from CD1 VGCV+, CD1 VGCV-, TK VGCV- groups were collapsed for statistical analyses and considered the "intact neurogenesis" cohort. Data from TK VGCV + mice were considered the "no neurogenesis" cohort. EPMdata were analyzed using a two-way ANOVA (intact neurogenesis/no neurogenesis x control/MSEW). Post hoc comparisons were made with Tukey HSD tests. Direct social interation data were analyzed using a three-way ANOVA when necessary (intact neurogenesis/no neurogenesis x control/MSEW x novel/familiar). When a three-way ANOVA was used post hoc comparisons were made with Holm-Šídák tests. A multiple linear regression was used to determine the effect of estrous cycle on female EPM behavior.

3. Results

3.1. Sex differences in EPM behavior are evident; males are more active than females

First, to examine baseline differences between males and females, we assessed activity levels by looking at total entries made to the arms of the EPM. A two-way ANOVA assessing the effect of sex on open arms, closed arms, and total arm entries revealed an interaction between sex x arm/total (Fig. 2). *Post hoc* tests show that male mice make more total entries on the EPM, entering both the open arms and closed arms significantly more than females. Given our observed sex differences on these measures, datasets from each sex were analyzed separately.

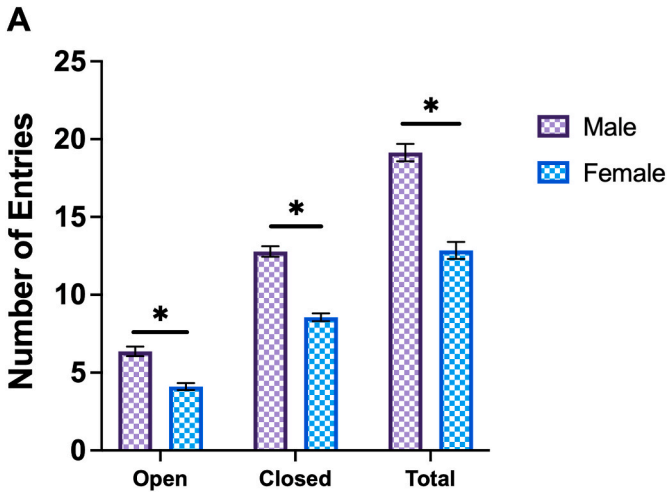


Fig. 2. Sex differences in behavior on the EPM with males showing greater activity than females. Entries to open arms, closed arms and total entries, the last being a measure of overall activity levels, were greater in males than females (two-way ANOVA; sex x arm/total: $F_{(2,582)} = 10.77$, $p = <0.0001$; Tukey *post hoc*: open arms, $p = 0.0007$; closed arms, $p = <0.0001$; total, $p = <0.0001$). *$p = <0.05$. Bars represent mean + SEM.

3.2. In male mice, inhibiting adult neurogenesis after MSEW decreases avoidance behavior

To assess the influence of abGCs on avoidance behavior after MSEW, we used transgenic GFAP-TK mice with inhibited adult neurogenesis following administration of VGCV and tested mice on the EPM. Unexpectedly, our results showed that MSEW males with intact neurogenesis did not exhibit more avoidance behavior compared to control-reared males with intact neurogenesis. MSEW males with intact neurogenesis did not show differences in the percent of time spent in the open arms compared to control males with intact neurogenesis (Fig. 3A). However, inhibiting adult neurogenesis had differential effects in male mice that experienced MSEW versus control rearing (Fig. 3A). Using a two-way ANOVA, we found a statistically significant interaction between the rearing group and neurogenesis status. *Post hoc* tests revealed that MSEW males with no neurogenesis spent a significantly greater percentage of time in the open arms of the EPM compared to control males with no neurogenesis and MSEW males with intact neurogenesis, suggesting reduced avoidance behavior.

We also measured a preference for open arms by the percentage of total entries made to open arms. A two-way ANOVA assessing the effect of rearing group and neurogenesis status on percent of total entries to the open arms revealed a statistically significant interaction (Fig. 3B). Control and MSEW males with intact neurogenesis did not differ in the percentage of entries made to the open arms while MSEW males with no neurogenesis entered the open arms significantly more than control males with no neurogenesis (Fig. 3B). Taken together, increased time and entries into the open arms suggest decreased avoidance behavior in male MSEW mice with no neurogenesis.

3.3. In female mice, inhibiting 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 (Luine and Frankfurt, 2013; Pentkowski et al., 2018). Additionally, studies in our lab have shown that avoidance behavior in female C57 mice following MSEW is increased only during diestrus (Laham et al., 2020). As a result, we lavaged female mice 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 arms as well as entries to the open arms based on the estrous stage. Significant regression equations were found, however, estrous stage was not a significant predictor of either of these measures (linear regression; percent time in open arms: $F_{(1,131)} = 1.639$, $p = 0.2027$; percent entries to open arms: $F_{(1,139)} = 0.0381$, $p = 0.8454$). Therefore, data from each estrous stage were averaged within each mouse and the resulting dataset was analyzed using two-way ANOVA. We found that control females with intact neurogenesis did not differ significantly from MSEW females with intact neurogenesis in the percent of time spent in the open arms (Fig. 4A) Reducing abGCs in females had no apparent effect on percent time in the open arms in the MSEW or control mice (Fig. 4A). Additionally, rearing group and neurogenesis status did not associate with the percent of entries made to the open arms (Fig. 4B). These data show that there are no obvious differences in avoidance behavior in female mice following MSEW with or without reduced numbers of abGCs.

Studies have reported adaptation to the avoidant properties of the EPM with repeated testing (Tucker and McCabe, 2017; Schrader et al., 2018). Thus, we analyzed whether there were any effects of rearing strategy or neurogenesis status in the first test only, on time and entries to the open arms, regardless of estrous cycle stage. A two-way ANOVA did not reveal any significant differences in the percent of time females spent in the open arms during the first test (rearing strategy x neurogenesis status: $F_{(1,59)i} = 0.3715$, $p = 0.5445$). Finally, the same analysis was done on percent of total entries made to the open arms, and no

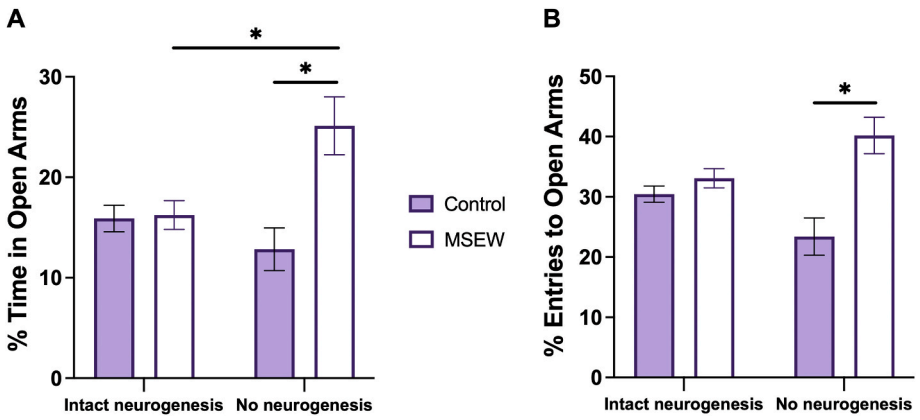


Fig. 3. In males, reduction of adult-born neurons after MSEW decreases avoidance behavior. **A)** Percent time on the open arms of EPM, a measure of avoidance behavior, was higher in MSEW males with no neurogenesis compared to control males with no neurogenesis and MSEW males with intact neurogenesis (two-way ANOVA; rearing group x neurogenesis status: $F_{(1,115)} = 8.822$, $p = 0.0036$; Tukey *post hoc*: MSEW "no neurogenesis" vs MSEW "intact neurogenesis", $p = 0.0154$; MSEW "no neurogenesis" vs Control "no neurogenesis", $p = 0.0037$). **B)** Percent entries to open arms of EPM. MSEW males with no neurogenesis males entered the open arms significantly more than control males with no neurogenesis (two-way ANOVA; rearing group x neurogenesis status: $F_{(1,119)} = 9.992$, $p = 0.0020$; Tukey *post hoc*: control "no neurogenesis" vs MSEW "no neurogenesis", $p = 0.0002$). Bars represent mean + SEM. *p=<0.05.

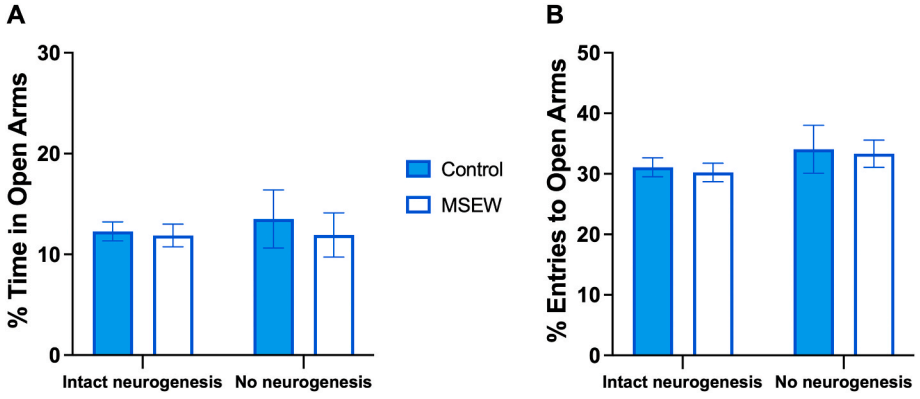


Fig. 4. In females, reducing abGCs did not alter avoidance behavior in MSEW or control mice **A)** Percent time spent in the open arms of EPM. There were no significant main effects or interactions between rearing group, or neurogenesis status (two-way ANOVA; rearing group x neurogenesis status: $F_{(1,58)} = 0.1292$, $p = 0.7205$). **B)** Percent of entries to open arms of EPM. There was no effect of the rearing group or neurogenesis status (two-way ANOVA; rearing group x neurogenesis status: $F_{(1,69)} = 0.0007$, $p = 0.9784$). Bars represent mean +SEM. *p=<0.05.

significant effects were found (two-way ANOVA; rearing strategy x neurogenesis status: $F_{(1,69)i} = 0.3330$, $p = 0.8557$).

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

Given the role of abGCs in social memory (Monteiro et al., 2014;

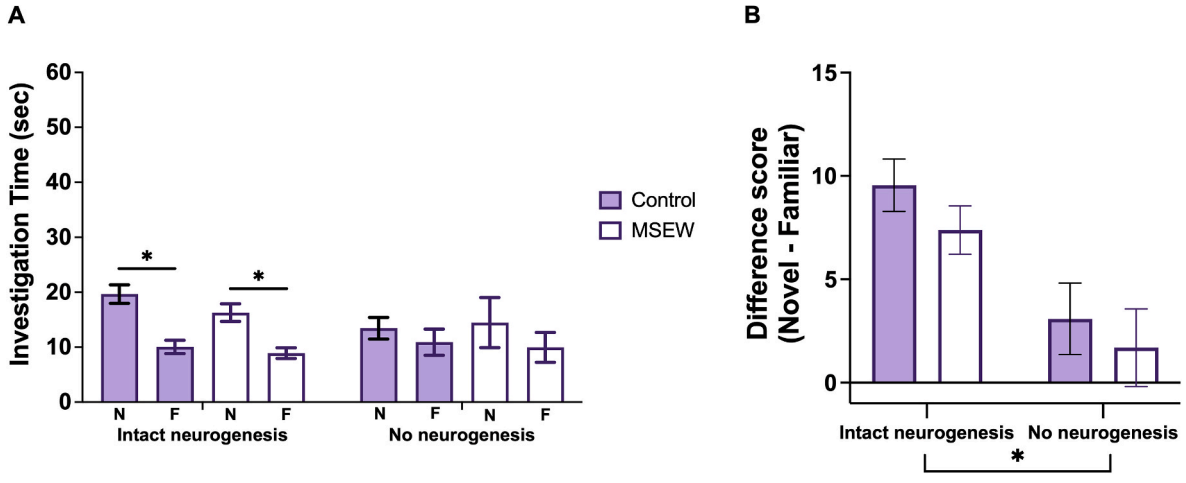


Fig. 5. MSEW does not alter social discrimination in male mice with intact neurogenesis but reducing adult neurogenesis causes significant impairment. **A)** Control males with intact neurogenesis display a significant reduction in time investigating the novel animal in trial 1 to the familiar animal in trial 2. MSEW males with intact neurogenesis also significantly reduced the time spent investigating the novel stimulus animal in trial 1 to the familiar stimulus animal in trial 2. Control males with no neurogenesis as well as MSEW males with no neurogenesis showed no difference in investigation times in trial 1 and trial 2 (three-way ANOVA; neurogenesis x trial:$F_{(1,85)} = 6.550$, $p = 0.0123$; Tukey *post hoc*: intact neurogenesis: control novel vs intact neurogenesis: control familiar, p= <0.0001; intact neurogenesis: MSEW novel vs intact neurogenesis: MSEW familiar, p= <0.0001) **B)** Difference score (novel trial investigation time minus familiar trial investigation time). Mice with no neurogenesis exhibit lower difference scores regardless of rearing group (two-way ANOVA; neurogenesis status: $F_{(1,79)} = 12.31$, $p = 0.0007$). Bars represent mean +SEM. N, novel; F, familiar.

Garrett et al., 2015; Pereira-Caixeta et al., 2017, 2018; Cope et al., 2020), we investigated whether MSEW impaired social memory function and whether reducing abGCs would impact any such effects. As described earlier, social memory function is defined as lower investigation times for familiar than novel stimulus mice. Using a three-way ANOVA, we found a significant two-way interaction of neurogenesis status and trial (Fig. 5A) *Post hoc* tests revealed that MSEW and control males with intact neurogenesis appeared to exhibit normal social discrimination function. These groups of mice significantly decrease their investigation time from novel to familiar (Fig. 5A). However, males with no neurogenesis from both MSEW and control-rearing groups showed impaired social discrimination ability in that they did not exhibit differences in the amount of time spent investigating stimulus mice between trials (Fig. 5A). Furthermore, this effect is illustrated by the difference score, calculated by subtracting the familiar trial investigation time from the novel trial investigation time. A two-way ANOVA revealed a main effect of neurogenesis, suggesting that mice lacking abGCs exhibit lower difference scores regardless of rearing group (Fig. 5B).

3.5. VGCV reduces abGCs in the dentate gyrus of TK mice

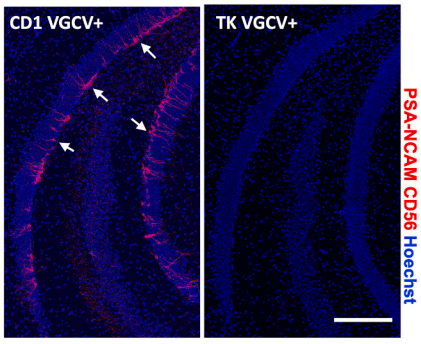
We verified that abGCs were almost completely eliminated in male and female MSEW and control-reared TK mice administered VGCV. For this analysis, all groups were analyzed separately. We stained a randomly selected subset of mice for PSA-NCAM, a marker of immature granule cells, and found that both control and MSEW CD1 VGCV-/+ mice,as well as control and MSEW TK VGCV- mice, had substantial numbers of immature abGCs in the dentate gyrus. Control and MSEW TK VGCV + mice had almost no immature abGCs in the dentate gyrus (Fig. 6). This observation verified 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.

4. Discussion

This study was designed to explore whether abGCs buffer against MSEW-induced increases in avoidance behavior. Based on our previous findings, we hypothesized that MSEW would increase avoidance behavior in both male and diestrus female mice (Murthy et al., 2019; Laham et al., 2020). Given the literature on adult neurogenesis and avoidance behavior (Revest et al., 2009; Hill et al., 2015; Anacker et al., 2018), we further hypothesized that eliminating abGCs would potentiate the negative effects of MSEW. Our results were not consistent with either of these hypotheses. First, we found that neither male nor female mice in either stage of estrous we tested (estrus or diestrus) as well as of either genotype we tested (TK or CD1 littermates) showed an increase in avoidance behavior after MSEW. Second, we found that reducing abGCs had no effect on control or MSEW females but produced an unexpected reduction in avoidance behavior in MSEW males. MSEW males with reduced neurogenesis spent more time in the open arms and made more entries to the open arms than control males with reduced neurogenesis. 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 male mice, abGCs may serve to stabilize the system after MSEW, dampening the expression of potentially high-risk behavior.

Our findings that MSEW did not increase avoidance behavior in either genotype or sex were surprising given previous results from our lab and others showing such an effect in males and diestrus females (George et al., 2010; Carlyle et al., 2012; Murthy et al., 2019; Laham et al., 2020). Similarly, we found no MSEW effect on social memory in males, which was unexpected given previous results from our lab (unpublished observations) and others (Franklin et al., 2011; Emmons et al., 2021), 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, we find that regardless of genotype, rearing group, and VGCV 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; Li and Graham, 2017). This sex difference may potentially contribute to obscuring MSEW effects on avoidance behavior. 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 defensive behavior in which mice with high defensive behavior at baseline do not show further stress-induced increases perhaps because they are already close to maximal on those measures (Bosch et al., 2006; Füchsl et al., 2014).

A



B

Control	Male	Female
CD1 VGCV-	✓	✓
CD1 VGCV+	✓	✓
TK VGCV-	✓	✓
TK VGCV+	X	X

MSEW	Male	Female
CD1 VGCV-	✓	✓
CD1 VGCV+	✓	✓
TK VGCV-	✓	✓
TK VGCV+	X	X

Fig. 6. Treatment with VGCV dramatically reduced the number of immature neurons in the ventral dentate gyrus. **Left**, Confocal images of PSA-NCAM labeled cells (arrows, red) in 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 = 100 μm. **Right**, Table depicting different experimental groups with verification of the presence of immature neurons (✓) in all groups but TK VGCV + males and females, which showed no immature neurons (x). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

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 (Berman et al., 2014; Orso et al., 2019) 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 and Domschke, 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 (Priebe et al., 2005; McEwen, 2008; Tang et al., 2014).

Given previous studies linking abGCs to the regulation of stress responses and defensive behavior (Revest et al., 2009; Snyder et al., 2011; Hill et al., 2015; Anacker et al., 2018), as well as evidence that abGCs participate in recovery from deleterious consequences of stress (Alves et al., 2017; Schoenfeld et al., 2019) in mice, a major goal of this study was to test whether abGC reduction would exacerbate MSEW-induced increases in avoidance behavior. We found that 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 defensive behavior but that abGCs buffered against these changes, a possibility that is consistent with views of adult neurogenesis serving to promote adaptive brain function and stress coping (Lyons et al., 2010; 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 some 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. Our results suggest that at least for TK mice, MSEW only alters defensive 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 (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 defensive 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, we found an expected impairment in social discrimination in both control and MSEW TK mice with reduced abGCs. These findings are consistent with previous work from our laboratory (Cope et al., 2020) and others (Monteiro et al., 2014; Garrett et al., 2015) and may reflect changes in social memory or social novelty detection. In addition, the findings 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.

4.1. Conclusions

Although the present study 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. Our results show that for the strains of mice we 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.

Bruce McEwen's pioneering studies on adaptive and maladaptive stress responses shed considerable light on the findings we obtained from our experimental work. McEwen's concept of allostasis, whereby organisms respond to stress in ways that maintain homeostasis (McEwen and Stellar, 1993; McEwen, 1998; McEwen and Wingfield, 2003; McEwen and Akil, 2020), has its roots in early life experience, which can help individuals to best predict responses and outcomes (Danese and McEwen, 2012; McEwen, 2020). McEwen and colleagues have shown through numerous experimental and theoretical studies that stress-induced outcomes can vary dramatically depending on genetics and environmental factors, which can contribute to, or protect against, the build-up of allostatic load (McEwen, 1998; Nasca et al., 2019). Since stress-induced pathology emerges when homeostatic mechanisms break down due to excessive allostatic load, mechanisms of resilience are of obvious interest (McEwen et al., 2015; McEwen, 2016, 2020; Nasca et al., 2017, 2019; McEwen and Akil, 2020). McEwen was a major proponent of the concept that there are two sides to the stress story, the positive and the negative (McEwen, 2020). Relatedly, McEwen's work was crucial for our understanding of how experiences with objective similarity can produce vastly different outcomes depending on qualities inherent to the individual, the circumstances in which the stressful experience occurs, and events that preceded as well as those that followed the experience. Due to McEwen's insights and research findings on mechanisms of resilience and individual differences in stress responsiveness, we have a much richer context within which to place the current results.

CRediT authorship contribution statement

Renée C. Waters: Conceptualization, data acquisition, Formal analysis, Writing – original draft, Writing – review & editing. **Hunter M. Worth:** data acquisition, Formal analysis, Writing – review & editing. **Betsy Vasquez:** data acquisition, Formal analysis, Writing – review & editing. **Elizabeth Gould:** Conceptualization, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors (Waters, Worth, Vasquez and Gould) declare they have no conflicts of interest regarding the manuscript entitled "Inhibition of adult neurogenesis reduces avoidance behavior in male, but not female, mice subjected to early life adversity" submitted to *Neurobiology of Stress* for the special issue dedicated to Bruce McEwen.

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