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Perineuronal Nets, Inhibitory Interneurons, and Anxiety-Related Ventral Hippocampal Neuronal Oscillations Are Altered by Early Life Adversity

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ABSTRACT

BACKGROUND: In humans, accumulated adverse experiences during childhood increase the risk of anxiety disorders and attention-deficit/hyperactivity disorder. In rodents, the ventral hippocampus (vHIP) is associated with anxiety regulation, and lesions in this region alter both anxiety-like behavior and activity levels. Neuronal oscillations in the vHIP of the theta frequency range (4–12 Hz) have been implicated in anxious states and derive in part from the activity of inhibitory interneurons in the hippocampus, some of which are enwrapped with perineuronal nets (PNNs), extracellular matrix structures known to regulate plasticity. We sought to investigate the associations among early life stress–induced anxiety and hyperactivity with vHIP neuronal oscillations, inhibitory interneurons, and PNNs in mice.

METHODS: We used repeated maternal separation with early weaning (MSEW) to model accumulated early life adversity in mouse offspring and studied the underlying cellular and electrophysiological changes in the vHIP that are associated with excessive anxiety and hyperactivity.

RESULTS: We found increased anxiety-like behavior and activity levels in MSEW adult males, along with increased theta power and enhanced theta–gamma coupling in the vHIP. MSEW mice showed reduced intensity of parvalbumin as well as increased PNN intensity around parvalbumin-positive interneurons in the vHIP. We further observed that MSEW increased orthodenticle homeobox protein 2, a transcription factor promoting PNN development, in the choroid plexus, where it is produced, as well as in parvalbumin-positive interneurons, where it is sequestered.

CONCLUSIONS: These findings raise the possibility of causal links among parvalbumin-positive interneurons, PNNs, orthodenticle homeobox protein 2, and MSEW-induced anxiety and hyperactivity.

Keywords: Anxiety, Early life stress, Interneurons, Perineuronal nets, Theta rhythm, Ventral hippocampus

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Childhood maltreatment is known to substantially increase the risk of the child's developing neuropsychiatric conditions in adulthood, including anxiety and mood disorders (1–3). In fact, more than 50% of diagnosed cases of anxiety disorders and clinical depression occur in individuals who report having experienced early life adversity (4). Attention-deficit/hyperactivity disorder is often comorbid with anxiety disorders in adults, and childhood maltreatment has been shown to increase susceptibility to both attention-deficit/hyperactivity disorder and excessive anxiety (5). Some studies in humans suggest that exposure to stress during a sensitive period in development may be predictive of negative outcomes (6–8), but the preponderance of evidence, including a recent large study, indicates that the accumulation of multiple bouts of early life stress exposure is most damaging in terms of long-term psychiatric problems (7–10). To cover both the sensitive period and cumulative aspects of early life adversity, we selected a multiple-hit model of early life stress in mice, maternal separation with early weaning (MSEW), where mouse pups are subjected to daily maternal separations of increasing length during the first 16 days of life followed by weaning 4 days earlier than typically reared laboratory mice (11,12). This developmental manipulation has been shown to increase both anxiety levels and hyperactivity in adulthood (11), suggesting that it has strong translational validity (see the Supplement for further discussion).

The ventral hippocampus (vHIP) of rodents has been linked to anxiety regulation. Bilateral lesions in this area have been shown to reduce anxiety-like behavior in rodents (13–15), whereas optogenetic or pharmacological stimulation of the region has the opposite effect (16–18). Several studies have suggested that neuronal oscillations in the theta frequency (4–12 Hz) range in the vHIP and the medial prefrontal cortex (mPFC) are important for anxiety regulation, with decreases in theta power in the vHIP occurring after anxiolytic drug administration (19) and with increases in theta power in the vHIP and mPFC as well as increases in theta synchrony between the vHIP and mPFC occurring during anxious states (20). Furthermore, in humans,

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increased self-reported anxiety during threat is associated with increased theta rhythm in the anterior hippocampus (21), a region that is considered analogous to the vHIP in rodents (22). Despite these associations, the electrophysiological profile of rodents that display increased anxious behavior after early life adversity has not been studied.

Inhibitory interneuron subtypes have been implicated in theta rhythm in the hippocampus and neocortex (23–27). Optogenetic studies of the hippocampus have shown that parvalbumin-positive (PV^+) interneurons are the main effectors of intrinsic theta rhythm, while somatostatin-positive (SST^+) interneurons modulate the entrainment of intrinsic theta by controlling external inputs to the hippocampus (25). Many PV^+ interneurons are encapsulated by specialized extracellular matrix structures termed perineuronal nets (PNNs). PNNs are thought to limit plasticity and are responsible for the closure of the critical period for ocular dominance in the visual cortex (28). PNN degradation has been shown to reduce PV^+ interneuron activity (29) and to reduce theta frequency synchrony between brain regions involved in fear memory (30). Experience has been shown to alter PNN expression, but the effects are complex, depending on the type of experience, the developmental stage examined, and the brain region. For example, early life trauma has been shown to reduce PNN expression in the basolateral amygdala (31), enriched environment rearing reduces PNNs in the cerebellum (32) but increases them in the CA2 region of the hippocampus (33), and enriched environment exposure in adulthood increases or decreases PNN expression depending on the brain region and presence of other experiences (34). PNNs have been linked to the transcription factor orthodenticle homeobox protein 2 (OTX2) (35), the expression of which coincides with the maturation of PV^+ cells and the closure of the critical period of plasticity in mice. In adults, OTX2 is produced in the choroid plexus, released into the cerebrospinal fluid, and accumulates in PV^+ cells where it facilitates PNN formation. No previous studies have investigated whether PNNs and OTX2 are altered in PV^+ cells of the hippocampus after early life adversity.

Studies have shown that adult-generated neurons in the hippocampus can modulate neuronal oscillations in that their elimination by focal irradiation or by transgenic means increases rhythmic firing (36). Some previous studies have reported that early life adversity reduces the production of new neurons in the dentate gyrus (37), raising the possibility that changes in neuronal oscillations and potentially anxiety may involve reductions in this form of plasticity. In addition, adult-generated neurons also form connections with PV^+ inhibitory interneurons (38,39). Despite these compelling associations, no previous studies have investigated whether changes in inhibitory interneurons, PNNs, and adult-generated neurons occur in association with early life stress–induced alterations in neuronal oscillations and anxiety-like behavior.

We investigated these possibilities by performing behavioral analyses and local field potential (LFP) recordings in the vHIP of adult mice subjected to MSEW and found consistent increases in anxiety-like behavior and hyperactivity along with increases in theta power in a novel environment, as well as enhanced theta–gamma coupling in both familiar and novel environments. This was accompanied by a reduction in densities of PV^+ and SST^+ interneurons, but no decrease in immature neurons, in the granule cell layer of MSEW mice. Further analysis of PV^+ interneurons revealed increased PNN intensity surrounding PV^+ cells and increases in OTX2 within these cells. These findings suggest interrelationships among PV^+ interneurons, PNNs, OTX2, and MSEW-induced anxiety and hyperactivity.

METHODS AND MATERIALS

Maternal Separation With Early Weaning

Twenty litters of C57BL/6J mice from two breeding cohorts were exposed to one of two rearing conditions at postnatal day 2 (P2): 1) nonhandled controls; or 2) maternal separation for 4 hours daily from P2 to P5 and 8 hours daily from P6 to P16. Pups in the latter group were weaned at P17 while control litters were weaned at the typical age of P21 (11). At age P60 to P70, mice underwent behavior testing (see Supplemental Methods).

Behavior

Anxiety and activity testing were carried out as detailed in the Supplemental Methods.

Histochemistry

Two hours after behavioral analyses were conducted, mice were perfused and sections through the hippocampus were stained for doublecortin (DCX), Ki67, calretinin (CR), PV, SST, OTX2, c-Fos, and *Wisteria floribunda* agglutinin (WFA) using immunohistochemical and histochemical methods. Antibodies are detailed in Supplemental Table S1 (see Supplemental Methods for more details).

Cell Count and Density Analyses

The vHIP was analyzed separately because it is functionally distinct from the dorsal hippocampus and has been implicated in anxiety regulation (22). DCX^+, $Ki67^+$, and glutamate deoxycarboxylase 67–positive ($GAD67^+$) cells from the granule cell layer and interneuron subtypes across layers of the dentate gyrus (DG), CA1, and CA3 were counted using Stereo Investigator software (Microbrightfield Bioscience, Williston, VT). Densities were determined by dividing the total number of positive cells by the volume of the region outlined.

WFA and OTX2 Intensity Analyses

Confocal images were analyzed with ImageJ where every PV^+/WFA^+ cell was outlined and the area and maximum intensity (mean gray value of WFA or OTX2 $\times$ area of the WFA or OTX2 stain, respectively) for each PV^+/WFA^+ cell was determined according to previously published protocols (40,41) (see Supplemental Methods for more details).

Surgery

Insulated stainless steel electrodes (0.005″; Plastics One, Roanoke, VA) were implanted in the vHIP CA1 (3.3 mm posterior, 3.45 mm lateral, and 4.2 mm depth). The electrode was placed in the left hemisphere because this hemisphere has been specifically shown to be affected in MSEW mice (42).

LFP Recordings and Analysis

One week after surgery, mice from cohort two underwent habituations and behavioral testing. LFP recording data were collected on the fourth day from two separate recording sessions. First, mice were exposed to a prehabituated familiar environment for a period of 10 minutes followed by an hour of rest and then exposed to a novel environment for a period of 10 minutes. Power spectra were calculated from data acquired from mice during segments of movement only (4–15 cm/s). Any animal without a minimum of 5 seconds of movement during the task was excluded from analysis. For phase amplitude coupling analyses (43), all animals regardless of movement (moving or stationary) were considered for analysis (see Supplemental Methods for details).

Statistical Analysis

Electrophysiology data were analyzed as described above using custom MATLAB (The MathWorks, Inc., Natick, MA) and R scripts (code available upon request to the corresponding author). For all other measures, unpaired two tailed Student's *t* or Mann-Whitney *U* tests were performed on each data set following determination of homogeneity of variance with Levene's test. All statistical tests and *p* values are listed in Supplemental Tables S2 and S3.

RESULTS

MSEW Results in Increased Anxiety-like Behavior in Males in the Elevated Plus Maze

We observed increased anxiety-like behavior in two cohorts of MSEW male mice compared with male controls. MSEW mice in cohort one showed increased anxiety in the elevated plus maze (EPM), with a significantly lower percentage of entries into the open arms (Figure 1B). MSEW mice in cohort one also spent significantly more time in the closed arms (Figure 1C). To verify the reliability of this manipulation, we repeated behavioral tests with a second cohort of mice. MSEW mice from cohort two also displayed greater anxiety in the EPM as they made significantly lower percent entries into the open arms (Figure 1D), although no differences were observed in the amount of time spent in the open or closed arms between groups (Figure 1E). No measurable changes in anxiety-like behavior were observed in MSEW female mice compared with same-sex controls (Supplemental Figure S1). Because our aim was to search for neural correlates of MSEW-increased anxiety, we focused our subsequent studies on males.

MSEW Results in Increased Activity Levels in a Novel Environment

Consistent with previous work (11), we found that MSEW male mice were more active in a novel environment than controls. MSEW male mice exhibited greater locomotion in a novel testing arena in that they covered a larger distance (Figure 2A) as well as showed an increase in the percentage of time spent moving during the duration of test (Figure 2B).

MSEW Alters vHIP Neuronal Oscillations in Both Familiar and Novel Environments

When tested in a familiar environment, no differences were observed between MSEW and control groups in theta (4–12 Hz), beta (12–20 Hz), or gamma (30–80 Hz) power

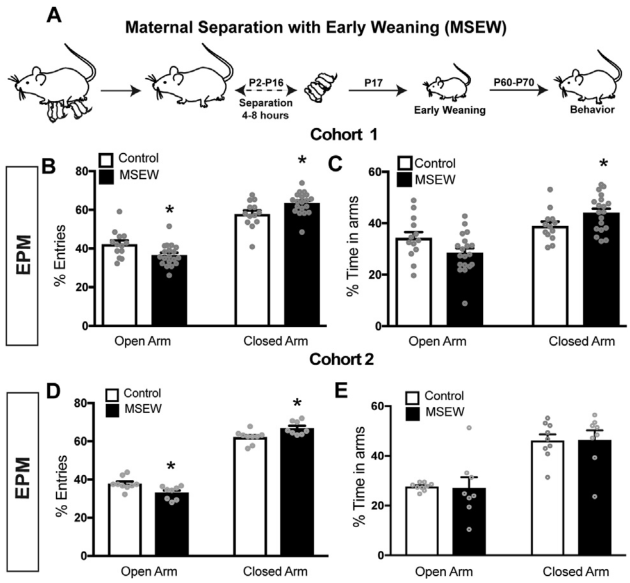

Figure 1. **(A)** Timeline depicting the maternal separation with early weaning (MSEW) paradigm. **(B)** MSEW male mice from cohort one displayed increased anxiety-like behavior in the elevated plus maze (EPM), making significantly lower percent entries into the open and higher percent entries into closed arms. **(C)** MSEW mice spent significantly more time in the closed arms of the EPM. **(D)** MSEW male mice from cohort two displayed increased anxiety-like behavior in the EPM, making significantly lower percent entries into the open arms and higher percent entries into closed arms. **(E)** MSEW mice from cohort two did not show any differences in the percent of time spent in the open or closed arms. *$p < .05$. P, postnatal day.

(Figure 3D–F). In addition, delta (1–4 Hz) power was not altered (Supplemental Figure S2A). By contrast, phase amplitude coupling analysis in the familiar environment showed that MSEW male mice displayed a greater modulation of gamma oscillations by theta phase (Figure 3G, H). When tested in a novel environment, MSEW mice showed significantly greater theta power (Figure 3K) as well as alpha/beta power (Figure 3L) compared with control mice. Delta (Supplemental Figure S2B) and gamma power (Figure 3M) were not substantially different between groups. Phase amplitude coupling analysis showed that the amplitude of gamma oscillations was also significantly

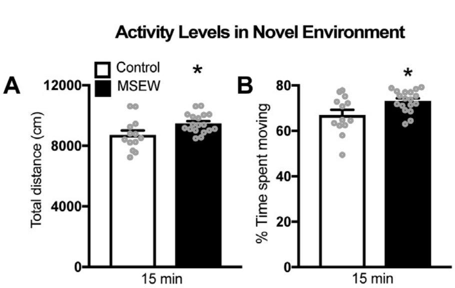

Figure 2. **(A)** Maternal separation with early weaning (MSEW) male mice displayed greater activity in a novel environment as they showed increased locomotion (total distance) compared with control mice. **(B)** MSEW mice showed increased time spent active (% time spent moving) during the testing period compared with control mice. *$p < .05$.

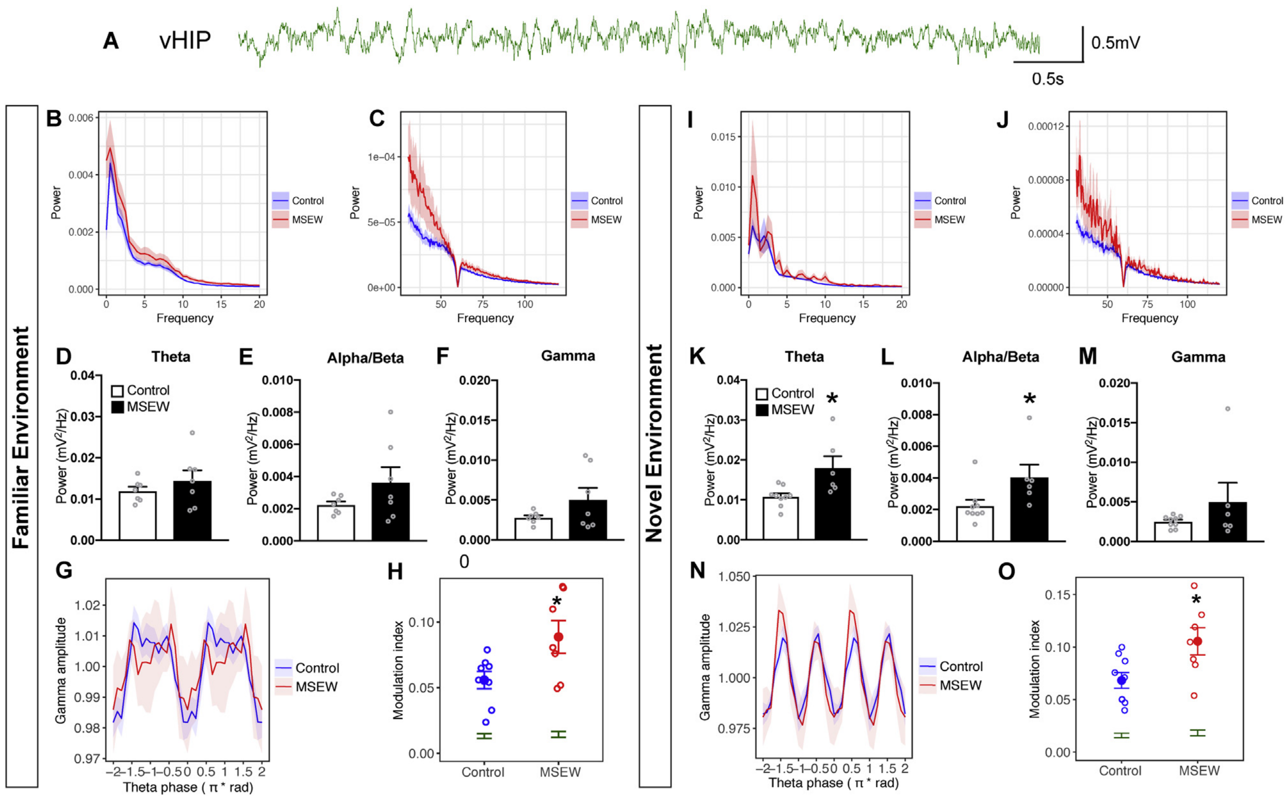

Figure 3. **(A)** Raw local field potential trace from the ventral hippocampus (vHIP). **(B, C)** High and low frequency power spectra plots from maternal separation with early weaning (MSEW) male mice during 10 minutes in the familiar environment. In the familiar environment there were no substantial differences in theta **(D)**, alpha/beta **(E)**, or gamma **(F)** power between groups. **(G)** Phase amplitude coupling analysis plotting normalized gamma power as a function of theta phase in the familiar environment. **(H)** Analysis of theta–gamma phase amplitude coupling in the familiar environment revealed that MSEW mice had significantly greater modulation compared with controls. Permutation tests showed significantly greater modulation above chance for both treatment conditions (green). **(I, J)** High and low frequency power spectra plots from MSEW mice during 10 minutes in the novel environment. **(K, L)** In the novel environment, theta and alpha/beta frequency power were significantly increased in MSEW mice compared with control mice. **(M)** No substantial changes in gamma power were observed between groups in the novel environment. **(N)** Phase amplitude coupling analysis plotting normalized gamma power as a function of theta phase in the novel environment. **(O)** MSEW significantly increased theta–gamma modulation relative to control mice in the novel environment. Permutation tests showed significantly greater modulation above chance for both treatment conditions (green). *$p < .05$.

modulated by the phase of theta oscillations in MSEW mice in a novel environment when compared with control mice (Figure 3N, O). These data suggest that vHIP theta–gamma coupling is inherently enhanced in MSEW mice compared with control mice, occurring in both familiar and novel environments, but increases in theta and alpha/beta power are observed only when MSEW mice are in a novel environment.

MSEW Leads to a Reduction in the Densities of PV$^+$ and SST$^+$ Cells but No Overall Decrease in Interneuron Densities

Because inhibitory interneurons play roles in theta rhythm and theta–gamma coupling (23,25), we examined their presence in the hippocampus of MSEW mice and control mice. Analyses of the DG, CA1, and CA3 revealed that the density of PV$^+$ interneurons in MSEW mice was significantly reduced in the ventral DG (vDG) compared with control mice (Figure 4B), while the densities in the CA1 and CA3 seemed to remain unaltered (Figure 4B′, B″). PV intensity within PV$^+$ neurons of the vDG was also reduced (Supplemental Figure S5A), suggesting that the decrease in cell density was caused by the reduced expression of PV as opposed to a loss of interneurons caused by cell death. SST$^+$ interneuron density was also significantly reduced in the vDG of MSEW mice (Figure 4D), while the CA1 and CA3 regions showed no differences in this measure (Figure 4D′, D″). It is likely that MSEW-induced decreases in densities of PV$^+$ and SST$^+$ interneurons are not the result of cell death because there was no overall difference in the density of cells stained with the pan-interneuron marker GAD67 (Supplemental Figure S5B). No significant change in density was observed in the CR$^+$ interneuron subpopulation in the DG, CA1, or CA3 regions (Figure 4F–F″).

Mice were perfused 2 hours after the EPM test to allow for maximal accumulation of the protein products of *c-Fos*, an immediate early gene used as a proxy for neuronal activation, in interneurons (44). No differences in the percentage of PV$^+$ or SST$^+$ cells colabeled with c-Fos in the DG or CA1 were observed (Figures 4H, H′, J, and J′), indicating that overt

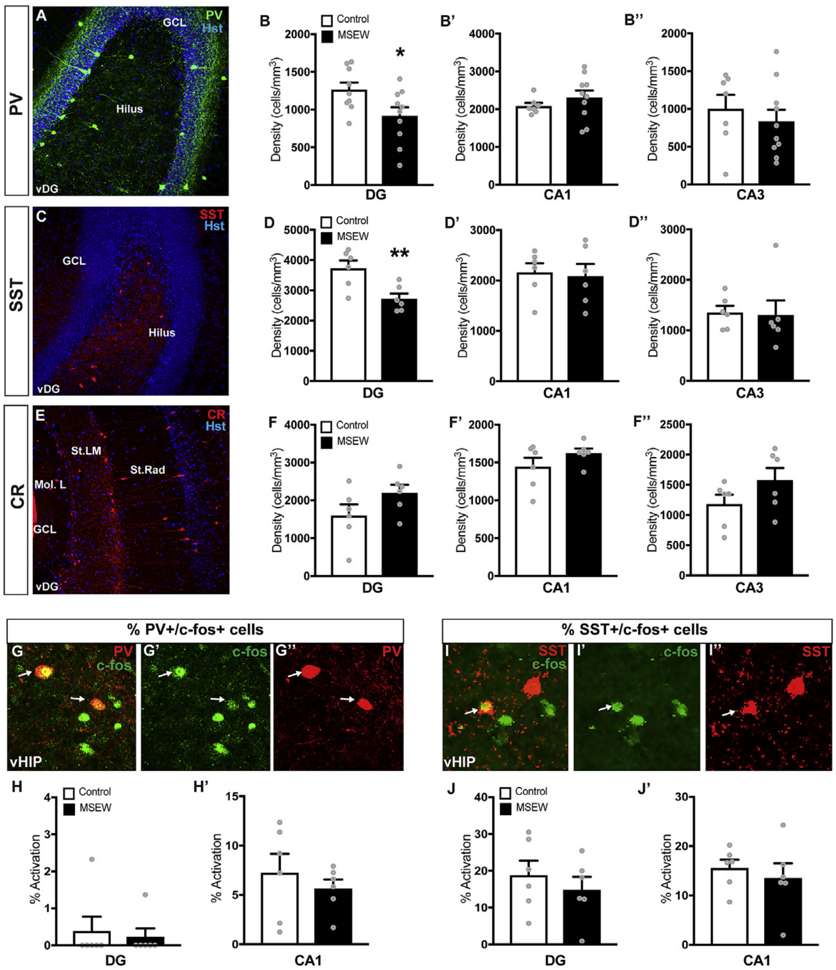

Figure 4. **(A)** Parvalbumin positive (PV^+) interneuron distribution in ventral dentate gyrus (vDG). **(B–B″)** Maternal separation with early weaning (MSEW) significantly reduced PV^+ interneuron density in the vDG but not in the CA1 or CA3 of the ventral hippocampus (vHIP). **(C)** Somatostatin-positive (SST^+) interneuron distribution in the vDG. **(D–D″)** MSEW significantly reduced SST^+ interneuron density in the vDG but not in the CA1 or CA3 of the vHIP. **(E)** Calretinin (CR^+) interneuron distribution in the vDG. **(F–F″)** MSEW did not alter CR^+ interneuron densities in the DG, CA1, or CA3 of the vHIP. **(G–G″)** c-Fos^+ cells that are PV^+ (arrows) in the vHIP after exposure to the elevated plus maze. **(H, H′)** No difference in the percentage of PV^+ cells that are $cFos^+$ in the DG or CA1 of the vHIP was observed. **(I–I″)** c-Fos^+ cell that is SST^+ (arrow) in the vHIP after exposure to the elevated plus maze. **(J, J′)** No difference in percentage of SST^+ cells that are c-Fos^+ in the DG or CA1 of the vHIP was observed. *$p < .05$, **$p < .01$. GCL, granule cell layer; Hst, Hoechst 33342.

differences in the population of PV^+ or SST^+ interneurons activated are not likely the cause of altered neuronal oscillations and behavior.

MSEW Increases the Intensity of PNN Labeling Around PV^+ Interneurons

PNNs are involved in the plasticity and activity of inhibitory interneurons (45). WFA, a plant-based lectin stain that is commonly used to label PNNs, was used along with PV immunolabeling to visualize PNNs around PV^+ cells in the brain (Figure 5A, B). SST^+ and CR^+ interneurons were not found to be surrounded by WFA^+ PNNs (Figure 5C, D). In the granule cell layer of the vDG, we observed that PV^+ cells in MSEW mice had significantly greater WFA intensity (Figure 5E) compared with control mice. However, no significant differences in the intensity of WFA was observed between groups in the vCA1 (Figure 5G). The overall density of WFA^+ cells in either region was also not affected between groups (Figure 5F, H). No differences were observed in the percentage of PV^+ cells that were WFA^+ in the vCA1 or the vDG between control mice and MSEW mice (Supplemental Figure S4).

MSEW Increases OTX2 Labeling Intensity

OTX2 produced in the choroid plexus binds to PNNs in the visual cortex where it facilitates the expression of PNNs (28). MSEW mice showed a significant increase in immunofluorescence intensity of OTX2 in the choroid plexus where it is produced when compared with control mice (Figure 6B). To verify that the increase in OTX2 was also present in PV^+/WFA^+ cells in the vDG, sections were triple-labeled for OTX2, PV, and WFA, and OTX2 intensity in PV^+/WFA^+ cells in the vDG was analyzed (Figure 6C–C″). As in the choroid plexus, MSEW mice showed increased intensity of OTX2 in PV^+/WFA^+ cells (Figure 6D), while PV^+/WFA^- cells showed no such increase (Figure 6E). Previous studies have shown that OTX2 is not synthesized in the vHIP (46), and our findings suggest that PV^+/PNN^+ cells in the vHIP sequester OTX2 in greater amounts after MSEW.

MSEW Has No Effect on the Density of New Neurons or Proliferating Cells

Adult-generated neurons affect neuronal oscillations in the hippocampus (36) and have been linked to stress-induced anxiety-like behavior (47–49), and therefore we examined the numbers of DCX^+ immature neurons and $Ki67^+$ proliferating

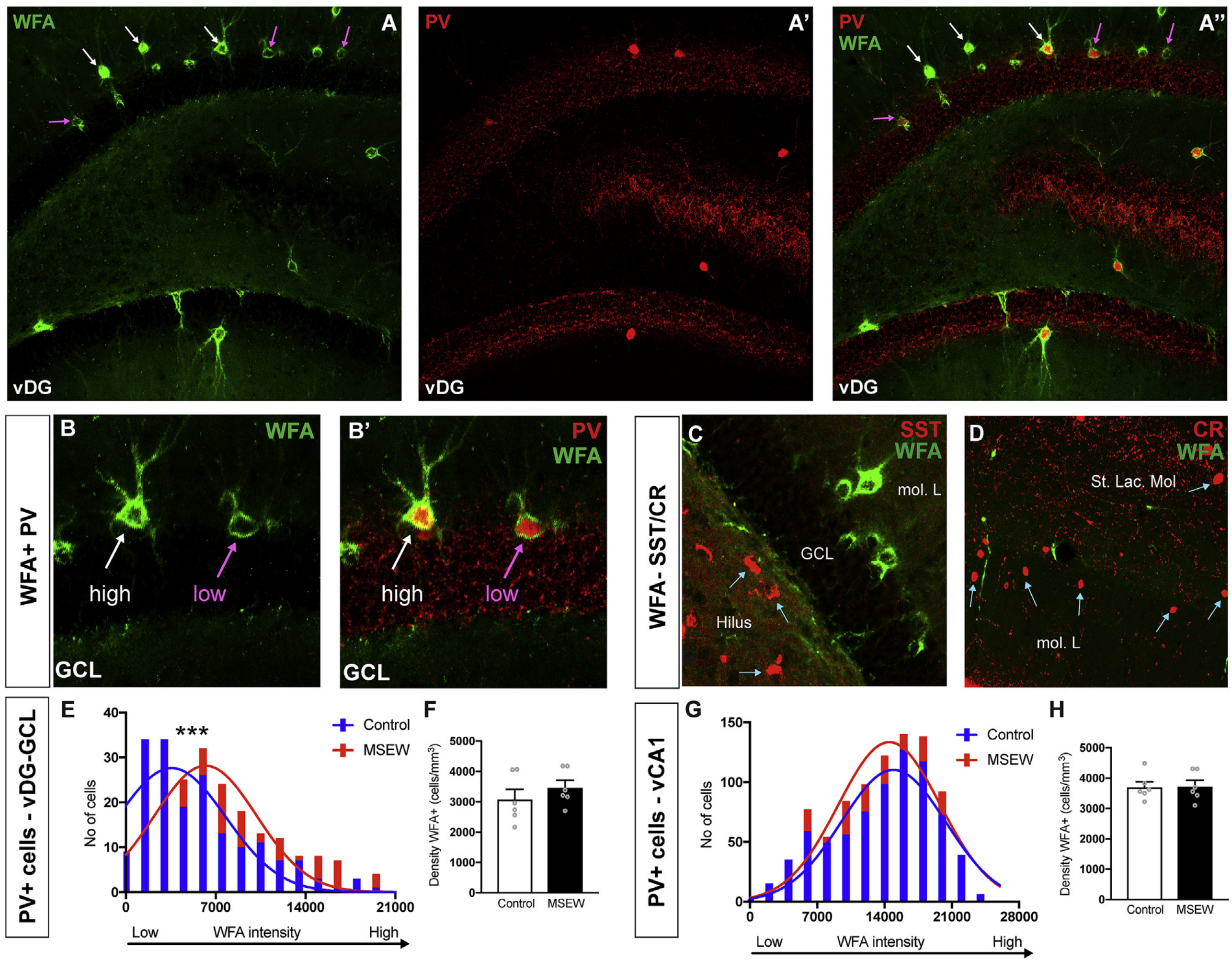

Figure 5. **(A–A″)** Parvalbumin-positive (PV^+) cells and *Wisteria floribunda* agglutinin–positive (WFA^+) perineuronal nets (PNNs) in the ventral dentate gyrus (vDG) of maternal separation with early weaning (MSEW) mice. **(B, B′)** Higher magnification images depicting high and low intensity WFA-stained PNNs surrounding PV^+ cells. **(C, D)** Somatostatin-positive (SST^+) and calretinin-positive (CR^+) interneurons are not surrounded by WFA^+ PNNs. **(E)** MSEW mice showed a significant increase in intensities of WFA-labeled PNNs in the vDG–granule cell layer (GCL). **(F)** Graph showing no substantial differences between groups in the density of WFA^+ cells in the vDG–GCL. **(G)** Graph showing no substantial differences between groups in the intensities of WFA-labeled PNNs in the ventral CA1 (vCA1). **(H)** Graph showing no substantial differences between groups in the density of WFA^+ cells in the vCA1. $^*p < .05$, $^{***}p < .001$.

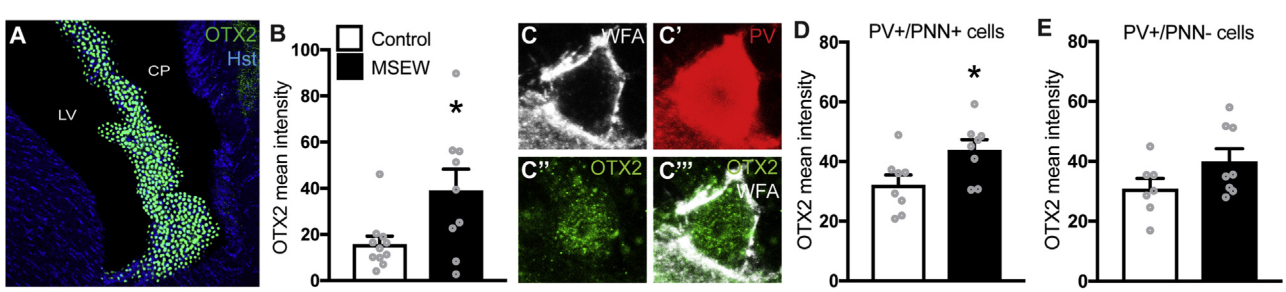

Figure 6. **(A)** Choroid plexus (CP) stained with orthodenticle homeobox protein 2 (OTX2) in the lateral ventricle (LV). **(B)** Graph showing increased OTX2 labeling intensity in the choroid plexus of maternal separation with early weaning (MSEW) mice. **(C–C‴)** Cell colabeled for *Wisteria floribunda* agglutinin (WFA), parvalbumin (PV), and OTX2 in the ventral dentate gyrus–granule cell layer. **(D)** Graph showing increased OTX2 labeling intensity in PV^+ WFA^+ cells in the ventral dentate gyrus of MSEW mice. **(E)** PV^+ WFA^- cells do not show an equivalent increase in OTX2 labeling in the ventral dentate gyrus of MSEW mice. $^*p < .05$. Hst, Hoechst 33342; PNN, perineuronal net.

cells in the vDG of MSEW and control mice. No differences were detected in DCX^+ or $Ki67^+$ cell densities between MSEW and control mice (Supplemental Figure S3A, B).

DISCUSSION

Our findings confirmed previous studies that adult male mice subjected to MSEW exhibit an increase in anxiety-like behavior and activity levels (11). We further showed that MSEW increased theta power in the vHIP when in a novel environment, but not in a familiar environment, as well as increased phase amplitude coupling between theta and gamma in both settings. The analysis of cellular subtypes within the vHIP revealed that MSEW resulted in reduced densities of PV^+ and SST^+ interneurons in the vDG but revealed no change in densities of CR^+ interneurons, $GAD67^+$ interneurons, DCX^+ immature neurons, or $Ki67^+$ proliferating cells. Further analysis of PV^+, SST^+, and CR^+ subtypes revealed that only PV^+ interneurons were surrounded by PNNs, which were present in increased intensities around PV^+ interneurons in the vDG of MSEW mice. Reduced PV expression in PV^+ cells was also observed after MSEW. These changes were accompanied by an increase in labeling intensity of the transcription factor OTX2 in the choroid plexus as well as in PV^+ interneurons in the vDG. Taken together, our findings show that early life adversity produces altered neuronal oscillations in the vHIP of adult male mice and further demonstrate cellular changes in interneurons that may be causally linked to the electrophysiological and behavioral alterations.

MSEW is an adaptation of the maternal separation paradigm developed to produce reliable increases in anxiety-like behavior in adult mice (11). Early weaning by itself results in increased anxiety in both mice and rats (50,51). The combination of early weaning with maternal separation also results in hyperactivity in mice (11). We did not observe any changes in anxiety-like behavior in female mice that were subjected to MSEW. The lack of an increase in anxiety among female rodents subjected to early life stress is consistent with several other studies [as reviewed by Murthy and Gould (52)] and surprising given that women are more susceptible to anxiety and depressive disorders than men (53). These inconsistent findings raise questions about whether traditional methods of assessing anxiety in male rodents may not be adequate for female rodents (52). It is also relevant to note that while overall significant differences in behavior were observed in two separate cohorts of male mice, within each study it was clear that not all mice were adversely affected by MSEW. These findings are also consistent with the work of others (52) and raise interesting questions about individual differences in resilience and susceptibility, the mechanisms of which will be the focus of future work.

Our data showing increased vHIP theta power in the novel environment are consistent with reports showing increased theta power and increased coherence between the mPFC and vHIP during heightened states of anxiety (20). In addition, enhanced theta–gamma coupling in MSEW mice when in either environmental setting indicates an inherent alteration of the electrophysiological profile. Behavioral tasks can modulate phase amplitude coupling, which has previously been implicated in sensory integration, memory processes, and attentional selection (54–56). Specifically, theta–gamma coupling in the dorsal hippocampus has been implicated in cognitive function (57), and this coupling in the amygdala has been linked to periods of heightened anxiety (58). Increased theta–gamma coupling has also been associated with greater dysfunctional attention/arousal behaviors in children with attention-deficit/hyperactivity disorder (59). While the involvement of PV^+ interneurons in theta–gamma coupling has been previously established (23), our study is the first to report enhanced theta–gamma coupling in the vHIP in the context of early life adversity and anxiety/hyperactivity.

Adult neurogenesis in the hippocampus has been implicated in some anxiety- and depression-related behaviors (60,61). Previous work showed that maternal separation stress reduced adult neurogenesis in the rat hippocampus (37,62–64), although other studies failed to show this (65). New neurons may be involved in modulating neuronal oscillations in the hippocampus as ablation of adult neurogenesis destabilizes network activity in vivo (36). While we did not observe differences in DCX^+ and $Ki67^+$ cell densities between MSEW mice and control mice, postsynaptic connections of new neurons to other cell types may be altered. Immature neurons project to interneurons in the DG and reports have documented the role of interneurons in modulating neuronal circuitry controlling hippocampal neurogenesis, as well as the activity of new neurons in vivo (38,39). It is possible that altered connections between new neurons and interneurons in the DG adversely affect neuronal networks, resulting in the altered electrophysiological profile of MSEW mice.

Interneurons, especially the PV^+ subtype, play an important role in the regulation of neuronal oscillations in the brain (24–27) and have also been implicated in neuropsychiatric disease (66,67). PV^+ interneurons appear to be the main generators of intrinsic theta oscillations in the hippocampus (24). In fact, disconnecting PV cell activity from the fast spiking inhibitory network in the hippocampus reduces both theta power and theta–gamma coupling (23) These reports along with our data suggest that increased rhythmic inhibitory inputs to PV^+ cells may underlie the increased theta power and theta–gamma coupling observed in MSEW mice. The reduction in PV^+ interneuron densities we observed in the MSEW hippocampus is likely caused by decreased PV protein levels rather than the loss of neurons given our data on PV expression and $GAD67^+$ cell densities, as well as previous studies showing reductions in PV protein levels caused by early life adversity in the mPFC of juvenile mice (68,69). Along these lines, it is worth noting that experimentally induced theta bursts reduce PV expression in other brain regions (70,71), raising the possibility that the changes we observed in PV intensity are the result of, as opposed to being responsible for, the altered electrophysiological profile observed with MSEW mice. Assessing this possibility will be the focus of future studies.

SST^+ interneurons, on the other hand, have been demonstrated to indirectly contribute to the entrainment of theta oscillations in the hippocampus (25) and are responsible for preferentially driving beta (15–30 Hz) frequency oscillations in other brain regions (72). We observed an increase in alpha/beta frequency oscillation power in the vHIP of MSEW mice, which could be related to decreased SST^+ interneuron intensities and possibly activity of this cell type. However, the exact role of SST^+ interneurons in MSEW-induced changes in neuronal oscillations needs to be more completely investigated in future studies.

Our findings reporting increased intensities of WFA-labeled PNNs around PV^+ interneurons suggest an altered plasticity of PV^+ cells because PNNs have been known to regulate synaptic and cellular plasticity of the cells they encapsulate (45), and digestion of PNNs around PV^+ cells has been shown to reduce the fast spiking properties and excitability of PV^+ cells (73). In fact, the maturation of PNNs around PV^+ cells in the visual cortex coincides with the closure of the critical period for the development of binocular vision; degradation of PNNs has been shown to reopen this critical period in the visual cortex (74,75). PNN degradation also reduces PV^+ interneuron activity and alters gamma- and theta-range neuronal oscillations in rats (29). PNN degradation has also been shown to reduce theta synchrony between the amygdala and the association visual cortex—brain regions that are involved in fear memory recall (30). However, no previous studies have investigated how early life adversity impacts PNNs, and our findings raise the possibility that increased PNNs around PV^+ cells may drive the increase in theta oscillations and the elevated anxiety and hyperactivity observed in MSEW mice.

OTX2 is a transcription factor that is known to be important in the regulation of the critical period of plasticity in the visual cortex of developing mice (46), and its expression coincides with the maturation of PV^+ cells and the closure of the critical period (35). In addition, OTX2 has been shown to specifically accumulate in PV^+ cells in the visual cortex through exogenous transfer from the choroid plexus, the region where it is produced (35,46). Our findings showed an increase in intensity of labeling of OTX2 in the choroid plexus, suggesting an increase in OTX2 production in MSEW mice. We hypothesized that this could lead to an increase in accumulation of OTX2 in cells of the vHIP, where OTX2 is not made (46) but may work in a non–cell autonomous way as it does elsewhere in the brain (28). Our observations showed that MSEW mice displayed increases in OTX2 labeling intensity in PV^+ PNN^+ cells in the vHIP. Continual binding of OTX2 to PNNs has been shown to be necessary for synaptic stability in the adult visual cortex, and blocking OTX2 uptake into PV^+ cells in adults has been demonstrated to reduce PNN expression and reinstate juvenile levels of plasticity (28). Our results are consistent with an OTX2-mediated mechanism of action for modulating PNNs and therefore PV^+ cell plasticity in the vHIP. Additional discussion of the role of cell autonomous OTX2 in stress resilience (76) can be found in the Supplement.

These findings show that early life adversity results in an altered electrophysiological profile in the vHIP in adulthood, which is associated with an increase in anxiety and hyperactivity in males. The data presented here further indicate that OTX2, PNNs, and PV^+ interneurons are affected by MSEW and that these changes may participate in altering neuronal oscillations underlying anxiety and hyperactivity. Understanding such mechanisms is important in the identification of novel targets to develop circuit-level interventions for countering the negative impact of early life adversity on neuropsychiatric disease.

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