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Prenatal alcohol exposure alters synaptic activity of adult hippocampal dentate granule cells under conditions of enriched environment

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Abstract

Prenatal alcohol exposure (PAE) results in fetal alcohol spectrum disorder (FASD), which is characterized by a wide range of cognitive and behavioral deficits that may be linked to impaired hippocampal function and adult neurogenesis. Preclinical studies in mouse models of FASD indicate that PAE markedly attenuates enrichment-mediated increases in the number of adult-generated hippocampal dentate granule cells (aDGCs), but whether synaptic activity is also affected has not been studied. Here, we utilized retroviral birth-dating coupled with whole cell patch electrophysiological recordings to assess the effects of PAE on enrichment-mediated changes in excitatory and inhibitory synaptic activity as a function of DGC age. We found that exposure to an enriched environment (EE) had no effect on baseline synaptic activity of 4 week or 8 week old aDGCs from control mice, but significantly enhanced the excitatory/inhibitory ratio of synaptic activity in 8 week old aDGCs from PAE mice. In contrast, exposure to EE significantly enhanced the excitatory/inhibitory ratio of synaptic activity in older pre-existing DGCs situated in the outer dentate granule cell layer (i.e., those generated during embryonic development; dDGCs) in control mice, an effect that was blunted in PAE mice. These findings indicate distinct electrophysiological responses of hippocampal DGCs to behavioral challenge based on cellular ontogenetic age, and suggest that PAE disrupts EE-mediated changes in overall hippocampal network activity. These findings may have implications for future therapeutic targeting of hippocampal dentate circuitry in clinical FASD.

Keywords

Neurogenesis; Electrophysiology; Fetal alcohol spectrum disorder; Environmental Enrichment

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Introduction

Fetal alcohol spectrum disorder (FASD) is a prevalent condition (2–5% in U.S.) that encompasses a range of clinical outcomes, from severe mental retardation as a consequence of high dose alcohol exposure (fetal alcohol syndrome), to more subtle behavioral abnormalities as a consequence of moderate gestational alcohol exposure (Riley et al., 2011) (May et al., 2009). Few empirically tested therapeutic interventions exist for mitigating the effects of FASD (Kodituwakku and Kodituwakku, 2011). Common behavioral problems in clinical FASD include those associated with hippocampal dysfunction that impact cognition and mood (Mattson et al., 2011). These behavioral disorders are thought to be due, in part, to long-lasting neurochemical and electrophysiological alterations in hippocampal circuitry (Berman and Hannigan, 2000).

The hippocampal dentate is a unique brain structure comprised of a heterogeneous population of DGCs of various cellular ages which exhibit ontogenetic age-dependent differences in network function (Kim et al., 2012). The adult hippocampal subgranular zone continuously generates cohorts of immature DGCs, which display unique connectivity, hyper-excitability and plasticity during a critical period between 4–8 weeks of cellular age. As their maturation progresses, aDGCs eventually resemble older, less active granule cells that are generated during embryonic and early postnatal development (dDGCs), in terms of both connectivity and electrophysiological properties (Laplagne et al., 2006). Although immature aDGCs only constitute an estimated 5–6% of the total DGC population, they exert unique and profound effects on hippocampal network activity, and are critical for the encoding of certain forms of episodic memory (Gu et al., 2012; Nakashiba et al., 2012; Piatti et al., 2013). Optogenetic silencing of immature aDGCs impairs spatial memory (Gu et al., 2012), whereas silencing of older, more mature DGCs enhances spatial learning performance (Nakashiba et al., 2012), further underscoring cellular age-dependent differences in network function. Recent work has also elucidated unique presynaptic inputs onto immature aDGCs, which are temporally regulated during cellular maturation and dynamically regulated by experience (Bergami et al., 2015; Vivar et al., 2015).

A number of studies using preclinical rodent models of FASD have demonstrated that prenatal alcohol exposure (PAE) exerts long-lasting impairments in the production of newborn DGCs in adulthood (Boehme et al., 2011; Choi et al., 2005; Gil-Mohapel et al., 2010; Helfer et al., 2009; Ieraci and Herrera, 2007; Kajimoto et al., 2013; Klintsova et al., 2007; Redila et al., 2006). We have previously demonstrated that moderate PAE had no effect on the production of aDGCs in mice exposed to standard laboratory housing conditions, but severely impaired the neurogenic response to social and physical enrichment (Choi et al., 2005; Kajimoto et al., 2013). Although prior studies have demonstrated that PAE can exert long-lasting effects on DGC function (Berman and Hannigan, 2000; Valenzuela et al., 2012), those studies did not take into account functional differences based on ontogenetic cellular age. Here, we utilized retroviral birth-dating coupled with whole cell patch electrophysiological recordings to assess the effects of PAE on enrichment-mediated changes in excitatory and inhibitory synaptic activity of DGCs in adult hippocampus. Our findings demonstrate that PAE alters baseline synaptic activity under conditions of enrichment in a manner that is dependent upon DGC maturational age.

Materials and Methods

Animals and PAE

All animal experiments were performed in accordance with protocols approved by the University of New Mexico Animal Care and Use Committee. C57/BL6J male mice (Jackson Laboratory, Bar Harbor, ME) housed under reverse 12-hour dark / 12-hour light cycle (lights off at 08:00 hours) were used for all experiments. PAE was performed using a previously described limited access paradigm of maternal drinking (Brady et al., 2012; Kajimoto et al., 2013). Briefly, 60 day old female mice were subjected to a ramp-up period with 0.066% saccharin containing 0% ethanol (2 days), 5% ethanol (2 days) and finally 10% ethanol for 4 hrs per day from 10:00–14:00 daily beginning 2 weeks prior to pregnancy, and continued throughout gestation. Average daily alcohol consumption was 6.98 ± 0.76 gm/kg/d (n=24 dams). This paradigm has previously been shown to result in an average daily blood ethanol concentration (BEC) of ~88 mg/dl after 4 hours of drinking, with ethanol consumption rate predictive of BEC (Brady et al., 2012; Kajimoto et al., 2013). Female mice offered 0.066% saccharin without ethanol during the same period of time throughout pregnancy served as controls. There was no effect of alcohol consumption on the number of pups per litter (Control litter size; 7.09 ± 1.59 pups and PAE litter size; 6.50 ± 1.55 pups) or the percentage of male vs. female offspring. On the day of birth, ethanol and saccharin concentrations were gradually decreased, with return to water only by postnatal day 5. All offspring were gender segregated at weaning and placed into standard housing or enriched environment, as described below. Given the technical challenges for patch clamp recordings across multiple treatment groups, only male pups were used for further study to avoid variability due to gender-specific effects of PAE.

Retrovirus injection

Between postnatal days 40–50, all PAE and control offspring received stereotaxic injection of replication-deficient murine retrovirus to confer expression of green fluorescent protein (GFP) to hippocampal DGC progenitor cells, targeting the dorsal and ventral hilar regions of the hippocampal dentate, as previously described (Ge et al., 2006). Briefly, 0.5 μ l of retrovirus was stereotaxically delivered to 4 sites targeting the dorsal and ventral hippocampal subgranular zone bilaterally, using a 1 μ l Hamilton syringe. Stereotaxic coordinates were measured from bregma as follows: -2 mm AP, ± 2.0 mm L, and -2 mm DV for dorsal hippocampus and -3 mm AP, ± 2.6 mm L and -2.5 mm DV for sites in the ventral hippocampus.

Enriched Environment (EE)

Standard housing included a standard mouse cage (28 cm \times 18 cm \times 13 cm) without running wheels or toys (3 mice/cage). EE included a larger cage (48 cm \times 27 cm \times 20 cm) with 2 running wheels and multiple objects (ladder, tunnel and hanging toys that were changed out daily), and 6 mice/cage (Supplementary Figure 1). Mice were housed under EE for 4 or 8 weeks until sacrifice. Mice were weighed weekly. There was a significant main effect of housing on weight ($p < 0.001$), but no effects of alcohol treatment (Supplementary Figure 2). Previous studies using EthoVision™ Motion Tracking demonstrated no difference between

PAE and control mice in the duration of time spent in the various EE cage zones (Kajimoto et al., 2013).

Electrophysiological recordings

Mice were anesthetized with ketamine (250 mg/kg, i.p.), transcardially perfused with pre-oxygenated (95% O₂/5% CO₂) cold cutting solution containing (in mM): 220 sucrose, 2 KCL, 1.25 NaH₂PO₄, 26 NaHCO₃, 12 MgSO₄, 10 glucose, 0.2 CaCl₂. Brains were removed, incubated for 2 minutes in pre-oxygenated (95% O₂/5% CO₂) ice-cold cutting solution, and bisected in the midsagittal plane, with the right hemisphere used for electrophysiological recordings. Transverse hippocampal slices (250 μm thick) were prepared using a vibratome and allowed to recover in artificial cerebrospinal fluid (ACSF) as described previously (Diaz et al., 2014). GFP⁺ aDGCs were detected using an Olympus BX51WI microscope equipped with epifluorescence illumination, whereas GFP⁻ dDGCs were identified based on their position in the outermost dentate granule cell layer (Mathews et al., 2010; Muramatsu et al., 2007). All recordings were performed with an Axopatch 200B amplifier and Clampex 9.2 software (Molecular Devices, Sunnyvale, CA,) as previously described (Diaz et al., 2014). Miniature spontaneous excitatory postsynaptic currents (mEPSCs) were detected using an internal pipet solution containing (in mM) 120 K-gluconate, 15 KCL, 4 MgCl₂, 0.1 EGTA, 10 HEPES, 4 MgATP, 0.3 NaGTP, 7 phosphocreatine, 5 QX-314 Br (pH 7.4, 290–300 mOsm), with 100 μM of picrotoxin and 0.5 μM tetrodotoxin (TTX) added to the recording ACSF. Miniature spontaneous inhibitory postsynaptic currents (mIPSCs) were detected using the following internal pipet solution (in mM): 19 K-gluconate, 121 KCl, 5 NaCl, 4 MgCl₂, 0.1 EGTA, 10 HEPES, 4 MgATP, 0.3 NaGTP, 10 phosphocreatine, 5 QX-314 Cl (pH 7.4, 300–310 mOsm), in the presence of 3 mM of kynurenic acid and 0.5 μM TTX added to the recording ACSF. Event data were analyzed using the Mini Analysis Program (Synaptosoft, Decatur, GA). 3–5 total DGCs were recorded for each mouse (for both mEPSC and iPSC, which were performed in separate recording sessions), with at least one of these cells from the GFP⁺ aDGC population and one from GFP⁻ dDGC population in the outermost region of the dentate granule cell layer. Recordings were averaged for each mouse, and n=number of mice (see statistics below).

Intracellular biocytin cell filling and staining

Where indicated, dDGCs recorded from the outermost region of the dentate granule cell layer were backfilled with biocytin for morphological analysis using 0.5% of biocytin (Sigma) added to the internal pipet solution. The slices were then incubated in the recording chamber for 15–20 minutes to allow biocytin to fill distal processes, followed by fixation in 4% paraformaldehyde in 0.1M phosphate-buffered saline (PBS) overnight. Slices were washed with PBS for 30 minutes, permeabilized with 0.4% Triton-X100 in PBS for 30 minutes and incubated with Cy-3-conjugated Streptavidin (0.125 μg/ml in PBS containing 1% BSA) for 90 minutes. Slices were mounted on glass slides and coverslipped with Fluoromount-G™ (Electron Microscopy Sciences, Hatfield, PA).

Morphological analysis

All images were taken using a Zeiss 510 META confocal microscope. Dendritic branching complexity was analyzed using Sholl analysis of biocytin-filled dDGCs. Briefly, an image stack was acquired using a 20× objective with 1 μm optical intervals, and compressed into a single image plane by applying the maximum intensity projection function. All dendrites from a single biocytin-filled neuron were traced using NeuroLucida software (MBF Bioscience, Inc. Williston, VT). Dendritic intersections crossing each concentric ring, (beginning 10 μm from the center of the cell soma), were counted manually, with concentric rings spaced at 10 μm intervals. For spine density measurements, the left hemisphere of each brain was immediately fixed with 4% PFA overnight, followed by overnight cryoprotection with 30% sucrose in PBS. Transverse hippocampal sections (60 μm thickness) were obtained using a freezing sliding knife microtome. Sections were mounted on glass slides and coverslipped with Fluoromount-GTM. Confocal image stacks were taken using a 100× oil objective with 0.2 μm optical section intervals. For each mouse, >50 μm of distal dendrite segments were sampled.

Statistics

Data were analyzed by two-way ANOVA with post-hoc multiple comparison (Sidak's or Tukey tests) using Prism (Graph Pad, San Diego, CA). N's represent the number of mice (n=6 mice per group; with whole cell patch electrophysiological recordings from 3–4 neurons per mouse, (which included at least 1 GFP– and 1 GFP+ cell in each recording session). To avoid litter effects, each mouse within any given treatment/housing group was taken from a different litter. Data are expressed as means ± S.E.M., with p<0.05 considered statistically significant.

RESULTS

PAE facilitates excitatory synaptic activity in aDGCs following EE

Utilizing the limited access PAE model, we previously demonstrated that exposure to moderate levels of alcohol throughout gestation significantly impaired the numerical neurogenic response to enriched environment (EE) (Choi et al., 2005; Kajimoto et al., 2013). To evaluate the impact of prenatal alcohol on functional synaptic activity in newly generated aDGCs in this PAE/EE paradigm, we applied a retroviral labeling and birth-dating approach coupled with whole cell patch clamp recordings. Following stereotaxic delivery of GFP-retrovirus, which targets dividing hippocampal progenitors, PAE and control mice were exposed to standard housing (SH) or EE conditions. Whole cell patch electrophysiological recordings of GFP+ aDGCs were performed at 4 and 8 weeks post retroviral injection, a period that spans a critical window of cellular maturation in which aDGCs are thought to uniquely influence hippocampal network activity (Piatti et al., 2013). Experimental design and GFP+ aDGC labeling is shown in Figure 1A and 1B, respectively.

Glutamatergic excitatory synaptic transmission—As shown in Figure 1C and 1D, spontaneous miniature excitatory postsynaptic currents (mEPSCs) were readily detectable in both 4 and 8 week-old GFP+ aDGCs from all mice, as indicated by whole cell patch electrophysiological recordings in ACSF containing picrotoxin (100 μM;

representative traces are depicted in Figs. 1C,1D). The mEPSCs were abolished by 3 mM kynurenatate (representative lower trace in Figs. 1C, 1D), indicating that these events were mediated by ionotropic glutamate receptors.

As anticipated, the frequency of spontaneous mEPSCs increased ~2-fold between 4 and 8 weeks in GFP⁺ aDGCs from both control-SH and PAE-SH groups ($p < 0.05$; Figs. C₁,D₁), reflecting the maturation and synaptic integration of aDGCs that occurs during this period (Ge et al., 2006). At 4 weeks, there were no significant effects of EE on excitatory synaptic transmission in any group (Fig. C₁–C₃). However, at 8 weeks, aDGCs from PAE-EE mice displayed an approximate 2-fold increase in mEPSC frequency that was not observed in aDGCs from control-EE mice (Fig. 1D₁; treatment \times housing interaction ($F(1, 20) = 5.056$, $p = 0.04$). PAE-EE aDGCs also displayed a small but significant decrease in mEPSC amplitude (Fig. 1D₂) and an increase in mEPSC decay time (Fig. 1D₃) at 8 weeks, neither of which were observed in control-EE mice (post hoc analysis, $p < 0.03$ for both amplitude and decay, PAE-EE vs. PAE-SH). These observations indicate that PAE significantly enhances action potential-independent glutamatergic synaptic transmission in aDGCs under conditions of EE in an ontogenetic age-dependent manner. Although the net effect of increased frequency and decay time of mEPSCs may be partially offset by reduced amplitude, all changes in glutamatergic neurotransmission in response to EE were only observed in PAE mice, indicating a potential impact of PAE on network activity.

GABAergic inhibitory synaptic transmission—As shown in Figure 2, spontaneous miniature inhibitory postsynaptic currents (mIPSCs) were also readily detectable in GFP⁺ aDGCs in all mice, with a slight increase in frequency between 4 and 8 weeks as assessed by whole cell patch electrophysiological recordings in ACSF containing 3 mM kynurenatate (Fig 2). Miniature IPSCs were blocked in the presence of 100 μ M bicuculline (Figs. 2A,B, bottom traces), indicating that these events were mediated by GABA_A receptors. In contrast to glutamatergic excitatory transmission described above, EE had no significant influence on mIPSC frequency (Figs. 2A₁,B₁) or decay (Figs. 2A₃,B₃) in 4 or 8 week old GFP⁺ aDGCs in any group. However, EE induced a small but significant decrease in the amplitude of mIPSCs in 8 week-old aDGCs recorded from control mice ($F(1, 20) = 11.39$; $p = 0.003$), which was not observed in PAE mice ($p = 0.005$).

Dendritic spine density in adult-generated DGCs—Environmental enrichment had no significant impact on distal spine density of GFP⁺ aDGCs in PAE mice (PAE-SH, 1.72 ± 0.055 vs. PAE-EE, 1.69 ± 0.22 spines/ μ m) or control mice (control-SH, 1.68 ± 0.15 vs. control-EE, 1.87 ± 0.21 spines/ μ m; means \pm SEMs, $n = 6$ mice/group; 8 weeks).

PAE attenuates excitatory/inhibitory ratio of synaptic currents in dDGCs following EE

To compare synaptic activity of newly generated DGCs (aDGCs) with that of older, pre-existing DGCs generated during embryonic and early postnatal development (dDGCs), we recorded from GFP⁺ neurons located in the outermost region of the dentate granule cell layer during each electrophysiological recording session. Previous studies have demonstrated that a cell's birthdate correlates with its subsequent location within the granule cell layer; DGCs born during embryonic and early postnatal development comprise the

outermost dentate granule layer compared to adult-generated cells that layer within the inner and middle regions (Mathews et al., 2010; Muramatsu et al., 2007).

Glutamatergic and GABAergic synaptic transmission—Spontaneous mEPSCs were readily detectable in GFP⁻ dDGCs recorded in the outermost dentate granule cell layer from all mice, and these were blocked by 3 mM kynureate (Fig. 3A). As shown in Figure 3B, dDGCs displayed an approximate 2-fold increase in glutamatergic synaptic current frequency (mEPSC) after EE in control mice. Although there was a trend toward increased frequency of mEPSCs in PAE-EE mice compared to PAE-SH mice, this did not reach statistical significance ($p=0.062$). Two-way ANOVA revealed that both the frequency ($F(1, 20) = 16.07$; $p<0.001$) and decay of mEPSCs ($F(1, 20) = 5.39$; $p=0.031$) were significantly affected by the housing conditions in control mice, with no significant effect on amplitude (Figure 3B–D). Thus, EE stimulated enhanced action potential-independent glutamatergic neurotransmission in dDGCs from control mice, which was attenuated in PAE mice.

Bicuculline-sensitive mIPSCs were readily detectable in dDGCs from all mice (Fig. 3E). As shown in Figure 3F, EE stimulated a significant (~2-fold) increase in mIPSC frequency in dDGCs recorded from PAE-EE mice ($p=0.002$). Although there was a trend toward increased frequency of mIPSC in control-EE mice, this did not reach statistical significance ($p=0.058$). Two-way ANOVA revealed that both the frequency ($F(1, 20) = 19.55$, $p<0.001$) and amplitude ($F(1, 20) = 4.367$, $p=0.049$), but not the decay, of mIPSCs were significantly influenced by housing in PAE mice (Fig. 3 F–H). The amplitude of mIPSC was significantly increased by EE only in PAE mice ($p= 0.018$). These data suggest that dDGCs display a trend toward increased GABA_A receptor-mediated synaptic input in both control and PAE mice following EE, which only reached statistical significance in the PAE group.

Dendritic branching—EE increased branching complexity in distal dendrites of dDGCs in control mice (control-EE), and this effect was abolished by PAE (PAE-EE) (Figure 4). As shown in Figure 4D–E, two-way ANOVA revealed significant housing ($F(1, 20) = 7.3$; $p= 0.014$) and treatment ($F(1, 20) = 4.86$; $p= 0.039$) effects on distal dendritic segments >170 μm from soma (Fig 4D–E). Post-hoc analysis confirmed that the effect of EE on dendritic branching was only significant in control mice (Fig. 4E, $p<0.01$). In addition, total dendritic length was increased by EE treatment only in control mice (Fig. 4F, $p<0.05$). Therefore, distal dendritic complexity is enhanced by environmental stimulation in control mice, an effect that is significantly attenuated by PAE.

Discussion

In this study, we asked whether PAE results in long-lasting changes in synaptic activity in adult hippocampal dentate granule cells following exposure to enriched environment. We used a GFP-retroviral labeling/birth-dating approach coupled with whole cell patch electrophysiological recordings to assess synaptic activity in adult-generated DGCs (aDGCs) at 4 and 8 weeks of age following exposure to standard or enriched housing conditions (SH vs. EE). To assess synaptic activity in older, pre-existing DGCs generated during embryonic and early postnatal development (dDGCs), we performed whole cell patch electrophysiological recordings from GFP⁻ cells in the outermost dentate granule cell layer

where these cells make up the vast majority of DGCs (Mathews et al., 2010). Our results demonstrate distinct electrophysiological responses to EE based on cellular ontogenetic age, and provide evidence that PAE disrupts EE-mediated changes in hippocampal network activity.

Adult-Generated DGCs

We previously demonstrated in the limited access PAE paradigm that exposure to moderate levels of alcohol throughout gestation has no effect on adult neurogenesis under SH conditions, but abolishes the numerical neurogenic response to EE (Kajimoto et al., 2013). Here, we extend those findings to demonstrate altered synaptic activity in aDGCs from PAE-EE mice that is characterized, in part, by heightened excitatory synaptic transmission. Importantly, this effect was dependent upon aDGC maturational state, observed in 8 week but not 4 week old aDGCs from PAE-EE mice. Using rabies virus-based retrograde transynaptic tracing methods, Bergami et al., recently demonstrated that EE exposure between 2–6 weeks following the birth of aDGCs profoundly affects the pattern of monosynaptic input by both local interneurons and long-distance cortical neurons (Bergami et al., 2015). These changes were not observed in 13 week old aDGCs, indicating important changes in network connectivity that occur with a specific time window of aDGC maturation, corresponding to the well-known critical period of enhanced synaptic plasticity of aDGCs previously described (Ge et al., 2007; Kheirbek et al., 2012). Our observation of heightened excitatory neurotransmission in response to EE in aDGCs from PAE, but not from control mice, may represent a compensatory hyper-responsiveness to EE either due to a lower number of aDGCs in PAE-EE compared to their control-EE counterparts, or a disruption in afferent connectivity during a critical period of cellular maturation.

Interestingly, the heightened excitatory response in PAE-EE mice was not associated with increased spine density. Previous studies have shown that neither EE, nor exercise, has a significant impact on total spine density in aDGCs, although both promote increased density of mushroom spines, which are thought to represent sites of enhanced excitatory synaptic transmission (Piatti et al., 2011; Zhao et al., 2015; Zhao et al., 2006). On the other hand, training on discrete spatial tasks stimulates enhanced dendritic complexity and spine density in aDGCs in proportion to the intensity of cognitive demand (Lemaire et al., 2012; Tronel et al., 2010). Although few studies have assessed the electrophysiological correlates of these behavioral paradigms and anatomical changes, a recent study demonstrated a profound effect of EE on the pattern of monosynaptic input to aDGCs that was paradoxically associated with a *reduction* in frequency of spontaneous excitatory currents, suggesting a dissociation of spine density and excitatory input (Bergami et al., 2015). It is currently unknown whether the increased mEPSC frequency in aDGCs from PAE-EE mice reflects a persistent increase of presynaptic glutamate release, activation of existing silent synapses, and/or increased number of synapses. Further investigation will be required to parse out these mechanisms.

In addition to increased frequency of mEPSCs in aDGCs from PAE-EE mice, we also observed small but significant changes in the glutamatergic and GABAergic amplitude and decay, indicating qualitative alterations in synaptic transmission. Several mechanisms could

underlie alteration of current amplitude and decay, including changes in receptor expression or subunit composition, resulting in altered gating kinetics (Koike-Tani et al., 2005; Rouach et al., 2005; Sommer et al., 1990). Indeed, PAE is known to influence the expression and function of NMDA and GABA receptors within the adult hippocampus (Allan et al., 1998; Samudio-Ruiz et al., 2010; Savage et al., 1992), but these have not been studied in aDGCs or within the context of combined PAE and EE.

Regardless of the underlying mechanisms, the enhancement of basal excitatory synaptic transmission following EE in aDGCs from PAE mice raises the question of whether this response is physiologically relevant for hippocampal function. In previous studies, we demonstrated that the impaired neurogenic response to EE in PAE mice is associated with delayed learning in an A-B contextual discrimination learning task, a behavior that is thought to be reliant on neurogenic function (Kajimoto et al., 2013). However, in those studies, PAE-EE mice eventually learned the task to perform at the same level as control-EE mice. This suggests that the surviving subpopulation of aDGCs in PAE-EE mice are functionally competent to mediate this behavioral improvement, albeit over a slower time course compared to control-EE mice.

Mature DGCs in outer region of dentate granule cell layer (dDGCs)

Previous extracellular slice electrophysiological studies have yielded conflicting results regarding the effect of EE on synaptic transmission in dDGCs (Eckert and Abraham, 2013). We are aware of only a few studies that have used patch-clamp techniques to study EE effects on spontaneous excitatory or inhibitory currents in hippocampal DGCs generated during embryonic or early postnatal development, and these focused on the CA1 region. Parsley et al (2007) reported that EE had no significant effect on either the amplitude or the frequency of mEPSCs in CA1 pyramidal neurons (Parsley et al., 2007). In contrast, Malik and Chattarji (2012) found that EE increases the frequency but not the amplitude of mEPSCs in CA1 pyramidal neurons (Malik and Chattarji, 2012). Here, we demonstrate that EE produces a similar effect on mEPSC frequency in dDGCs and also increases the decay time of these events. However, in contrast to our findings of enhanced excitatory synaptic transmission in aDGCs from PAE-EE mice, we found electrophysiological evidence for the *suppression* of this EE-mediated effect on mEPSCs in dDGCs from PAE mice. PAE not only attenuated the EE-mediated increase in basal excitatory synaptic transmission in dDGCs, but also exacerbated inhibitory neurotransmission as indicated by an increase in mIPSC frequency that reached significance only in PAE-EE mice. Several studies have demonstrated impaired LTP within DGCs from rodent models of PAE, as measured by field excitatory postsynaptic potential potentiation (Brady et al., 2013; Sutherland et al., 1997; Varaschin et al., 2010). In addition, PAE has been shown to attenuate EE-mediated enhancement of dendritic spine density in CA1 pyramidal neurons (Berman and Hannigan, 2000). Our studies add to these findings, to demonstrate an imbalance between excitatory and inhibitory synaptic transmission in favor of the latter in PAE-EE mice, which is correlated with impaired morphological plasticity in dDGCs.

Conclusion

The overall findings from this study indicate that PAE impairs the responsiveness of the dentate gyrus to EE-stimulated inputs. In newly generated aDGCs, the increased excitatory transmission in 8 week old cells perhaps reflects increased response to EE in the context of significantly fewer numbers of aDGCs in the PAE group. By contrast, our data suggest a blunted responsiveness to EE in older DGCs that are generated earlier in development (dDGCs), with a concomitant increase in GABAergic transmission. Together, both of these effects suggests that PAE significantly impairs the dentate responsiveness to EE, which may have therapeutic implications in FASD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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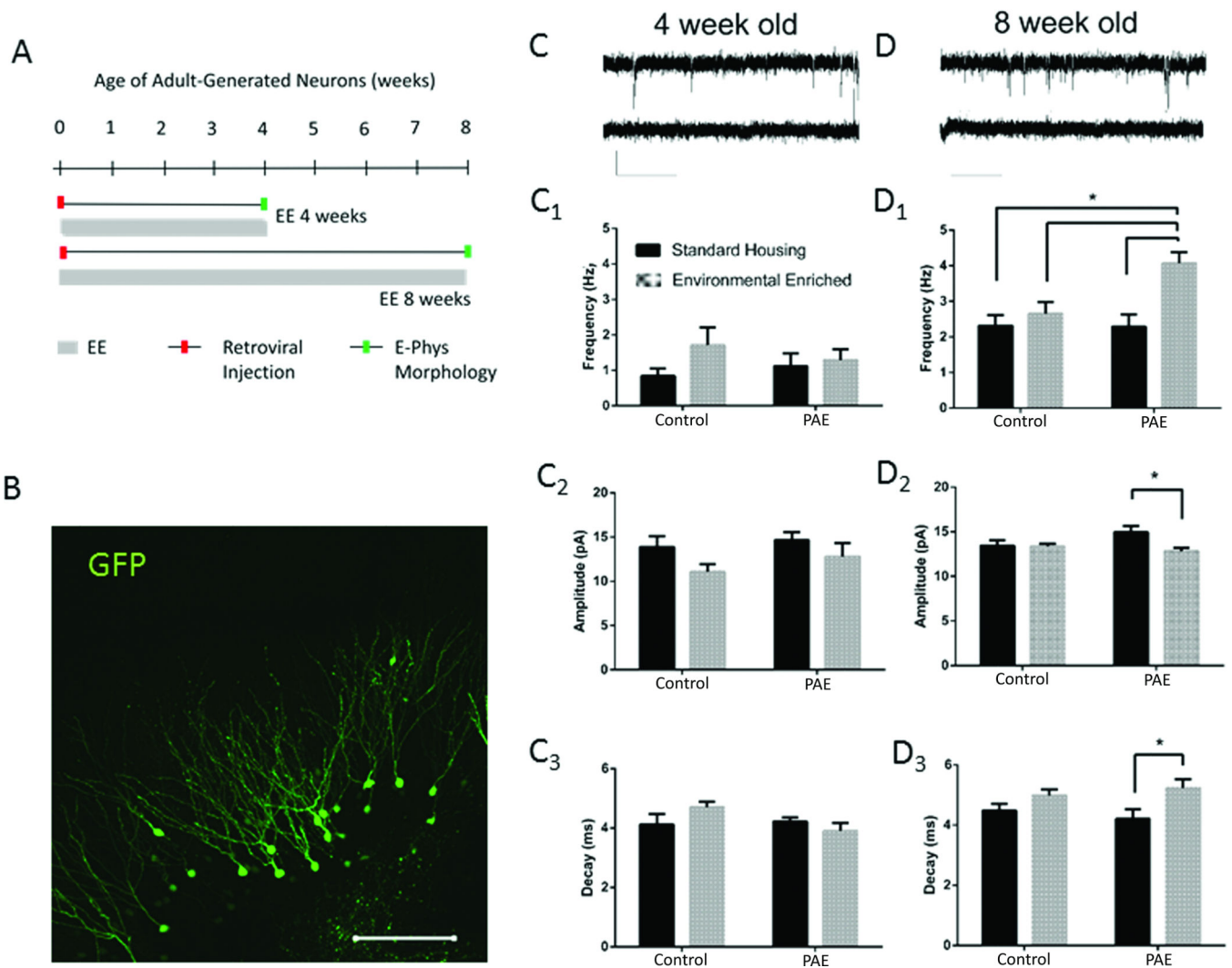
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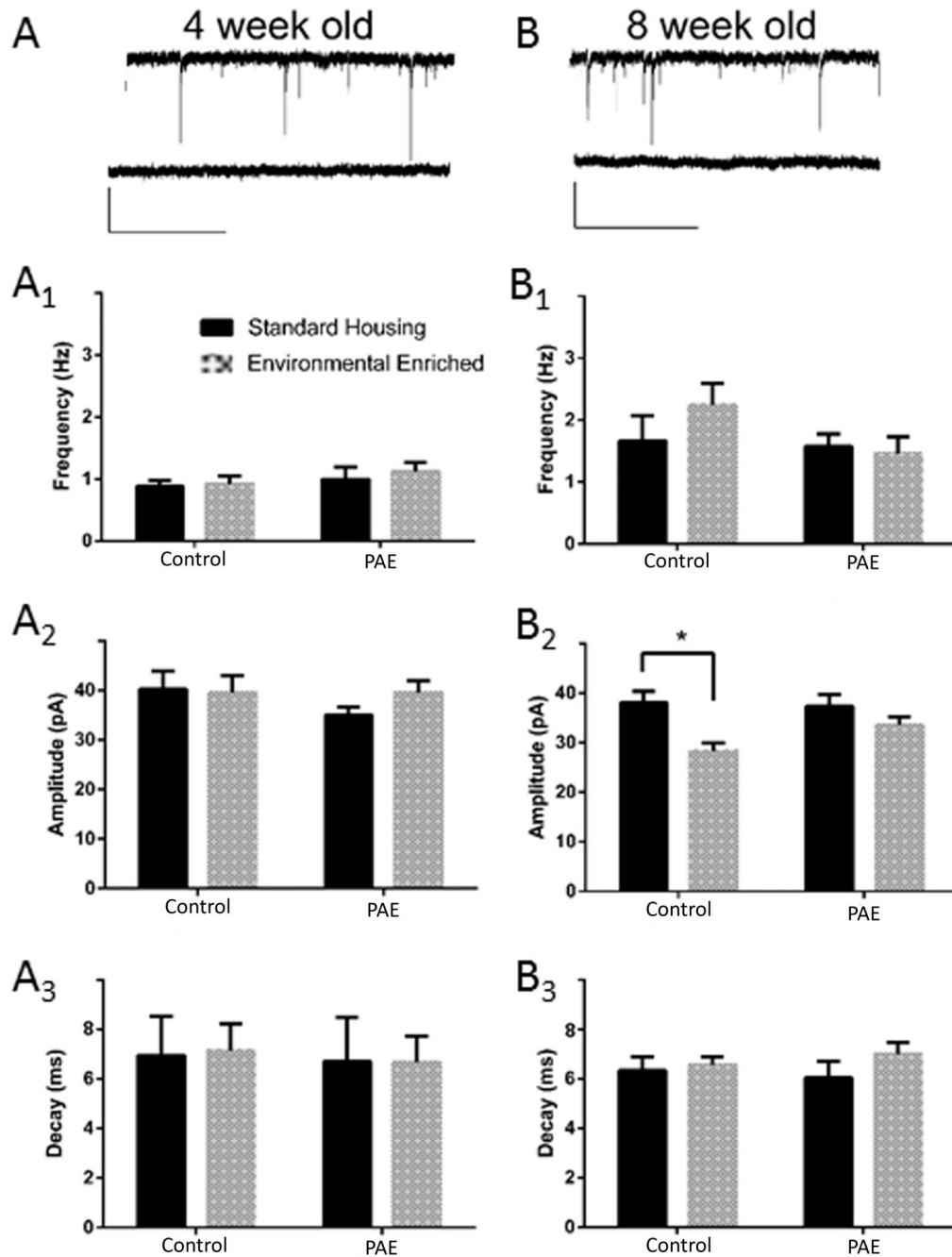


Figure 2. mIPSC Recordings from GFP⁺ Adult-Generated DGCs

(A and B) Whole cell patch traces recordings from individual GFP⁺ aDGCs at 4 wpi (A) or 8 wpi (B) in the absence (top trace) or presence (bottom trace) of 100 μ M bicuculline. Scale bar = 40 pA and 2 s. Frequency, amplitude and decay values for mIPSCs recorded from GFP⁺ aDGCs at 4 (A₁–A₃) and 8 (B₁–B₃) wpi. Graphed data represent means \pm SEM, * $p < 0.05$ using Sidak's post-hoc multiple comparison, $n = 6$ mice/group.

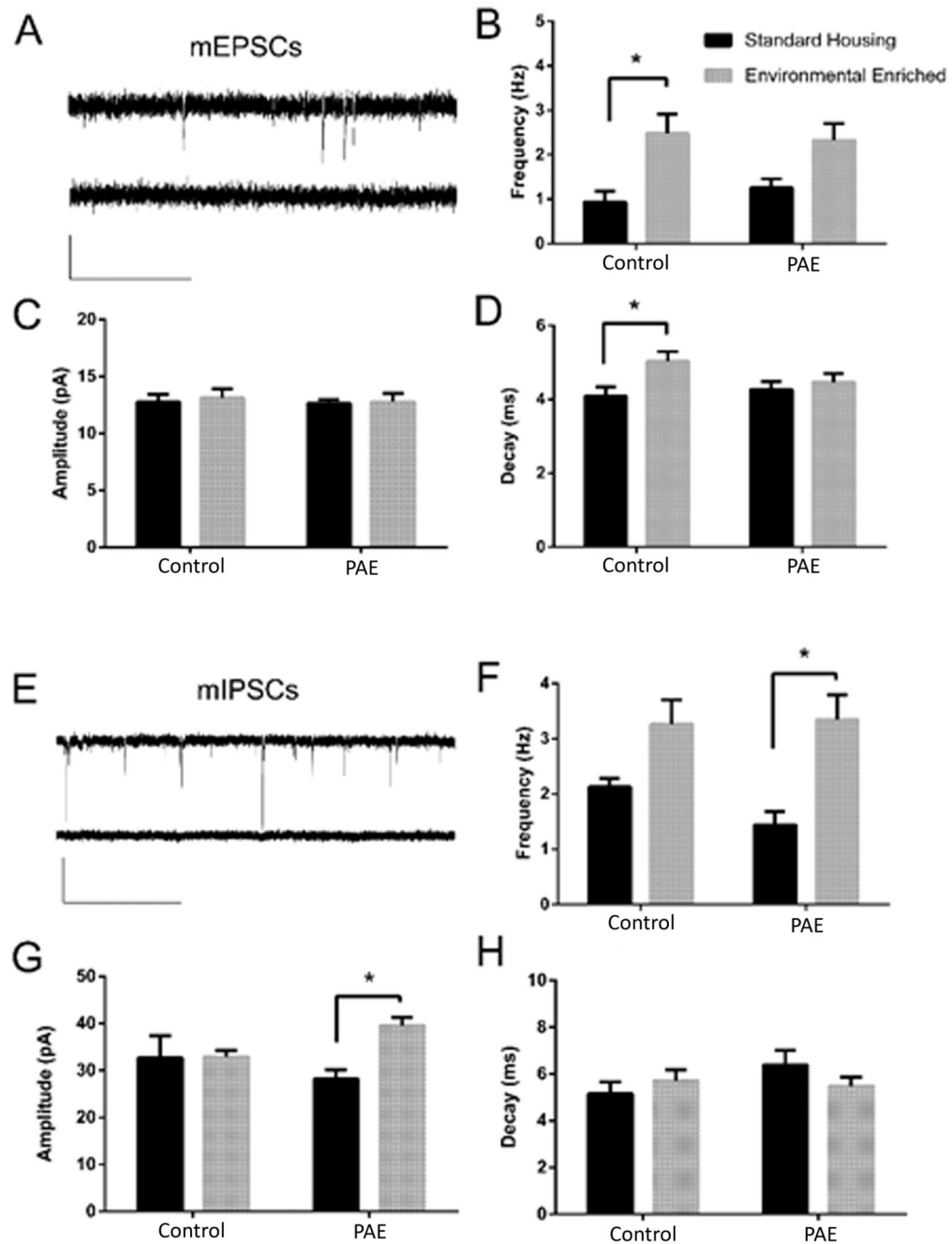


Figure 3. mEPSC and mIPSC Recordings from dDGs

(A) mEPSC trace recordings from an individual DGC in the absence (top) or presence (bottom) of 3 mM kynurenate. Scale bar = 20 pA and 1 s. (B–D) Frequency, amplitude and decay values for mEPSCs (E) sample traces of mIPSC recordings from an individual DGC at in the absence (top) or presence (bottom) of 100 μ M bicuculline. Scale bar = 40 pA and 2 s. (F–H) Frequency, amplitude and decay values for mIPSCs. * $p < 0.05$, Sidak's multiple comparison, $n = 6$ mice/group.

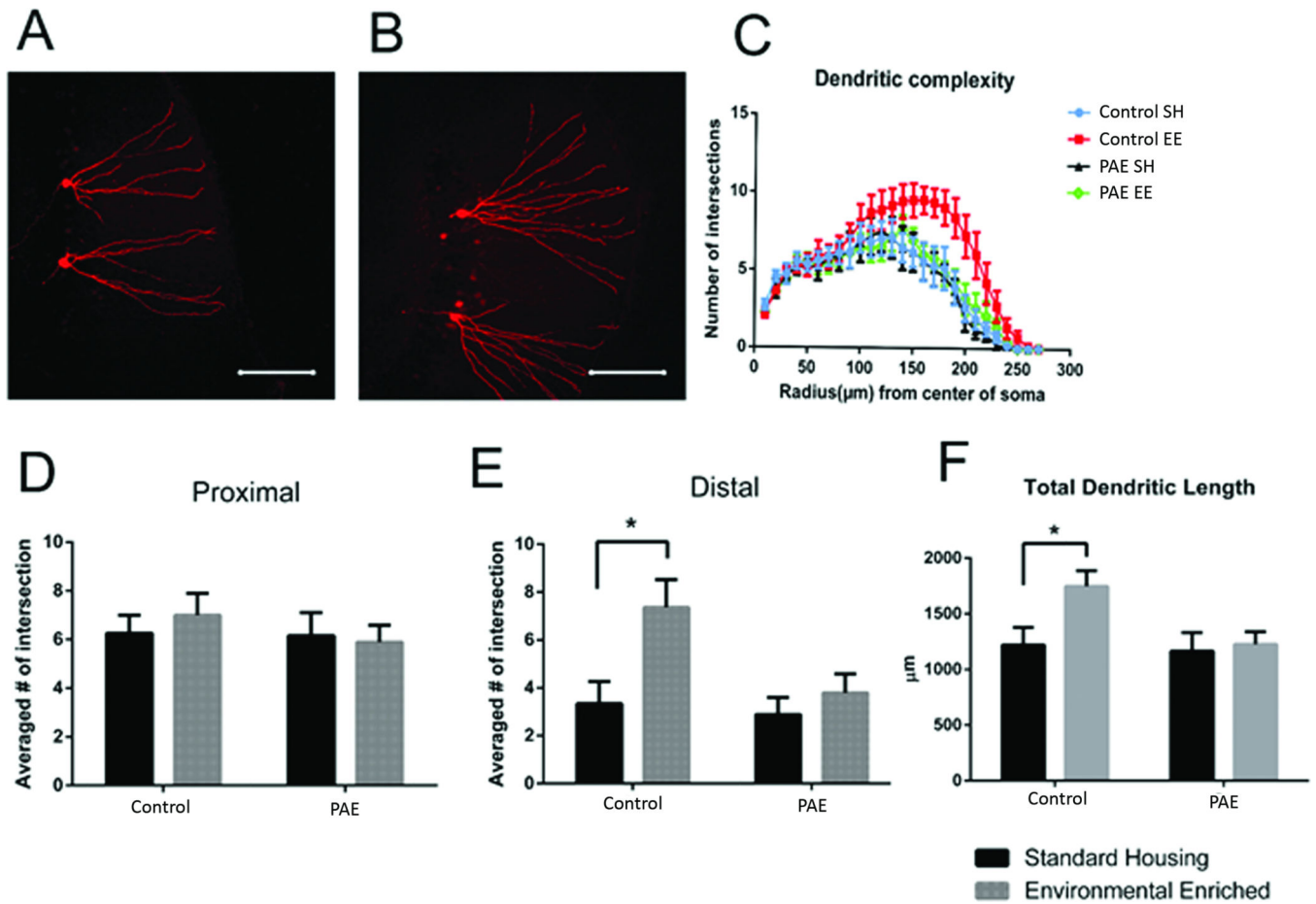


Figure 4. Dendritic Complexity in dDGCs

(A, B) Representative images of dendritic morphology from control mice housed under standard (A) vs. EE (B) conditions. (C) Sholl analysis of dendritic branching (D) Branching complexity of proximal dendrites (60–110 μm). (E) Branching complexity of distal dendrites (170–220 μm). (F) Total dendritic length. * $p < 0.05$, Sidak's multiple comparison, $n = 6$ mice/group.