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Prenatal Exposure to Alcohol Induces Functional and Structural Plasticity in Dopamine D1 Receptor-Expressing Neurons of the Dorsomedial Striatum

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Abstract

Background—Prenatal alcohol exposure (PAE) is a leading cause of hyperactivity in children. Excitation of dopamine D1 receptor-expressing medium spiny neurons (D1-MSNs) of the dorsomedial striatum (DMS), a brain region that controls voluntary behavior, is known to induce hyperactivity in mice. We therefore hypothesized that PAE-linked hyperactivity was due to persistently altered glutamatergic activity in DMS D1-MSNs.

Methods—Female Ai14 tdTomato-reporter mice were given access to alcohol in an intermittent-access, 2-bottle choice paradigm before pregnancy, and following mating with male D1-Cre mice, through the pregnancy period, and until postnatal day (P) 10. Locomotor activity was tested in juvenile (P21) and adult (P133) offspring, and alcohol conditioned place preference (CPP) was measured in adult offspring. Glutamatergic activity in DMS D1-MSNs of adult PAE and control mice was measured by slice electrophysiology followed by measurements of dendritic morphology.

Results—Our voluntary maternal alcohol consumption model resulted in increased locomotor activity in juvenile PAE mice, and this hyperactivity was maintained into adulthood. Furthermore, PAE resulted in a higher alcohol-induced CPP in adult offspring. Glutamatergic activity onto DMS D1-MSNs was also enhanced by PAE. Finally, PAE increased dendritic complexity in DMS D1-MSNs in adult offspring.

Conclusions—Our model of PAE does result in persistent hyperactivity in offspring. In adult PAE offspring, hyperactivity is accompanied by potentiated glutamatergic strength and afferent connectivity in DMS D1-MSNs, an outcome that is also consistent with the observed increase in alcohol preference in PAE offspring. Consequently, a PAE-sensitive circuit, centered within the D1-MSN may be linked to behavioral outcomes of PAE.

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Keywords

Fetal alcohol exposure; dorsal striatum; synaptic plasticity; dendritic spines

Introduction

Prenatal alcohol exposure (PAE) can result in a cluster of neurobehavioral and other developmental disabilities that are collectively termed fetal alcohol spectrum disorders (FASD)(Riley et al., 2011, Mattson et al., 2011). FASD is common and its worldwide prevalence in the general population is estimated at ~2.3% with a high of 11.3% in South Africa (Roozen et al., 2016). A recent Texas study reported that 8.4% of proportionately sampled newborns had biochemical evidence for PAE (Bakhireva et al., 2017), and in the US, FASD may account for between 1% and 5% of school-aged children (May et al., 2018). Attention deficit-hyperactivity disorder (ADHD) was found to be a very common co-morbid disorder in children with FASD (Lange et al., 2017), and hyperactivity has also been reported in animal models of PAE (Shea et al., 2012, Idrus et al., 2014, Hausknecht et al., 2005). Though ADHD and FASD exhibit overlap in behavioral indices (Infante et al., 2015), medications commonly used for ADHD are less effective in managing hyperactivity in FASD children (Frankel et al., 2006), and FASD children exhibit somewhat different patterns of cerebral cortical activation in response-inhibition tasks compared to ADHD children (Kodali et al., 2017) suggesting that neural mechanisms mediating loss of impulse control and hyperactivity due to PAE may differ from those mediating other forms of ADHD.

A few studies have suggested that ADHD-like hyperactivity following PAE is associated with abnormal synaptic plasticity within the cerebral cortex and the striatum (Robbins, 2002, Sonuga-Barke et al., 2016, Emond et al., 2009). The striatum is the major nucleus of the basal ganglia and gates all the cortical inputs to the basal ganglia (Gunaydin and Kreitzer, 2016). The dorsomedial part of the striatum (DMS) controls voluntary behavior and has been strongly implicated in neurobiology of alcohol and substance use disorders (Gittis and Kreitzer, 2012, Volkow and Morales, 2015, Wang et al., 2007, Cheng et al., 2017, Ma et al., 2018). Prenatal and adult exposure to alcohol has been shown to alter plasticity in DMS neurons (Rice et al., 2012, Yin et al., 2007, Wang et al., 2015), though the cellular substrate for PAE in the DMS is unclear. The principal cells of the DMS, the medium spiny neurons (MSNs), can be divided into two neuronal populations with little overlap: D1 and D2-MSNs (Gerfen and Surmeier, 2011, Maia and Frank, 2011, Sippy et al., 2015, Santana et al., 2009). D1-MSNs are known to mediate “Go” actions (Gerfen and Surmeier, 2011, Maia and Frank, 2011, Sippy et al., 2015, Cheng et al., 2017) and overactivation of D1-MSNs in the dorsal striatum results in a hyperactivity in mice (Kravitz et al., 2012, Freeze et al., 2013, Kravitz et al., 2010). Previous studies have shown that PAE results in increased glutamatergic transmission in the basolateral amygdala (Baculis and Valenzuela, 2015) and medial prefrontal cortex (Louth et al., 2016), supporting the hypothesis that PAE facilitates excitatory neurotransmission in the DMS as well. Our previous studies found that excessive alcohol consumption in adult rodents selectively increased the activity of glutamatergic inputs onto D1-MSNs and altered the morphology of the D1-MSNs in the DMS (Wang et

al., 2015, Cheng et al., 2017). We also found that interfering with the activity of D1-MSNs in the adult DMS resulted in altered alcohol intake and preference (Wang et al., 2015, Ma et al., 2018, Cheng et al., 2017). Thus, we hypothesized that PAE would cause glutamatergic and morphological plasticity in DMS D1-MSNs.

Collectively, our data show that in a voluntary consumption model, prenatal alcohol exposure results in increased alcohol preference, and as predicted, hyperactivity in affected offspring. Moreover, PAE resulted in increased glutamatergic activity and significant augmentation of dendritic complexity in D1-MSNs of the DMS, a group of neurons that have been implicated previously in both locomotor and alcohol seeking behaviors in the adult.

Materials and Methods

Reagents

α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) was obtained from Sigma (Saint Louis, MO). Cyclothiazide and Tetrodotoxin (TTX) were purchased from Tocris Bioscience (Minneapolis, MN). Alexa Fluor 594-conjugated streptavidin was purchased from Invitrogen (Carlsbad, CA). All other reagents were obtained from Sigma.

Animals

Drd1a-Cre (D1-Cre) mice were obtained from the Mutant Mouse Regional Resource Center. Ai14 mice were purchased from the Jackson Laboratory. Mouse genotypes were determined by PCR analysis of tail DNA (Wei et al., 2018, Cheng et al., 2017). Before breeding, mice were housed in the same-sex colonies under a 12 h light/dark cycle with lights on at 11:00 P.M. and food and water available ad libitum. The light/dark cycle we used for all behavioral tests was the same as that of the breeding conditions. All behavioral tests were conducted during the dark phase of the light/dark cycle. All animal procedures in this study were approved by Texas A&M University Institutional Animal Care and Use Committee. All the procedures were conducted in agreement with the Guide for the Care and Use of Laboratory Animals, National Research Council, 1996.

Intermittent-Access to Alcohol 2-Bottle-Choice Drinking Procedure and Breeding

Individually housed, ~8-week old female Ai14 mice were randomly assigned and counterbalanced based on weight, to one of two drinking groups: a control group with free access to tap water only, or an alcohol drinking group with free access to both water and a 20% alcohol solution (vol/vol in tap water). The alcohol group was housed in the same room with controls.

To establish high levels of alcohol consumption in alcohol group mice, we employed an intermittent alcohol access, two-bottle choice drinking procedure as described previously (Wang et al., 2015, Cheng et al., 2017, Wei et al., 2018). Briefly, female mice were given 24-h concurrent access to one bottle of 20% alcohol in water (vol/vol) and one bottle of water starting at 1:30 P.M. on every other day, with 24-hr periods of alcohol deprivation between the alcohol-drinking sessions. Alcohol solutions were prepared by mixing alcohol (190

proof pure alcohol, KOPTEC) with tap water. The placement (left or right) of the bottles was alternated between each session to prevent side preference. The weight of water and alcohol bottles was measured 24 h after the start of each drinking session. This paradigm has been reported by others to induce high levels of alcohol intake, up to 30 g/kg/day at 20% vol/vol in female mice (Hwa et al., 2011), reaching peak blood alcohol concentrations above 100 mg/dl.

Following the 6-week excessive alcohol drinking and withdrawal period, female Ai14 mice were mated overnight with 8–12-week-old D1-Cre males. During mating, only water was available to prevent males from consuming alcohol. Females were examined for the presence of a vaginal plug at the end of the mating period, indicating gestational day 0, and males were removed. If no vaginal plug was found in the cage, we allowed the females a maximum of 2 additional overnight mating sessions to ensure the pregnancy. For other experiments in this study, we did not assess estrous cycle stages of female mice. Successfully impregnated females were re-exposed to 10% alcohol (commonly used during pregnancy) in the two-bottle choice paradigm outlined above to decrease the potential toxicity to the infants (Kleiber et al., 2011, Sanchez Vega et al., 2013, Patten et al., 2014), through gestation and into the early postpartum period, corresponding to the 3rd trimester equivalent period of human fetal development, to postnatal day (P) 10 of pup development. After P10, alcohol was removed, and only water was provided to the female. Pups were weaned at P21 and housed with a maximum of five same-sex littermates for the duration of testing.

Locomotor activity

All pups were tested for locomotor activity in an open field box (16 inches × 16 inches × 15 inches, Hamilton Kinder) (Cheng et al., 2017). The traveled distance was detected as infrared beam crosses (16 beams per side per box) using activity monitors (Hamilton Kinder). Locomotion was tested for 30 min. At the end of testing, the mouse was removed and returned to its home cage. The surface and walls of the open field box were wiped clean with water and 30% isopropanol. Female and male mice were tested in different open field boxes.

Conditioned Place Preference

The conditioned place preference method was reported in our previous study (Cheng et al., 2017). Briefly, different visual and tactile cues distinguish the two chambers: black/white stripes with a “rod” flooring in the first chamber, and black/white dots with a metal plate flooring with holes in the second chamber. Each experiment consisted of three steps. For the first step (day 1, preconditioning), each mouse was placed in the neutral hall and was permitted to explore both chambers for 30 min. For the second step (days 2 to 9, conditioning), the mice were administered 20% alcohol (i.p.,2 g/kg) and immediately placed into one of the given chambers and confined for 5 min on days 3, 5, 7, and 9. On alternate days (conditioning days 2, 4, 6, and 8), mice were administered saline and immediately placed into the opposite chamber and confined for 5 min. For the third step (day 10, post-conditioning test), mice were placed in the center of the neutral hall and allowed free access to both chambers for 30 min. The total time spent in each chamber and the locomotion activity was recorded.

Preparation of Acute Striatal Slices and Electrophysiology Recordings

Slice preparation—Slice preparation was described previously (Wang et al., 2015, Cheng et al., 2017, Ma et al., 2018). For the present study, we usually prepared slices from two mouse brains per day, one in the late morning and the other one in the late afternoon. Briefly, coronal sections of the striatum (250 μm) were sliced using a vibratome (VT1200s, Leica) in an ice-cold cutting solution containing the following (in mM): 40 NaCl, 143.5 sucrose, 4 KCl, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 0.5 $CaCl_2$, 7 $MgCl_2$, 10 glucose, 1 sodium ascorbate, and 3 sodium pyruvate, saturated with 95% O_2 and 5% CO_2. Slices were then incubated in a 1:1 mixture of cutting solution and an external solution at 32°C for 45 min before being transferred to a chamber that contained the external solution. The external solution was composed of the following (in mM): 125 NaCl, 2.5 KCl, 2.5 $CaCl_2$, 1.3 $MgCl_2$, 1.25 NaH_2PO_4, 25 $NaHCO_3$, and 10 glucose, saturated with 95% O_2 and 5% CO_2. Slices were stored in the external solution at room temperature until use.

Whole-cell recording—Individual slices were placed in a recording chamber, and cells in the DMS were visualized using epifluorescence microscopy (Examiner A1; Zeiss). Whole-cell recordings were made using a Multiclamp 700A amplifier (Molecular Devices). Electrodes (4–6 MΩ) contained the following (in mM): 115 cesium methanesulfonate, 15 HEPES, 0.6 EGTA, 8 TEA-Cl, 4 MgATP, 0.3 NaGTP, 7 Na_2CrPO_4, Ph 7.2–7.3, and 0.5% biocytin with an osmolarity of 270–280 mOsm. AMPA induced currents and AMPAR-mediated mEPSCs were measured as described previously (Wang et al., 2010b, 2012). Specifically, AMPA (5 μM) was bath-applied for 30 s. mEPSCs were recorded in the presence of 1 μM TTX, 100 μM picrotoxin, and 1.3 mM external Mg^{2+} with neurons clamped at −70 mV.

Histology

Post-recording biocytin-staining and confocal imaging have been described previously (Wang et al., 2015). Briefly, immediately after electrophysiology recording, DMS sections containing biocytin-filled neurons were fixed in 4% paraformaldehyde at 4°C overnight. Sections were then incubated with Alexa Fluor 594-conjugated streptavidin for 72 h. Micrographs of overall dendritic branches and the soma of biocytin-filled neurons were acquired with a 40 X oil immersion objective at the vertical interval of 1 μm. A confocal microscope (Fluorview-1200, Olympus) was used to image fluorescent sections. EGFP was excited by the 470 nm laser.

Morphological Analysis

Biocytin-filled neurons were traced using Simple Neurite Tracer module in ImageJ (Fiji) (Longair et al., 2011, Ferreira et al., 2014). Dendritic branches were quantified with Sholl analysis (Sholl, 1953). The center of each concentric spheres was defined as the center of the soma. The starting radius was 10 μm, and the ending radius was 160 μm from the center with an interval of 10 μm between radii.

Statistical Analysis

Data from male and female mice were combined for analysis and not assessed for sex differences. All data were analyzed using unpaired *t* tests and two-way ANOVA with repeated measures (two-way RM ANOVA), followed by the Student-Newman-Keuls (SNK) *post hoc test*. Statistical analysis was conducted by OriginLab and SigmaPlot programs. mIPSCs were analyzed using Mini Analysis software (Synaptosoft Inc.). All data were expressed as the Mean ± SEM.

Results

Characterization of maternal volunteer alcohol drinking using the two-bottle choice paradigm

To model a natural human drinking pattern and establish high drinking level in mice, we initially trained adult female mice to drink 20% alcohol using the intermittent access two-bottle choice procedure for over 6 weeks (Ron and Barak, 2016, Ma et al., 2018, Cheng et al., 2017) (Figure 1a). Alcohol was not available during mating to prevent interruption of mating (Figure 1a). During pregnancy, the alcohol concentration was decreased to 10% to avoid premature pregnancy termination, and alcohol was available until P10 (Kleiber et al., 2011) (Figure 1a). During the drinking session, both water and alcohol bottles were available. To assess whether adult female mice achieved a high level of alcohol drinking in the pre-pregnancy period, alcohol intake was measured at the end of the drinking session in last two weeks of the training period (week 5 and 6). We found that the alcohol consumption was maintained at a high level (~20 g/kg/24 h) (Ron and Barak, 2016, Cheng et al., 2017), and did not change across weeks 5 and 6 (Figure 1b; $t_{(3)} = -0.94$, $p = 0.42$). Importantly, alcohol preference of pre-pregnant mice was more than 60%, and did not change across weeks 5 and 6 (Figure 1c; $t_{(3)} = 1.45$, $p = 0.24$). To examine whether mice underwent dehydration or malnutrition during the training, which could also impact the development of the offspring, water intake and body weight were also measured in weeks 5 and 6. We did not find any significant change in their water intake nor body weight (Figure 1d and 1e; for 1d: $t_{(3)} = -1.82$, $p = 0.17$; for 1e: $t_{(3)} = -2.14$, $p = 0.12$). These results demonstrate that excessive alcohol drinking using intermittent access to alcohol two-bottle choice establishes high alcohol intake and preference in adult female mice without causing dehydration or malnutrition.

Prenatal exposure to alcohol elevates locomotor activity in childhood mice

To examine hyperactivity in juvenile (P21) PAE mice, we measured locomotor activity in the open field for 30 min (Sanchez Vega et al., 2013). Compared to the age-matched water control, PAE mice exhibited a longer overall traveled distance (Figure 2a; main effect of time: $F_{(5,90)} = 2.65$, $p = 0.028$; main effect of prenatal treatment: $F_{(1,18)} = 8.06$, $p = 0.011$; time × prenatal treatment interaction: $F_{(5,90)} = 2.97$, $p = 0.016$). For the first 10 min and the last 5 min, we observed a significant increase of travel distance in the PAE group than the age-matched control group (Figure 2a; 5 min: $q = 6.3$, $p = 0.00015$; 10 min: $q = 3.13$, $p = 0.031$; 30 min: $q = 3.13$, $p = 0.031$). Also, the total 30-min traveled distance was significantly higher in the PAE group compared to the age-matched water group (Figure 2b; $t_{(18)} = -2.84$, $p = 0.011$). Interestingly, the velocity of movement was lower in PAE mice

than in their age-matched water controls (Figure 2c; $t_{(18)} = 2.22$, $p = 0.04$). However, PAE mice moved for a longer time period than their age-matched water controls (Figure 2d; $t_{(18)} = -3.18$, $p = 0.0052$). We also measured anxiety-liked behavior and found that PAE mice presented the periphery area of the open-field arena for significantly less time than control mice (Figure 2e; $t_{(18)} = 3.79$, $p = 0.0013$). Taken together, these results suggest that prenatal exposure to alcohol causes hyperactivity in juvenile (P21) mice, but with decreased movement velocity and lower anxiety.

PAE mice exhibit higher alcohol conditional place preference and preserve hyper-locomotor activity in adult age

It has been reported that prenatal exposure to cocaine induces CPP to cocaine in adult mice (Malanga et al., 2007). To assess whether prenatal exposure to alcohol can induce CPP to alcohol in adulthood, we performed an alcohol CPP test in PAE adult mice (at P133), and compared performance to age-matched water control. Mice were tested in a customized CPP apparatus with a neutral chamber and two test chambers that have different visual and tactile cues (Figure 3a). All mice were permitted to freely explore all chambers in the CPP apparatus before the conditioning (pre-conditioning). Each mouse was then conditioned in the alcohol-associated chamber and the saline-associated chamber for 8 days (conditioning). On the last day, the time that each mouse spent in both alcohol- and saline-chamber was recorded (post-conditioning). The preferences for each chamber in both pre- and post-conditioning days were measured as the ratio of time in the alcohol- over the saline-chamber. We found that the PAE adult mice showed higher preference ratio than their age-matched water controls in post-conditioning day, but not in the pre-conditioning day (Figure 3b; $F_{(1,10)} = 5.93$, $p = 0.035$; for post-conditioning: $q = 4.12$, $p = 0.013$; for pre-conditioning: $q = 1.96$, $p = 0.19$). Additionally, the PAE adult mice presented higher preference ratio in their post- than pre-conditioning day ($q = 4.35$, $p = 0.012$). Next, we tested whether the PAE mice continued to exhibit hyper-locomotor activity in adulthood (P133). We found that the PAE mice exhibited higher overall total traveled distance than their water controls (Figure 3c; $t_{(10)} = -3.26$, $p = 0.0086$). Additionally, the moving time of adult PAE mice is slightly longer than that of their age-matched water controls, but this difference is not significant (Figure 3d; $t_{(10)} = -1.52$, $p = 0.18$). In summary, our results suggest that prenatal exposure to alcohol produces a higher alcohol CPP and that hyperactivity is still preserved in adult age PAE mice.

Prenatal exposure to alcohol produces an increase in AMPAR-mediated glutamatergic transmission in D1-MSNs

Our recent study reveals that high alcohol preference in adult, non-PAE mice was strongly associated with enhancement of the glutamatergic activity selectively on DMS D1-MSNs (Wang et al., 2015, Cheng et al., 2017). Also, excitation of D1-MSNs induced hyperactivity (Kravitz et al., 2012, Freeze et al., 2013, Kravitz et al., 2010). Thus, we reasoned that the alcohol CPP and hyperactivity observed in adult PAE mice is driven by the enhancement of glutamatergic activity on D1-MSNs.

To investigate this possibility, we prepared brain slices from adult PAE and control D1-Cre;Ai14 mice and performed whole-cell electrophysiology. We first measured AMPA-

receptor (AMPAR) activity in D1-MSNs and found that bath application of AMPA (5 μM) induced a significantly larger current in D1-MSNs of PAE mice than their age-matched water controls (Figure 4a and b; for 4b: $t_{(15)} = -3.28$, $p = 0.0051$). Next, to examine whether the glutamatergic synaptic transmission was affected by PAE, we measured miniature excitatory postsynaptic currents (mEPSCs). The mPESCs recorded from PAE mice showed significantly higher frequency and amplitude than those in age-matched control mice (Figure 4c). This was demonstrated by a rightward shift of the cumulative probability distributions of mEPSC amplitudes recorded from PAE mice (Figure 4d), and a significant increase in the mean amplitude of mPESCs (Figure 4d, inset; $t_{(30)} = -2.07$, $p = 0.047$). We also observed a leftward shift of cumulative probability distributions of mEPSC inter-event intervals (Figure 4e), and a significant increase in the mean frequency of mEPSCs from D1-MSNs of PAE mice compared to age-matched controls (Figure 4e, inset; $t_{(30)} = -3.11$, $p = 0.0041$). Taken together, these results suggest that PAE causes a long-term increase in glutamatergic afferents onto D1-MSNs.

Prenatal exposure to alcohol increases dendritic complexity of D1-MSNs of the DMS

Given that AMPAR-mediated glutamatergic plasticity has been associated with morphological changes in neurons (Kasai et al., 2010), we examined whether the complexity of dendritic arborization was altered by PAE in the DMS D1-MSNs. To visualize the overall dendritic branches and the soma of the D1-MSNs from above recording, the neuronal tracer biocytin was applied through the patching pipette into a patched D1-MSN, and biocytin-labeled D1-MSNs were imaged using confocal microscopy (Figure 5a). The number of dendritic processes was measured by Sholl analysis in concentric spheres centered on the soma (Wang et al., 2015). As shown in Figure 5b, dendrites that were 10–100 μm from the soma exhibited more intersections in DMS D1-MSNs from PAE mice than that in their age-matched water controls (Figure 5b; $F_{(16,231)} = 3.79$, $p = 0.000003$). Furthermore, the total length of DMS D1-MSNs was significantly higher in PAE mice compared to their age-matched water controls (Figure 5c; $t_{(16)} = -3.16$, $p = 0.0061$). We also observed the total number of dendritic branches of D1-MSNs was significantly increased in the PAE mice than that in their age-matched water controls (Figure 5d; $t_{(16)} = -3.67$, $p = 0.0021$). Taken together, our findings reveal that prenatal exposure to alcohol changes the dendritic complexity in the DMS D1-MSNs.

Discussion

The present study confirmed that adult female mice voluntarily and stably consume high levels of alcohol in a two-bottle choice paradigm and exhibit preference for alcohol. Thus, the two-bottle choice paradigm in mice can be used to model voluntary alcohol consumption during pregnancy in human populations. Using this paradigm, we show that, as reported by others (Shea et al., 2012, Idrus et al., 2014, Kim et al., 2013), PAE results in hyperactivity in exposed offspring during the juvenile period, and that hyperactivity persists into adulthood. Adult PAE mice also exhibited higher conditioned place preference to alcohol, compared to non-PAE controls. Importantly, we discovered that PAE increased AMPAR activity in DMS D1-MSNs in adult offspring. Furthermore, we found that prenatal exposure to alcohol increased total length and number of branches of DMS D1-MSNs in adult offspring. Our

findings suggest that PAE triggers a long-term functional and structural plasticity in DMS D1-MSNs, potentially contributing to hyperactivity in both juvenile and adult offspring.

Hyperactivity is often a co-morbid condition in individuals diagnosed with a FASD (Lange et al., 2017). Consistent with data from human populations as well as with the results reported in a number of preclinical studies (Sanchez Vega et al., 2013, Patten et al., 2014, Mantha et al., 2013), we also observed an increase in locomotor activity in juvenile PAE offspring. Interestingly, despite the overall increase in the traveled distance, we also found that PAE juveniles exhibit decreased movement velocity. The latter data are consistent with preclinical evidence that PAE also disturbs musculoskeletal development and motor control circuits (Kleiber et al., 2011, Sylvain et al., 2010), and with clinical evidence for gait disturbances in FASD children (Taggart et al., 2017). More importantly, we found that PAE mice moved for a longer time, as compared with their water controls; this may account for the increased travel distance of PAE mice, despite their decreased speed of movement. An increase in the percentage of time spent moving by PAE juveniles is consistent with the hyperactivity component of ADHD. Although we observed a reduction of traveled distance over time in PAE group, habituation and fatigue in these mice could explain the data. In contrast, there was little decrement in distance traveled in the control mice, indicating that, as a group, control mice did not exhibit significant habituation or fatigue during the test period. In our study, PAE offspring appeared to show less anxiety, since they spent less time in the periphery of the open-field arena compared to control offspring. Our data are in contrast to a few studies which showed that prenatal exposure to alcohol increased anxiety-like behavior (Hellemans et al., 2008, Kleiber et al., 2011, Hausknecht et al., 2005). However, other studies have reported that PAE for more restricted 1st and 2nd trimester-equivalent exposure periods (Mantha et al., 2013, Fish et al., 2016) result in increased exploratory behavior in the central zone of the open field arena. Moreover, PAE reportedly impairs the development of serotonin neurons in fetal mice (Zhou et al., 2001), which contributes to the facilitation of anxiety-like behavior. The inconsistency in data between different research groups may be due in part, to the timing and dose of alcohol exposure. Other differences in outcome may be partly explained by contextual components of experimental design. For example, Hellemans and colleagues (2008) pre-exposed PAE mice to a stress paradigm, before evaluating anxiety behaviors. However, reduced anxiety may also be due to other developmental consequences of PAE. For example, children with FASD exhibit deficits in sensory processing (Franklin et al., 2008), which may impair adaptation to anxiogenic environments.

It has been reported that the maternal 10% alcohol exposure procedure during pregnancy period can alter the epigenotype and the phenotype of offspring (Kaminen-Ahola et al., 2010). More importantly, this report indicated that this epigenotype change could be preserved in adult age. In line with this study, we found that the PAE adult offspring continued to exhibit indices of hyperactivity. We also found that adult PAE offspring demonstrated higher alcohol-induced CPP than their age-matched water controls. This finding is in line with previous reports which state that prenatal exposure to cocaine results in a higher conditioned place preference (Malanga et al., 2007, Pautassi et al., 2012).

Previously, we reported that excessive alcohol drinking increases AMPAR and NMDAR activity in adult mice (Wang et al., 2015, Cheng et al., 2017). Here, we found that PAE enhanced the AMPAR-mEPSC amplitude in DMS D1-MSNs of adult offspring. In line with our results, others have also reported that PAE enhances AMPAR function in other basal forebrain regions as well (Hsiao and Frye, 2003). Moreover, another study reported that high-frequency stimulation induced an abnormal AMPAR-mediated LTP in the dorsal striatum of PAE adult mice and could be blocked by the application of D1R antagonist (Zhou et al., 2012). In our study, we also found that PAE resulted in an increase of the frequency of AMPAR-mediated mEPSCs in D1-MSNs in the DMS of adult offspring. These data suggest that PAE may result in increased glutamatergic release from presynaptic terminals onto D1-MSNs, and are consistent with previous research showing that, in alternate contexts, i.e., neurogenesis, PAE preferentially facilitates glutamatergic activity to facilitate an imbalance in excitatory signaling (Kim et al., 2010).

Enhancement of mEPSC frequency may attribute to the increased complexity of dendritic branching, where glutamatergic synapses are located (Kerchner and Nicoll, 2008). Cycles of alcohol consumption and withdrawal increase arborizations, the total number, and the total length of dendrites of D1-MSNs (Wang et al., 2015). In this study, we found an increase of dendritic arborizations, as well as the total number and the total length of dendritic branches of D1-MSNs in PAE adult mice, which is likely to account for the enhanced mEPSC frequency in D1-MSNs in response to prenatal exposure to alcohol. It should be noted that another study which was not able to document changes in morphology of striatal MSNs (Rice et al., 2012), achieved lower levels of PAE and did not discriminate between D1 and D2 sub-populations of MSNs. Additionally, Rice et al used a male rat model of PAE instead of our mixed-sex mouse model, and the blood alcohol concentration may have been lower in rats than in the mice. Further studies will be needed to define thresholds for PAE activation of D1-MSNs. Interestingly methylphenidate, the psychostimulant commonly used to treat ADHD has been shown to increase spine density on D1-MSNs (Kim et al., 2009), suggesting that PAE may developmentally program the excitability of a brain circuit important for controlling activity and attention, and perhaps, explain the decreased efficacy of anti-ADHD medications in managing FASD. Lastly, we note that one limitation of the current study is that while we used a mixed-sex study model, our study was not statistically powered to assess sex differences due to PAE.

In summary, our results suggest that prenatal exposure to alcohol induced hyperactivity in both juvenile and adult offspring, and alcohol preference in adult offspring. More importantly, the PAE-induced hyperactivity and alcohol preference in adult offspring may be linked to functional and morphological change in D1-MSNs in the DMS.

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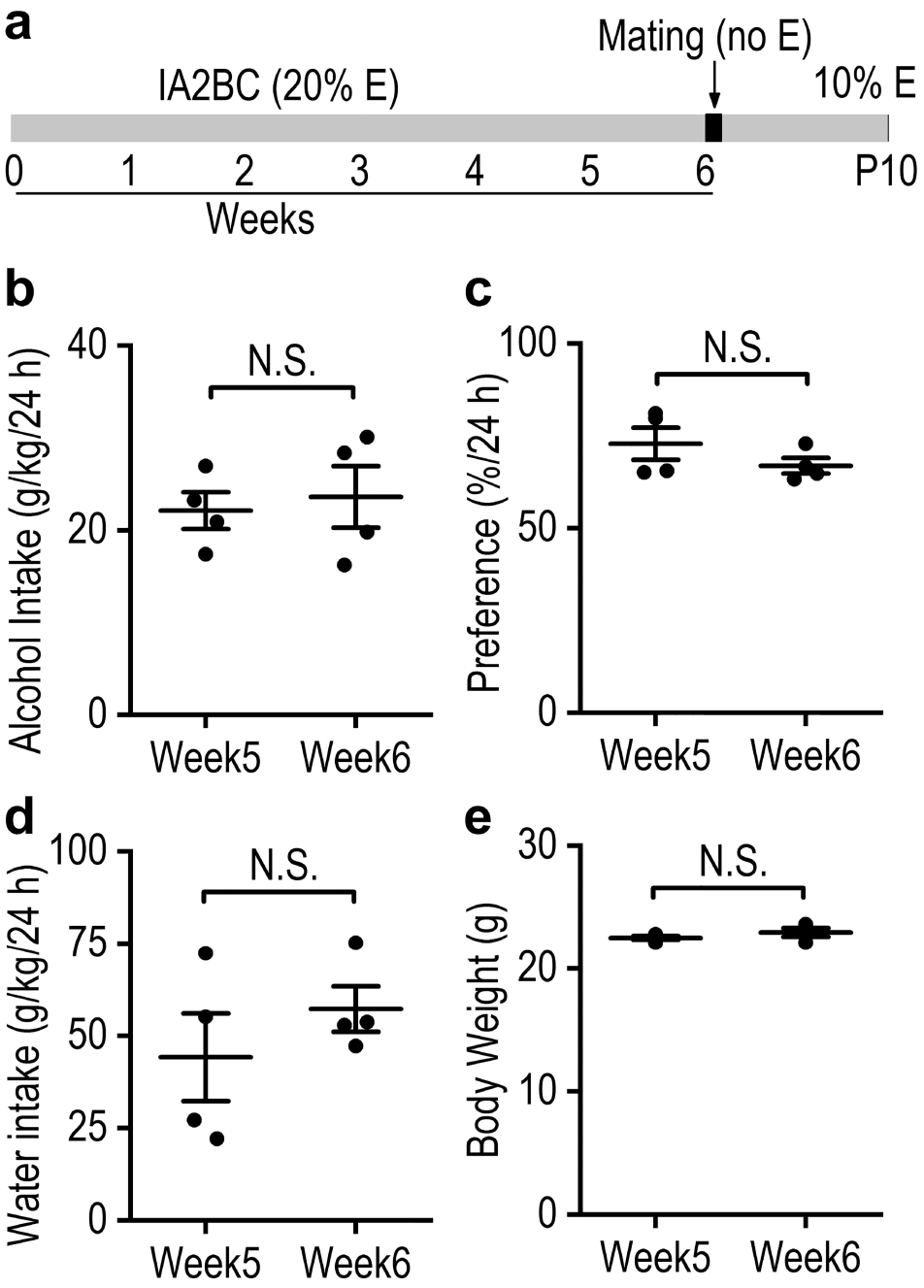

Figure 1. A voluntary, intermittent access alcohol-drinking paradigm established a high level of alcohol consumption and preference

(**a**) Schematic of experimental design. Female Ai14 mice were trained to establish a high level of alcohol drinking with the intermittent access to 20% alcohol (20% E) two-bottle choice paradigm (IA2BC). To avoid fetal effects due to paternal alcohol consumption, no alcohol (no E) was available during the mating period. After that, IA2BC was reinstated, with 10% alcohol (10% E) through the period of pregnancy and into the post-partum period, until postnatal day 10 (P10). (**b, c**) Female mice achieved a high level of alcohol intake (b) and preference (c) in 24 h, which were remained at the same level in the last two weeks,

week 5 and 6. Not significant (N.S.), $p > 0.05$, unpaired *t* test. (**d, e**) Water intake and the body weight of female mice did not change in the last 2 weeks, i.e., week 5 and 6 of the IA2BC paradigm. Not significant (N.S.), $p > 0.05$, unpaired *t* test. n = 4 female mice for **b**–**e**.

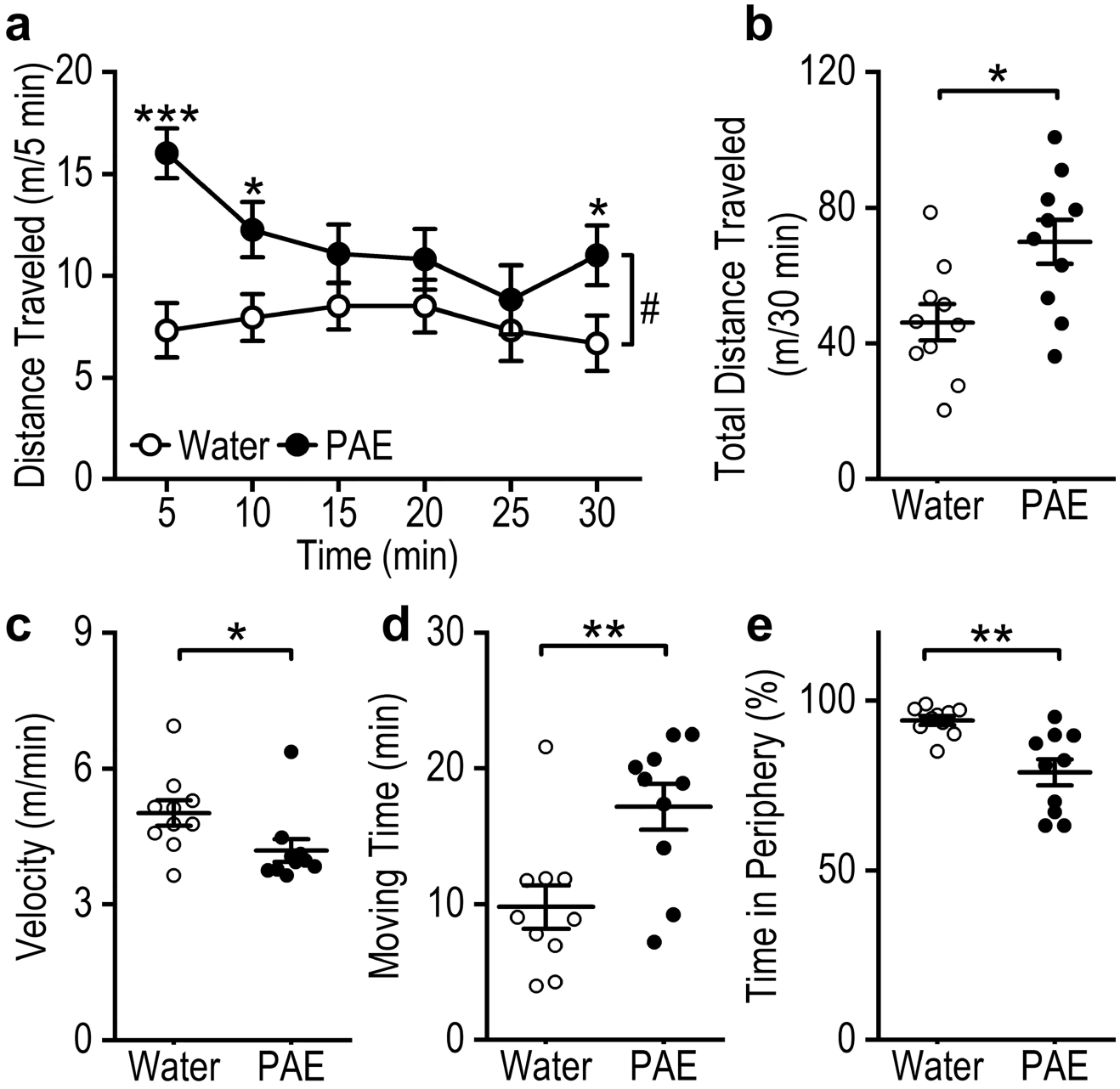

Figure 2. Prenatal exposure to alcohol increases locomotor activity

(**a**) Time course of locomotor activity demonstrated that prenatal alcohol exposure (PAE) mice traveled more distance than their age-matched water controls at 5, 10 and 30 min. $^{\#}p < 0.05$, two-way RM ANOVA; $^{*}p < 0.05$ and $^{***}p < 0.001$ versus the water group at the same time points, post-hoc SNK test. (**b**) The cumulative distance traveled during the 30 min period was higher in PAE mice compared to water controls. $^{*}p < 0.05$ by unpaired *t* test. (**c**) PAE mice demonstrated a decreased velocity of movement compared to age-matched water controls. $^{*}p < 0.05$, unpaired *t* test. (**d**) PAE mice demonstrated a greater moving time than age-matched water controls. $^{**}p < 0.01$, unpaired *t* test. (**e**) PAE mice spent less time at the periphery of the open field arena than their age-matched water controls. $^{**}p < 0.01$,

unpaired *t* test. n = 10 mice (7 males and 3 females) from 3 litters (Water); 10 mice (8 males and 2 females) from 4 litters (PAE) in **a**–**e**.

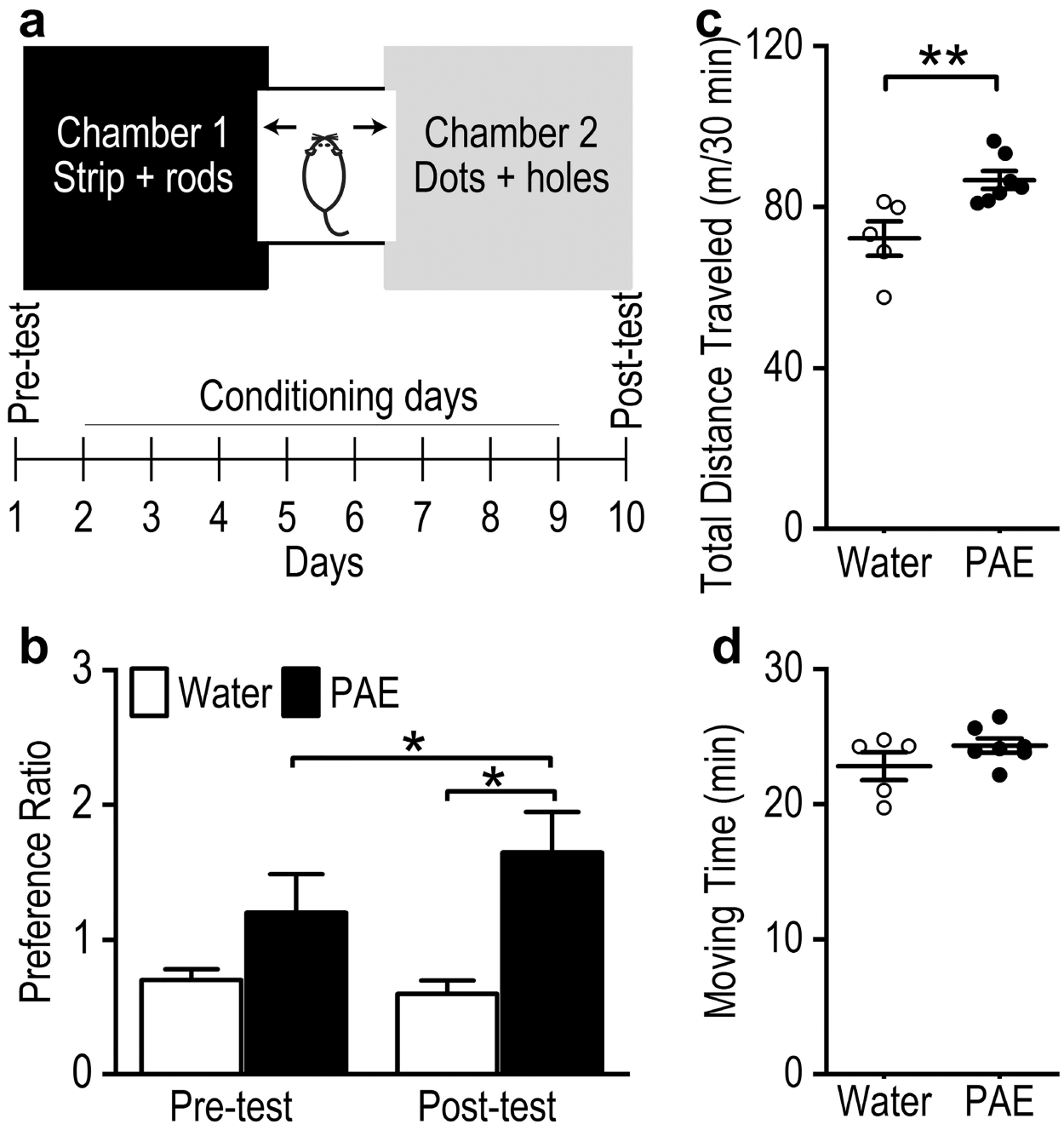

Figure 3. Prenatal alcohol exposure results in conditioned place preference for alcohol, and increased locomotor activity in adult (P133) offspring

(**a**) Schematic of experimental procedures. The diagram illustrates the design of the customized CPP chambers and the timeline for the CPP experimental protocol. (**b**) Adult PAE mice exhibited a higher preference ratio on the post-conditioning test, compared to the pre-conditioning test. PAE mice also demonstrated a higher preference ratio on the post-conditioning test compared to age-matched water controls. Preference ratio = time spent in alcohol chamber/time spent in the saline chamber. *$p < 0.05$, two-way RM ANOVA; *$p < 0.05$, post-hoc SNK test. (**c**) Adult PAE mice showed a higher cumulative distance traveled

in a 30-min testing session compared to their age-matched water controls. $^{**}p < 0.01$, unpaired *t* test. (d) Adult PAE mice showed a slightly (but not significant) higher moving time, as compared with their age-matched water controls. $p > 0.05$, unpaired t test. n = 5 mice (3 males and 2 females) from 3 litters (Water); 7 mice (5 males and 2 females) from 4 litters (PAE) in **b**–**d**.

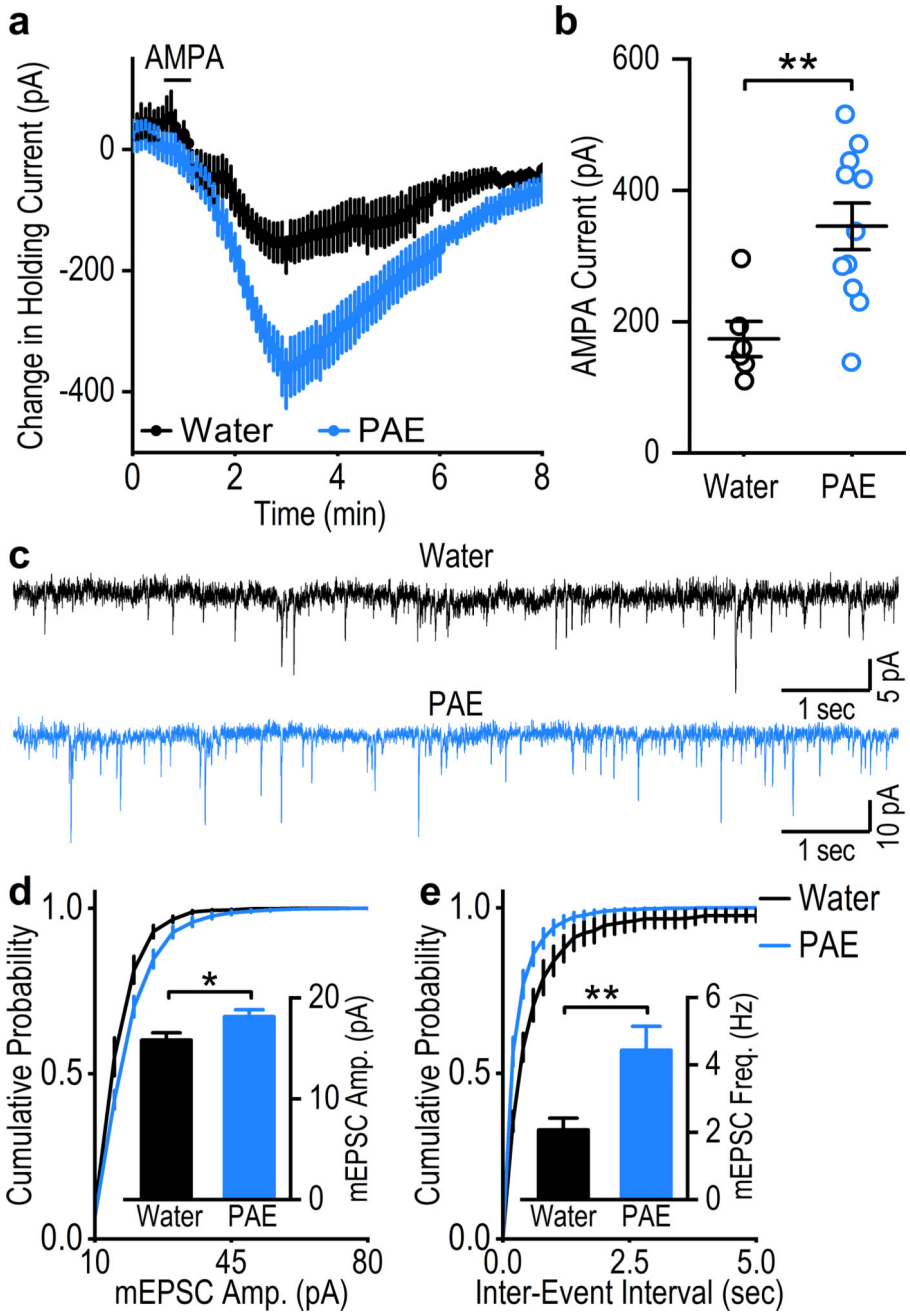

Figure 4. Prenatal exposure to alcohol increases AMPAR-mediated glutamatergic transmission in DMS D1-MSNs of adult offspring

(**a**) PAE produced a long-lasting increase in the peak amplitude of the AMPA-induced current. AMPA (5 μm) was bath-applied. (**b**) The peak amplitude of AMPA-induced current was higher in PAE groups compared to age-matched controls. $^{**}p < 0.01$, unpaired *t* test. n = 6 neurons from 5 mice (4 males and 1 female) that were derived from 3 litters (Water); 11 neurons from 7 mice (6 males and 1 female) that were derived from 4 litters (PAE) in **a** and **b.** (**c**) Representative mEPSC traces of D1-MSNs from water and PAE groups. (**d**) PAE increased the amplitude of mPESCs as shown in cumulative probability plots for the mEPSC

inter-event interval from water and PAE mice. Inset, bar graph represents the mean mEPSC amplitude in control and PAE groups. *$p < 0.01$, unpaired *t* test. (**e**) Prenatal exposure to alcohol increased the frequency of mEPSCs as shown in the cumulative probability plots for mEPSC amplitude from control and PAE mice. Inset, bar graph represents the mean mEPSC frequency in control and PAE groups. **$p < 0.01$, unpaired *t* test. n = 11 neurons from 5 mice (2 males and 3 females) that were derived from 3 litters (Water); 21 neurons from 7 mice (5 males and 2 females) that were derived from 4 litters (PAE) in **d**–**e**.

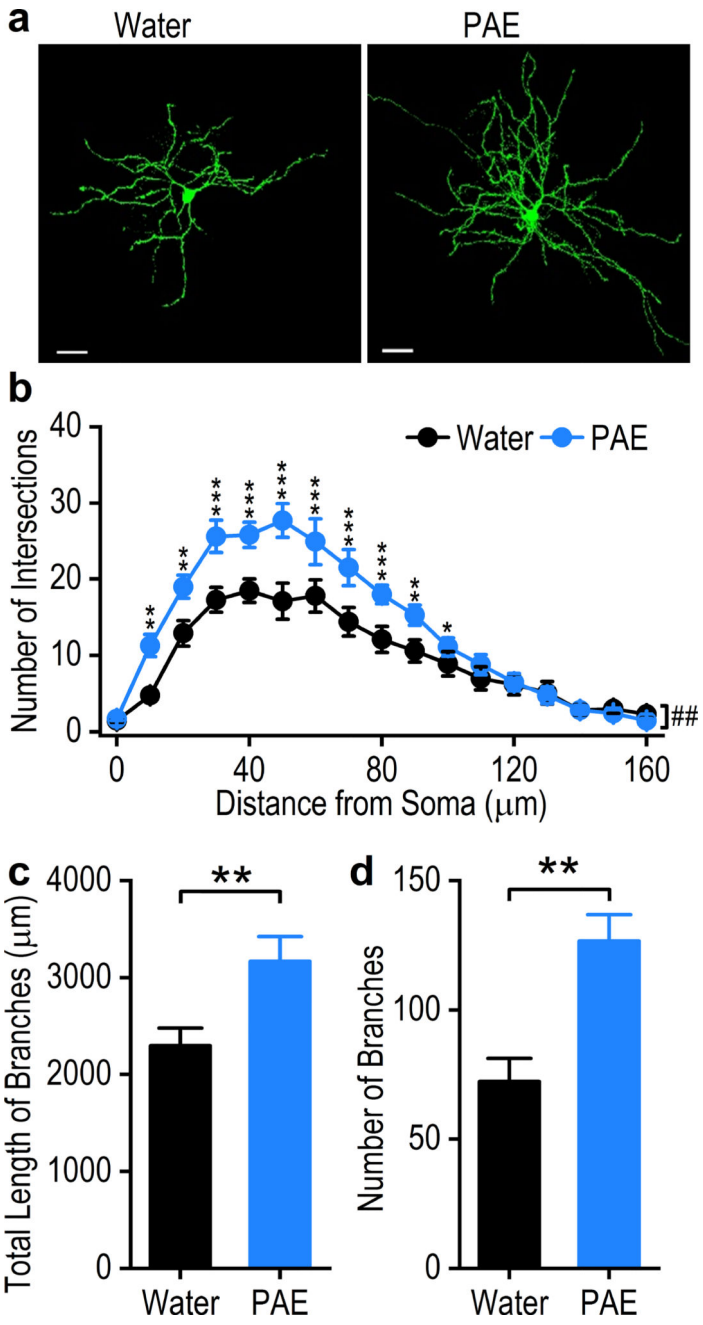

Figure 5. Prenatal exposure to alcohol results in increased dendritic length and branching in DMS D1-MSNs of adult offspring

(**a**) Representative confocal images illustrate dendritic branches and soma of D1-MSNs from PAE mice and their water controls. (**b**) A three-dimensional Sholl analysis revealed significantly more intersections of the dendritic process at 10 – 100 μm from soma of D1-MSNs in PAE mice, compared to their age-matched controls. $^{\#\#}p < 0.01$, two-way RM ANOVA; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, SNK test. (**c**) D1-MSNs from PAE mice exhibited increased lengths of dendritic branches compared to controls. $^{**}p < 0.01$, unpaired *t* test. (**d**) The total number of branches was increased in D1-MSNs of PAE mice compared

to water controls. $^{**}p < 0.01$, unpaired *t* test. n = 8 neurons from 5 mice (3 males and 2 females) that were derived from 3 litters (Water); 10 neurons from 7 mice (6 males and 1 female) that were derived from 4 litters (PAE) (b–d).