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Ventral Hippocampus Modulates Anxiety-Like Behavior in Male But Not Female C57BL/6 J Mice

Cheng Wang,[a] Yu Zhang,[a] Shan Shao,[a] Shuang Cui,[a] You Wan[a,b,c,d,*] and Ming Yi[a,b,]**

[a]*Neuroscience Research Institute, Peking University, 38 Xueyuan Road, Beijing 100083, China*

[b]*Department of Neurobiology, School of Basic Medical Sciences, Peking University, 38 Xueyuan Road, Beijing 100083, China*

[c]*Key Laboratory for Neuroscience, Ministry of Education/National Health Commission, Peking University, 38 Xueyuan Road, Beijing 100083, China*

[d]*Co-innovation Center of Neuroregeneration, Nantong University, Nantong 226001, China*

Abstract—Remarkable sex difference has been observed in emotional processing including anxiety. The hippocampus, its ventral pole in particular, modulates anxiety-like behavior in rodents. However, most researches have been performed in male animals only, leaving hippocampal modulation of anxiety in females poorly defined. In the present study, we showed that excitotoxic lesioning of the ventral hippocampus with ibotenic acid produced anxiolytic effects in three behavioral tests (novelty-suppressed feeding, marble burying, and elevated-plus maze) in male but not female C57BL/6 J mice. Locomotion in the open field remained similar after lesioning in either sex. More c-Fos-positive neurons were observed in the ventral hippocampus in male than in female mice after exploration in an elevated plus-maze, indicating stronger enrollment of this region in anxiety-like behavior in males. These results reveal significant biological sex difference in ventral hippocampal modulation on anxiety in mice and provide a new sight for anxiety modulation and hippocampal function.

Key words: ventral hippocampus, anxiety, sex difference.

INTRODUCTION

Anxiety disorders are prevalent (Kessler et al., 2010) and cover 7.3% of the population (Baxter et al., 2013). Significant sex difference has been observed in mood disorders: women are more likely than men to experience anxiety (Kessler et al., 1994; Craske and Stein, 2016) or depression (Nolen-Hoeksema and Girgus, 1994) from early adolescence through adulthood. Brain regions in the limbic system underlie emotional processing (Rosen and Schulkin, 1998). In rodents, ventral hippocampus shows dense connection with affect-related regions including amygdala, prefrontal cortex and hypothalamus (Bannerman et al., 2004; Fanselow and Dong, 2010). Excitotoxic lesioning of the ventral hippocampus in rats reduces anxiety-related behaviors in elevated plus-maze and hyponeophagia tests (Kjelstrup et al., 2002; Bannerman et al., 2003), whereas optogenetic activation of granule cells in the ventral dentate gyrus suppresses innate anxiety in mice (Kheirbek et al., 2013). Furthermore, local field potentials in the ventral hippocampus show increased correlation in theta-frequency oscillations with medial prefrontal cortex in anxiogenic environments in mice (Adhikari et al., 2010). Optogenetic activation and inhibition of basolateral amygdala to ventral hippocampus projections increase and decrease anxiety-related behaviors in mice, respectively (Felix-Ortiz et al., 2013). More recently, “anxiety cells” have been discovered in ventral hippocampal CA1 in mice (Jimenez et al., 2018).

However, most of these studies were performed only in male animals. To our knowledge, effects of ventral hippocampal lesioning on anxiety in female rodents have not been reported. Previous work has shown that males have larger hippocampal (Ruigrok et al., 2014; Meyer et al., 2017) and amygdala (Giedd et al., 1996; Goldstein et al., 2001; Ruigrok et al., 2014) volumes, as well as greater within-hemispheric connectivity (Ingalhalikar et al., 2014), than females in both mice and humans. The present study aims to explore potential sex difference in hippocampal modulation of anxiety, by examining anxiety-like behavior in male and female C57BL/6 J mice with excitotoxic lesioning of the ventral hippocampus and assessing hippocampal neuronal activation with c-Fos staining after an anxious experience.

*Correspondence to: Key Laboratory for Neuroscience, Ministry of Education/National Health Commission, Peking University, 38 Xueyuan Road, Beijing 100083, China Tel.: +86 137 1882 3306.
E-mail addresses: ywan@hsc.pku.edu.cn (You Wan) mingyi@hsc.pku.edu.cn (Ming Yi)

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EXPERIMENTAL PROCEDURES

Animals

Adult weight-matched (26–30 g) male and female C57BL/6 mice at the age of 6–8 weeks were housed 4–6 per cage under a 12 h light/dark cycle with food and water provided *ad libitum* (except before the novelty-suppressed feeding test). Corn cobs were laid in the cage as bedding. All experiments were conducted in a blind manner and in accordance with the Institutional Animal Care and Use Committee of Peking University Health Science Center (LA2016061). All mice were handled for three days before behavioral experiments. 32 male and 36 female mice were used in behavioral experiments. Mice were randomly allocated to sham and lesion groups (16 male and 18 female mice in each group). For c-Fos staining, 6 male and 6 female mice were used.

Hippocampal lesioning

Mice were anesthetized with 1% pentobarbital sodium, and positioned in a stereotaxic instrument (RWD Life Science, Shenzhen, China); 0.4 μl of ibotenic acid (pH 7.4, 10 mg/ml, Sigma-Aldrich, USA) was injected into bilateral ventral hippocampus (AP -3.40 mm, ML ±3.0 mm, DV -3.2 mm relative to Bregma) (Paxinos and Franklin, 2001) through a 1-μL Hamilton microsyringe within 5 min, at a flow rate of 0.08 μl/min. Sham lesion was performed by injecting the same amount of normal saline at the same flow rate. After stereotaxic injection, the needle was left in place for another 5 min to allow for drug diffusion before slow withdrawal. Mice were allowed to recover for 7 days before further experiments.

Novelty-suppressed feeding test

The timeline of behavioral experiments was shown in Fig. 1. After recovery from surgery, mice were first examined in the novelty-suppressed feeding test as previously reported (Zhang et al., 2017). The apparatus consisted of a chamber (40 × 40 cm) filled with cob bedding materials at a depth of 5 cm. Under 300 lx illumination, a standard chow pellet (5 g) was placed on a piece of white filter paper (10 cm in diameter) positioned in the center of the arena. Each mouse was left single in a new cage for 30 min after 24 h food deprivation before being placed in a random corner of the chamber facing the chamber wall. The amount of time passed before the mouse approached and ate the pellet was recorded. After the first bite or a 10 min cut-off time, the mouse was transferred to the prior new cage. A new single food pellet, which was weighed in advance, was placed in the cage. The mouse was allowed to eat the food pellet for 5 min. After that, the mouse was returned to its home cage, and the food pellet was weighed again to obtain the amount of food consumed.

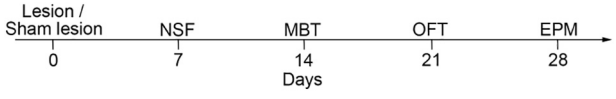

Fig. 1. Timeline of behavioral experiments. NSF: novelty-suppressed feeding test, MBT: marble burying test, OFT: open field test, EPM: elevated plus-maze test.

Marble burying test

The marble burying test (Zhang et al., 2017) was performed 7 days after the novelty-suppressed feeding test. Twenty marbles, with 4 × 5 arrangement, were put on cob bedding materials at a depth of 5 cm in home cage. Each mouse was left single in its home cage for 30 min before placed in a random corner of the test apparatus facing the chamber wall. A marble with two-thirds or more in the cob bedding was treated as a buried marble. The number of buried marbles was counted after 30 min.

Open field test

7 days after the marble burying test, each mouse was placed in a 60 × 60 × 60 cm open field chamber with 30 lx illumination and allowed to explore freely for 5 min (Jiang et al., 2018). Locomotive activity was videotaped and the total distance traveled in the field was measured using the SMART software (v2.5.21, Panlab). The chamber was cleaned by 75% ethanol between tests.

Elevated plus-maze test

Seven days after the open field test, mice performed the elevated plus-maze test (Zheng et al., 2017). The maze consisted of two open (5 × 30 cm) and two closed arms (same size with 15 cm walls) and was placed 50 cm above the floor. In a room with 30 lx illumination, each mouse was placed onto the central area heading towards the same open arm. Animal activity was videotaped and the time spent in open arms and the percent of entries into open arms in the following 5 min were assessed by hand-score. The maze was cleaned by 75% ethanol between tests.

Estrus cycle identification

The estrus cycle of female mice consisted of proestrus, estrus, metestrus and diestrus phases, and was defined by vaginal cytology after each behavioral test. Proestrus and estrus were allocated to high-estradiol phase (HEP), while metestrus and diestrus were allocated to low-estradiol phase (LEP). Vaginal smears were obtained by toothpicks scraping gently in vagina after each behavioral test. 95% alcohol was used to fix vaginal smears for 15 min after drying in air for 15 min. Harris–Shorr staining was carried out to stain vaginal epithelial cells. Vaginal smears were put in Shorr stain solution for 10 min, then put in 95%, 95%, 100% and 100% alcohol for 1–2 min in turn, finally put in xylol for 10 min. After that, vaginal smears were sealed by neutral gum, dried in a 37 °C oven. Vaginal cytology was observed with a light microscope (Leica DMI 4000B, Germany) as previously described (Nelson et al., 1982).

Histology of hippocampal lesioning

After the elevated plus-maze test, mice were anesthetized with 1% pentobarbital sodium (50 mg/kg, *i.p.*), intracardially perfused with 50 ml normal saline and 50 ml 4% paraformaldehyde (PFA, in 0.1 M phosphate buffer, pH 7.4) in turn. Brains were post-fixed with PFA for 24 h, and cryoprotected in 20% and 30% sucrose solutions in turn. Coronal brain sections were cut at 30-μm using a cryostat microtome (Leica 1950, Germany) and mounted on positive charged plus slides, which were dried in 37 °C oven for a week. Slides were rehydrated through phosphate buffer saline and stained in Cresyl violet solution for 8 min, then put in 75%, 75%, 80%, 80%, 90%, 100% and 100% alcohol in turn (1 min for each step). Finally, slides were put in xylol for 2 min twice and sealed by neutral gum. Lesion sites in ventral hippocampus were confirmed by microscopic inspection (Leica DMI 4000B, Germany).

Immunofluorescence and quantification of c-Fos expression

To assess hippocampal neuronal activation, male and female mice were sacrificed either 90 min after a 5-min exploration in the elevated plus-maze (anxiety group) or directly from the home cage (control group). Estrus cycle of female mice were identified before sacrifice. To exclude influences from the estrus cycle (Marcondes et al., 2001), we selected female mice showing similar levels of open arm exposure to males, representing similar level of innate anxiety. Of the 6 female mice used for c-Fos staining, 3 were in the estrus phase and 3 were in the metestrus phase. Procedures of perfusion and post-fixing were similar to described above. 30-μm sections were sliced coronally through ventral hippocampus, amygdala and prelimbic cortex. Free-floating sections were washed in PBS (5 min × 3 times), blocked for 60 min with blocking-buffer containing 3% bull serum albumin and 0.3% triton X-100 dissolved in PBS, and incubated with the rabbit anti-c-Fos (Cell Signaling Technology 2250S, USA) in 4 °C for 24 h. The primary antibody was dissolved 1:200 in blocking-buffer. Sections were then washed in PBS (10 min × 3 times) and incubated with Alexa Fluor 594-conjugated goat anti-rabbit antibody (Invitrogen A11032, USA) at room temperature for 60 min, followed by PBS-washing (10 min × 3 times), and finally mounting and cover-slipping on microscope slides after incubated with DAPI (1: 1000). The secondary antibody was dissolved 1: 500 in blocking-buffer. Images were taken on a Leica STED laser scanning microscope using a 10× objective for quantification of c-Fos immunoreactivity in brain regions. Identical image

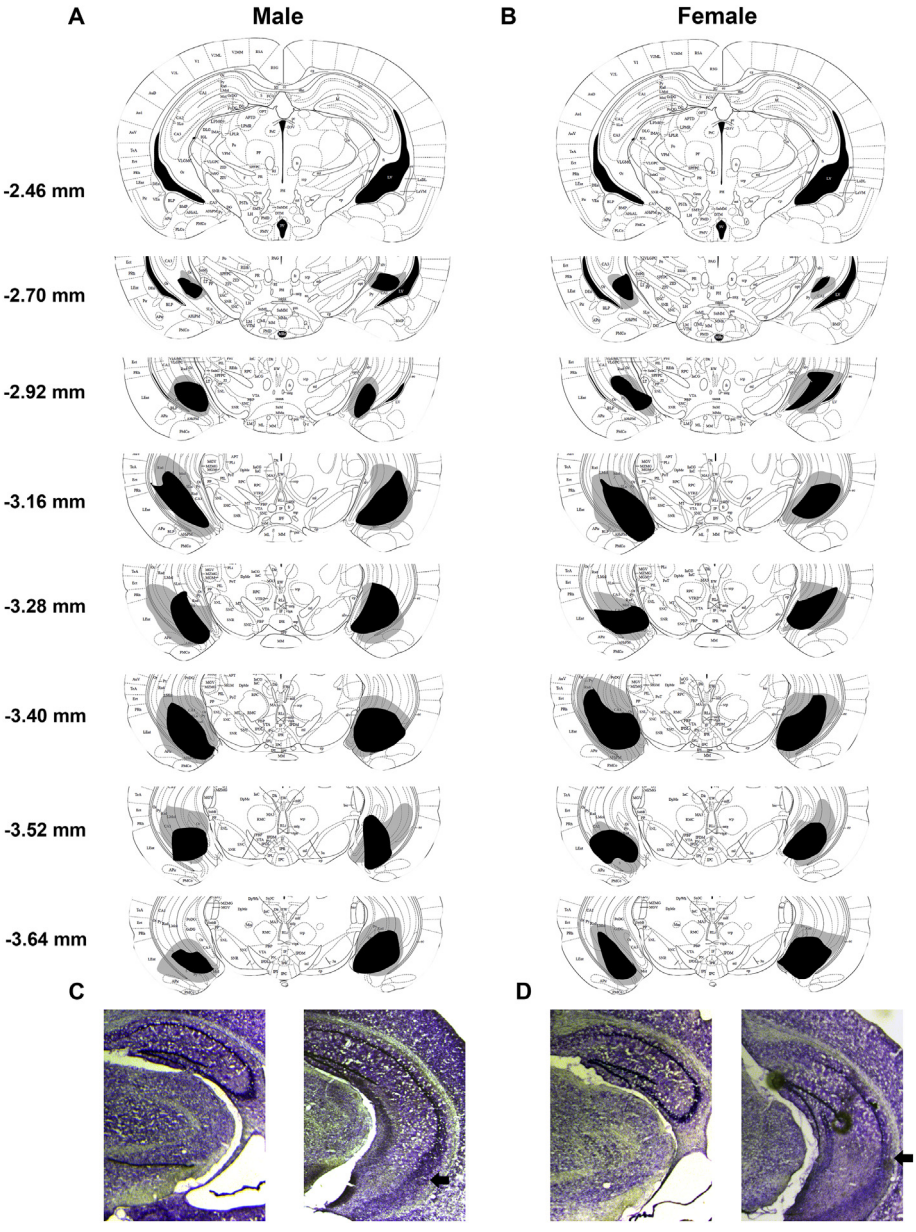

Fig. 2. Histology of ventral hippocampal excitotoxic lesion. Schematic of ventral hippocampal lesion size in male (A) and female mice (B). Coronal sections were made from AP −2.5 mm to AP −3.6 mm relative to Bregma. Gray and black zones in schematic figure represented maximum and minimum lesion areas, respectively. No lesions were detected in the slice of −2.46 mm in either male or female mice. (C, D) Representative slices of dorsal and ventral hippocampus from male (C) and female (D) mice after ibotenic acid lesioning. Dorsal hippocampus was intact (left), whereas ventral hippocampus was lesioned (right). Filled arrows indicated borders between intact and damaged tissues.

acquisition settings were maintained for all subsequent imaging of c-Fos.

For quantification of c-Fos expression, three slices were counted for ventral hippocampal CA1 (AP -3.3 mm, −3.4 mm, and − 3.5 mm relative to Bregma), basolateral amygdala (AP -1.5 mm, −1.6 mm, and − 1.7 mm relative to Bregma) and prelimbic cortex (AP 1.9 mm, 1.8 mm, and 1.5 mm relative to Bregma), respectively. c-Fos expression was quantified for each of these brain regions by ImageJ (Version 1.52, National Institutes of Health, USA). The numbers of c-Fos-positive neurons in left and right hemispheres was pooled since no significant differences were observed (data not shown).

Statistical analysis

All data were analyzed and plotted using the GraphPad Prism 6 software. Data were expressed as mean ± SEM (standard error of the mean). Males and females were analyzed separately. The assumption of normality and equality of variance was tested by Shapiro–Wilk test and F-test, respectively. All data met the normal distribution and had equal variances in each group. Unpaired Student's t test was performed in males. Two-way ANOVA with Bonferroni *post hoc* test was performed in females, with lesion and estrus cycle as the two factors. Probability values of $P < .05$ were considered to represent significant differences.

RESULTS

Reduced anxiety in male, but not female, mice with ventral hippocampal lesions

To examine biological sex differences in hippocampal modulation of anxiety-like behavior, we first performed excitotoxic lesions of bilateral ventral hippocampus by local injection of ibotenic acid. Fig. 2A and Fig. 2B showed schematic illustration of damages from male and female mice, respectively. The lesion extended throughout bilateral ventral hippocampus and included all cell fields, with minimal extrahippocampal damages. 2 male and 4 female mice were excluded from analysis due to incorrect lesion site, resulting in 30 male and 32 female mice in final statistical analysis. Male and female mice had an average of 56% (40%–77%) and 60% (37%–70%) bilateral tissue loss, respectively. No significant differences of lesion size were observed between males and females [$t_{(60)} = 1.07$, $P = .291$, unpaired t test].

In the novelty-suppressed feeding test, bilateral ventral hippocampal excitotoxic lesioning shortened the feeding latency in male mice [$t_{(28)} = 2.21$, $P = .036$, unpaired t test, Fig. 3A], indicating reduced anxiety. By sharp contrast, in female mice, the same lesion did not produce anxiolytic effects in this test [lesion effect: $F_{(1, 28)} = 0.23$, $P = .64$; interaction: $F_{(1, 28)} = 0.38$, $P = .541$, Fig. 3B], despite a significant effect of the estrus cycle [$F_{(1, 28)} = 597.00$, $P < .001$, two-way ANOVA with Bonferroni *post hoc* test] as previously reported (Mora et al., 1996; Seeman, 1997; Gangitano et al., 2009). No significant differences in food consumption were observed in either biological sex [male: $t_{(28)} = 0.30$, $P = .765$, unpaired t test, Fig. 3C; female: estrus cycle effect: $F_{(1, 28)} = 0.33$, $P = .568$; lesion effect: $F_{(1, 28)} = 0.05$, $P = .818$; interaction: $F_{(1, 28)} = 0.02$, $P = .899$, two-way ANOVA with Bonferroni *post hoc* test, Fig. 3D], indicating similar levels of hunger and motivation. The same conclusion could be reached after food consumption was normalized to body weight [male: $t_{(28)} = 0.52$, $P = .607$, unpaired t test; female: estrus cycle effect: $F_{(1, 28)} = 0.25$, $P = .623$; lesion effect: $F_{(1, 28)} = 0.46$, $P = .505$; interaction: $F_{(1, 28)} = 1.01$, $P = .323$, two-way ANOVA with Bonferroni *post hoc* test].

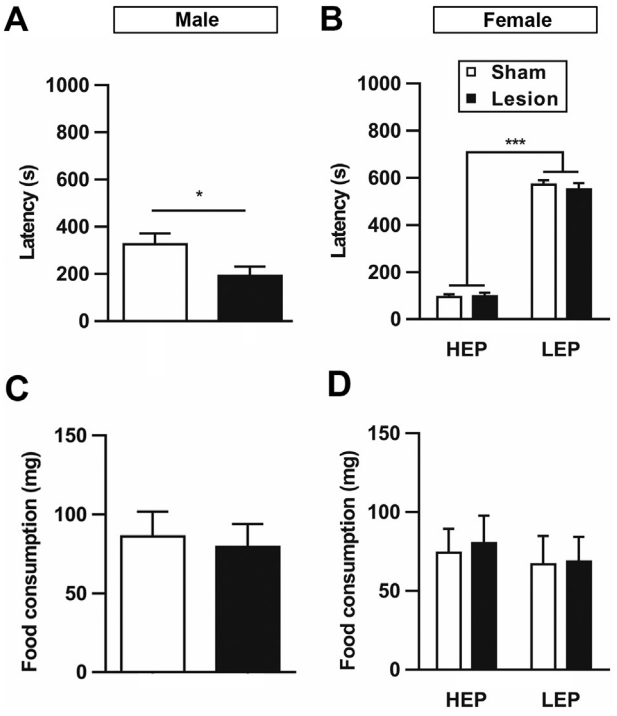

Fig. 3. Ventral hippocampal lesioning relieves anxiety-like behavior in the novelty-suppressed feeding test in male but not female mice. Bilateral ventral hippocampal lesioning decreased feeding latency in the novelty-suppressed feeding test in male (A) but not female (B) mice, despite significant influences from estrogen levels in females. Similar amount of food was consumed in two groups of male (C) and female (D) mice. *$P < .05$, **$P < .01$, ***$P < .001$, unpaired t test or two-way ANOVA with Bonferroni's test. n = 15/group for males, n = 8/group for females in HEP and LEP, respectively. HEP: high-estradiol phase, LEP: low-estradiol phase.

In the marble burying test, bilateral ventral hippocampal excitotoxic lesion resulted in fewer buried marbles in male mice [$t_{(28)} = 2.59$, $P = .016$, unpaired t test, Fig. 4A], indicating decreased anxiety level. Again, we failed to observe such effects in female mice, despite a significant effect of the estrus cycle [estrus cycle effect: $F_{(1, 28)} = 108.80$, $P < .001$; lesion effect: $F_{(1, 28)} = 0.22$, $P = .642$; interaction: $F_{(1, 28)} = 2.55$, $P = .121$, two-way ANOVA with Bonferroni *post hoc* test, Fig. 4B].

In the elevated plus-maze test, male mice spent more time [$t_{(28)} = 0.29$, $P = .023$, Fig. 5A] and exhibited more entries into the open arms [$t_{(28)} = 2.53$, $P = .018$, unpaired t test, Fig. 5C] after ventral hippocampal excitotoxic lesions.

This effect was not observed in female mice [time in open arms: estrus cycle effect: $F_{(1, 28)} = 23.66$, $P < .001$; lesion effect: $F_{(1, 28)} = 0.01$, $P = .973$; interaction: $F_{(1, 28)} = 0.11$, $P = .741$, Fig. 5B; entry into open arms: estrus cycle effect: $F_{(1, 28)} = 5.68$, $P = .024$; lesion effect: $F_{(1, 28)} = 0.54$, $P = .469$; interaction: $F_{(1, 28)} = 0.07$, $P = .797$, two-way ANOVA with Bonferroni *post hoc* test, Fig. 5D].

Bilateral ventral hippocampal excitotoxic lesions did not affect exploring behavior in male [$t_{(28)} = 0.78$, $P = .441$, unpaired t test, Fig. 5E] or female [estrus cycle effect: $F_{(1, 28)} = 2.52$, $P = .124$; lesion effect: $F_{(1, 28)} = 0.08$, $P = .778$; interaction: $F_{(1, 28)} = 0.02$, $P = .904$, Fig. 5F] mice, indicated by similar total arm entries in the plus maze. This was further confirmed by the open field test, where lesion effects were observed in neither biological sex [male: $t_{(28)} = 0.86$, $P = .398$, unpaired t test, Fig. 6A; female: estrus cycle effect: $F_{(1, 28)} = 0.05$, $P = .828$; lesion effect: $F_{(1, 28)} = 3.70$, $P = .065$; interaction: $F_{(1, 28)} = 0.51$, $P = .482$, two-way ANOVA with Bonferroni *post hoc* test, Fig. 6B].

Overall, these findings indicate anxiolytic effects of ventral hippocampal excitotoxic lesions in male but not female mice.

Stronger ventral hippocampal enrollment under innate anxiety in male than female mice

Several brain regions are involved in anxiety modulation, such as ventral hippocampus (Kjelstrup et al., 2002; Bannerman et al., 2003), amygdala (Davis, 1992; Davidson, 2002) and prefrontal cortex (Jinks and McGregor, 1997; Shah et al., 2004; Stern et al., 2010). One possible mechanism of the biological sex difference revealed above is that an anxiogenic context activated more hippocampal neurons in males than females. We observed increased c-Fos expression in ventral hippocampal CA1 [vCA1; exposure effect: $F_{(1, 31)} = 367.30$, $P < .001$; biological sex effect: $F_{(1, 31)} = 20.84$, $P < .001$; interaction: $F_{(1, 31)} = 25.31$, $P < .001$, Fig. 7A-C], basolateral amygdala [exposure effect: $F_{(1, 31)} = 10.82$, $P = .003$; biological sex effect: $F_{(1, 31)} = 4.22$, $P = .048$; interaction: $F_{(1, 31)} = 2.73$, $P = .109$, Fig. 7D-F] and prelimbic cortex [exposure effect: $F_{(1, 31)} = 277.20$, $P < .001$; biological sex effect: $F_{(1, 31)} = 0.01$, $P = .960$; interaction: $F_{(1, 31)} = 0.03$, $P = .868$, Fig. 7G-I] in both male and female mice. More interestingly, we detected significantly more c-Fos-positive neurons in vCA1 in males than females (Fig. 7C), indicating stronger enrollment of ventral hippocampal neurons in male mice under innate anxiety.

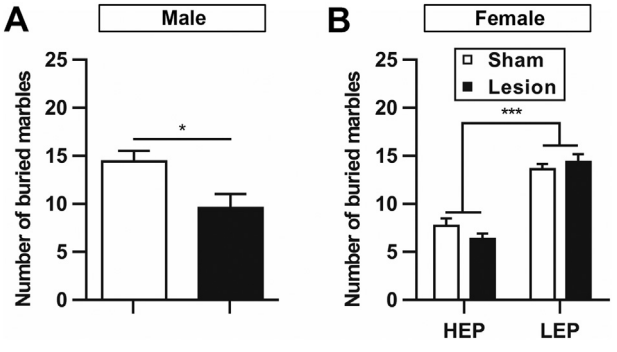

Fig. 4. Ventral hippocampal lesioning relieves anxiety-like behavior in the marble burying test in male but not female mice. Bilateral ventral hippocampal lesioning resulted in fewer marbles buried in male (A) but not female (B) mice, despite a significant effect of estrogen levels in females. *$P < .05$, **$P < .01$, ***$P < .001$, unpaired t test or two-way ANOVA with Bonferroni's test. n = 15/group for males, n = 8/group for females in HEP and LEP, respectively. HEP: high-estradiol phase, LEP: low-estradiol phase.

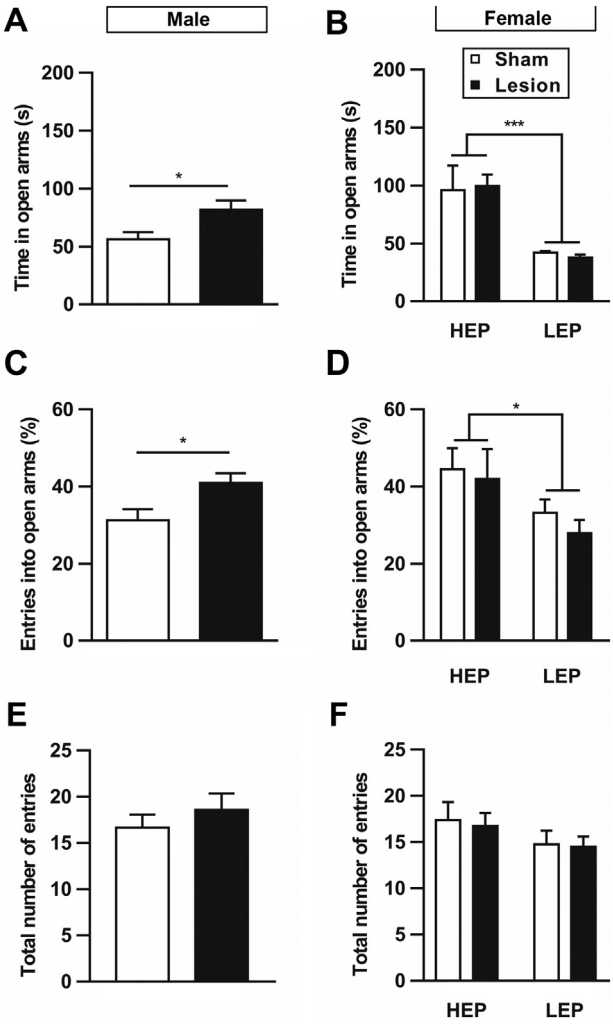

Fig. 5. Ventral hippocampal lesioning relieves anxiety-like behavior in the elevated plus-maze test in male but not female mice. Bilateral ventral hippocampal lesioning increased time spent in open arms in male (A) but not female (B) mice, and increased entries into open arms in male (C) but not female (D) mice. Bilateral ventral hippocampal lesioning had limited effect on total arm entries in male (E) or female (F) mice. *$P < .05$, **$P < .01$, ***$P < .001$, unpaired t test or two-way ANOVA with Bonferroni's test. n = 15/group for males, n = 8/group for females in HEP and LEP, respectively. HEP: high-estradiol phase, LEP: low-estradiol phase.

DISCUSSION

Substantial evidence supports sex difference in anxiety disorders (Kessler et al., 1994; Nolen-Hoeksema and Girgus, 1994; Craske and Stein, 2016). But the neural correlates remain insufficiently understood. In the present study, we

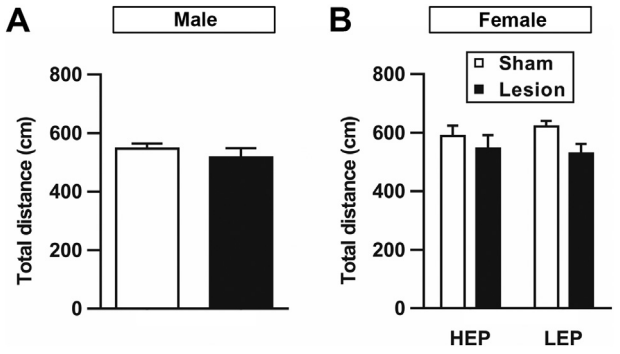

Fig. 6. Ventral hippocampal lesioning does not affect locomotive activity in the open field test. Bilateral ventral hippocampal lesioning did not affect distance traveled in the open field in male (A) or female (B) mice. *$P < .05$, **$P < .01$, ***$P < .001$, unpaired t test or two-way ANOVA with Bonferroni's test. n = 15/group for males, n = 8/group for females in HEP and LEP, respectively. HEP: high-estradiol phase, LEP: low-estradiol phase.

show that excitotoxic lesioning of ventral hippocampus yields anxiolysis in novelty-suppressed feeding, marble burying and elevated plus-maze tests in male C57BL/6 J mice (Figs. 3-5), consistent with previous reports (Bannerman et al., 2002; McHugh et al., 2004). By sharp contrast, the same lesion did not affect anxiety in female mice.

Sex hormones are important factors underlying biological sex difference in anxiety. The fluctuation of estrogen in the menstrual cycle, concomitant with alteration in levels of progesterone, androgens and their metabolites, increases susceptibility of women to develop affective disorders (Roca et al., 2003; Walf and Frye, 2006; Sahingoz et al., 2011). Our findings indicate that the estrus cycle has a robust effect on anxiety level in C57BL/6 J mice, showing low and high levels of anxiety in high- and low-estradiol phases, respectively (Figs. 3-5). This is consistent with a number of previous studies using the marble burying test in Wistar (Fernandez-Guasti and Picazo, 1992; Schneider and Popik, 2007) and Long–Evans rats (Llaneza and Frye, 2009), the elevated-plus maze test in Sprague–Dawley (Mora et al., 1996; Diaz-Veliz et al., 1997), Wistar (Marcondes et al., 2001) and Long–Evans rats (Walf and Frye, 2007), and the elevated T-maze test in Wistar rats (Gouveia Jr. et al., 2004). The hippocampus shows high-level expression of sex hormone receptors. Systemic, intra-hippocampal and intra-amygdala administration of estradiol all produces anxiolysis in rats (Frye and Walf, 2004; Walf and Frye, 2006). Estradiol binds to estrogen receptors α and β, which are widely distributed in hippocampus, amygdala, hypothalamus and other regions in rodents and humans (Shughrue et al., 1997; Osterlund et al., 2000), and exerts its anxiolytic effect at least partly through upregulating brain-derived neurotrophic factor expression in the brain (Gourley et al., 2008; Deltheil et al., 2009; Bath et al., 2012). However, the present study carefully differentiated between high and low estrogen level phases in the female, but did not observe different lesioning effects in either phase (Figs. 3-5). These findings indicate that the biological sex difference of hippocampal modulation of anxiety in the present study is independent of estrogen levels.

Human neuroimaging studies have revealed significantly stronger activation of hippocampus and amygdala under stress (Seo et al., 2011), as well as stronger correlation between trait anxiety and white matter tract integrity of the temporal lobe (Montag et al., 2012), in males than females. In the present study, we observed higher expression of c-Fos in vCA1, basolateral amygdala and prelimbic cortex in both male and female mice after the elevated-plus maze test, indicating enrollment of these brain regions in task-induced anxiety. However, we noticed significantly greater c-Fos expression in vCA1 in male than in female mice, despite similar levels of anxiety in the elevated plus-maze test (Fig. 7C). vCA1 and basolateral amygdala have robust reciprocal connections (O'Donnell and Grace, 1995; Pikkarainen et al., 1999) and participate in anxiety modulation in mice (Felix-Ortiz et al., 2013). It is possible that vCA1 is more strongly modulated by basolateral amygdala in male than in female mice after anxiety tests. Both ventral hippocampus and basolateral amygdala exert inhibition on the hypothalamic–pituitary-adrenocortical (HPA) axis (Jacobson and Sapolsky, 1991; Bhatnagar et al., 2004), and disinhibition of the HPA axis induces secretion of glucocorticoids and initiates numerous physiological effects (Herman and Cullinan, 1997; Herbert et al., 2006). Female HPA axis shows relative inability of adaptation, demonstrated by deficits in glucocorticoid receptor regulation in female but not male rats following chronic stress (Bourke et al., 2013). The deficits may result from distinct ventral hippocampus enrollment in anxiety in different sexes. However, we need to note that the present study checks c-Fos expression only in the elevated plus-maze test, thus could not exclude the possibility of a test-specific phenomenon unless other anxiety tests are carried out.

The weaker hippocampal involvement in anxiety-like behavior in female mice indicates alternative neural substrate of anxiety modulation in females. One possible candidate is the prefrontal cortex. Neuroimaging studies have shown stronger enrollment of prefrontal areas in anxiety tests in women (Hakamata et al., 2009; Marumo et al., 2009; Seo et al., 2017). However, we did not observe stronger activation in the prelimbic cortex in our study (Fig. 7I), which might be caused by different subregion included: only the prelimbic area was examined in the present study, whereas neuroimaging studies included all prefrontal subregions.

Neurons in vCA1 are heterogenous and different vCA1 subpopulations project to different downstream targets such as amygdala, infralimbic cortex and hypothalamus (Fanselow and Dong, 2010). Several studies have demonstrated hippocampal modulation of anxiety behavior by temporal and reversible manipulation of ventral hippocampal circuits. Inhibiting ventral hippocampal inputs to medial prefrontal cortex elicits anxiolysis (Padilla-Coreano et al., 2016; Parfitt et al., 2017), while inhibiting inputs to lateral septum (Parfitt et al., 2017) or activating inputs to lateral hypothalamus (Jimenez et al., 2018) elicits anxiogenic effects in mice. These seemingly conflicting data are consistent with the finding that distinct sub-populations of ventral CA1 pyramidal neurons target different downstream regions

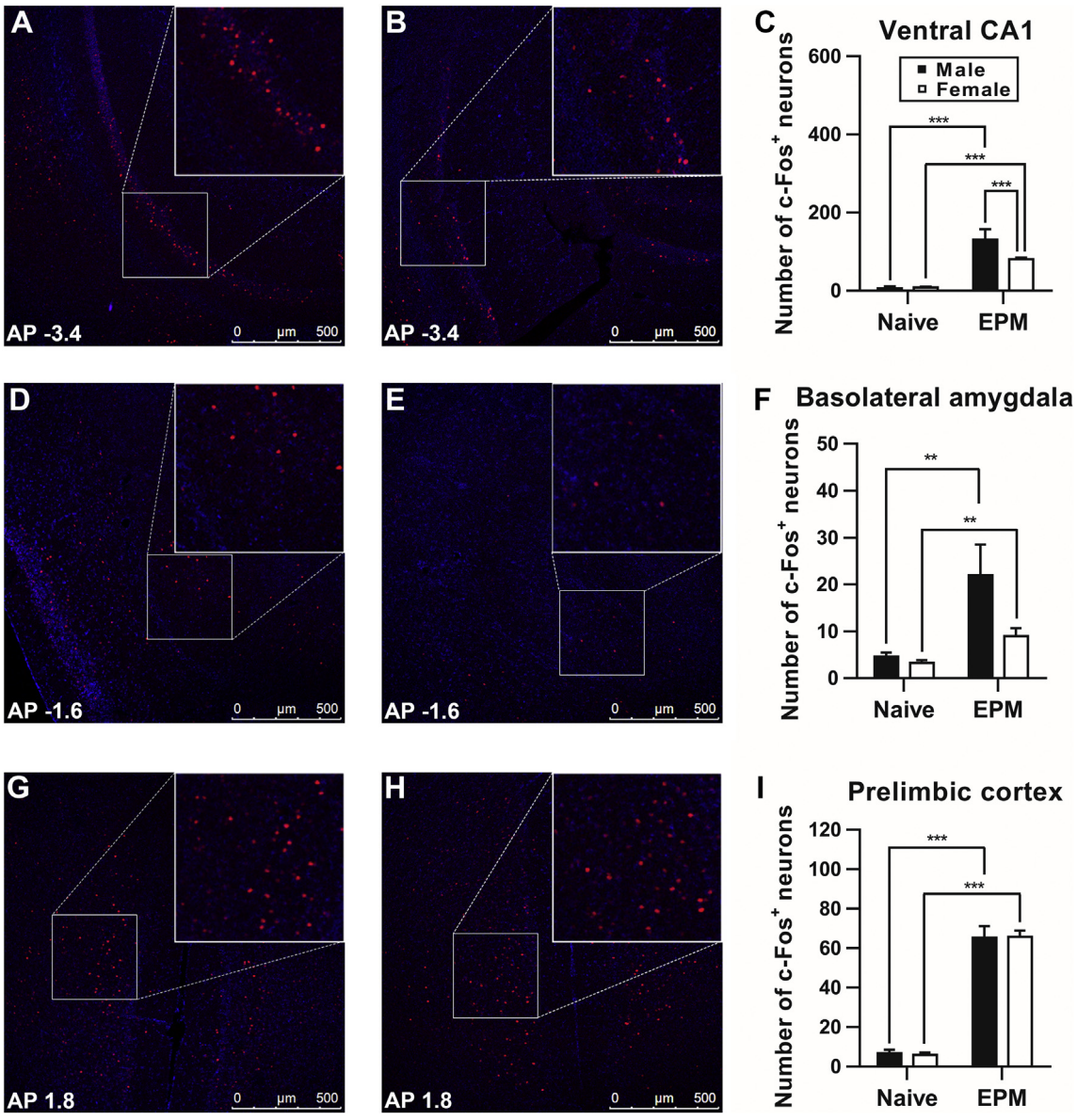

Fig. 7. Stronger enrollment of ventral hippocampus in innate anxiety in male mice. (A, B) Representative images of vCA1 (AP -3.4 mm relative to Bregma) c-Fos expression in males (A) and females (B) after exploration in an EPM. (C) Increased c-Fos expression in the vCA1 of both sexes after EPM exploration, especially in males. (D, E) Representative images depicting quantification of basolateral amygdala (AP -1.6 mm relative to Bregma) c-Fos expression in males and females after the elevated plus-maze test. (F) Increased c-Fos expression in the basolateral amygdala of both sexes after EPM exploration, with similar expression levels between males and females. (G, H) Representative images of prelimbic cortex (AP 1.8 mm relative to Bregma) c-Fos expression in males and females after the elevated plus-maze test. (I) Increased c-Fos expression in the prelimbic cortex of both sexes after EPM exploration, with similar expression levels between males and females. Scale bar: 100 μm. $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$, two-way ANOVA with Bonferroni's test, 3 mice/group, 3 slices/mouse. EPM: elevated plus-maze.

(Cenquizca and Swanson, 2007). However, most of these studies are performed in male animals. The present study did not dissect specific hippocampal subpopulations or circuits in anxiety modulation, leaving it an open question for further research.

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CONFLICT OF INTEREST

The authors declare no competing financial interests.

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