

## ARTICLE OPEN



# Environmental enrichment improves deficits in hippocampal neuroplasticity and cognition in prenatally aripiprazole-exposed mouse offspring

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Aripiprazole has become one of the most commonly prescribed antipsychotics, including in pregnant women, owing to a broad range of indications for psychiatric disorders and relatively few metabolic side effects. Compared with that of other antipsychotics, data regarding the safety of gestational aripiprazole exposure for offspring neurodevelopment are limited. This study investigated how prenatal exposure to aripiprazole affects the hippocampal neuroplasticity of adult offspring and whether any such effect can be reversed by environmental enrichment. Aripiprazole was administered to pregnant C57BL/6 N mice from embryonic days 6–16. Key findings revealed that aripiprazole exposure (3.0 mg/kg) persistently impaired hippocampal plasticity and related cognitive function in adult male offspring, including reduced adult neurogenesis, dendrite retraction and spine loss of granule cells in the dentate gyrus and recognition memory deficits. The proteomics results revealed decreased hippocampal levels of dopamine and cAMP-regulated phosphoprotein 32 kDa (DARPP-32), a key regulatory molecule of dopamine signaling. In addition, lower concentrations of dopamine and higher concentrations of serotonin in the hippocampus were detected in aripiprazole-exposed mice via HPLC with electrochemical detection. Notably, environmental enrichment reversed the disruption of spatial memory function and partially improved impaired hippocampal neuronal plasticity in prenatally aripiprazole-exposed mouse offspring. Our results provide insight into the long-term negative effects of early-life exposure to aripiprazole on hippocampal plasticity and behavior, which may be related to disturbances in the dopamine and serotonin transmitter systems. As a relatively “natural” intervention, environmental enrichment has potential for future clinical application.

*Translational Psychiatry* (2025)15:102; <https://doi.org/10.1038/s41398-025-03335-1>

## INTRODUCTION

Pregnant women are a high-risk group for mental illness. Owing to the significant impact of the illness on the social functioning of patients, proactive treatment is necessary once the disease is diagnosed. Research from more than 10 countries has shown a notable increase in the use of antipsychotics, particularly second-generation antipsychotics (SGAs), during pregnancy [1, 2]. Although limited evidence currently suggests that the use of SGAs during pregnancy does not pose a significant risk of congenital malformations [3], prospective studies [4, 5] and meta-analyses [6] have indicated that the use of antipsychotics during pregnancy can lead to short-term neurodevelopmental delays in offspring. Research on the long-term effects of prenatal exposure to these compounds on offspring neurodevelopment is limited, and the existing data in terms of quantity and quality do not permit definitive conclusions [7, 8]. However, animal studies have repeatedly indicated that prenatal exposure to first- and second-generation antipsychotics can cause long-term neural plasticity abnormalities and associated functional impairments in offspring [9–13]. Therefore, the long-term effects of using antipsychotics during pregnancy on offspring

neurodevelopment deserve attention and further in-depth investigation.

Aripiprazole is a relatively novel antipsychotic that has been increasingly used during pregnancy because of its minimal impact on female metabolism and the endocrine system. It has become one of the most frequently prescribed antipsychotics during pregnancy, second only to quetiapine [14]. Nonetheless, there is a serious lack of safety data concerning the use of aripiprazole in pregnant women. Although subsequent studies have not revealed a heightened risk of teratogenicity compared with other antipsychotics [15], a recent large-scale birth cohort study with a 14-year follow-up indicated a potential increase in the risk of neurodevelopmental disorders in offspring (1.36 [1.14–1.63]) associated with the use of aripiprazole during pregnancy [16]. The embryotoxicity of aripiprazole cannot be excluded. However, owing to ethical concerns, conducting case-control studies is nearly impossible. Therefore, there is an urgent need for relevant basic research to investigate the long-term effects of prenatal exposure to aripiprazole on offspring neurodevelopment.

Currently, research on the effects of prenatal aripiprazole exposure on offspring is limited. Investigations have revealed

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Received: 29 August 2024 Revised: 23 February 2025 Accepted: 18 March 2025

Published online: 28 March 2025

that exposure to different doses of aripiprazole during gestation may reduce the body weight of fetal rats, persist postnatally [17], and increase the expression of genes associated with apoptosis in the hippocampus [12]. Moreover, prenatal aripiprazole exposure could interact with 7-dehydrocholesterol reductase (DHCR7) mutants, consequently impairing neural development in embryos [18]. Research on other antipsychotics, including our previous findings, suggested that prenatal exposure to drugs such as haloperidol [19], risperidone [9, 11], and quetiapine [10, 20] could have long-term adverse effects on offspring brain structure and function, particularly those affecting hippocampus-mediated cognitive function. This might be related to ongoing neurogenesis in the dentate gyrus (DG) of the hippocampus and its crucial role in cognitive regulation [21]. Although the definitive mechanisms have not yet been fully elucidated, it is widely believed that the effects of prenatal exposure to antipsychotic medications on offspring neurodevelopment might be related to their pharmacological effects [5, 10, 19]. The monoaminergic neurotransmitter systems targeted by antipsychotics undergo development during early embryogenesis [22]. These systems not only facilitate neural transmission but also play critical roles in brain development by interacting with growth factors and cytokines, thereby influencing neuron and glial cell proliferation, maturation, and migration, as well as neuronal differentiation and apoptosis during specific developmental stages [23–25]. Consequently, exposure to aripiprazole during critical periods of brain development may affect offspring brain neurodevelopment and plasticity through the monoaminergic neurotransmitter system, leading to corresponding cognitive abnormalities.

In this study, we first evaluated the long-term behavioral consequences of different doses of gestational aripiprazole exposure on adult mouse offspring. On the basis of the results and the equivalent dose conversion between mice and humans, the intermediate dose (3 mg/kg) was selected for investigation of its biological effects and possible mechanisms. Proteomic analysis was used to explore the changes in the protein composition of the hippocampus in aripiprazole-exposed mice. In combination with other methods, exposure to aripiprazole during pregnancy led to disruption of the dopamine and serotonin neurotransmitter systems in the hippocampus of the offspring, particularly the downregulation of dopamine and cAMP-regulated phosphoprotein 32 kDa (DARPP-32), a key regulatory molecule of dopamine signaling. We then demonstrated that embryonic exposure to aripiprazole disrupted the plasticity of dentate neurons in adult mouse offspring. Finally, the effects of environmental enrichment (EE) [26], a relatively 'natural' intervention, on the negative effects of prenatal aripiprazole exposure on offspring neurodevelopment were tested.

## METHODS

Below is an overview of the key points. For more details, please refer to the supplementary materials.

### Animals

Adult male and female C57BL/6N mice (8 weeks old; Vital River Laboratories, Beijing, China) were used. After habituation for 1 week, male and female mice (1:1) were caged together overnight for mating. If a vaginal plug was detected, it was considered a pregnancy and recorded as embryonic day 0 (E0) and then single-housed until delivery.

### Groups and choice of doses

Aripiprazole (Ari) was purchased from Sigma–Aldrich (St. Louis, MO) and was dissolved in 1% Tween-80 with saline to the desired concentration. The principle of drug dosage selection refers to the study of Li and colleagues [27]. According to the equivalent dose conversion of human and mouse drugs [28], 1 mg/kg, 3 mg/kg and 10 mg/kg were selected.

The pregnant dams were randomly exposed to Ari or vehicle (Veh) daily from E6–E16 [11], which is a sensitive and critical period for brain development [29]. All the substances were administered intraperitoneally (i.p.) at a volume of 10 mL/kg. The body weights of the pregnant mice were recorded daily during the drug treatment period. The day of birth was designated postnatal day 0 (PND 0). Pups were weighed once a week and were weaned on PND 28. Only male offspring were included in this study.

### Behavioral testing

The Any Maze 4.98 spontaneous activity video analysis system (Stoelting, Wood Dale, IL) was used to monitor and automatically record the mouse's activity track. All behavioral tests were conducted between 09:00 am and 02:00 pm. All the experimental procedures, including open field (OF) test, elevated plus-maze (EPM) test, spatial object recognition (SOR) task, and novel object recognition (NOR) task, were carried out in accordance with the protocols outlined in our previously published work [30]. The SOR task and NOR task were scored by an investigator blind to treatment conditions. The comprehensive procedures are delineated in the supplementary experimental procedures.

### Liquid chromatography coupled with mass spectrometry (LC–MS/MS) proteomics analysis

All analyses were detected on a Thermo Fusion LUMOS mass spectrometer. A linear gradient was employed at a flow rate of 0.3 µL/min. Mobile phase A consisted of 0.1% formic acid, while mobile phase B consisted of 80% acetonitrile and 0.1% formic acid. For details, see the supplemental experimental procedures. Differential protein screening was conducted using a threshold of a 1.2-fold difference (FC = fold change) and  $p < 0.05$ .

### Dopamine and serotonin levels in the hippocampus

Dopamine (DA) and serotonin (5-HT) levels in the hippocampus of offspring were measured via high-performance liquid chromatography-electrochemical detector (HPLC-ECD), following a previously described method [31]. For details, see the supplemental experimental procedures.

### Golgi-Cox staining and analysis of dendrites and spines

Golgi-Cox staining and the subsequent analysis of dendritic structures and spines followed the procedures outlined in our previous study [32]. Detailed methods are provided in the supplementary experimental procedures.

### Environmental enrichment (EE)

The enriched environment box had dimensions of 36 × 25 × 60 cm, and it was made of acrylic. The cage was divided into three layers, connected by stairs and pipes, and equipped with wheels, swings, pipes, houses, and other amenities. Toys of various shapes and colors were placed inside the cage. The mice in the cage had free access to food and water. To maintain novelty, the type, number, and location of the toys were changed weekly. Additionally, to increase social opportunities, 5–6 mice were housed together in each enriched environment box. The control mice were kept in regular cages with 3–4 mice per cage. To address the progressive abnormalities in neural development and to mitigate aggression associated with adult co-housing, EE was initiated at weaning (PND 28) and maintained until the end of behavioral assessments (PND 78).

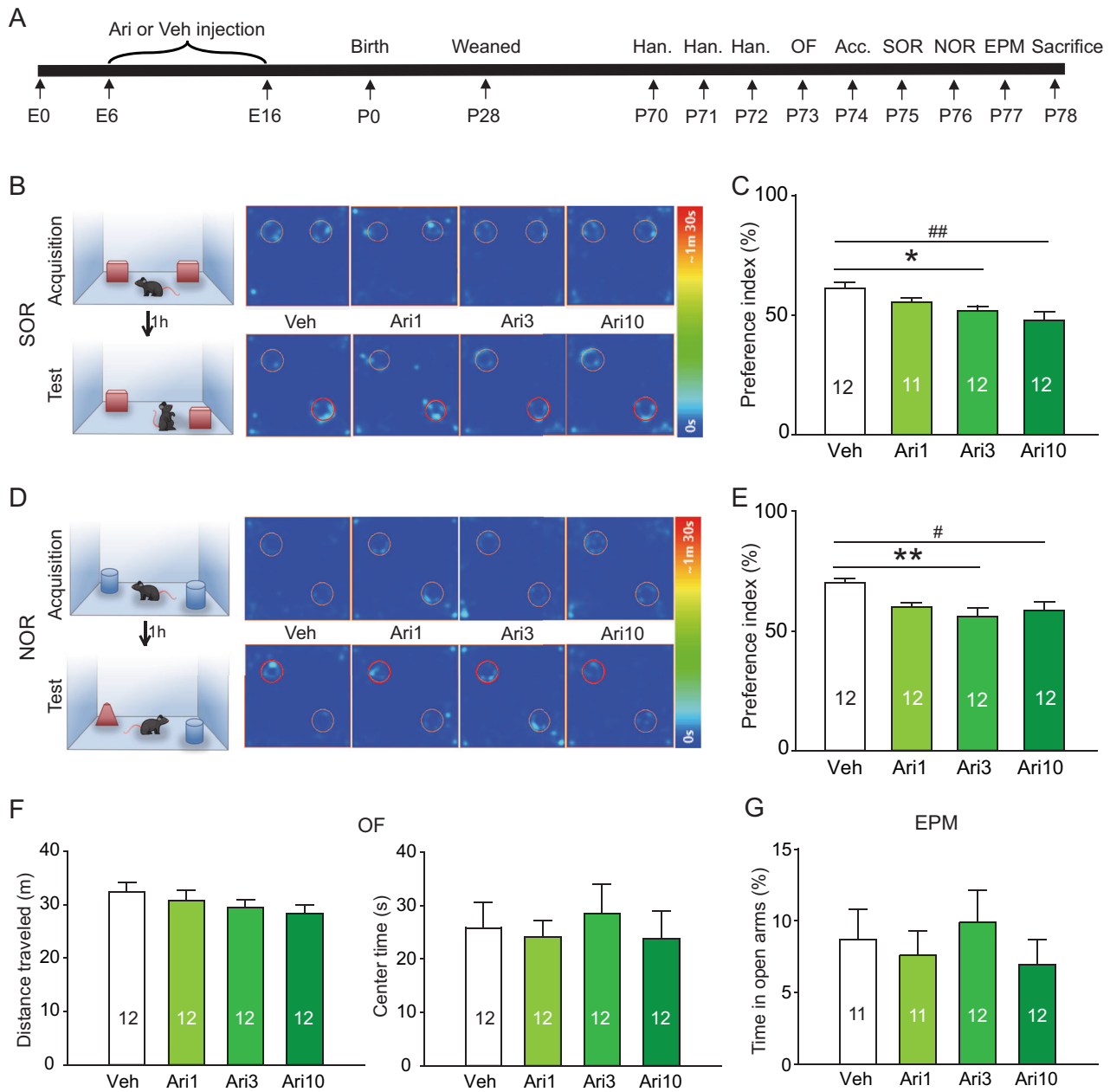
### Statistical analysis

SPSS 19.0 (SPSS, Chicago, IL) was used for the statistical analysis. Statistical significance was defined as  $p < 0.05$ . All the statistical data, including statistical methods,  $p$  and  $F/t$  values, are presented in Table S1.

## RESULTS

### Prenatal exposure to aripiprazole (Ari) impaired recognition memory in adult mice

Exposure to high-dose aripiprazole (10 mg/kg) during pregnancy led to a decrease in the number of pups, whereas the fertility of the mice in the medium-dose (3 mg/kg) and low-dose (1 mg/kg) groups did not exhibit any abnormalities (Figure S1A). Maternal body weight gain was not significantly affected by aripiprazole administration during pregnancy (Figure S1B). However, there was



**Fig. 1** Prenatal exposure to aripiprazole (Ari) impaired recognition memory in adult mice. **A** Flowchart of prenatal aripiprazole administration, behavioral assessment, Golgi-Cox staining, and immunohistochemistry procedures. **B** Schematic diagram of exploration time in the spatial object recognition (SOR) test. **C** In the SOR test, the spatial recognition ability of the 3 mg/kg aripiprazole (Ari3) and 10 mg/kg aripiprazole (Ari10) groups, but not the 1 mg/kg aripiprazole (Ari1) group, was impaired. **D** Schematic diagram of exploration time in the novel object recognition (NOR) test. **E** In the NOR test, only Ari3 and Ari10 mice exhibited recognition memory deficits. **F** In the open field (OF) test, the total distance traveled (left) and the time spent in the center zone (right) in adult offspring did not differ among the three aripiprazole-administered groups. **G** Anxiety levels evaluated via the elevated plus maze (EPM) were comparable among the three groups. \* $P < 0.05$ , \*\* $P < 0.01$ , Ari3 group vs vehicle (Veh) group. # $P < 0.05$ , ## $P < 0.01$ , Ari10 group vs Veh group. E embryonic, P postnatal, Han handling, Acc. acclimation. In this and subsequent figures, the number of animals in each group is indicated in the bar groups.

a significant difference in the weight of offspring, which was primarily observed in the high-dose and low-dose groups (Figure S1C).

In the SOR test, both the medium- and high-dose groups were unable to discriminate displaced objects from nondisplaced objects in male offspring (Figure S2A). Both of these treatment groups performed worse than the control group did, whereas spatial memory ability remained unaffected in the low-dose group (Fig. 1A–C). Similarly, in the NOR test, although all four groups of mice were able to identify novel objects (Figure S2B), further

comparison of the preference indices indicated that the medium- and high-dose groups presented a significantly lower preference than the control group did (Fig. 1D, E). These findings suggest that exposure to aripiprazole (3 and 10 mg/kg) during pregnancy disrupted hippocampus-dependent learning and memory performance in adult offspring.

To assess anxiety levels in prenatally aripiprazole-exposed offspring, the OF and EPM tests were used. The total distance traveled and center time in the OF (Fig. 1F), as well as the percentage of time spent in the open arms (Fig. 1G), were similar

among the four groups. Therefore, prenatal exposure to different doses of aripiprazole did not affect anxiety-related behaviors in adult offspring. Moreover, there were no differences in the total distance traveled in the SOR, NOR, OF and EPM tests, nor in the total number of arm entries in the EPM test (Figure S2C–F), indirectly suggesting that prenatal exposure to aripiprazole does not affect the motor function in male offspring.

In conclusion, since both the medium- and high-dose groups of aripiprazole resulted in hippocampus-dependent cognitive disruption in offspring, the 3 mg/kg dose given to mice corresponds to 14.58 mg/day for humans on the basis of equivalent dose conversion [28], which falls within the normal dose range for humans. Additionally, D2 receptor occupancy was greater than 70% in the striatum [33]. However, the 10 mg/kg dose of aripiprazole exceeded the maximum clinical dose. Therefore, to provide a better experimental basis for clinical medication, subsequent experiments focused on the 3 mg/kg exposure dose group.

### Exposure to aripiprazole during pregnancy led to disturbances of the dopaminergic and serotonergic systems in the hippocampus of adult offspring

The effects of prenatal exposure to aripiprazole on these neurotransmitter systems, including receptor mRNA expression, neurotransmitter levels, and signal transduction, in the hippocampus of offspring were investigated. In addition, we found that the weight of the hippocampus was significantly reduced in the group exposed to aripiprazole (Figure S3A). LC–MS/MS analysis was performed to explore the impact of maternal aripiprazole exposure on protein expression in the hippocampus of offspring. A total of 4668 proteins were co-expressed in both groups, with 199 proteins increased and 174 proteins decreased in the aripiprazole group (Figure S3B). Hierarchical clustering analysis was used to analyze the differential protein expression patterns (Figure S3C).

KEGG pathway analysis identified several differentially expressed molecules. In the dopaminergic synaptic pathway, the differentially expressed molecules, including Ppp1r1b, Ppp1cb, Ppp2r5a, Ppp2r5d, and Ppp3cb, are predominantly involved in the DARPP-32 (Ppp1r1b) cascade. Downstream molecules such as Grin2a (GluN2A) and Grin2b (GluN2B), which play important regulatory roles in hippocampal synaptic plasticity and learning and memory processes [34, 35], were also affected (Fig. 2A and Table S2). DARPP-32 was significantly decreased, which was confirmed by both immunohistochemical staining and quantitative mRNA analysis. (Fig. 2B–D). Dopamine levels in the hippocampus were decreased in the aripiprazole group (Fig. 2E). However, prenatal exposure to aripiprazole did not affect the mRNA expression of dopamine receptor subtypes (*D1a*, *D2*) in the offspring hippocampus (Fig. 2F, G).

The differentially expressed molecules within the serotonergic synaptic pathway are detailed in Figure S4A and Table S3, although they lack specificity for serotonergic signaling. We examined the expression levels of GSK-3 $\beta$ , a key regulatory molecule in this pathway [36], and found no changes in the protein or mRNA expression levels of GSK-3 $\beta$  in the hippocampus (Figure S4B–C and Fig. 2H). In terms of 5-HT neurotransmitter levels in the hippocampus, we observed an increase in the offspring exposed to aripiprazole (Fig. 2I). The mRNA expression level of the 5-HT<sub>1a</sub> receptor showed a marginal decrease, whereas the expression level of the 5-HT<sub>2a</sub> receptor remained unchanged (Fig. 2J, K).

### Prenatal exposure to aripiprazole impaired the plasticity of dentate granule cells in adult male mice

The signaling systems mediated by DA and 5-HT play crucial roles in the differentiation of the forebrain and the formation of neural circuits during neurodevelopment. To investigate the impact of

prenatal exposure to aripiprazole on adult neurogenesis in offspring, we performed immunostaining for Ki-67, a marker of cellular proliferation, and doublecortin (DCX), a marker of early neurogenesis. The results revealed that prenatal exposure to aripiprazole significantly reduced the density of Ki-67-positive cells (Fig. 3A–C) and DCX-positive cells (Fig. 3D–F) in the DG (including both DGsp and DGip) of adult offspring, suggesting that prenatal exposure to aripiprazole impairs neurogenesis in adult offspring.

With respect to the structural plasticity of granule cells in the DG, a significant decrease in dendritic length (Fig. 3G, H) and the number of intersections of granule cells (Fig. 3I) were observed in the adult offspring. Additionally, prenatal exposure to aripiprazole reduced the density of total dendritic spines of granule cells in the adult offspring, with pronounced effects on the thin and mushroom subtypes but no effect on the stubby spines (Fig. 3J–L). No significant differences were observed in the DGsp or DGip (Figure S5).

### Effects of environmental enrichment on brain developmental deficits induced by prenatal exposure to aripiprazole

The multiple subtle impairments in brain development caused by aripiprazole exposure during pregnancy may be attributed to perturbations in the monoamine neurotransmitter system. The complexity of the monoamine system makes it difficult to find a suitable drug intervention that targets these specific effects. Therefore, we have taken an alternative approach by exploring interventions that focus on improving phenotypic outcomes. EE has been shown to be a potentially promising intervention for increasing adult neurogenesis and promoting dendritic development. We found that EE reversed the disruption of spatial object recognition in offspring caused by gestational aripiprazole exposure (Fig. 4A–D). Additionally, EE did not influence anxiety levels in the offspring (Fig. 4E, F). Considering the previous biological changes observed, we further investigated the potential mechanisms by which EE mitigates cognitive deficits in mice.

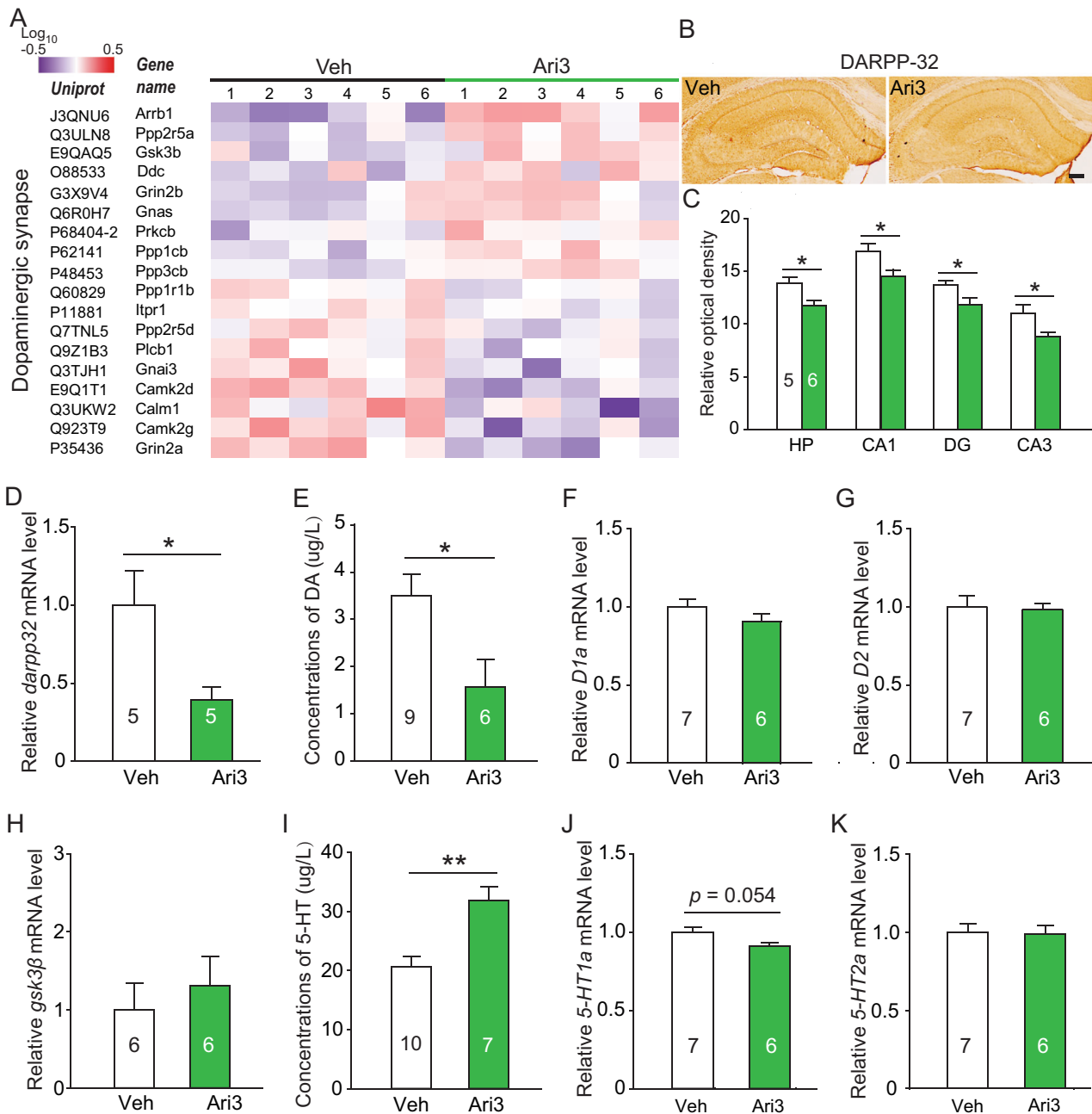
Consistent with previous findings in the prenatal aripiprazole administration model, a decrease in DARPP-32 protein levels in the hippocampus of offspring was detected, whereas EE resulted in an increase of DARPP-32 protein levels, but interaction effects were not detected (Fig. 4G, H). In terms of adult neurogenesis, we observed that EE had no effect on the decrease in the density of Ki-67-positive cells in the DG of offspring caused by maternal administration of aripiprazole (Figure S6A–B). As we observed above, a decrease in the density of DCX-positive cells in the DG in offspring was induced by aripiprazole exposure during pregnancy. Notably, EE led to an increase in DCX-positive cell density, although no interaction effect was found (Fig. 5A, B).

Furthermore, we found that EE reversed the reduction in the dendrite length of the dentate granule cells of offspring mice caused by aripiprazole exposure during pregnancy (Fig. 5C, D). Similarly, EE reversed the decrease in dendritic complexity in the DG of offspring caused by gestational aripiprazole exposure (Fig. 5E). In terms of the density of the dendritic spines of granule cells, EE was able to reverse the decrease in the density of the dendritic spines (Fig. 5F, G). These findings indicate that EE intervention can reverse the defects in the structural plasticity of dentate granule cells in prenatally aripiprazole-exposed mouse offspring.

### DISCUSSION

In this study, we investigated the impact of prenatal exposure to aripiprazole on the hippocampal development of adult male offspring, from the molecular and cellular levels to behavior. Our findings revealed that maternal exposure to aripiprazole disrupted hippocampus-dependent cognitive tasks and inhibited hippocampal neurogenesis, dendritic retraction, and spine loss in granule cells of the DG in adult male offspring. These observed impairments may be attributed to disturbances in the DA and



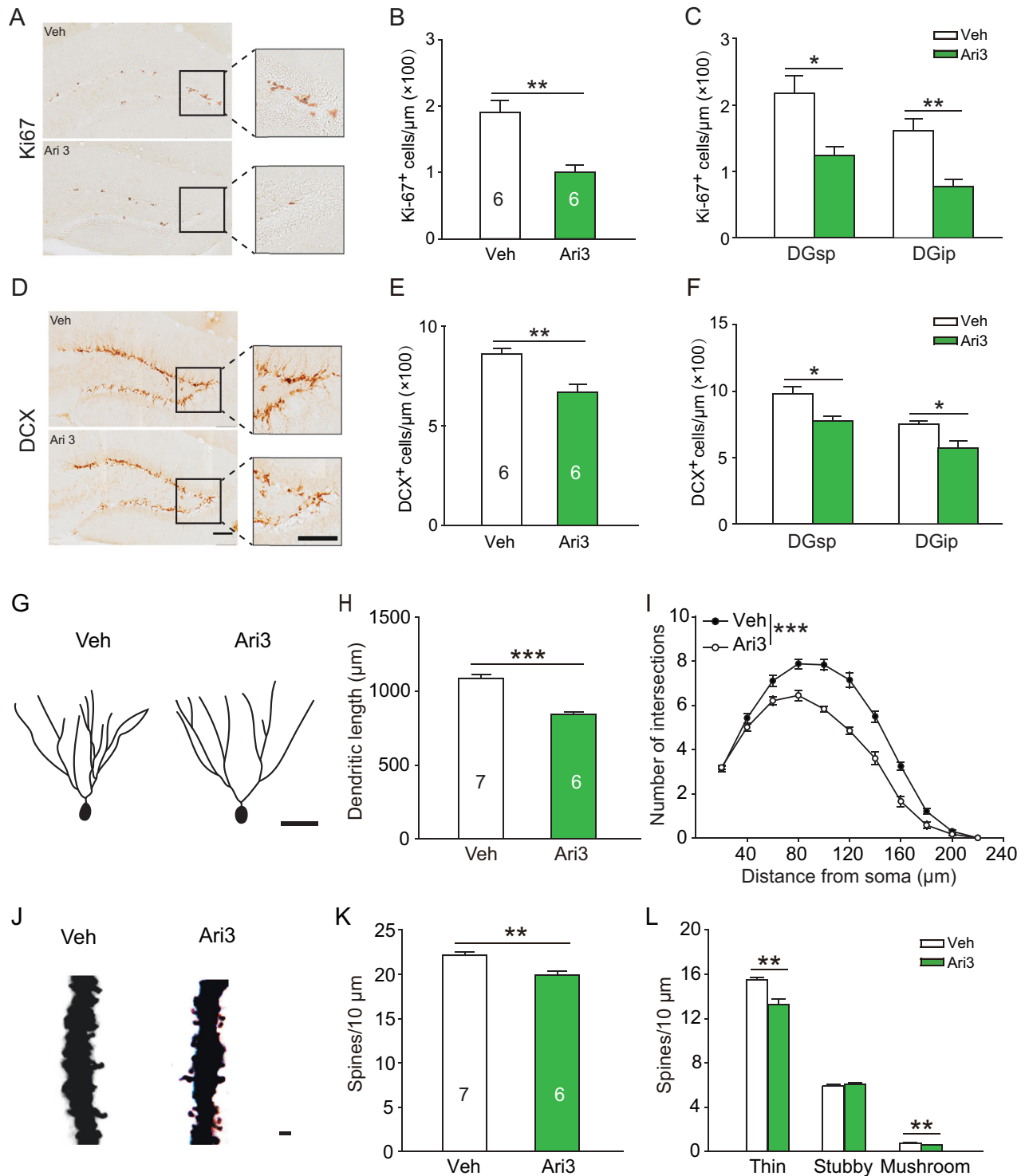


**Fig. 2** Exposure to aripiprazole during pregnancy led to disturbance of the dopamine and 5-HT neurotransmitter system in the hippocampus of adult offspring. **A** Heatmap displaying differentially expressed molecules related to the dopaminergic synapse between Ari3 and Veh. **B** Representative sections showing the immunoreactivity of DARPP-32 in the hippocampus of both groups. Immunohistochemical staining. Scale bar = 200  $\mu$ m. **C** Bar plots illustrating the relative optical density of DARPP-32 in different subregions of the hippocampus. **D** Relative mRNA levels of *darpp32* in the hippocampus are shown. **E** Dopamine neurotransmitter levels were significantly decreased in the hippocampus of the aripiprazole-exposed group. **F, G** Aripiprazole exposure during pregnancy had no effect on the mRNA expression levels of hippocampal dopamine receptor subtypes in offspring. **H** Aripiprazole exposure during pregnancy had no effect on the mRNA levels of *gsk3 $\beta$*  in hippocampus. **I** Level of serotonin neurotransmitter was significantly decreased in hippocampus of aripiprazole-exposed group. **J, K** Exposure to aripiprazole during pregnancy resulted in a down-regulation trend of *5-HT1a* receptor mRNA expression levels in the offspring, but not *5-HT2a*. \* $P < 0.05$ , \*\* $P < 0.01$ , Ari3 group vs vehicle (Veh) group. Uniprot universal protein.

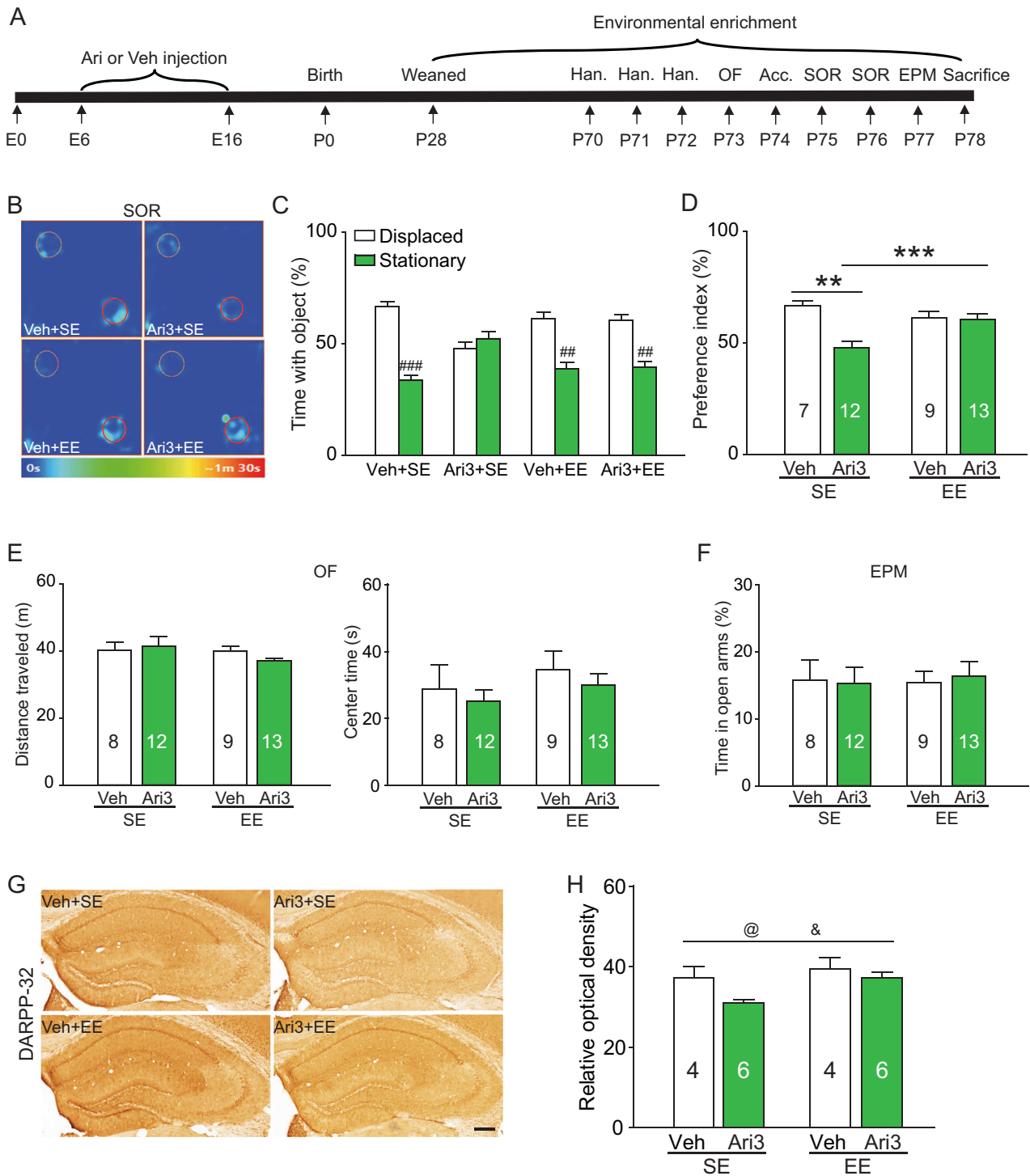
5-HT neurotransmitter systems. We also demonstrated that EE, as a natural intervention, effectively reversed the impairments in the cognitive function and structural plasticity of dentate granule cells in male offspring caused by prenatal exposure to aripiprazole.

We observed that exposure to aripiprazole at a high dose (10 mg/kg) during pregnancy resulted in a reduction in the number of pups born, which aligns with the findings of Thiago and colleagues [18]. However, the effect on male offspring body

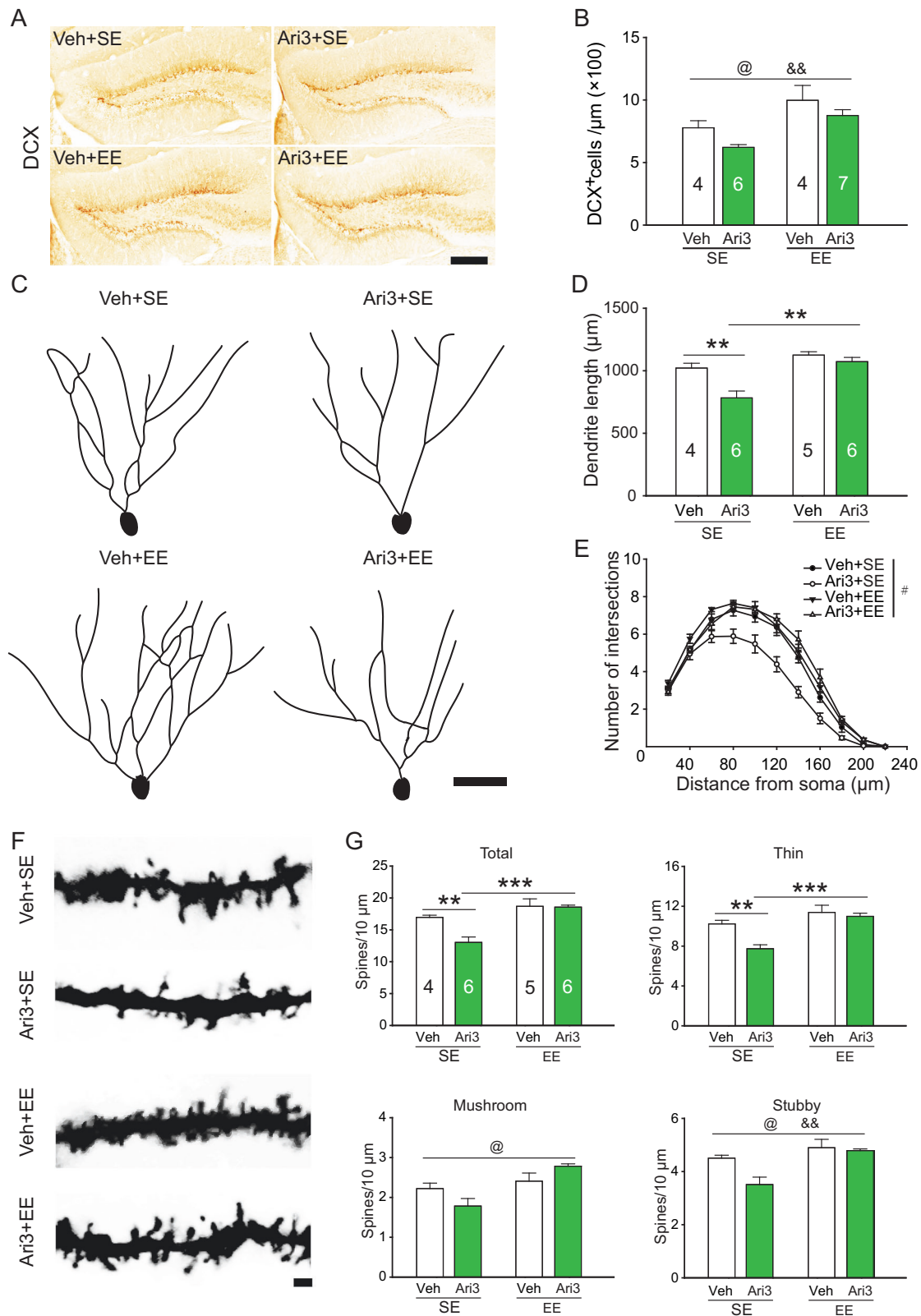
weight in our study did not align with Singh's findings [17]. Singh and colleagues reported a decrease in offspring body weight with embryonic administration of aripiprazole at doses of 2 mg/kg, 3 mg/kg, and 5 mg/kg in rats. The discrepancy may be attributed to the fact that our study included only male offspring, whereas their study included both male and female offspring. This difference could be because antipsychotics have a delayed effect on sexual maturation in female offspring [37]. Furthermore, our



**Fig. 3 Effects of prenatal aripiprazole exposure on adult neurogenesis and the dendritic architecture of dentate gyrus (DG) granule cells.** **A** Representative sections showing the immunoreactivity of Ki-67-positive neurons in the DG of the 2 groups. Scale bar = 100  $\mu$ m. **B**, **C** Exposure to aripiprazole significantly decreased the density of Ki-67-positive cells in the whole DG **B**, suprapyramidal blade (DGsp) and infrapyramidal blade (DGip) **C** in adult offspring. **D** Representative sections showing the immunoreactivity of doublecortin (DCX)-positive neurons in the DG of the 2 groups. Scale bar = 100  $\mu$ m. **E**, **F** The density of DCX-positive cells in the whole DG **E**, DGsp and DGip **F** was also decreased. **G** Representative tracings of Golgi-impregnated granule cells reconstructed by NeuroLucida in the 2 groups. Scale bar = 50  $\mu$ m. **H**, **I** Aripiprazole exposure significantly reduced the length of dendrites **H** and the complexity of dendrites **I**. **J** Representative photomicrographs of dendrite segments. Scale bar = 2  $\mu$ m. **K**, **L** Prenatal aripiprazole (Ari) exposure evoked a decrease in the total number of spines **K** as well as thin and mushroom spines **L**. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , Ari3 group vs vehicle (Veh) group.



**Fig. 4** Environmental enrichment reversed the deleterious effects on hippocampus-dependent cognitive function in adult offspring induced by gestational aripiprazole exposure. **A** Experimental timeline of prenatal aripiprazole administration, EE, behavioral assessment, Golgi-Cox staining, and immunohistochemistry procedures. **B** Heatmap of exploration time in the spatial object recognition (SOR) test. **C** Comparison of the percentage of time spent exploring objects in each group of mice in the SOR test. **D** EE ameliorated aripiprazole-induced deficits in spatial memory. **E**, **F** EE had no effect on anxiety-related behavior, as measured by the OF test and EPM. **G** Representative sections showing the immunoreactivity of DARPP-32 in the hippocampus of the four groups. Scale bar = 200  $\mu$ m. **H** Aripiprazole administration prenatally decreased the expression of DARPP-32, whereas EE increased the expression level of DARPP-32. \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , comparisons between two groups; ## $P < 0.01$ , ### $P < 0.001$ , within-group comparisons; @ $P < 0.05$ , significant aripiprazole effect; & $P < 0.05$ , significant intervention effect. SE standard environment, EE environmental enrichment.



study revealed that maternal exposure to aripiprazole had no effect on anxiety levels in offspring, which is generally in line with previous studies investigating other antipsychotics, including haloperidol [11], risperidone [11, 38], and asenapine [39]. When examining offspring cognitive performance, the evidence to date

has been inconsistent, but most studies have reported that exposure to either first- or second-generation antipsychotics during pregnancy can lead to hippocampus-dependent cognitive impairment in adult offspring [9–11]. This is generally in line with our own findings.



**Fig. 5 Environmental enrichment ameliorated the negative effects of prenatal aripiprazole exposure on the plasticity of the dentate gyrus (DG).** **A** Representative sections showing the immunoreactivity of doublecortin (DCX)-positive neurons in the DG. Scale bar = 200  $\mu$ m. **B** Aripiprazole exposure during pregnancy decreased the density of DCX-positive cells in the DG, whereas EE increased the density of DCX-positive cells. **C** Representative tracings of Golgi-impregnated granule cells reconstructed by NeuroLucida in the four groups. Scale bar = 50  $\mu$ m. **D, E** EE reversed the aripiprazole-induced reduction in the length and complexity of dendrites. **F** Representative photomicrographs of dendrite segments. Scale bar = 2  $\mu$ m. **G** EE prevented the aripiprazole-induced loss of total, thin and mushroom spines on DG dendrites. For stubby spines, aripiprazole exposure during pregnancy decreased the density, whereas EE increased it.  $^{**}P < 0.01$ ,  $^{***}P < 0.001$ , comparisons between two groups;  $^{\oplus}P < 0.05$ , significant aripiprazole effect;  $^{\otimes}P < 0.01$ , significant intervention effect;  $^{\#}P < 0.05$ , significant interaction effect. SE standard environment, EE environmental enrichment.

To the best of our knowledge, this study is the first to report that maternal exposure to aripiprazole can lead to perturbations in the hippocampal DA and 5-HT transmitter systems in offspring, including changes in transmitter levels and signaling pathways. In our study, we observed a reduction in DA levels in the offspring. This may be attributed to a decrease in midbrain dopaminergic projections, as previous studies have shown that prenatal exposure to haloperidol can lead to a reduction in midbrain dopaminergic neuron activity [40], and the hippocampus primarily receives dopamine from midbrain dopaminergic projections [41]. Additionally, it has been suggested that low levels of DA transmitters, particularly those binding to D2 receptors [42], may interfere with the induction and maintenance of long-term potentiation (LTP), a process associated with learning and memory [43]. We also observed a decrease in DARPP-32 expression in the hippocampus of the offspring. DARPP-32 is distributed primarily in neurons receiving dopaminergic projections and plays a bidirectional regulatory role in protein phosphorylation and dephosphorylation through its phosphorylation at different sites [44]. Research has suggested that the activation of DARPP-32 is necessary for the induction of long-term depression (LTD) and LTP [45]. Additionally, DARPP-32 can influence synaptic plasticity by regulating the interaction between  $\beta$ -adducin and actin, thereby affecting the stability of the cytoskeleton in spines and dendrites [46]. In the brains of patients with schizophrenia, the expression of DARPP-32 is significantly reduced [47], and haplotype variations in the gene encoding DARPP-32 have been found to be closely related to working memory encoding and emotional processing [48]. These findings suggest that prenatal exposure to aripiprazole may impact offspring behavior and brain outcomes by affecting the expression and function of DARPP-32.

In addition to its effects on the DA system, maternal exposure to aripiprazole also disrupted the 5-HT transmitter system in offspring. This disruption manifested as an increase in 5-HT levels and a downward trend in the mRNA expression of 5-HT<sub>1A</sub> receptors. These bidirectional alterations were consistent with previous studies [49, 50]. Previous *in vivo* studies have shown that the administration of 5-HT<sub>1A</sub> agonists in the rat hippocampus leads to decreased 5-HT levels, which may be attributed to the dual localization and effects of 5-HT<sub>1A</sub> receptors [50]. Although increasing extracellular 5-HT concentrations through the induction of 5-HT release or blockade of 5-HT reuptake via pharmacological methods has been shown to maintain or improve spatial cognitive function in animals, abnormalities in downstream signaling pathways may impede these effects [51]. Moreover, there is strong interplay between the DA and 5-HT systems, which mutually interact with each other. Future studies should delve deeper into these interactions via updated assays and methodologies. The impact of monoamine transmitter systems on neuroplasticity has been extensively documented [52, 53]. In our current study, we discovered that maternal exposure to aripiprazole markedly impaired neurogenesis in the DG. This outcome potentially arises from irregularities within the DA and 5-HT transmitter systems in adult offspring.

In our study, we observed that maternal exposure to aripiprazole resulted in a reduction in the dendritic length and

complexity of granule cells in the offspring's DG, findings that are consistent with our previous studies on haloperidol and risperidone [11]. Similarly, Singh et al. reported that maternal exposure to risperidone [9] and quetiapine [10] reduced the thickness and area of hippocampal subregions in the fetal brain, disrupted cell structure and induced apoptosis-related neurodegeneration. Notably, 5-HT and its receptors also play crucial roles in hippocampal neuronal structure and plasticity. Rojas and colleagues [54] demonstrated that 5-HT administration in an *in vitro* culture environment led to a reduction in the length and complexity of hippocampal principal neurons within 24 h, which was mediated through the 5-HT<sub>1A</sub> receptor. In our study, we found that the increase in 5-HT levels in the hippocampus of adult offspring could contribute to structural changes in DG cells. Additionally, dendritic spines, with their diverse morphologies, serve important functions [55]. These structural changes may underlie the observed cognitive impairments in adult offspring. The proportion of thin dendritic spines is closely associated with cognitive performance [56], and the absence of mushroom-type dendritic spines significantly impacts neuronal function [57]. Alterations in the number of dendritic spines, which reflect changes in neural circuit connectivity, often accompany synaptic remodeling. The formation of new synapses may enhance the memory storage capacity of the brain more effectively than changes in synaptic strength [58]. In our study, maternal aripiprazole exposure significantly reduced the density of thin and mushroom-type spines in the DG, thereby diminishing dendritic plasticity. These effects may underlie the observed cognitive impairments in the adult offspring.

Multiple lines of evidence have demonstrated that EE can enhance learning and memory capabilities [59, 60]. EE has been shown to modify neuronal structure, augment synaptic plasticity, and improve cognitive function [61]. Additionally, EE stimulates glial cell proliferation and promotes neurogenesis [62]. Furthermore, EE exerts significant effects on various neurotransmitter systems [63, 64]. In our study, we found that EE reversed the cognitive deficits in offspring induced by maternal exposure to aripiprazole. Consistent with previous research, postnatal EE has been reported to mitigate learning and memory disruptions caused by maternal stress [65]. Moreover, our findings revealed that EE increased the expression of DARPP-32, a protein crucial for the regulation of dopamine signaling. This might be a key mechanism underlying the beneficial effects of EE. Research has demonstrated that EE can modulate dopamine signaling within the mesolimbic circuit by altering the expression and phosphorylation status of DARPP-32 [66, 67]. These modifications, in turn, have significant implications for cognitive functions and other behavioral outcomes [68, 69].

Previous studies have indicated that adult neurogenesis is a complex, multistage process, and EE has been shown to have differential effects on various aspects of this process. Specifically, some studies have shown that EE predominantly enhances the survival of newborn neurons while exerting limited effects on the proliferation of neural precursor cells. Conversely, voluntary wheel running primarily promotes precursor cell proliferation [59]. Golo et al. [70] also reported that EE increases the density of

DCX-positive cells but does not affect the density of Nestin-positive cells. In the present study, we found that maternal exposure to aripiprazole resulted in decreased proliferation of neonatal neurons (Ki-67-labeled cells in the mitotic phase) in the DG of offspring. However, EE did not counteract this reduction; rather, it promoted the density of DCX-positive cells in the migratory phase. Notably, EE alone can increase the number of DCX-positive cells in normal healthy animals, which may limit the interaction between aripiprazole exposure and EE. Our findings suggest that EE can nonspecifically mitigate the reduction in DCX-positive cells in the DG of adult offspring induced by prenatal aripiprazole exposure.

Furthermore, we observed that EE reversed the aberrant granule cell plasticity in the DG caused by maternal aripiprazole exposure, which was consistent with previous findings [71]. Gonçalves et al. [72] longitudinally assessed the effects of EE (from PND 7–60) on the dendritic structure of the DG via in vivo imaging and demonstrated that, compared with control animals, animals exposed to EE exhibited greater dendritic length and complexity. These structural modifications are believed to contribute to the observed improvements in learning and memory tasks. In clinical practice, given the beneficial effects of early skin-to-skin contact [73] and high-quality parent-child interactions [74] as forms of environmental enrichment on children's neurodevelopment, it is essential to provide early enriched environmental stimuli to children at risk. Additionally, enhanced monitoring and timely adjustment of environmental enrichment strategies should be implemented to reduce their risk of developmental disorders.

There are some limitations in the present study. First, since female offspring were not included in this study, the conclusions are limited to male offspring. Future research should explore the effects on female offspring and the potential differences between the sexes. Second, clinically, aripiprazole is typically administered orally. In this study, the drug was administered by intraperitoneal injection. Although the absolute oral bioavailability of aripiprazole is 87% [75], the higher bioavailability associated with intraperitoneal administration means that the effects observed in this study may not be fully extrapolated to clinical practice. Future studies should use oral gavage to validate the findings from intraperitoneal administration. Last, the samples used for detecting monoamine neurotransmitter levels were taken from the entire hippocampus, rather than specifically from the dorsal hippocampus. Given the functional differences between the dorsal and ventral hippocampus, future research should employ more advanced techniques, such as neurotransmitter probe technology, to investigate the precise effects on monoamine neurotransmitter levels in different subregions of the hippocampus.

In conclusion, our results provide multifaceted evidence of the long-term negative effects of maternal exposure to aripiprazole on hippocampal plasticity and cognitive performance in adult male offspring, which may be related to disruption of the dopamine and serotonin transmitter systems. Environmental enrichment has beneficial effects and could be used as an intervention in the future.

## DATA AVAILABILITY

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request, subject to ethical approvals and data protection regulations.

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## ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (grant Nos. 82071528, 81571312, 82171529, and 82271569). The Graphical Abstract was created with BioRender.com.

## AUTHOR CONTRIBUTIONS

Han Wang: Investigation, Formal analysis, Methodology, Writing - original draft. Ji-Tao Li: Investigation, Formal analysis, Methodology, Writing - original draft. De-Nong Liu: Investigation, Methodology. Meng Sun: Investigation, Formal analysis, Methodology.

Xian-Qiang Zhang: Resources, Investigation. Chen-Chen Zhang: Conceptualization, Investigation, Writing. Tian-Mei Si: conceptualization, funding acquisition, supervision, writing - review & editing. Yun-Ai Su: Conceptualization, Funding acquisition, Investigation, Supervision, Writing - review & editing.

### COMPETING INTERESTS

The authors declare no competing interests.

### ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was conducted exclusively on animal subjects. Experimental design and reporting strictly adhered to the ARRIVE Guidelines 2.0 (Animal Research: Reporting of In Vivo Experiments). All animal procedures were approved and monitored by the Institutional Animal Care and Use Committee of Peking University (Approval No. LA2014169).

### ADDITIONAL INFORMATION

**Supplementary information** The online version contains supplementary material available at <https://doi.org/10.1038/s41398-025-03335-1>.

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