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Impact of intrauterine hypoxia on adolescent and adult cognitive function in rat offspring: sexual differences and the effects of spermidine intervention

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Intrauterine hypoxia (IUH) affects the growth and development of offspring. It remains unclear that how long the impact of IUH on cognitive function lasts and whether sexual differences exist. Spermidine (SPD) has shown to improve cognition, but its effect on the cognitive function of IUH offspring remains unknown. In the present study we investigated the influence of IUH on body weight and neurological, motor and cognitive function and the expression of APP, BACE1 and Tau5 proteins in brain tissues in 2- and 4-month-old IUH rat offspring, as well as the effects of SPD intervention on these parameters. IUH rat model was established by treating pregnant rats with intermittent hypoxia on gestational days 15–21, meanwhile pregnant rats were administered SPD (5 mg·kg⁻¹·d⁻¹;ip) for 7 days. Neurological deficits were assessed in the Longa scoring test; motor and cognitive functions were evaluated in coat hanger test and active avoidance test, respectively. We found that IUH decreased the body weight of rats in both sexes but merely impaired motor and cognitive function in female rats without changing neurological function in the rat offspring of either sex at 2 months of age. For 4-month-old offspring, IUH decreased body weight in males and impaired neurological function and increased cognitive function in both sexes. IUH did not affect APP, BACE1 or Tau5 protein expression in either the hippocampus or cortex of all offspring; however, it increased the cortical Tau5 level in 2-month-old female offspring. Surprisingly, SPD intervention prevented weight loss. SPD intervention reversed the motor and cognitive decline caused by IUH in 2-month-old female rat offspring. Taken together, IUH-induced cognitive decline in rat offspring is sex-dependent during puberty and can be recovered in adult rats. SPD intervention improves IUH-induced cognitive and neural function decline.

Keywords: prenatal hypoxia; intrauterine hypoxia; rat offspring; neurological function; motor defects; cognitive defects; sexual differences; spermidine intervention

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INTRODUCTION

Intrauterine hypoxia (IUH) is the most frequent intrauterine condition occurring in high-altitude pregnancy, preeclampsia, placental insufficiency, and any inflammatory condition during pregnancy caused by gestational diabetes or maternal obesity [1, 2]. Clinical data have shown that fetuses that experience IUH have a lower birth weight and a preterm birth rate of approximately 60%, among which 20% ~ 50% of neonates die within a few months and more than 25% of newborns experience permanent brain damage [3]. Importantly, IUH is a high-risk factor for substantial long-term neurological morbidity, including cognitive and learning difficulties, developmental delay, severe seizures, mental retardation, attention-deficit hyperactivity disorder (ADHD), and autism [4, 5].

A previous study reported that male adolescent rat offspring exposed to prenatal hypoxia showed memory decline [6]. In addition, hypoxic/ischemic male adolescent rat offspring showed cognitive deficits [7]. However, how IUH induces neurological damage is largely unknown. The excessive release of reactive

oxygen species is believed to be one of the mechanisms underlying the brain injury induced by hypoxia/ischemia [8, 9]. The antioxidant stress response may have the potential to treat or prevent IUH-induced neurological injury in offspring. However, whether the effect of IUH on cognitive function is temporary or permanent and whether the effects of IUH on cognitive function exhibit sex differences are largely unknown.

A large amount of evidence has shown that intrauterine environment influences the later development of neurodegenerative diseases, such as Alzheimer's disease (AD) [10]. The pathological hallmarks of Alzheimer's disease (AD) are amyloid plaque (A β) deposition and tau hyperphosphorylation [11]. It has been reported that the cleavage of APP by BACE1 is the basis for A β formation and that A β is considered an inducer of tau hyperphosphorylation [12]. Importantly, the total tau (T-tau) level in cerebrospinal fluid (CSF) was found to reflect axonal degeneration and tangle pathology in AD [13]. Furthermore, chronic brain hypoxia induced by chronic brain hypoperfusion (CBH) promotes cognitive decline, which is closely associated with A β deposition

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and tau hyperphosphorylation, by upregulating the expression of APP, BACE1 and Tau5 [14]. However, whether IUH exposure could lead to A β deposition and tau hyperphosphorylation by upregulating the expression of APP, BACE1 and Tau5 is unexplored thus far.

Spermidine (SPD), one of the well-studied metabolites of polyamines (PAs), has prominent cardioprotective and neuroprotective effects and stimulates anticancer immunosurveillance in aged rodent models by preserving mitochondrial function, exhibiting anti-inflammatory properties, and preventing stem cell senescence [15]. Interestingly, mice receiving SPD had a significantly prolonged life span [16, 17]. In addition, an intramygdalar or intrahippocampal microinjection of SPD increased fear conditioning and improved memory persistence in healthy adult rats [18, 19]. Under pathological conditions, low concentrations of SP, SPD, and PU were found to protect SHSY5Y cells against the toxic conformational species of A β by promoting the structural transition of A β toward its less toxic fibrillar state [20]. A previous study reported that SPD ameliorated the neonatal rat heart injury induced by IUH by inhibiting oxidative stress [21]. Importantly, a randomized controlled trial indicated that dementia patients who received an SPD-rich extract supplement showed moderately enhanced memory performance compared with control groups [22]. These studies suggested that SPD may have the potential to protect against IUH-induced cognitive decline in offspring. However, studies on this issue are lacking.

The present study found that IUH decreased the body weight of female and male offspring and impaired motor function and cognition in females without changing neurological function in either sex or cognition in 2-month-old male offspring rats. For 4-month-old adult offspring, IUH decreased body weight only in males and increased the risk of neurological injury and increased cognitive function without changing motor ability in both females and males. The expression of APP, BACE1 and Tau5 proteins in IUH-exposed 2- and 4-month-old rats did not change, except that the Tau5 level in cortices was increased in the 2-month-old female IUH group. Interestingly, the application of SPD to maternal rats under hypoxia prevented weight loss in female and male 2-month-old rat offspring and in male 4-month-old adult rat offspring. SPD prevented the motor and cognitive decline induced by IUH in female 2-month-old rat offspring.

MATERIALS AND METHODS

Animals

All experiments were carried out following the National Institutes of Health guidelines, and all procedures were approved by the local ethics review committee of Harbin Medical University (China). Male and female Wistar rats (3 months old) were purchased from the Harbin Medical University Experimental Center. Rats at a female: male ratio of 2:1 were randomly mated in one cage, and pregnancy was detected via a vaginal smear obtained the following morning, which was examined for the presence of sperm, or via the observation of a copulatory plug in the vagina, which signified day 0 of pregnancy. The pregnant rats were housed in rooms with controlled humidity (60%), controlled temperature (21 °C), and a 12:12 h light: dark cycle.

IUH model

From 15 to 21 days of pregnancy, the rats in the hypoxia group were placed inside a plexiglass chamber with a maternal oxygen supply of 10% for 4 h every day. The percentage of oxygen in the plexiglass chamber was monitored by continuous infusion of a nitrogen gas and air mixture with an oxygen analyzer (Pro OX120; BioSpherix, New York, NY). We placed calcium chloride in the chamber to absorb excess carbon dioxide and water vapor for humidity control to avoid carbon dioxide retention and acidosis. This experimental procedure of IUH induction was carried out

according to a previous study [21]. Prior to term (day 22), SPD was administered intraperitoneally (ip) to the rats in the hypoxia group for 7 days (gestational days 15–21). The control rats were maintained under room air conditions (21% O₂) throughout pregnancy. The animals were randomly divided into three groups: (1) anormoxic control group, control mother+0.9% saline (1 mL/kg every day; ip); (2) an IUH group, IUH + 0.9% saline (1 mL/kg every day; ip); and (3) an IUH-SPD (5 mg/kg every day; ip) group. After birth, newborn pups from the above three groups were reared with their mothers and housed under room air conditions.

Longa scoring for neural function

Neurological examinations were performed according to Longa scoring: a score of 0 indicated no neurological deficit; a score of 1, left front forelimb flexion when lifting the tail, unable to fully extend, indicated a mild focal neurological deficit; a score of 2, turn to the left when walking, indicated a moderate focal neurological deficit; a score of 3, dumping to the left while walking, indicated a severe focal neurological deficit; and a score of 4, unable to walk autonomously, indicated decreased consciousness [23].

Coat hanger test

The rats were placed hanging at the center of the horizontal bar (horizontal diameter, 3 mm; length, 35 cm; height, 40 cm from the ground) with forepaws. The body position was observed for 30 s for each rat. If the animal fell from the hanger within 10 s, a score of 0 was given; animals with two front paws on the hanger received 1 point; animals that attempted to climb the hanger were given 2 points; animals with forepaws and at least one hind paw on the hanger were given 3 points; animals with all four paws and their tail wrapped around the hanger were given 4 points; and animals that tried to escape to the end of the hanger were given 5 points [24].

Active avoidance tests

Cognitive performance in a stimulus avoidance test was evaluated using a shuttle box (STT-100, Chengdu Taimeng Software Co. Ltd., China). The apparatus was divided into two identical shuttle compartments of the same size (24.3 cm × 23 cm × 30 cm) connected by a gate (11.5 cm × 4.5 cm). A conditioned stimulus (CS, coincident presentation of a 3.6 w light and a 90 dB sound) was delivered 10 s before the unconditioned stimulus (US, a 2 V electrical foot shock) and with a 5 s overlap. At the end of the stimulus presentation, both the CS and US were terminated, and the cycle began in the other compartment. Animals were subjected to 3 daily 30-cycle sessions with a 20 s cycle interval. On the fourth day, the cycle was performed without electric foot shock. An avoidance response was recorded when the animal avoided the US by running into the dark compartment within 10 s after the onset of the CS [25].

Western blot analysis

Brain tissue samples were harvested and stored at –80 °C. Total protein samples for Western blot analysis were extracted from the hippocampi or cortices of rats, and the detailed preparation protocol was described as follows. Frozen brain tissues were lysed in a solution containing 40% SDS, 60% RIPA, and 1% protease inhibitor. The homogenate was then centrifuged at 16700 × *g* at 4 °C for 30 min, and the supernatants (containing cytosolic and membrane fractions) were collected. The concentration of proteins was detected spectrophotometrically using a BCA kit (Universal Microplate Spectrometer; Bio-Tek Instruments, Winooski, VT, USA). Protein samples were fractionated by 10% SDS-PAGE gels and then transferred onto a nitrocellulose membrane. Anti-APP (1:1000, MAB348, Millipore, MA, USA), anti-BACE1 (1:1000, ab2077, Abcam, Dedham, UK), and anti-Tau5

(1:1000, AHB0042, Invitrogen, Rockford, USA) were used as primary antibodies. β -Actin (1:1000, AT-09, ZSGB-BIO, China) was selected as an internal control. Blots were imaged with an Odyssey Infrared Imaging System (Licor, USA) and quantified with Odyssey v1.2 software by measuring the protein intensity (area \times optical density) in each group. The final results were expressed as fold changes compared with the control values.

Statistical analysis

Data are expressed as the mean \pm SEM. Student's *t* test was used for statistical analysis between two groups. One-way analysis of variance was used for the statistical evaluation of differences among groups. When a significant difference existed among three or more groups, the groups were assessed with a post hoc multiple comparison analysis using Tukey's method. All statistical analyses were performed using SAS 9.1. *P* values < 0.05 were considered statistically significant.

RESULTS

SPD relieves motor dysfunction in 2-month-old female IUH rat offspring

Body weight loss is a common consequence of IUH in offspring in the clinic [26, 27]. A previous study reported that IUH caused body weight loss in 1-week-old neonatal rat offspring, and SPD treatment effectively prevented this loss [21]. To observe the effects of IUH on rat offspring at the adolescent stage, 2-month-old IUH rat offspring were used [28]. In the present study, we found that the IUH-induced body weight loss could extend to 2 months in both female and male rat offspring (Fig. 1a, b). Similarly, SPD markedly reversed the decrease in body weight (Fig. 1a, b). Neurological deficits are a common outcome of neonatal brain ischemia/hypoxia [29, 30]. Humans and experimental animals with intrauterine hypoxia develop various neurological deficits [4]. Here, in the Longa scoring experiment, we found that the Longa score of all 2-month-old female (Fig. 1c) and male (Fig. 1d) rats in the control group was 0, while only 2 female and 1 male IUH rat offspring earned a score of 2, and the others all earned a score of 0 (Fig. 1c, d). The results suggest that IUH is a potential but not significant risk factor for damaged neurological function in adolescent rats. The coat hanger test is a common method to analyze muscle strength and coordination ability in animals [24]. This test is based on the ability of mice to climb and use their claws to grasp an object. Compared with those of the control group, the hanger scores of the 2-month-old female IUH offspring were reduced, and SPD reversed the decrease in the hanger scores of the IUH groups (Fig. 1e). These data indicated that IUH increased the risk of neurological damage in 2-month-old female offspring. Interestingly, although the effect of IUH on body weight was similar in 2-month-old male and female offspring (Fig. 1a, b), IUH had no effect on the hanger score of 2-month-old male offspring (Fig. 1f). These results suggest that although there is no sex difference in the effect of IUH on the body weight and Longa scores of 2-month-old rat offspring, its effects on motor function exhibit sex differences. Adolescent female rats are more susceptible to damage by IUH than adolescent male rats. As predicted, SPD can prevent the adverse effects of IUH on the body weight and motor function of adolescent offspring.

The effect of SPD on learning and memory in 2-month-old IUH rat offspring

To evaluate whether IUH has sex-specific effects on the cognitive function of rat offspring and whether SPD is effective, we performed an active avoidance (AAV) test. The results indicated that IUH had no effect on the percentage of active avoidance response (Fig. 2a) but increased the latency of active avoidance response (Fig. 2b) of 2-month-old female offspring on the last training day of the training process. During the test period, IUH

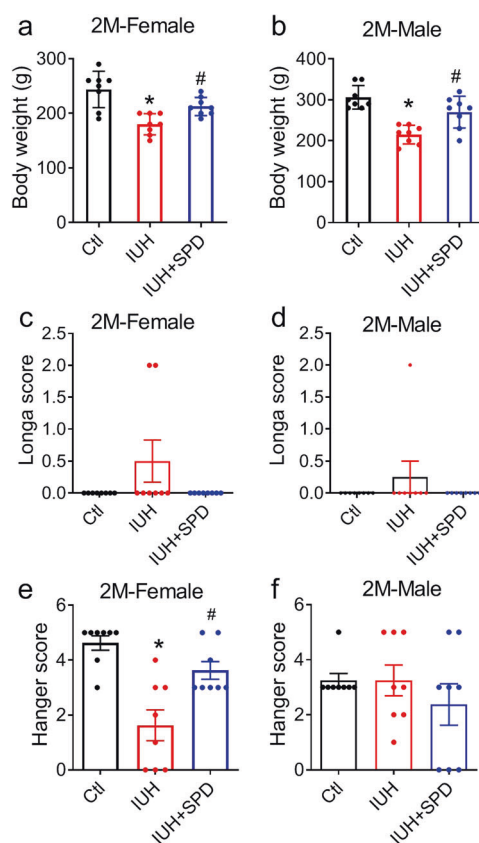


Fig. 1 The effects of SPD on body weight, Longa score and coat hanger score of 2-month-old IUH rat offspring. **a** SPD prevented IUH-induced body weight decreases in 2-month-old female offspring rats. **b** SPD reversed IUH-induced body weight decreases in 2-month-old male offspring rats. **c** IUH had no effect on the neurological function of 2-month-old female offspring rats. **d** IUH did not affect the neurological function of 2-month-old male offspring rats. **e** SPD reversed IUH-induced motor ability decline in 2-month-old female offspring rats. **f** IUH had no effect on the motor ability of 2-month-old male offspring rats. $n = 8$ for each group. * $P < 0.05$, vs the control group; # $P < 0.05$, vs the IUH group.

decreased the percentage of AAV response (Fig. 2c) and increased the latency of the IUH group (Fig. 2d). Markedly, SPD treatment rescued all these changes in 2-month-old IUH female offspring (Fig. 2b–d). Unlike female rats, although IUH resulted in a decreased percentage of conditional response times in male IUH rats compared with control rats on the third day of the training period, which was prevented by SPD intervention (Fig. 2e), the percentage of active avoidance response in the test period was not affected (Fig. 2g). Additionally, IUH had an effect on the latency of the active avoidance response either in the training period (Fig. 2f) or in the test period (Fig. 2h). Interestingly, SPD pretreatment in pregnant rats could effectively prevent the cognitive decline of adolescent female IUH rat offspring but did not influence the normal cognition of male rat offspring. These data implied that the damaging effects of IUH on offspring also involve sex differences in the adolescent period. SPD treatment could improve the decrease in cognition but could not further increase learning and memory ability.

Body weight, neurological function and motor function of 4-month-old IUH offspring and the effects of SPD intervention Since 2-month-old rats can be used to model adolescent humans, 4-month-old rats are considered almost adults [31]. We then investigated whether the harmful influence of IUH on adult rats differed depending on sex. To our surprise, we found that body

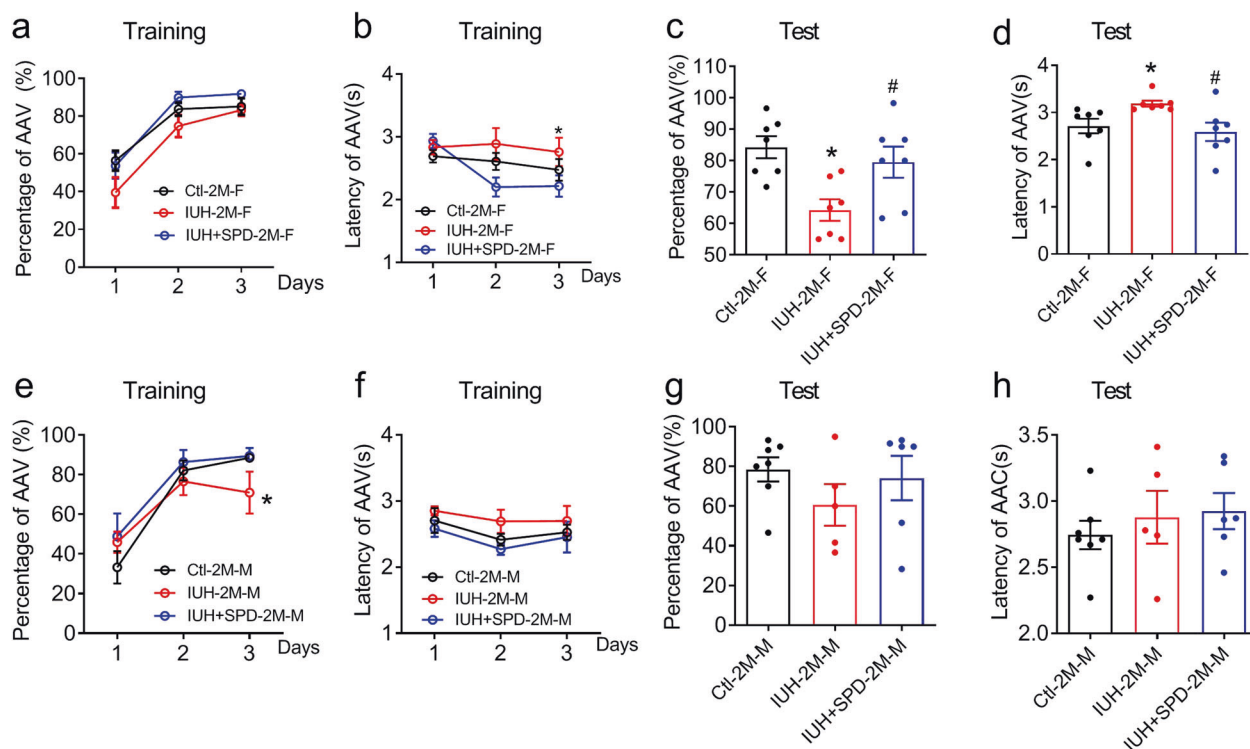


Fig. 2 The cognitive function of 2-month-old female and male rat offspring that experienced IUH and the effects of SPD. IUH did not affect the percentage of the active avoidance response (a) or the latency of the active avoidance response (b) of 2-month-old female rats during the training process. c SPD reversed the IUH-induced decrease in the percentage of the active avoidance response of 2-month-old female rats during the test process. d SPD reversed the IUH-induced increased latency of the active avoidance response of 2-month-old female rats during the test process. IUH did not affect the percentage of the active avoidance response (e) or the latency of the active avoidance response (f) of 2-month-old male rats during the training process. IUH did not affect the percentage of the active avoidance response (g) or the latency of the active avoidance response (h) of 2-month-old male rats during the test process. $n = 8$ for each group. * $P < 0.05$, vs the control group; # $P < 0.05$, vs the IUH group.

weight was significantly reduced in 4-month-old male IUH offspring (Fig. 3b) but not in 4-month-old female IUH offspring compared with control offspring (Fig. 3a). In contrast to the results of 2-month-old rat offspring, we found that IUH significantly damaged neurological function in both male and female rats as evidenced by Longa scoring (Fig. 3c, d). The results of the coat hanger test showed that there were no significant differences between the IUH and control groups (Fig. 3e, f). Similar to the data of 2-month-old offspring, SPD application dramatically rescued the neurological deficits induced by IUH but had no effect on unimpacted function (Fig. 3a–f). These findings suggest that there was a sex-dependent difference in the effect of IUH on the body weight of 4-month-old offspring but not on neurological function or motor function.

The effect of SPD on learning and memory in 4-month-old IUH rat offspring

We continued to observe the effects of IUH on learning and memory in adult offspring. Unexpectedly, we found that in the training period, the percentage of AAV response was markedly higher and the latency of AAV response was shorter in the IUH group than in the control group in both male and female rat offspring (Fig. 4a, b, e, f). In the test period, although IUH did not affect the percentage of AAV response of 4-month-old female offspring (Fig. 4c), it significantly reduced the latency of AAV response compared with the control condition (Fig. 4d). In male rat offspring, IUH not only increased the percentage of AAV response but also shortened the latency of AAV response (Fig. 4g, h). SPD did not affect the learning and memory of the female and male IUH groups (Fig. 4a–h). This result indicated that IUH can

improve the learning and memory ability of 4-month-old male and female offspring and that SPD has no effect on these outcomes.

The effect of SPD on the expression of APP, BACE1 and Tau5 in 2- and 4-month-old IUH rat offspring

In recent years, a large amount of evidence has shown that adverse maternal factors during pregnancy not only affect brain development but also influence the later development of neurodegenerative diseases, including Parkinson's disease (PD) and AD [10]. Amyloid plaque formation depends on its substrate protein, APP, and its cleavage enzyme, BACE1, and amyloid plaque formation and tau protein hyperphosphorylation are common characteristics of senile dementia and can be initiated by brain hypoxia [32, 33]. However, whether these pathological phenomena are related to the adverse effects of IUH on offspring during adolescence and adulthood is still unclear. Here, we evaluated the effects of IUH on the expression of APP, BACE1 and Tau5 and the effects of SPD intervention. The Western blot results showed that IUH had no effect on the expression of APP or BACE1 in the hippocampi and cortices of 2-month-old female and male offspring (Fig. 5a, b, d, e). For 2-month-old female offspring, IUH increased the level of Tau5 in the cortex without changing the level in the hippocampus (Fig. 5c). The expression level of Tau5 showed no difference among control, IUH and SPD-treated 2-month-old IUH male offspring (Fig. 5f). These findings suggest that the decreased learning and memory in 2-month-old IUH female rats might be associated with the upregulated Tau5 level, but the protective role of SPD might not be related to Tau5. Then, we examined the expression of related proteins in the hippocampi

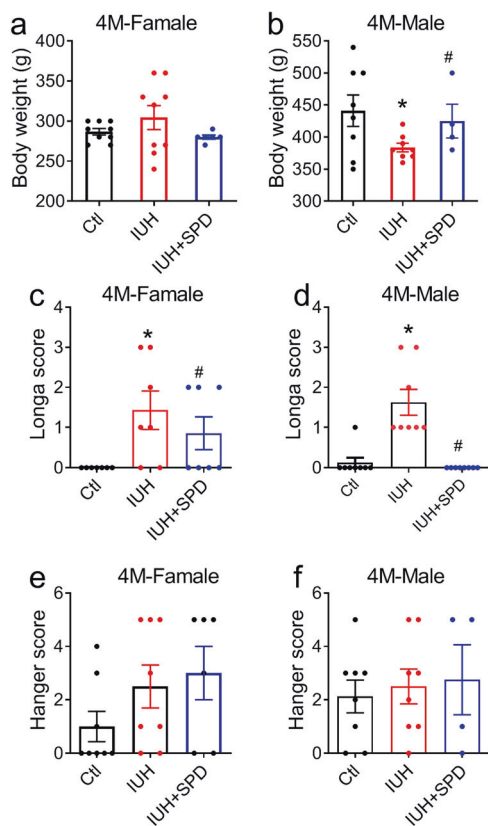


Fig. 3 The effects of SPD on body weight, Longa score and coat hanger score of 4-month-old IUH offspring rats. **a** IUH had no effect on the body weight of 4-month-old female offspring rats. **b** SPD prevented the IUH-induced decrease in body weight in 4-month-old male offspring rats. **c** SPD reversed IUH-induced impairment of neurological function in 4-month-old female offspring rats. **d** SPD reversed IUH-induced impairment of neurological function in 4-month-old male offspring rats. **e** IUH did not affect the motor ability of 4-month-old female offspring rats. **f** IUH had no effect on the motor ability of 4-month-old male offspring rats. $n = 7$ for female rats, $n = 8$ for male rats. * $P < 0.05$, vs the control group; # $P < 0.05$, vs the IUH group.

and cortices of 4-month-old offspring. There were no changes in APP, BACE1 or Tau5 expression in the hippocampi and cortices of the 4-month-old IUH male and the 4-month-old female IUH offspring compared with control offspring (Fig. 6a–f).

DISCUSSION

Many studies have indicated that IUH is a high-risk factor for cognitive decline and neuropsychiatric diseases, such as seizures, ADHD and autism [4, 5]. However, whether there are sex differences in IUH-associated nervous system disorders and whether the outcome is temporary or permanent are still unclear. We first reported that, during adolescence (2 months of age), IUH impaired the cognitive function of female rats but not male rats as evaluated by an active avoidance test. Surprisingly, we found that cognitive function was significantly increased in both male and female IUH rat offspring compared with control group rats. As expected, SPD not only improved IUH-induced weight loss and neurological deficits but also reversed cognitive impairment in female rat offspring at the age of 2 months but without affecting the increased cognitive function of rat offspring at the age of 4 months. All these results provide important information that the influence of IUH on offspring differs depending on sex and developmental stage (adolescence vs adulthood). SPD could

markedly prevent all the adverse effects of IUH on offspring but had no adverse effects on unimpacted function. These results suggested that SPD may be a good strategy to prevent the onset of IUH-induced damage in offspring.

Previous clinical studies reported that limited nutrient supply (or growth constraint) hinders growth early in life and then induces rapid weight gain due to the hypothesized “thrifty mechanisms”. This phenomenon is called catch-up growth, which occurs early in life and is believed to be a major risk factor for later obesity, type 2 diabetes, and cardiovascular diseases in epidemiological studies [34, 35]. In our study, we found that body weight was decreased in 2-month-old female IUH rat offspring but recovered to control levels in 4-month-old IUH female rat offspring even without SPD. This phenomenon might be explained by the phenomenon of catch-up growth, as hypoxia exposure during late pregnancy affected general fetal growth, but weight recovered to the control level with age when the rats were exposed to improved oxygen supply.

In this study, we found that IUH did not affect the neurological function of adolescent rat offspring (2 months old) but did affect the neurological function of adult rats (4 months old). Cerebral cortex ischemia/infarction could lead to neurological deficits [36]. Longa scoring is based on neurological behavioral performance; therefore, the peripheral nervous system function of the animal might directly affect the result. A previous study reported that the muscle contractile properties of rat offspring with prenatal ischemia induced by unilateral ligation of the uterine artery were not changed in the P28 group but were significantly decreased in adult offspring [37]. Therefore, the different effects of IUH on the neurological function of offspring at different ages might be due to the late maturation of muscle. IUH impaired the motor function of female rats but did not change that of male rats at the age of 2 months or that of both female and male rats at the age of 4 months. Therefore, it is no surprise that there is also a sex difference in cognitive behavior, specifically that IUH-induced cognitive decline in female rats but not in male rats at the age of 2 months. This result was supported by a clinical trial that reported that 7-year-old girls with chronic placental hypoxia showed greater inhibition and lower verbal IQ than boys, suggesting that girls are more vulnerable to chronic placental hypoxia [38]. However, the mechanism underlying the sex difference in IUH-induced motor dysfunction and cognitive deficits needs to be explored further.

Previous studies have shown that IUH offspring at 1 month of age, considered to be equivalent to the human juvenile stage, displayed body weight reduction and various sensory-motor reactions [39]. At 1.5 and 2 months of age, which is considered equivalent to the human adolescent state, IUH offspring showed impairments in learning and memory ability compared with control offspring [6, 7, 40]. In addition, IUH male offspring did not demonstrate any changes in the acquisition or retention of spatial memory at 4 months of age, which is considered equivalent to the adult stage in humans [41]. However, these studies merely focused on male animals. Since cognitive development is not equal for each kind of cognitive ability at the juvenile stage, it is difficult to objectively evaluate the changes in cognitive ability due to IUH injury or individual genetic factors. In the present study, in contrast to those studies [6, 7], we evaluated the sex differences in the cognition of 2- and 4-month-old IUH rat offspring. We found that the learning and memory ability of the male IUH rat offspring was not affected, but it was impaired in the female rat offspring at the age of 2 months. These controversial results may be due to the difference in the IUH protocol and cognition tasks. In our study, pregnant rats from day 15 to day 21 of pregnancy were kept in the hypoxia chamber with 10% oxygen supplementation for 4 h every day. Nevertheless, the pregnant rats used in the above research were kept in the hypoxia chamber with 10.5% oxygen supplementation from day 4 to day 21 of

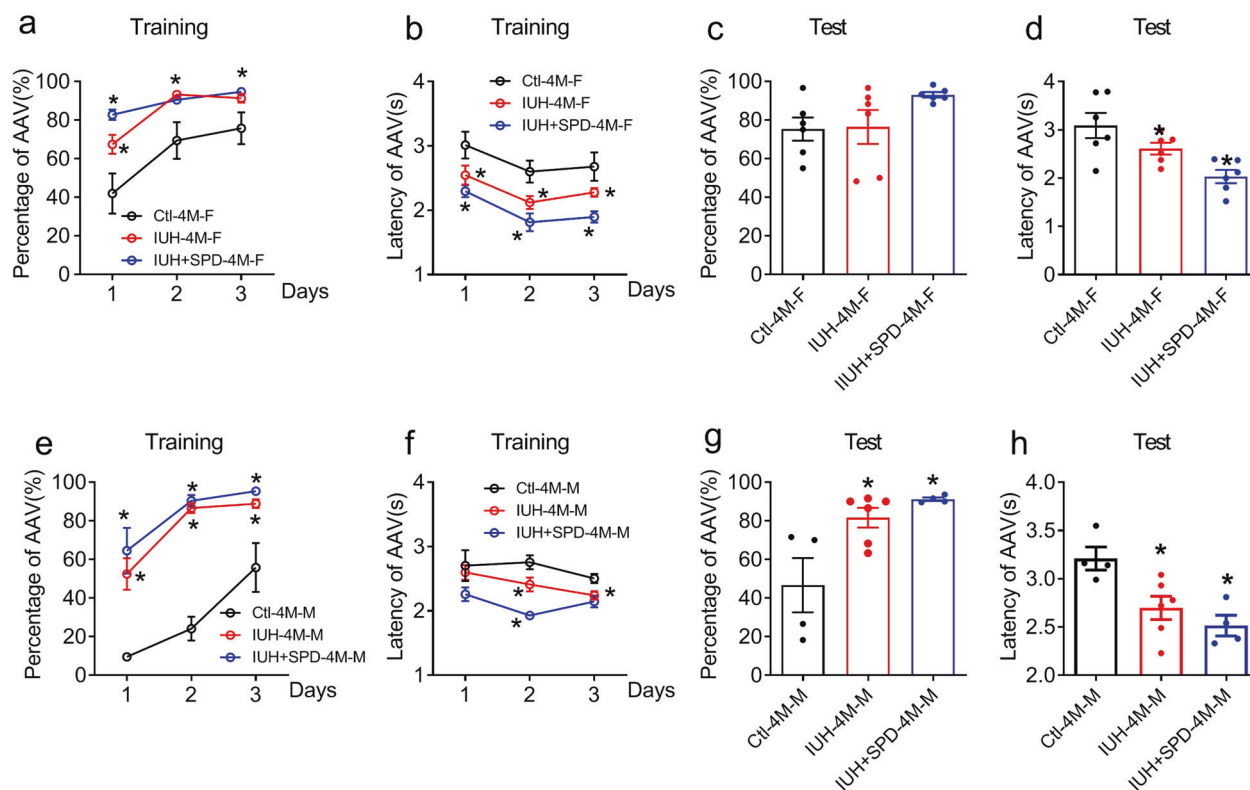


Fig. 4 The cognitive function of 4-month-old IUH female and male rat offspring and the effects of SPD. **a** IUH and SPD increased the percentage of the active avoidance response of 4-month-old female rats during the training process. **b** IUH and SPD shortened the latency of the active avoidance response of 4-month-old female rats during the training process. **c** IUH and SPD did not affect the percentage of the active avoidance response of 4-month-old female rats during the test process. **d** IUH and SPD shortened the latency of the active avoidance response of 4-month-old female rats during the test process. **e** IUH and SPD increased the percentage of the active avoidance response of 4-month-old male rats during the training process. **f** IUH and SPD shortened the latency of the active avoidance response of 4-month-old male rats during the training process. **g** IUH and SPD increased the percentage of the active avoidance response of 4-month-old male rats during the test process. **h** IUH and SPD shortened the latency of the active avoidance response of 4-month-old male rats during the test process. $n = 7$ for female rats, $n = 8$ for male rats. $*P < 0.05$, vs the control group.

gestation [6]. In another previous study, IUH was performed by unilateral ligation of the uterine artery of maternal rats [7]. To assess cognition, we used the active avoidance test, but a previous study used the Morris water maze to assess cognition [6]. The active avoidance test is a common method used to assess contextual memory, which is dependent on the connection between the amygdala and hippocampus [42, 43], and the Morris water maze is the common protocol to assess spatial memory, which is dependent on the hippocampus [44]. Even so, we used the same protocol to compare the sex-dependent influence of IUH on cognition. We first reported that female offspring are more sensitive to IUH than male offspring at the adolescent stage. Interestingly, when these female IUH offspring were 4 months old, they showed improved performance in the active avoidance test, and surprisingly, we found that cognitive function was significantly increased in both the male and female IUH offspring rats compared with the control offspring. Based on the theory of catch-up growth regarding body weight, we speculated that the IUH rat offspring may also have a compensatory ability regarding neural development deficits during growth in a normal environment. However, the molecular mechanism should be further identified.

A previous study reported that IUH might affect the development, differentiation and maturation of oligodendrocyte progenitor cells, which are pivotal for myelination, contributing to motor and cognitive decline [45]. Many experimental IUH offspring animals demonstrated dopaminergic system disturbances and abnormal neurotrophin signaling [46]. MRI research found that

IUH offspring mice showed hemispheric tissue loss and white matter injury, which is correlated with neurological deficits [47]. These are potential mechanisms contributing to the neurological and cognitive decline induced by IUH. However, other underlying mechanisms are unclear and need to be explored further.

A large amount of evidence has shown that intrauterine experiences influence the later development of neurodegenerative diseases, such as AD [10]. Senile plaques induced by amyloid plaque deposition and neurofibrillary tangles caused by tau protein hyperphosphorylation are two typical pathological hallmarks thought to cause cognitive impairment in AD [11]. A previous study reported that in 1-month-old APP/PS1 transgenic offspring mice, exposure to prenatal hypoxia resulted in higher levels of APP, lower levels of the $A\beta$ -degrading enzyme neprilysin, and increased $A\beta$ accumulation in the brain [48]. In the present study, we found that IUH did not change the expression of APP and BACE1 in the hippocampi and cortices of both 2- and 4-month-old IUH rat offspring. However, IUH increased the Tau5 level in the cortices of 2-month-old female offspring but did not change the expression of Tau5 in 2-month-old male offspring or in 4-month-old male and female offspring. These findings suggest that the increased Tau5 level might be associated with the cognitive decline in female 2-month-old rats induced by IUH.

SPD is a kind of polycationic aliphatic amine that shows protective action in aging-associated diseases, including cardiovascular diseases, cancer and neurodegenerative diseases, due to its anti-inflammatory properties, antioxidant functions, and mitochondrial metabolism enhancement [15]. SPD significantly

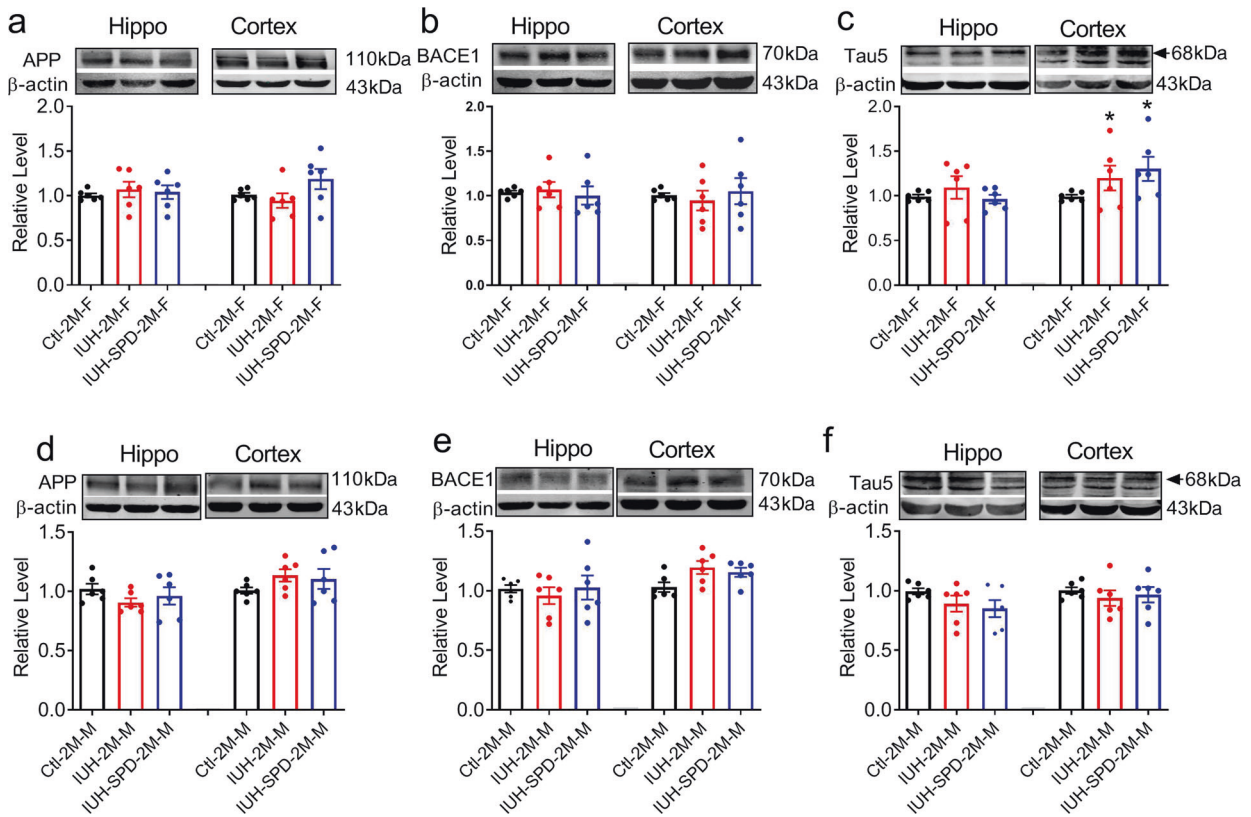


Fig. 5 Expression of APP, BACE1 and Tau5 in the hippocampi and cortices of sham-treated rats, IUH-treated rats, and SPD-treated IUH rats at the age of 2 months. IUH and SPD did not affect the expression of APP (a) or BACE1 (b) in the hippocampi or cortices of 2-month-old female rat offspring. c IUH did not change the expression of Tau5 in hippocampi but did increase the expression of Tau5 in cortices of 2-month-old female rat offspring. IUH did not affect the expression of APP (d), BACE1 (e) or Tau5 (f) in the hippocampi or cortices of 2-month-old male rat offspring. $n = 6$ for each group. $*P < 0.05$, vs the control group.

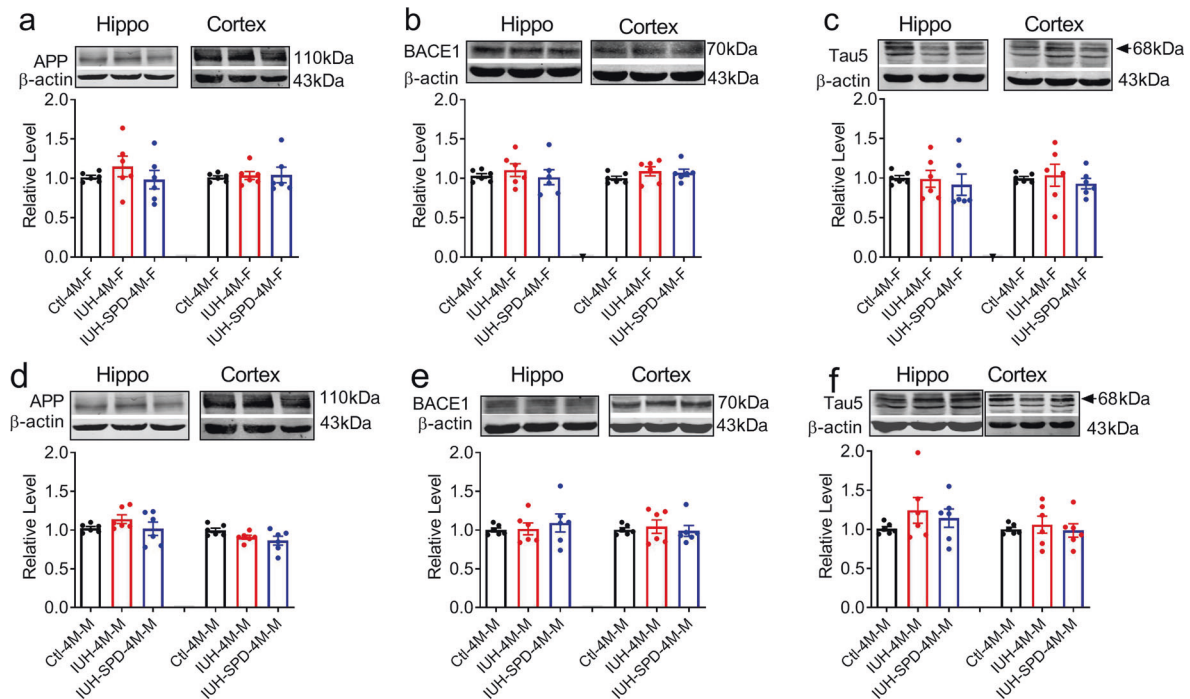


Fig. 6 The expression of APP, BACE1 and Tau5 in the hippocampi and cortices of sham-treated rats, IUH-treated rats, and SPD-treated IUH rats at the age of 4 months. IUH and SPD did not affect the expression of APP (a), BACE1 (b) or Tau5 (c) in the hippocampi or cortices of 2-month-old female rat offspring. IUH did not affect the expression of APP (d), BACE1 (e) or Tau5 (f) in the hippocampi or cortices of 2-month-old male rat offspring. $n = 6$ for each group.

reduced hippocampal CA1 cell death induced by in vitro ischemia, and SPD showed neuroprotective effects in an in vivo global forebrain ischemia model [49], which supports the protective role of SPD in hypoxia/ischemia-related injury. A previous study reported that SPD markedly reversed the neonatal rat heart injury induced by IUH by inhibiting oxidative stress [21]. Previous studies have reported that the intraperitoneal administration of 5–10 mg/kg every day SPD for 21 days exerted a potential neuroprotective effect in a 3-NP rat model of Huntington disease [50]. SPD (5 mg/kg ip) treatments in mice for 10 d led to a partial rescue of histological alterations in muscle defects [51]. In addition, SPD (5 mg/kg ip) treatments under hypoxic pregnant rats during the late stage of pregnancy prevented heart injury in neonatal rats that experienced maternal hypoxia [21]. Therefore, we chose the same dosage in our study. Here, we provide new evidence of a protective action of SPD that could alleviate the neurological and cognitive decline in adolescent IUH rat offspring but without affecting the increased cognitive function of rat offspring at the age of 4 months. Notably, although we found that upregulated Tau5 levels may be involved in the cognitive decline of 2-month-old IUH female rats with IUH; and that SPD could alleviate this symptom, our data suggested that the protective action of SPD was not associated with Tau5 levels. Even so, our findings suggested that SPD could be a good candidate drug to prevent the negative consequences of IUH in the nervous system.

In the present study, although we evaluated sex differences in cognitive changes in adolescent and adult rats exposed to IUH, we did not observe how IUH affected sex differences in aged rat offspring. We believe that it is very important to clarify the very long-term effects of IUH on cognition, even when the rat offspring reach the aged stage. In addition, we observed the action of SPD on IUH offspring through SPD treatment of the hypoxic mother. What about the influence of SPD on the cognition of IUH offspring when it is directly delivered to IUH offspring? Clarifications of these issues, together with the findings of the present study, would provide an important scientific basis to decrease the incidence of Alzheimer's disease.

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AUTHOR CONTRIBUTIONS

JA and YJZ designed the study. MM, LY, ZJ, LXL, YRW and TTL performed the experiments and analyzed the data. LY wrote the manuscript.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

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