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RESEARCH ARTICLE

Neurotoxicity of prenatal alcohol exposure on medullary pre-Bötzinger complex neurons in neonatal rats

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Abstract

Prenatal alcohol exposure disrupts the development of normal fetal respiratory function, but whether it perturbs respiratory rhythmical discharge activity is unclear. Furthermore, it is unknown whether the 5-hydroxytryptamine 2A receptor (5-HT_{2A}R) is involved in the effects of prenatal alcohol exposure. In the present study, pregnant female rats received drinking water containing alcohol at concentrations of 0%, 1%, 2%, 4%, 8% or 10% (v/v) throughout the gestation period. Slices of the medulla from 2-day-old neonatal rats were obtained to record respiratory rhythmical discharge activity. 5-HT_{2A}R protein and mRNA levels in the pre-Bötzinger complex of the respiratory center were measured by western blot analysis and quantitative RT-PCR, respectively. Compared with the 0% alcohol group, respiratory rhythmical discharge activity in medullary slices in the 4%, 8% and 10% alcohol groups was decreased, and the reduction was greatest in the 8% alcohol group. Respiratory rhythmical discharge activity in the 10% alcohol group was irregular. Thus, 8% was the most effective alcohol concentration at attenuating respiratory rhythmical discharge activity. These findings suggest that prenatal alcohol exposure attenuates respiratory rhythmical discharge activity in neonatal rats by downregulating 5-HT_{2A}R protein and mRNA levels.

***Key Words:** nerve regeneration; brain injury; prenatal alcohol exposure; pre-Bötzinger complex; respiratory depression; neonatal rats; medullary slice; medullary respiratory center; respiratory rhythmical discharge activity; respiratory neuron; 5-hydroxytryptamine 2A receptor; neural regeneration*

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Introduction

Numerous studies have shown that prenatal alcohol exposure induces a wide spectrum of structural and functional abnormalities in the central nervous system. It induces long-term respiratory depression after episodic hypoxia *in vitro* (Kervern et al., 2009; Cullere et al., 2015), and it perturbs newborn respiratory adaptation to a low oxygen environment (Dubois et al., 2008). In the present study, we recorded from medullary slices containing the pre-Bötzinger complex (preBötC), a region of the ventral respiratory group that is the key site of respiratory rhythm generation (Smith et al., 1991; Cinelli et al., 2013) and which generates respiratory rhythmical discharge activity (RRDA). The RRDA reflects the function of the preBötC (Ren et al., 2003; Chen et al., 2013). The 5-hydroxytryptamine 2A receptor (5-HT_{2A}R) expressed by preBötC neurons plays a significant role in generating and modulating the RRDA of the respiratory network (Liu et al., 2008; Niebert et al., 2011). In the early development of the nervous system, the 5-HT_{2A}R also participates in the development and maturation of the fetal respiratory center (Bou-Flores et al., 2000; Ozawa et al., 2002). Although a number of studies have focused on the respiratory center and the 5-HT_{2A}R, little is known about the role of preBötC 5-HT_{2A}R on RRDA in neonatal rats exposed to alcohol during the prenatal period. In the present study, we investigate the effect of prenatal alcohol exposure on RRDA and the role of the 5-HT_{2A}R using neonatal rat medullary slices.

Materials and Methods

Animals and drugs

A total of 36 specific-pathogen-free adult Sprague-Dawley rats, aged 14 weeks, comprising 24 female rats weighing 290 ± 17 g and 12 male rats weighing 330 ± 19 g, were provided by the Experimental Animal Center of Zhengzhou University in China (license No. SCXK (Yu) 2012-0002). Rats were housed at 22–25°C and 35–40% humidity, with a 12-hour light-dark cycle. Alcohol solutions of various concentrations (0%, 1%, 2%, 4%, 8% and 10% alcohol, v/v in water) (Tianjin Deen Chemical Reagent Co., Ltd., Tianjin, China) were used as the only source of water for female rats throughout the gestation

period (Abate et al., 2004; Miranda-Morales et al., 2014). Medullary slices from 2-day-old neonatal rats, weighing 5.67 ± 1.71 g, of either gender, were used for the recording experiments. Protocols were approved by the Experimental Animal Ethics Committee, Xinxiang Medical University, China.

The 5-HT_{2A}R agonist DOI (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in dimethyl sulfoxide and diluted in artificial cerebrospinal fluid. The final concentration of dimethyl sulfoxide (0.1%) had no effect on RRDA (Wang et al., 2005). The 5-HT_{2A}R-specific antagonist ketanserin (Sigma-Aldrich) was dissolved in artificial cerebrospinal fluid for perfusing, and the final concentration of DOI and ketanserin were 40 μM (Qian et al., 2008).

Medullary brain slice preparation and electrophysiological recording

Two-day-old neonatal rats were used to prepare medullary slices. Rats were deeply anesthetized with ether until the disappearance of nociceptive reflexes and were decapitated at the C_{3-4} spinal level. The brainstem and spinal cord were dissected as previously described (Smith et al., 1991; Qian et al., 2010). Dissection was performed in ice-cold artificial cerebrospinal fluid (NaCl 124 mM, KCl 5 mM, $CaCl_2$ 2.4 mM, $MgSO_4$ 1.3 mM, KH_2PO_4 1.2 mM, $NaHCO_3$ 26 mM and glucose 30 mM, pH 7.4) (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) equilibrated with carbogen (95% O_2 and 5% CO_2) (Henan Yuanzheng Science and Technology Co., Ltd., Zhengzhou, China). Dissection lasted for less than 3 minutes. The isolated brainstem was glued onto an agar block with the dorsal side facing downward and the blade at a 20° angle. An 850-μm transverse slice was cut, containing the preBötC, inferior olive, nucleus of the solitary tract, hypoglossal nucleus and nucleus ambiguus. The medullary slice was transferred to a recording chamber and continuously perfused with oxygen-saturated artificial cerebrospinal fluid at a rate of 5.0–6.0 mL/minutes at 27–29°C. The RRDA from hypoglossal nerve rootlets was recorded with a suction electrode. Signals were amplified and band-pass filtered (100.0 Hz–3.3 kHz). Data were sampled (5 kHz) and stored in the computer using a BL-420 biological signal processing system (Chengdu TME Technology, Chengdu, China). The RRDA parameters evaluated were inspiratory time (the time from start to finish of an inspiratory discharge), respiratory frequency (number of inspiratory discharges in a minute) and integral amplitude (the integral amplitude of an inspiratory discharge).

Western blot analysis for 5-HT_{2A}R expression

Protein was isolated from six medullary slices containing the preBötC for assessing 5-HT_{2A}R expression. Equal amounts of protein (60 μg) were loaded onto a 10% sodium dodecyl sulphate-polyacrylamide gel for electrophoresis and electrotransferred onto a polyvinylidene difluoride membrane (Millipore, Billerica, MA, USA). The membrane was blocked with 5% bovine serum albumin diluted in Tris-buffered saline containing Tween-20 for 1 hour, followed by incubation with primary antibodies (goat polyclonal antibody against 5-HT_{2A}R; 1:100; Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 4°C overnight. After washing, the membrane was incubated with secondary antibody (horseradish peroxidase AffiniPure rabbit anti-goat IgG; 1:2,000; EarthOx, Millbrae, CA, USA) for 1 hour at room temperature. GAPDH antibody (1:1,000; Santa Cruz Biotechnology) was blotted on the same membrane as an internal control to normalize relative density. Immunoreactive bands were visualized with enhanced chemiluminescence (Amersham, Piscataway, NJ, USA) and analyzed using Image J software (Molecular Dynamics IQ solutions, Molecular Dynamics Inc., Sunnyvale, CA, USA). 5-HT_{2A}R relative protein levels were calculated using the gray scale ratios of 5-HT_{2A}R and GAPDH. The data were normalized to those obtained for the 0% alcohol group, which was set at 100%.

Quantitative RT-PCR (qRT-PCR) analysis of 5-HT_{2A}R mRNA levels

Total RNA from six medullary slices containing the preBötC was extracted using the RNAiso Plus kit (Takara Biotechnology (Dalian) Co., Ltd.) following the manufacturer's protocol. Gene-specific primer pairs are listed in **Table 1**. The cDNA was synthesized using the PrimeScript II First Strand cDNA Synthesis Kit according to the manufacturer's instructions (Takara Biotechnology (Dalian) Co., Ltd.). The cDNA products were stored at −80°C. qRT-PCR was performed in a final volume of 50 μL. A 1-μL aliquot of RNA, 25 μL Taq premix (Takara Biotechnology (Dalian) Co., Ltd.), 1 μL forward primer, 1 μL reverse primer and RNase-free sterile water were added to a PCR tube to a final volume of 50 μL. Cycling parameters were as follows: Amplification was carried out with an initial denaturation stage of 95°C for 5 minutes, followed by 40 cycles. Each cycle consisted of denaturation at 95°C for 30 seconds, annealing at 60°C for 35 seconds, and extension at 72°C for 1 minute. GAPDH served as an internal control, and serial dilutions of the positive control were performed on each plate to create a standard curve. The amount of target gene was normalized to the reference GAPDH to obtain the relative threshold cycle. Quantification of PCR products was performed using the $2^{-\Delta\Delta Ct}$ method (Liebscher et al., 2005). To calculate relative mRNA amounts, the average target gene Ct values were subtracted from the GAPDH values to determine changes in Ct value. mRNA levels were normalized to the housekeeping gene, GAPDH, and the 0% alcohol group was set at 100%.

Statistical analysis

The data were analyzed using SPSS 13.0 software (SPSS, Chicago, IL, USA) and are expressed as the mean ± SD. Statistical comparisons were performed using one-way analysis of variance using the Student-Newman-Keuls test for *post hoc* comparisons. A value of $P < 0.05$ was considered statistically significant.

Results

Effects of prenatal alcohol exposure on RRDA

Compared with the 0% alcohol group, RRDA in the 1% and 2% alcohol groups did not change significantly ($P > 0.05$).

Table 1 Primer sequences for qRT-PCR

Gene		Primer sequences (5'–3')	Product size (bp)
5-HT_{2A}R	Forward	AAG CTG CAG AAT GCC ACC AAC	108
	Reverse	CCA GGT AAA TCC AGA TCG CAC A	
GAPDH	Forward	GGC ACA GTC AAG GCT GAG AAT G	163
	Reverse	ATG GTG GTG AAG ACG CCAGTA	

5-HT_{2A}R: 5-HT_{2A} receptor; GAPDH: glyceraldehyde phosphate dehydrogenase; qRT-PCR: quantitative reverse transcription-polymerase chain reaction.

RRDA declined with increasing alcohol concentration, in the 4% and 8% alcohol groups, with a decrease in respiratory frequency, shortened inspiratory time and reduced integral amplitude ($P < 0.05$, $P < 0.01$). RRDA in the 10% alcohol group was weaker than in the 8% alcohol group; however, it was so irregular that it could not be statistically analyzed (**Figure 1**).

Prenatal alcohol exposure reduced RRDA and decreased the effects of DOI and ketanserin

Slices from the 8% alcohol group showed reduced RRDA compared with the 0% alcohol group. Inspiratory time and integral amplitude were decreased (reductions of 27.52% and 16.16%, respectively), and RF was also decreased (reduction of 28.82%), compared with the 0% alcohol group. After treatment with DOI, RRDA in both groups increased. However, the magnitude of the change in RRDA was smaller in the 8% alcohol group than in the 0% alcohol group ($P < 0.05$). With ketanserin treatment, RRDA in both groups weakened. However, the magnitude of the change in RRDA was smaller in the 8% alcohol group than in the 0% alcohol group ($P < 0.05$; **Figure 2**).

Prenatal alcohol exposure decreased 5-HT_{2A}R mRNA and protein levels

To examine how alcohol may affect the preBötC and disturb respiratory rhythm generation, we measured 5-HT_{2A}R protein levels in the medullary slices in the 0–8% alcohol groups. 5-HT_{2A}R protein levels gradually diminished with increasing alcohol concentration in preBötC respiratory neurons of the respiratory center in the 4% and 8% alcohol groups ($P < 0.01$; **Figure 3**).

qRT-PCR analysis revealed a significant decrease in the levels of 5-HT_{2A}R mRNA in medullary slices from neonatal rats in the 8% alcohol group compared with the 0% alcohol group ($P < 0.01$). These results suggest that prenatal alcohol exposure may cause a decrease in 5-HT_{2A}R mRNA expression in the neonatal rat medulla (**Figure 4**).

Discussion

In this study, we found that RRDA in the 8% alcohol group was reduced compared with the 0% alcohol group, indicating that prenatal alcohol exposure decreases preBötC activity. It is known that the 5-HT_{2A}R contributes to the modulation of respiratory motor function. To examine the role of the 5-HT_{2A}R in mediating the effect of prenatal alcohol exposure on RRDA, we treated medullary slices with a 5-HT_{2A}R agonist, DOI, and an antagonist, ketanserin. DOI increased RRDA in the 0% and 8% alcohol groups, while ketanserin decreased RRDA in both groups. However, the magnitude of the change in RRDA after DOI or ketanserin treatment was smaller in the 8% alcohol group than in the 0% alcohol group. This suggests that prenatal alcohol exposure reduces the effects of the 5-HT_{2A}R on RRDA in neonatal rats.

Previous studies have shown that changes in the distribution or dysfunction of 5-HT_{2A}R in the respiratory center can lead to breathing disorders (Ozawa et al., 2002; Taylor et al., 2005). Prenatal alcohol exposure diminishes the regulatory effect of 5-HT_{2A}R on RRDA. However, whether prenatal alcohol exposure decreases the expression of 5-HT_{2A}R in respiratory neurons of the preBötC region of neonatal rats was still unknown. Therefore, we used western blot analysis and qRT-PCR to evaluate the expression of 5-HT_{2A}R. Our results suggest that prenatal alcohol exposure downregulates 5-HT_{2A}R mRNA and protein levels.

Previous studies have shown that prenatal alcohol exposure reduces the activity (Yanpallewar et al., 2010; Zhou et al., 2010; Hausknecht et al., 2015) and expression (Dobson et al., 2014; Bird et al., 2015) of various receptors in the central nervous system, and that it decreases the number of 5-HT neurons (Sliwowska et al., 2014). In our study, prenatal alcohol exposure downregulated 5-HT_{2A}R expression in the preBötC, suggesting that alcohol attenuates 5-HT_{2A}R activity, thereby reducing the excitability of neurons.

During embryogenesis, alcohol is cytotoxic, and results in neuronal degeneration, necrosis, delayed differentiation, structural damage and developmental defects in the central nervous system (Augustyniak et al., 2005; Chang et al., 2012; Luo, 2014). The neurotoxic effects of alcohol elicit DNA damage, consistent with our present finding that prenatal alcohol exposure downregulates 5-HT_{2A}R mRNA expression.

Chronic alcoholism reduces PaO_2 and SaO_2, resulting in a continuous hypoxic state (Nogués et al., 2008; Mao et al., 2014). A number of studies have shown that after chronic intermittent hypoxia, neurons in the respiratory center of the rat lower brainstem exhibit morphological abnormalities, including changes in cell volume, pyknosis, a reduction in organelles and mitochondria, and apoptosis. Furthermore, the longer the duration of intermittent hypoxia, the greater the degree of neuronal apoptosis was (Baker et al., 2001; Li et al., 2007).

Our findings show that prenatal alcohol exposure inhibits the RRDA, reduces the contribution of 5-HT_{2A}R to the RRDA, and decreases 5-HT_{2A}R protein and mRNA expression in preBötC neurons. Our results provide further evidence that prenatal alcohol exposure perturbs the development of the nervous system, including the respiratory center in the medulla oblongata.

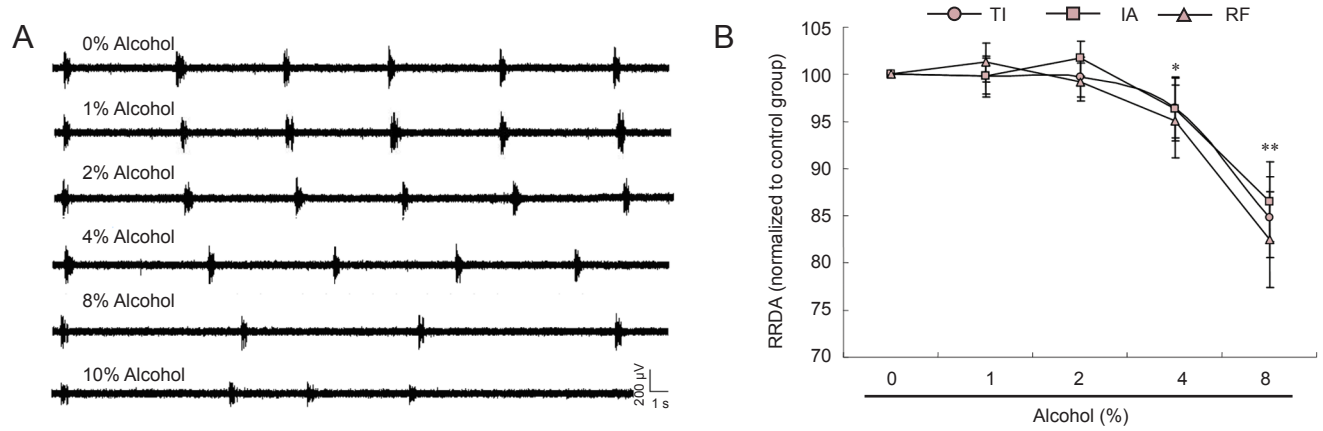

Figure 1 Impact of prenatal exposure to different concentrations of alcohol on the RRDA in neonatal rats.
The RRDA in the 0% alcohol group was set at 100% and was used to standardize the RRDA in each group. Compared with the 0% alcohol group, the RRDA in the 1% and 2% alcohol groups did not change significantly. However, with increasing alcohol concentration (4–8%), the TI shortened, and the IA and RF decreased. The RRDA was weaker in the 10% alcohol group than in the 8% alcohol group; however, it was so irregular that it could not be statistically analyzed. Calculation: (original data in 1–8% alcohol group/original data in 0% alcohol group) × 100%. Data are expressed as the mean ± SD ($n = 6$; one-way analysis of variance, followed by Student-Newman-Keuls *post hoc* test.). *$P < 0.05$, **$P < 0.01$, *vs.* 0% alcohol group. RRDA: Respiratory rhythmical discharge activity; TI: inspiratory time; IA: integral amplitude; RF: respiratory frequency; s: second.

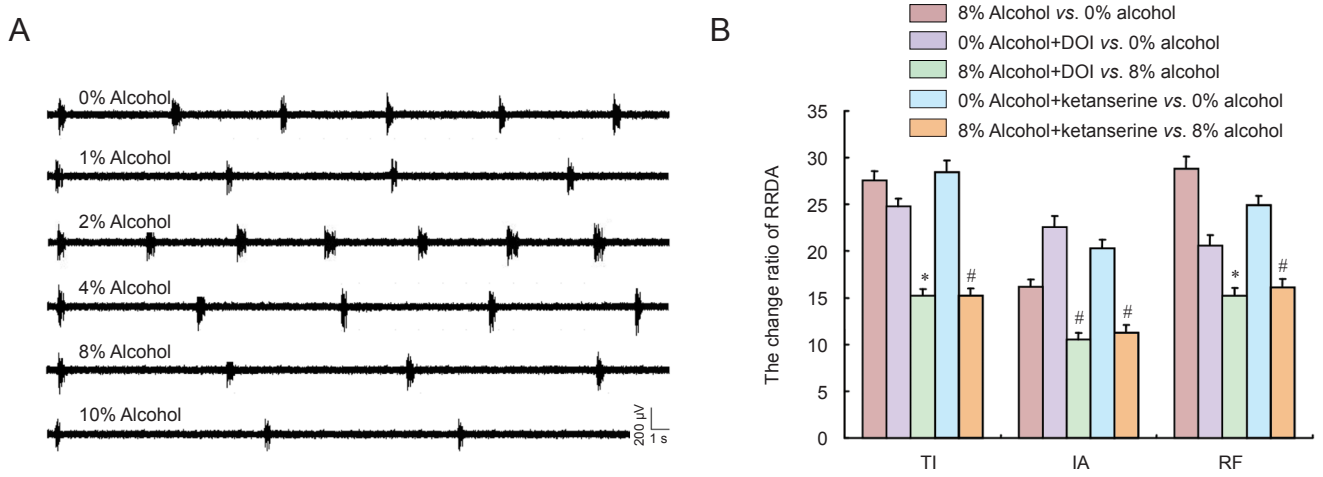

Figure 2 Effects of DOI and ketanserin on the RRDA in the 0% and 8% alcohol groups.
After treatment with DOI (40 μM), the RRDA in both groups strengthened; however, the percent change in the RRDA was smaller in the 8% alcohol group than in the 0% alcohol group. With ketanserin (40 μM) treatment, the RRDA in both groups weakened; however, the percent change in RRDA was smaller in the 8% alcohol group than in the 0% alcohol group. *$P < 0.05$, *vs.* (0% alcohol + DOI *vs.* 0% alcohol); #$P < 0.05$, *vs.* (8% alcohol + ketanserin *vs.* 8% alcohol). Data are expressed as the mean ± SD ($n = 6$; one-way analysis of variance, followed by Student-Newman-Keuls *post hoc* test). RRDA: Respiratory rhythmical discharge activity; TI: inspiratory time; IA: integral amplitude; RF: respiratory frequency; s: second.

Our data suggest that 5-HT_{2A}R may be a novel therapeutic target for prenatal alcohol exposure-induced respiratory depression. Nevertheless, our study has limitations. For example, although we investigated changes in 5-HT_{2A}R levels, we did not examine changes in its structure. Furthermore, we only analyzed the impact of prenatal alcohol exposure on the activity of respiratory neurons in 0–3-day-old neonatal rats and the role of 5-HT_{2A}R in this process, but we did not evaluate the dynamic changes that occur during the growth process. Additional studies are necessary to more comprehensively evaluate the effect of prenatal alcohol exposure on respiration in neonates and the role of 5-HT_{2A}R in this process. Future studies should investigate the following: (1) whether prenatal alcohol exposure causes 5-HT_{2A}R genetic mutations; (2) the impact of genetic mutations on 5-HT_{2A}R protein structure; and (3) how genetic mutations and protein structural changes affect 5-HT_{2A}R function.

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Author contributions: *ZBQ conceived and designed the study, and performed experiments. MLJ performed the experiment, analyzed the data, and wrote the paper. YHW performed experiment and analyzed the data. All authors approved the final version of the paper.*

Conflicts of interest: *None declared.*

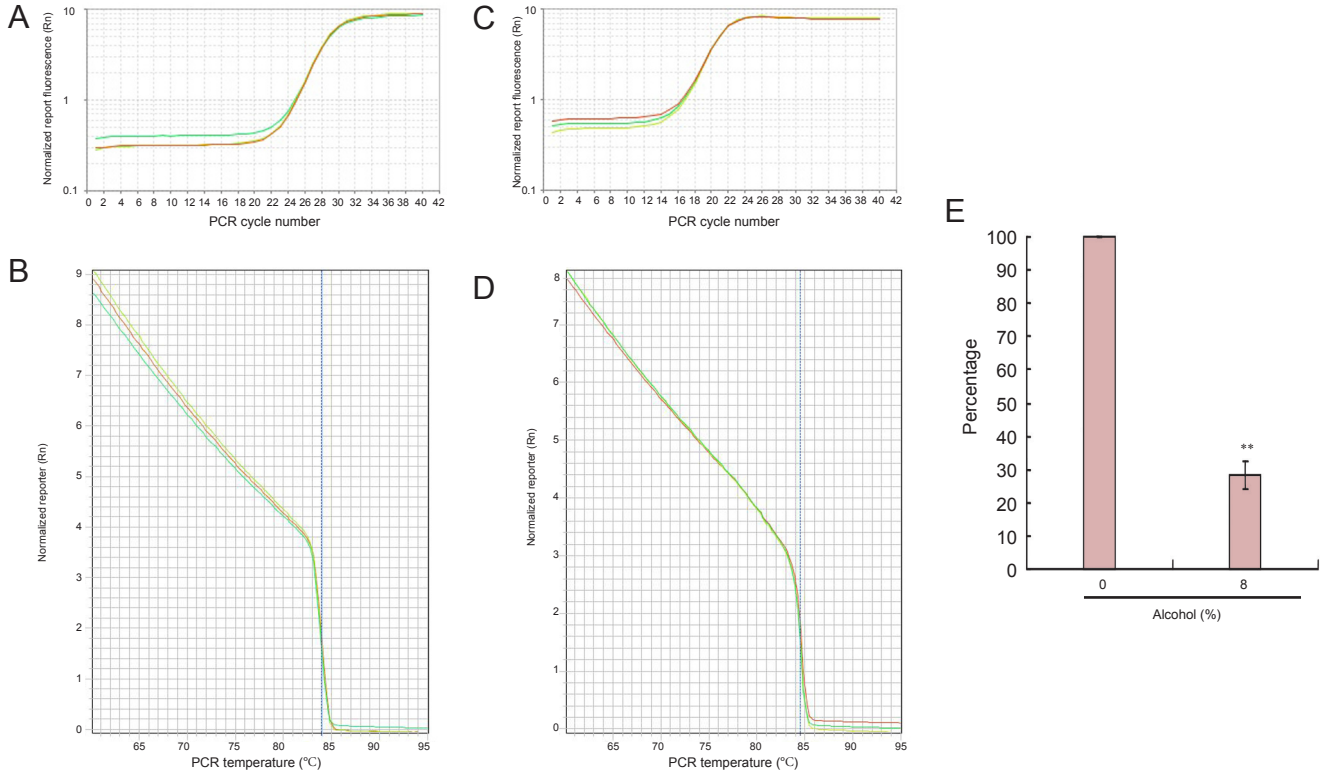

Figure 4 Prenatal alcohol exposure inhibited the expression of 5-hydroxytryptamine 2A (5-HT$_{2A}$) receptor mRNA in the medulla oblongata of neonatal rats (quantitative reverse transcription-polymerase chain reaction, qRT-PCR).
(A) Amplification plots for the 5-HT$_{2A}$ receptor in the preBötC in the 8% alcohol group. (B) The melting curve for the 5-HT$_{2A}$ receptor in the 8% alcohol group. (C) Amplification plots for GAPDH in the 8% alcohol group. (D) The melting curve for GAPDH in the 8% alcohol group. (E) Comparison of the expression levels of 5-HT$_{2A}$ receptor mRNA in the 0% and 8% alcohol groups. Data are normalized to those obtained in the 0% alcohol group. $^{**}P < 0.01$, *vs.* 0% alcohol group. Data are expressed as the mean ± SD ($n = 6$; one-way analysis of variance and Student-Newman-Keuls *post hoc* test).

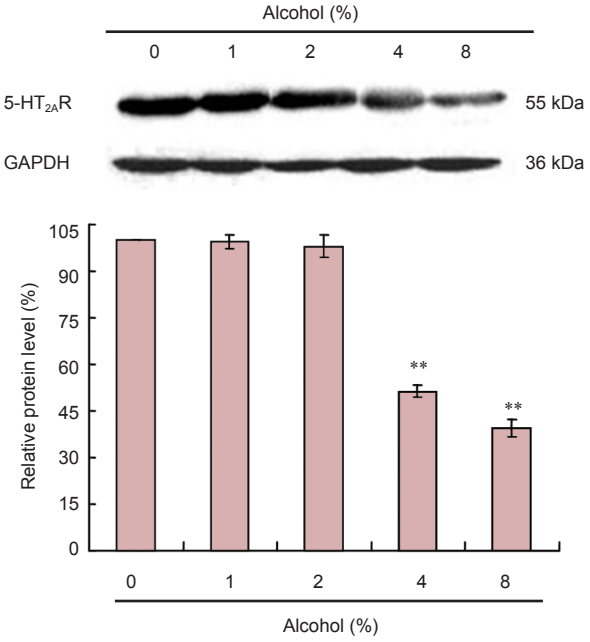

Figure 3 Comparison of 5-hydroxytryptamine 2A (5-HT$_{2A}$) receptor protein levels in the preBötC of neonatal rats prenatally exposed to different concentrations of alcohol (western blot assay).
Data are normalized to those obtained in the 0% alcohol group. $^{**}P < 0.01$, *vs.* 0% alcohol group. Data are expressed as the mean ± SD ($n = 6$; one-way analysis of variance and Student-Newman-Keuls *post hoc* test).

References

Abate P, Pepino MY, Spear NE, Molina JC (2004) Fetal learning with ethanol: correlations between maternal hypothermia during pregnancy and neonatal responsiveness to chemosensory cues of the drug. Alcohol Clin Exp Res 28:805-815.

Augustyniak A, Michalak K, Skrzydlewska E (2005) The action of oxidative stress induced by ethanol on the central nervous system (CNS). Postepy Hig Med Dosw 59:464-471.

Baker TL, Fuller DD, Zabka AG, Mitchell GS (2001) Respiratory plasticity: differential actions of continuous and episodic hypoxia and hypercapnia. Respir Physiol 129:25-35.

Bird CW, Candelaria-Cook FT, Magcalas CM, Davies S, Valenzuela CF, Savage DD, Hamilton DA (2015) Moderate prenatal alcohol exposure enhances GluN2B containing NMDA receptor binding and ifenprodil sensitivity in rat agranular insular cortex. PLoS One 10:e0118721.

Bou-Flores C, Lajard AM, Monteau R, De Maeyer E, Seif I, Lanoir J, Hilaire G (2000) Abnormal hrenic motoneuron activity and morphology in neonatal monoamine oxidase A-deficient transgenic mice: possible role of a serotonin excess. J Neurosci 20:4646-4656.

Chang GQ, Karatayev O, Liang SC, Barson JR, Leibowitz SF (2012) Prenatal ethanol exposure stimulates neurogenesis in hypothalamic and limbic peptide systems: possible mechanism for offspring ethanol overconsumption. Neuroscience 222:417-428.

Chen L, Zhang J, Ding Y, Li H, Nie L, Zhou H, Tang Y, Zheng Y (2013) Site-specific hydrogen sulfide-mediated central regulation of respiratory rhythm in medullary slices of neonatal rats. Neuroscience 233:118-126.

Cinelli E, Robertson B, Mutolo D, Grillner S, Pantaleo T, Bongianni F (2013) Neuronal mechanisms of respiratory pattern generation are evolutionary conserved. J Neurosci 33:9104-9112.

Cullere M, Macchione AF, Haymal B, Paradelo M, Langer MD, Spear NE, Molina JC (2015) Neonatal sensitization to ethanol-induced breathing disruptions as a function of late prenatal exposure to the drug in the rat: modulatory effects of ethanol's chemosensory cues. Physiol Behav 139:412-422.

Dobson CC, Thevasundaram K, Mongillo DL, Winterborn A, Holloway AC, Brien JF, Reynolds JN (2014) Chronic prenatal ethanol exposure alters expression of central and peripheral insulin signaling molecules in adult guinea pig offspring. Alcohol 48:687-693.

Dubois C, Houchi H, Naassila M, Daoust M, Pierrefiche O (2008) Blunted response to low oxygen of rat respiratory network after perinatal ethanol exposure: involvement of inhibitory control. J Physiol 586:1413-1427.

Hausknecht K, Haj-Dahmane S, Shen YL, Vezina P, Dlugos C, Shen RY (2015) Excitatory synaptic function and plasticity is persistently altered in ventral tegmental area dopamine neurons after prenatal ethanol exposure. Neuropsychopharmacology 40:893-905.

Kervern M, Dubois C, Naassila M, Daoust M, Pierrefiche O (2009) Perinatal alcohol exposure in rat induces long-term depression of respiration after episodic hypoxia. Am J Respir Crit Care Med 179:608-614.

Liebscher T, Schnell L, Schnell D, Scholl J, Schneider R, Gullo M, Fouad K, Mir A, Rausch M, Kindler D, Hamers FP, Schwab ME (2005) Nogo-A antibody improves regeneration and locomotion of spinal cord-injured rats. Ann Neurol 58:706-719.

Li L, Wang C, Zhang J (2007) Central mechanism of hypoxic rrespiratory depression. Yixue Zongshu 13:1341-1343.

Liu Q, Wong-Riley MT (2008) Postnatal changes in the expression of serotonin 2A receptors in various brain stem nuclei of the rat. J Appl Physiol 104:1801-1808.

Luo J (2014) Autophagy and ethanol neurotoxicity. Autophagy 10:2099-2108.

Mao KH, Jiang W, Liu YY, Chen Q, Chang E, Pao T (2014) Primary investigation into effect of chronic intermittent hypoxia on respiratory center at rat low brainstem. Linchuang Junyi Zazhi 42:5-7.

Miranda-Morales RS, Nizhnikov ME, Spear NE (2014) Prenatal exposure to ethanol during late gestation facilitates operant self-administration of the drug in 5-day-old rats. Alcohol 48:19-23.

Niebert M, Vogelgesang S, Koch UR, Bischoff AM, Kron M, Bock N, Manzke T (2011) Expression and function of serotonin 2A and 2B receptors in the mammalian respiratory network. PLoS One 6:e21395.

Nogués MA, Benarroch E (2008) Abnormalities of respiratory control and the respiratory motor unit. Neurologist 14:273-288.

Ozawa Y, Okado N (2002) Alteration of serotonergic receptors in the brain stems of human patients with respiratory disorders. Neuropediatrics 33:142-149.

Qian ZB, Wu ZH (2008) Role of 5-HT(2A) receptor in increase in respiratory-related rhythmic discharge activity by nikethamide in neonatal rat transverse medullary slices. Sheng Li Xue Bao 60:216-220.

Qian ZB, Ji ML, Wu ZH (2010) Nikethamide affects inspiratory neuron discharge in the nucleus retrofacialis medial region in brain slices from neonatal rats. Neural Regen Res 5:287-290.

Ren J, Lee S, Pagliardini S, Gérard M, Stewart CL, Greer JJ, Wevrick R (2003) Absence of Ndn, encoding the Prader-Willi syndrome-deleted gene necdin, results in congenital deficiency of central respiratory drive in neonatal mice. J Neurosci 23:1569-1573.

Sliwowska JH, Song HJ, Bodnar T, Weinberg J (2014) Prenatal alcohol exposure results in long-term serotonin neuron deficits in female rats: modulatory role of ovarian steroids. Alcohol Clin Exp Res 38:152-160.

Smith JC, Ellenberger HH, Ballanyi K, Richter DW, Feldman JL (1991) Pre-Botzinger complex: a brainstem region that may generate respiratory rhythm in mammals. Science 254:726-729.

Taylor NC, Li A, Nattie EE (2005) Medullary serotonergic neurones modulate the ventilatory response to hypercapnia, but not hypoxia in conscious rats. J Physiol 566:543-557.

Wang JL, Wu ZH, Pan BX, Li J (2005) Adenosine A1 receptors modulate the discharge activities of inspiratory and biphasic expiratory neurons in the medial region of Nucleus Retrofacialis of neonatal rat in vitro. Neurosci Lett 379:7-31.

Yanpallewar SU, Fernandes K, Marathe SV, Vadodaria KC, Jhaveri D, Rommelfanger K, Ladiwala U, Jha S, Muthig V, Hein L, Bartlett P, Weinshenker D, Vaidya VA (2010) Alpha2-adrenoceptor blockade accelerates the neurogenic, neurotrophic, and behavioral effects of chronic antidepressant treatment. J Neurosci 30:1096-1109.

Zhou R, Wang S, Zhu X (2010) Prenatal ethanol exposure attenuates GABAergic inhibition in basolateral amygdala leading to neuronal hyperexcitability and anxiety-like behavior of adult rat offspring. Neuroscience 170:749-757.

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