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Chronic hypoxia exposure during pregnancy is associated with a decreased active nursing activity in mother and an abnormal birth weight and postnatal growth in offspring of rats

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ABSTRACT

Stress during pregnancy is known to have a significant impact on animal's behavior and offspring development. The effects of gestational hypoxia on maternal behavior have not been studied. In the present study, we investigated the effects of gestational hypoxia exposure on dam's maternal behavior, offspring's growth and plasma corticosterone levels after parturition in rats. Altitude hypoxia (3 and 5 km) was simulated in the hypobaric chambers during the last week of pregnancy and the effects were compared to those found in controls exposed at sea level. We found that gestational hypoxia significantly decreased dam's arched-back nursing activity across the lactation period. The effect was more profound in 5 km group. Gestational hypoxia also altered other maternal behaviors such as blanket and passive nursing. Hypoxia exposure was associated with abnormal birth weight and postnatal growth in pups, with a significantly higher and lower birth weight than control found in 3 and 5 km groups, respectively, and accelerated growth in both stressed groups. Gestational hypoxia exposure significantly elevated plasma corticosterone levels in dams at the time of weaning and in pups across the measurement days. Taken together, the present results indicate that hypoxia, particularly severe hypoxia during the late phase of pregnancy has a significantly adverse impact on animal's behavior, endocrine function and offspring development. The higher birth weight found in the offspring of 3 km group suggests a compensatory system counteracting with the inhibitory effects of hypoxia on fetus growth at this altitude.

Introduction

Maternal behavior, characterized as a set of behavioral patterns displayed by female animals after parturition, plays an essential role for the survival of offspring (Everett-Hincks and Dodds, 2008; Grandinson, 2005). This behavior in rodents includes nursing (arched-back, blanket, and passive), nest building, licking and grooming of pups, pup retrieval, and maternal aggression (defense of offspring). The latency for initiation and the quality of maintenance of maternal behavior vary significantly among species as well as individually within a species. While the differences among species are largely determined by the unique genetic predispositions of each species, individual differences have been shown to be attributed mainly to the differences in individual neuronal and endocrinal states, particularly the responses of these systems to environmental factors (Weinstock, 2001).

Stress during pregnancy is one of common factors that significantly impact mother's maternal behavior and consequently the offspring development. Dams suffered from gestational stress, such as restraint, food restriction, noise exposure or predator presentation, are found to spend less time with their pups (Smith et al., 2004) and show less active nursing behavior compared to controls (Maccari et al., 1995; Melniczek and Ward, 1994; Moore and Power, 1986; Patin et al., 2002). Gestational stress also interferes with pregnancy outcomes such as increased spontaneous abortion, unbalanced sex ratio (de Catanzaro, 1988; Euker and Riegle, 1973; Guo et al., 1993; Patin et al., 2002), low birth weight (Fameli et al., 1995; Herrenkohl, 1979; Pollard, 1984), high mortality (Guo et al., 1993; Pollard, 1984) and retarded development of infant reflexes (Melniczek and Ward, 1994). The occurrences of these effects depend on the onset, intensity and duration of stress during the pregnant period (Henry et al., 1994; Pardon et al., 2000; Smith et al., 2004; Weinstock, 1997).

High-altitude exposure is also a common environmental stress that significantly compromises physiological functions in animals and humans. Ambient hypoxia associated with acute high-altitude exposure activates both hypothalamic–pituitary–adrenal (HPA) and hypothalamic–pituitary–gonadal (HPG) axes (Li et al., 1997; Wu

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and Du, 2001a), and increases dopamine release in the ventral striatum (Akiyama et al., 1991), while chronic hypoxia exposure is associated with increased oxytocin, vasopressin and prolactin levels in peripheral circulation, and increased oxytocin and vasopressin and decreased CRF levels in the hypothalamus (Kelestimur et al., 1991; Zhang and Du, 2000). Alterations in these systems have been shown to play significant roles in activation, expression and maintenance of maternal behavior in rats (Bridges, 1996; Doerr et al., 1981; Fameli et al., 1995; Meaney et al., 1991; Smith et al., 2004). Hypoxia exposure during pregnancy is known to have profound effects on both mothers and offspring, such as decreased motivation for food and weight gain in mothers, retarded intrauterine growth in the fetus and altered cognitive behavior in adult offspring (Tapanainen et al., 1994). It is not known currently, however, whether gestational hypoxia would modulate mother's maternal behavior as seen under other stress conditions.

In the present study, the effects of chronic hypoxia exposure during late pregnancy (days 14–20) on mother's maternal behavior activity and offspring's postnatal growth were investigated in rats. The adrenal cortical activities of both mothers and offspring were also assessed through measurement of plasma corticosterone levels. The aims of this study were to determine whether gestational hypoxia changes mother's maternal behavior patterns and whether such changes have a relationship with pup's growth and adrenal-corticoid activity.

Materials and methods

Animal preparation

Thirty female Sprague–Dawley rats (240 ± 10 g) were purchased from Scientific Research Center of Zhejiang Province. Upon arrival, they were housed in a temperature (22 ± 1 °C) and humidity (50–60%) controlled facility individually with standard rat pellets and water available *ad libitum*, under a 12 h light/dark cycle (light on at 07:00 a.m.). All procedures were approved by the Institutional Animal Care and Use Committee, and were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

After one week of acclimation, one of the sexually mature male rats (340 ± 10 g) was introduced into each cage and kept with a female overnight. On the following morning, the female rats were examined. The presence of vaginal plugs was considered indicative of pregnancy and deemed gestational day (GD) 1. The females were then again housed individually and their weight gain was periodically checked for further assurance of pregnancy.

Chronic hypoxia exposure

On GD14, 27 successfully pregnant rats were first randomly divided into three groups, one served as control and other two were used for hypoxia exposure. The rats in each group were then placed into a hypobaric chamber with food and water available *ad libitum*. Simulation of hypoxia for the two exposure groups was achieved through decompression of the chambers to elevations of the altitudes to 3 km (P_{O2}, 116 mm Hg, equivalent to 15.1% O_2) or 5 km (P_{O2}, 85 mm Hg, equivalent to 10.9% O_2). The altitude was elevated at a speed of 200 m/min until the desired levels were reached. The chamber for control group was not decompressed but ventilated at the atmospheric pressure equal to Hangzhou (0 km, P_{O2}, 158 mm Hg, 20.9% O_2). Animal cages in each group were cleaned, and food and water were replaced every day between 8:00 and 8:30 am. Thus, the exposure groups received approximately 23 h daily hypoxia treatment until GD20. Then the pregnant rats were carried out of the hypobaric chamber and returned to the facility. Each rat was closely watched for parturition.

Following parturition, the litter characteristics including pup number (litter size), survival rate, male–female ratio and pup weight in each litter were recorded, and the day was considered as postnatal day (PD) 1. The pup weight and survival rate in each litter were then continuously checked weekly until weaning.

Maternal behavior observation

The maternal behavior of each dam was observed on the PD 1, 3, 6, 10, and 15, five times per day, with three 15-min observations occurring in the light phase and two in the dark phase (9:00, 13:00, 17:00, 19:30, 21:30). The distribution of the observations was based on the findings that nursing in rats occurs more frequently during the light phase of the cycle (Illnerova and Sumova, 1997; Turek, 1994). We recorded the time that dams spent engaged in each of following behaviors: 1, active nursing; 2, passive nursing; 3, pup licking/grooming. Active nursing included arched-back and blanket nursing. Arched-back nursing represents the period that dam holds herself erect over the pups in a canopy posture, with her legs stretched out to provide balance. Blanket-nursing consists of periods when the dam grovels herself wholly on the pups with no support. Passive nursing represents the periods that dams were lying either on their back or side while the pups nursed actively. Pup licking and grooming included both anogenital and body licking whether the dam was nursing or not. In addition, the non-maternal behavior of mother, such as self-grooming, resting, eating and drinking were also observed.

Blood collection and corticosterone determination

Blood samples from mothers and pups were all collected between 09:30 and 10:30 am to avoid circadian variations. Mother's blood was collected on the day of weaning. Pup's blood samples were collected on postnatal days 10, 20, 40 and 70 respectively. On each of these days, a pup from each litter was randomly chosen. The rats were sacrificed through rapid decapitation. Trunk blood was collected into the ice-chilled anticoagulant centrifuge tube. The samples were then centrifuged at 4000 rpm for 30 min at 4 °C, and the collected plasma samples were stored at −20 °C until analysis.

Plasma corticosterone levels were measured using a fluorometric method previously described (Xiong and Suo, 1998; Zenker and Bernstein, 1958). Briefly, 0.1 ml of plasma samples from each animal and corticosterone standard solutions was first diluted four times with 0.4 M sodium hydroxide solution in the conical centrifuge tubes, followed by introduction of 5 ml of dichloromethane into each tube. The samples were then shaken for 2 min and the aqueous layer in each tube was carefully aspirated, followed by addition of 3 ml dichloromethane and fluorescence reagent respectively to each tube. The tubes were shaken again as described and then deposited 30 min at room temperature. The dichloromethane layer was then removed and the sulfuric acid layer was left in the tube at room temperature for 2 h for florescence development. The fluorescence density in each sample was measured using RF-5301PC(S) fluorometer at excitation and emission wavelengths of 470 and 520 nm, respectively. Corticosterone levels in each sample were calculated using the linear equation created with varying concentrations of the standard.

Data analysis

The data including pup body weight, survival rate and corticosterone levels in the three experimental groups were analyzed using repeated measure ANOVA of covariance (ANCOVA) with time as a within factor, treatment as a between factor and litter size as a covariate. Pups' body weights at birth and across postnatal days were measured individually and expressed as mean weight/litter. The hormone levels and the behavior data from dams in the three groups were

Table 1
Effects of gestational hypoxia on litter characteristics at parturition. Hypoxia significantly altered birth weight in both the 3 km and 5 km stressed groups. Data are expressed as mean ± SEM. * indicates significant ($P<0.05$) difference relative to the 0 km control group. N = 8–9 in each group.

Altitude (km)	0	3	5
Litter size	13.25 ± 0.94	13.25 ± 0.66	12.63 ± 0.60
Litter average birth weight (g)	6.78 ± 0.15	7.93 ± 0.18*	6.40 ± 0.47*
Litter male/female ratio	1.06 ± 0.32	1.06 ± 0.36	1.11 ± 0.41
Survival rate at birth (%)	97.87 ± 4.02	98.76 ± 0.41	92.2 ± 1.59

analyzed using either one way or two-way ANOVA whenever appropriate. Regression analysis was applied to explore potential relationships between the hypoxia challenges and the maternal behavior data, and between hypoxia challenges or maternal behavioral data and offspring development indices. When ANCOVA or ANOVA revealed a significant interaction, post hoc comparisons were applied using LSD honestly significant difference (Tukey's) test. The level of $P<0.05$ was considered as statistical significance.

Results

Effects of gestational hypoxia on litter characters

Table 1 shows litter characteristics in three experiment groups. Gestational hypoxia exposure at 3 km and 5 km showed differential effects on pup birth weight with an increase and a decrease found in 3 km and 5 km groups, respectively. One-way ANCOVA using litter size as covariate revealed a significant effect ($F_{(2,44)}=46.89$, $P<0.001$, Table 1). Post hoc analysis indicated that the birth weight in the 3 km group was significantly higher ($P<0.01$), while it was significantly lower in the 5 km group ($P<0.01$) compared to the controls. Gestational hypoxia did not affect the litter size, sex ratio and survival rate at birth significantly.

Effects of gestational hypoxia on dam's behaviors

Effects on maternal behavior

Arched-back nursing. All dams showed significantly more arched-back nursing during the light phase than during the dark phase. The overall time that hypoxia exposed dams engaged in this behavior was significantly lower than controls ($F_{(2,20)}=7.735$, $P<0.01$, Fig. 1). This difference was seen during both the light ($F_{(2,20)}=4.421$, $P<0.05$) and dark ($F_{(2,20)}=5.65$, $P<0.02$) phases. Post hoc multiple comparisons indicated that, during the light phase, both hypoxia exposure groups showed significantly less arched-back nursing than control group on PD3 and 6 ($P<0.05$), and the 5 km group showed less arched-back nursing than both 3 km and control groups on PD6 and 10 ($P<0.001$ *vs control*; $P<0.05$ *vs* 3 km group). During the dark phase, the less arched-back nursing in the hypoxia groups was found only on PD6 and 10, in a hypoxia intensity-dependent manner ($P<0.05$ *or* 0.001). Along with the lactation period, arched-back nursing was gradually decreased both in light and dark phases.

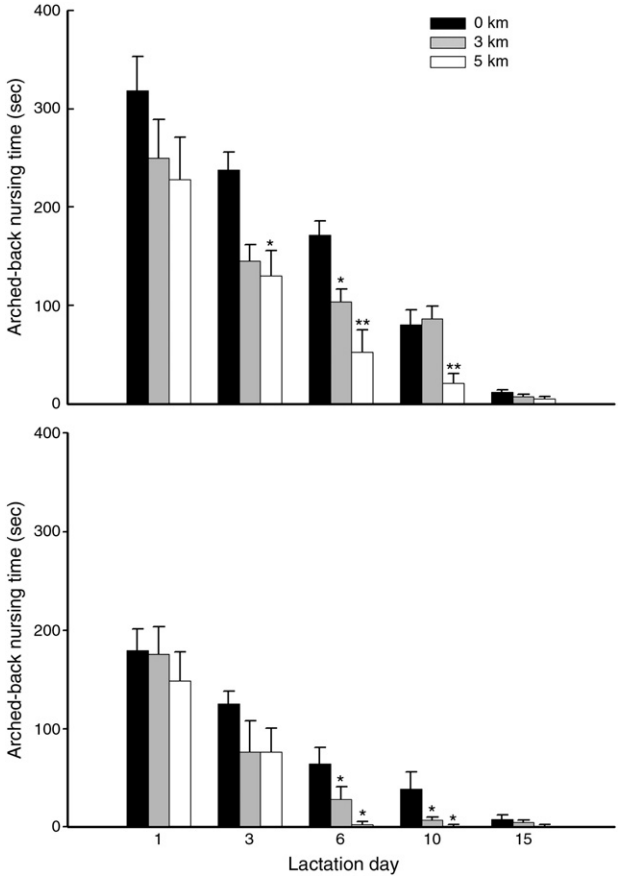

Fig. 1. Gestational hypoxia exposure during the last week of pregnancy significantly decreased the time that dams engaged in arched-back nursing during both light (top) and dark (bottom) phases across the lactation period. The reduction was more profound in the 5 km exposure group. Data are presented as mean + SEM. * indicates significant ($P<0.05$) difference relative to the 0 km control group. ** indicates significant ($P<0.05$) difference relative to both the 0 km control and 3 km stressed groups. N = 8–9 in each group.

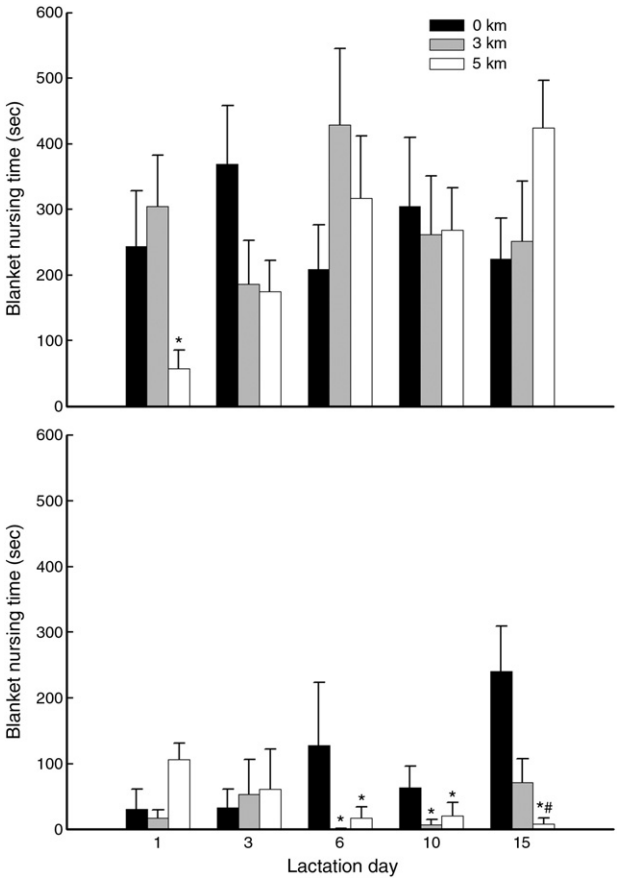

Fig. 2. Effects of gestational hypoxia on dam's blanket nursing behavior during the light (top) and dark (bottom) phases across lactation period. Data are presented as mean + SEM. * indicates significant ($P<0.05$) difference relative to the 0 km control group. # indicates significant ($P<0.05$) difference between the 5 km and 3 km groups. N = 8–9 in each group.

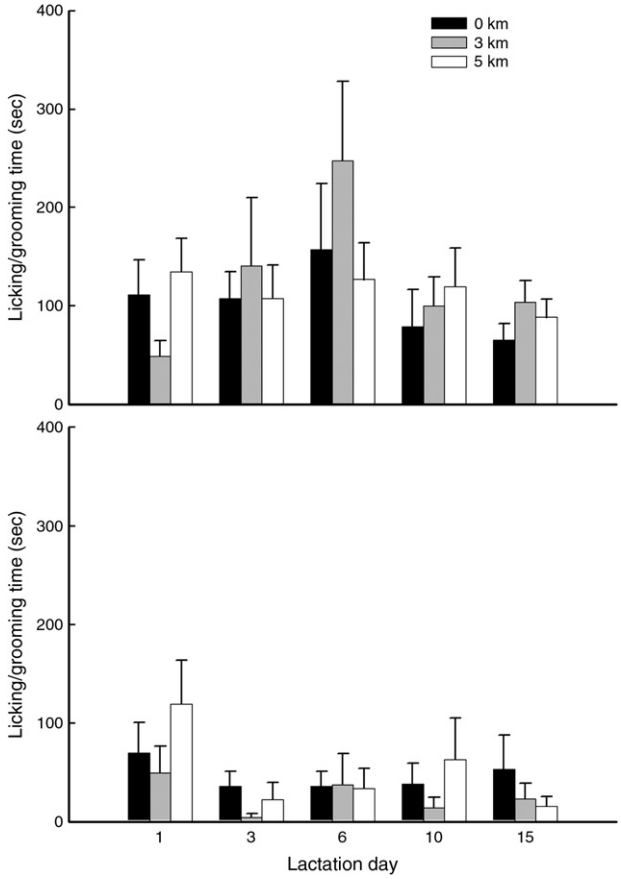

Fig. 3. Effects of gestational hypoxia on dam's pup licking/grooming during the light (top) and dark (bottom) phases across lactation period. Data are presented as mean + SEM. N = 8–9 in each group.

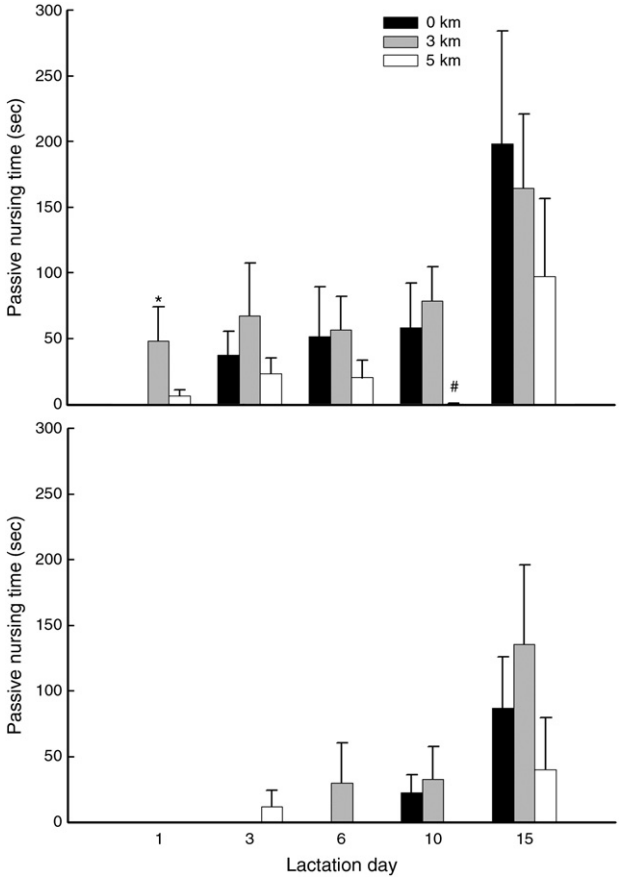

Fig. 4. Effects of gestational hypoxia on dam's passive nursing during the light (top) and dark (bottom) across lactation period. Data are presented as mean + SEM. * indicates significant ($P<0.05$) difference relative to the 0 km control group. # indicates significant difference between 5 km and 3 km groups. N = 8–9 in each group.

Blanket nursing. The time that dams spent engaged in blanket nursing varied significantly among three groups and individually within each group across testing days. ANOVA revealed a significant interaction during the dark ($F_{(2,23)} = 5.77$, $P<0.01$), but not the light ($F_{(2,23)} = 0.997$, $P = 0.38$). Post hoc multiple comparisons indicated that the time that dams engaged in this behavior was significantly less from lactation days 6 to 15 in the 3 km and 5 km groups than in controls ($P<0.05$, Fig. 2).

Pup licking/grooming and nesting. Pup licking/grooming displayed by dams in the three groups varied across the measurement days but no significant effects were found among the three groups during either the light ($F_{(2,23)} = 0.43$, $P = 0.61$) or dark ($F_{(2,23)} = 0.82$, $P = 0.45$, Fig. 3) phase.

Passive nursing. Dams spent significantly more time engaged in passive nursing during the light phase across test days ($F_{(2,23)} = 3.65$, $P<0.05$). There was no significant difference found during the dark phase among the three groups ($F_{(2,23)} = 2.82$, $P = 0.08$. Fig. 4).

Effects on non-maternal behavior

Self-grooming displayed by dams was considered as a non-maternal behavior during lactation period. We found that the occurrence of this behavior was significantly different between the three groups during the dark ($F_{(2,23)} = 4.34$, $P<0.05$. Fig. 5, lower panel), but not during the light ($F_{(2,23)} = 0.31$, $P = 0.73$) phase.

Effects of gestational hypoxia on pup growth and litter survival rate

Repeated measure ANCOVA of pup's body weight along with the lactation period revealed a greater body growth in stressed than in control pups ($F_{(2,44)} = 16.33$, $P<0.001$). No litter effects was found ($F_{(1,44)} = 0.01$, $P = 0.94$, Fig. 6). The acceleration of growth occurred 7 days after parturition, with significant higher body weights found on PD14 in 3 km group ($P<0.05$) and on PD21 in both hypoxia exposed groups ($P<0.05$).

Litter survival rate showed a decreased trend in all three groups over the lactation period but such decrease did not reach significant difference ($F_{(2,23)} = 0.89$, $P = 0.42$, Fig. 7).

Effects of gestational hypoxia on plasma corticosterone levels in dams and pups

Plasma corticosterone levels were 39.77 ± 3.63, 51.88 ± 10.54 and 88.64 ± 5.08 μg/100 ml in dams of 0, 3 and 5 km groups, respectively, at the time of weaning. One-way ANOVA revealed a significant difference among groups ($F_{(2,23)} = 8.69$, $P<0.01$). Post hoc comparisons indicated a significant elevation in the 5 km exposure group compared to control group ($P<0.01$), while the elevation in the 3 km group did not reach statistical significance ($P = 0.17$).

Corticosterone levels were also increased in the offspring of hypoxia groups and the increase was observed across lactation days and after weaning. ANCOVA indicated a revealed treatment–time interaction ($F_{(2,20)} = 15.33$, $P<0.001$) with no effect found on litter size. Post hoc multiple comparisons indicated that corticosterone levels in the 3 km groups were significantly higher than in corresponding controls while levels in 5 km groups were significantly higher than those in both the control and 3 km groups. Corticosterone levels in control pups did not differ across the test days. Corticosterone levels in both 3 and 5 km groups were gradually increased after weaning (Fig. 8).

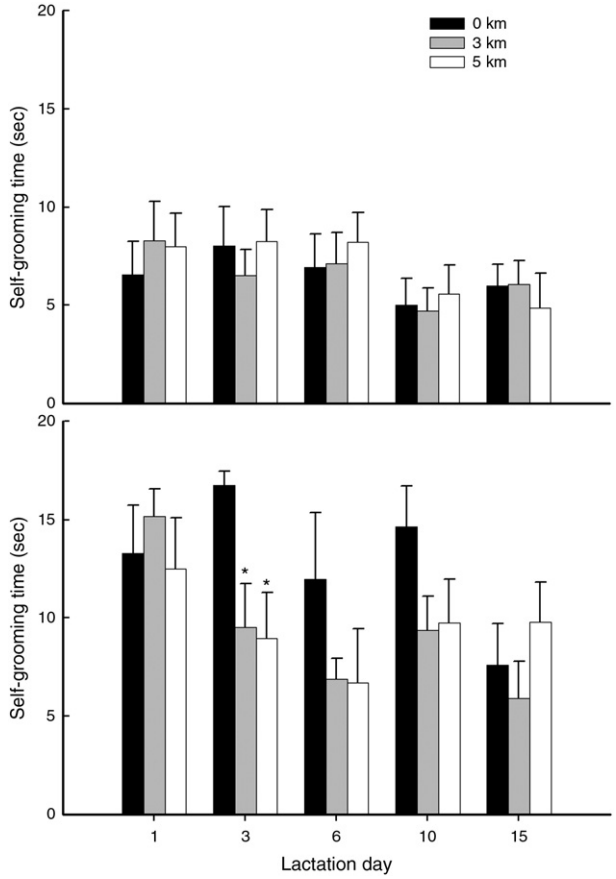

Fig. 5. Effects of gestational hypoxia on dam's self-grooming behavior during the light (top) and dark (bottom) phases across lactation period. Data are presented as mean + SEM. * indicates significant ($P<0.05$) difference relative to the 0 km control group. N = 8–9 in each group.

Relationships of dam's behavioral responses and offspring development outcome to gestational hypoxia challenges

To explore the potential relationships between the dam's behavior responses and corticosterone levels to hypoxia challenges, multiple regression analysis used hypoxia exposure as a dependent variable. We found that the time that dams spent engaged in arched-back nursing across lactation ($t=-6.76$, $P<0.001$) was, while the time

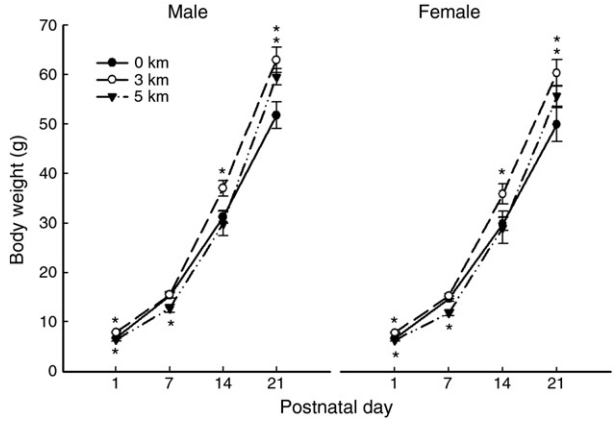

Fig. 6. Effects of gestational hypoxia on offspring postnatal growth. Chronic hypoxia exposure during the last week of pregnancy significantly increased growth rate in both male and female pups, with a growth acceleration starting at 7 days after birth. Data are presented as mean + SEM. * indicates significant ($P<0.05$) difference relative to the 0 km control group. N = 8 in each group.

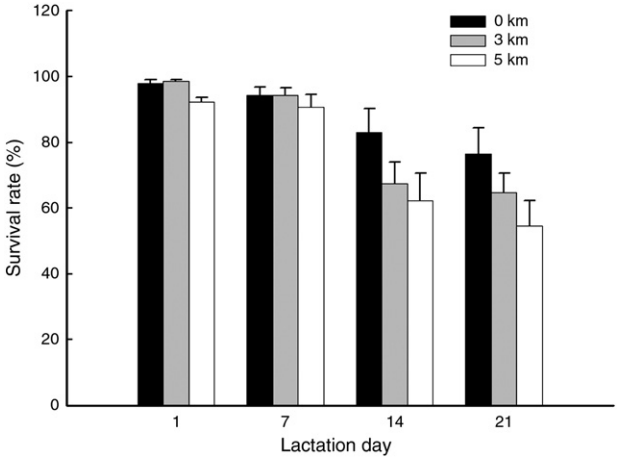

Fig. 7. Effects of gestational hypoxia on pups' survival rates across the lactation period. Data are expressed as the percentage of pups delivered on lactation day 1 and are presented as mean + SEM. N = 8–9 in each group.

that engaged in other behaviors ($t=-0.34$–0.29, $P=0.73$–0.91) and corticosterone levels at the time of weaning ($t=1.09$, $P=0.73$–0.91) were not, correlated to the hypoxia challenges. Single linear regression revealed significant negative correlations of arched-back nursing ($r=-0.92$, $P<0.001$) and passive nursing ($r=-0.44$, $P<0.05$) and positive correlations of corticosterone levels ($r=0.65$, $P<0.001$) to hypoxia intensities. Other dam's behaviors were not correlated ($r=-0.25$–0.07, $P=0.21$–0.73).

Multiple regression analysis of pup data indicated that pup corticosterone levels were correlated to both hypoxia challenges ($t=2.82$, $P<0.05$) and arched-back nursing activities ($t=-4.81$, $P<0.001$) while pup survival and growth rates were not ($P=0.41$–0.77). Single linear regression test indicated that corticosterone levels in pups were negatively correlated to arched-back nursing ($r=-0.87$–0.95, $P<0.001$) and positively correlated to hypoxia challenges ($r=0.65$–0.87, $P<0.001$) across testing days. Single linear regression test also revealed a significant correlation of pup growth rate to hypoxia challenges ($r=0.47$, $P<0.05$) and dam's arched-back nursing ($r=0.46$, $P<0.05$) in male but not in female pups (hypoxia, $r=0.35$, $P=0.08$; arched-back nursing, $r=0.29$, $P=0.14$).

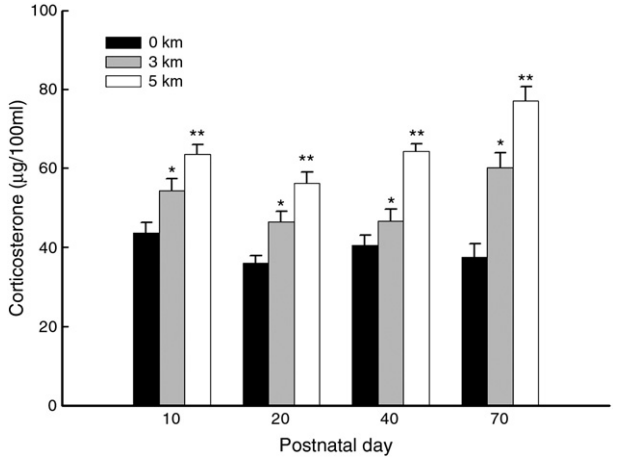

Fig. 8. Effects of gestational hypoxia on offspring plasma corticosterone levels across various postnatal days. Prenatal hypoxia significantly elevated pup's corticosterone levels in both the 3 km and 5 km exposure groups and across testing days. Data are presented as mean + SEM. * indicates significant ($P<0.05$) difference relative to the 0 km control group. ** indicates significant ($P<0.05$) difference relative to both the 0 km control and 3 km stressed groups. N = 6–12 in each group.

Discussion

In the present study, we have found that chronic hypoxia during the last week of pregnancy is associated with several adverse changes in both dams and offspring, including decreased maternal behavior activity in dams, disturbed growth patterns in offspring and elevated corticosterone levels in both dams and offspring. The decreased maternal activity resulted mainly from reduced arched-back and blanket nursing in both stressed groups with a more prominent effect seen in the 5 km group. The effects of hypoxia on offspring growth include altered birth weight at delivery and growth patterns during the neonatal period. The elevated corticosterone levels in pups were found throughout the test days. These findings are in general agreement with previous findings using other gestational stress procedures (Liu et al., 1997; Melniczek and Ward, 1994; Patin et al., 2002) and add to the lines of evidence suggesting gestational stress as a powerful modulator of animal's behavioral traits and offspring development.

In the present study, arched-back nursing activity significantly correlated to the intensity of hypoxia exposure. Arched-back nursing represents the major form of active nursing and play a crucial role in the survival of offspring, particularly during the early neonatal period (Smith et al., 2004). Studies in high LG-ABN rats have found that arched-back nursing occurs often concurrently with pup licking/grooming (Liu et al., 1997, 2000), and thus a positive relationship between these behaviors has been noticed in these dams. In the present study, we did not notice any significant differences in pup licking/grooming among the three groups although arched-back nursing in the dams varied during both light and dark phases across test days. A lack of correlation between pup licking and nursing has also been reported in dams stressed by gestational crowding (Moore and Power, 1986). While the factors that account for these disparate findings are not clear, the animal behavior phenotypes, the age of pups, and the behavioral measurement frequencies and durations may each have a significant influence.

Initiation and maintenance of maternal behavior are normally controlled by the neuroendocrine and physiological changes in and the environmental stimuli to the dams around parturition and stress is known to modulate these changes and the responses of dams to these stimuli (Stern, 1989, 1990). Corticosterone, a hormone that plays critical role in stress-induced dam's behavior, was elevated along with the gestational hypoxia challenges in dams at the time of weaning. This finding suggests a long term impact of hypoxia on dam's endocrine function. Currently, it is unknown whether this elevation is induced directly from gestational hypoxia exposure, or developed at parturition and lactation period. We have previously demonstrated in male rats that while acute hypoxia induces significant elevations of plasma corticosterone levels, these levels are not significantly different from controls after 7 days of chronic exposure. This lack of effect is explained in part as adaptive changes in the HPA axis which include the hormone synthesis, receptor expression and feedback regulation (Wu and Du, 2001a). However, it is well known that pregnancy can significantly compromise the adaptation systems in female and thus stress may be more easily to override this system in pregnant animals. More experiments would be necessary before a definitive conclusion can be drawn.

The elevations in corticosterone in stressed pups resulted most likely from the combined consequences of prenatal hypoxia exposure and reduced postnatal mother–pup interactions. The effects of gestational stress on offspring development depend not only on the nature of stress but also on the time of stress application during gestation (Ohkawa et al., 1991; Weinstock et al., 1988). The response of HPA axis in rat fetuses develops mainly during the last week of gestation (Boudouresque et al., 1988; Erisman et al., 1990; Ohkawa et al., 1991). Thus, gestational stress during this stage has been shown to modulate fetal HPA functions at all three levels (Boudouresque et al., 1988; Erisman et al., 1990; Hagerman and Villee, 1960). Therefore, stress during late stage of gestation may influence offspring *via* stress induced changes in hormone levels from both mother and fetuses themselves (Caldji et al., 1998). In addition, cross-fostering studies in rats have indicated that the quality and the quantity of maternal care that offspring receive also significantly influence offspring's corticoid functions (Liu et al., 2000). In our present study, stressed pups also received significant less maternal care from their mother as revealed by less active nursing, thus the elevations of corticosterone found in these pups may stem from the sum of circumstances including direct and indirect influences of prenatal hypoxia stress and reduced postnatal interaction with the mother. It would be interesting to test in future studies whether postnatal fostering would play a role in the normalization of corticoid functions induced by hypoxia stress.

Offspring growth rate is considered as one of the main predictors for developmental outcome (Weinstock, 2001). Thus, prenatal as well as postnatal stresses, that impact the offspring's neuronal development, endocrine state and behavior patterns, have been frequently associated with a low birth weight and a retarded postnatal growth (Champagne et al., 2003; Wadhwa et al., 2001). While the altered birth weight is mainly due to the mother's changes in nutritional and neuroendocrine state during gestation, the factors that affect postnatal development are more complex, which involve offspring's characteristics at birth and maternal care that offspring receives (Peters, 1988; Power and Moore, 1986). In agreement with previous studies under other gestational stress conditions (Baker et al., 2008; Kinsley and Svare, 1988), we have found that the birth weights of offspring in the 5 km hypoxia group are significantly lower than in controls. Hypoxia exposure is known to be anorexic even in high altitude acclimated rats (Norese et al., 2002). Chronic hypoxia exposure at 5 km has been shown to be associated with a persistent decrease in food intake and reduced weight gain during the entire periods of exposure in male rats (Chen et al., 2007). Thus, it is possible that the lower birth weight found in prenatally stressed pups resulted from the decreased food intake in stressed mother, which in turn limited the nutrient availability for their rapidly growing fetuses. In support of this, gestational hypoxia exposure and nutrient restrictions have been both associated with similar reduction of pup's birth weight, despite their differential effects on offspring's cardiovascular functions (Cianfarani et al., 1998; Williams et al., 2005). However, opposite to what we have found in 5 km group, gestational hypoxia exposure at 3 km altitude induced a significant increase in pup's birth weight. While the mechanisms underlying these effects of hypoxia need to be explored, this surprising finding suggests an adaptation system through which pregnant rats promote fetus's growth at this level of hypoxic stress.

Despite the opposite effects on birth weight, pups in both stressed groups showed accelerated postnatal growth than controls. This acceleration occurred primarily 7 days after parturition and was found in both male and female pups. This effect could not be attributed to the differences in maternal care that pups received since less maternal nursing found in stressed dams would lead to less food intake and slower development, and the maternal care would be more critical for pup growth during early postnatal period (Lemaire et al., 2000; Levine, 1994; Marais et al., 2008; Schanberg and Field, 1987). Pup growth can also be affected by the pup numbers that each mother nourishes. Dams with large litter size may reach a limit in their ability to nourish suckling young, and have less ability than those with fewer pups to fulfill the energy demands of growing offspring (Rogowitz and McClure, 1995). Differences in litter size would also affect the time that mothers spend interacting with each pup which is known to influence pup growth (Lemaire et al., 2000; Previc, 2007). In the present study, however, we did not notice any significant differences in the litter size and male–female ratio at birth among the control and stressed groups.

It is possible that the faster postnatal growth found in stressed pups is associated with their abnormal birth weights induced by gestational hypoxia exposure. Abnormal birth weights are strongly linked to postnatal development of metabolic disorders including obesity and diabetes (Gillman et al., 2003; Ong and Dunger, 2004; Seckl and Meaney, 2004). Clinical studies indicate that children born with birth weight 1 kg above the average, the likeliness for developing obesity increases nearly by 50% (Gillman et al., 2003). Children born with abnormally low birth weight frequently display catch-up growth, altered feeding behavior and increased risks for overweight or obesity (Leong et al., 2003; Ong and Dunger, 2004). Our findings in rats are in accordance with these clinical observations and point out a likely detrimental effect of gestational hypoxia on offspring development. More detailed studies at physiological, biochemical and behavior levels would be necessary to test whether such growth is actually reflecting a propensity for obesity in these stressed pups.

In summary, gestational hypoxia during the last week of pregnancy significantly reduced active nursing and elevated corticoid activity in dams after parturition, with more profound effects seen in 5 km exposure group. Gestational hypoxia is associated with abnormal birth weights and postnatal growth in pups. Gestational hypoxia exposure elevated pup's corticosterone levels across measurement days. The elevations in pups were more prominent after weaning. The present results indicate that hypoxia, particularly severe hypoxia exposure during the late phase of pregnancy has a significantly adverse impact on animal's behavior, endocrine function and offspring development.

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