Published in final edited form as:

Respir Physiol Neurobiol. 2009 February 28; 165(0): . doi:10.1016/j.resp.2008.11.004.

Gestational cigarette smoke exposure and hyperthermic enhancement of laryngeal chemoreflex in rat pups

Luxi Xia^a, Mardi Crane-Godreau^b, James C. Leiter^a, and Donald Bartlett Jr.^{a,*}

^aDepartment of Physiology, Dartmouth Medical School, One Medical Center Drive, Lebanon, NH 03756 USA

^bDepartment of Microbiology and Immunology, Dartmouth Medical School, Lebanon, NH 03756 USA

Abstract

Laryngeal chemoreflex (LCR) apnea occurs in infant mammals of many species in response to water or other liquids in the laryngeal lumen. The apnea can last for many seconds, sometimes leading to dangerous hypoxemia, and has therefore been considered as a possible mechanism in the Sudden Infant Death Syndrome (SIDS). We have found recently that this reflex is markedly prolonged in decerebrate piglets and anesthetized rat pups that are warmed 1–3 °C above their normal body temperatures. We intermittently exposed pregnant rats to cigarette smoke and examined the LCR in their four- to fifteen-day-old offspring under general anesthesia, with and without whole body warming. During warming, pups of gestationally smoke-exposed dams had significantly longer LCR-induced respiratory disruption than similarly warmed control pups. The results may be significant for the pathogenesis and/or prevention of SIDS as maternal cigarette smoking during human pregnancy and heat stress in infants are known risk factors for SIDS.

Keywords

Cigarette smoke; Pregnancy; Neonatal rats; Hyperthermia; Laryngeal chemoreflex; Sudden Infant Death Syndrome

1. Introduction

In recent years, the incidence of the Sudden Infant Death Syndrome (SIDS) has decreased substantially in the United States and elsewhere, largely as a result of educational campaigns favoring the supine posture for sleeping infants (“Back to Sleep”, “Face Up to Wake Up”). Despite this success, SIDS remains a major contributor to infant mortality (Heron, 2007) and a tragic event for the affected families. Further progress in the prevention of SIDS is likely to require an improved understanding of its pathogenesis.

Epidemiologists have used case-control methods to identify several risk factors for SIDS. In addition to the high risk of the prone sleeping position (Blair et al., 1996; Paterson et al., 2006), which remains poorly understood, maternal cigarette smoking during pregnancy and heat stress in the infant are both associated with high SIDS incidence (Anderson et al., 2005; Blair et al., 1996; Mitchell et al., 1993). The laryngeal chemoreflex (LCR)—apnea, swallowing, cough, bradycardia and redistribution of blood flow in response to water, gastric contents or other foreign liquids in the laryngeal lumen—is mediated by afferents in

the superior laryngeal nerves (SLNs) (Boggs and Bartlett, 1982; Storey and Johnson, 1975). This reflex is much more prominent in newborn animals and human infants than in adults and therefore has long been suspected as a cause of some cases of SIDS (Boggs and Bartlett, 1982; Leiter and Böhm, 2007; Pickens et al., 1988; St. Hilaire et al., 2007; Storey and Johnson, 1975; Thach, 2008).

We have recently found that in neonatal piglets and rat pups, the respiratory disruption associated with the LCR is greatly exaggerated in animals that are warmed 1–3 °C above their normal body temperatures (Curran et al., 2005; Xia et al., 2008a; Xia et al., 2007; Xia et al., 2006; Xia et al., 2008b). This effect of hyperthermia is reversible by returning the body temperature to normal. This combination of findings raises the question whether maternal tobacco smoke exposure during pregnancy interacts with postnatal hyperthermia to prolong and exaggerate the LCR in infants. We have therefore carried out experiments in which we replicated two epidemiological risk factors for SIDS (maternal smoking and thermal stress) in rat pups to test the hypothesis that the duration of the respiratory disruption associated with the LCR or its hyperthermic exaggeration is influenced by maternal exposure to cigarette smoke during pregnancy. We chose a moderate level of maternal smoke exposure in this initial study.

2. Methods

The Institutional Animal Care and Use Committee of Dartmouth College approved the protocols for these studies. Timed pregnant Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA), weighing 210–245 g were received on day 3 of pregnancy and kept in individual cages with constant availability of standard rat chow and water. Beginning the day following their arrival, half of the pregnant rats (exposed group) were exposed in a chamber for four hours per day, five days per week to combined mainstream and sidestream cigarette smoke generated by a Teague model TE-10z smoking machine. Smoke was generated from humidified 1R4F research cigarettes (Tobacco Health Institute, Lexington, KY), which were smoked 3 at a time with 35-ml puffs at 60-sec intervals. The smoke was diluted and aged in the machine and delivered to the exposure chamber together with an airflow adjusted to produce a total suspended particulate (TSP) concentration of approximately 30 mg/m³. The actual TSP in the exposure chamber, measured every hour during the exposure periods (Esposito et al., 2008; Yu et al., 2002), averaged 31.6 ± 1.3 mg/m³. In calibration tests conducted by the manufacturer shortly before these studies, this TSP level was associated with a nicotine concentration of approximately 4 mg/m³ and a carbon monoxide concentration of approximately 140 ppm. Mothers of rat pups in the control group were exposed to clean air in the same room. The TSP concentration in the control atmosphere was less than 1 mg/m³. On the day of delivery (day 21 of gestation), the smoke exposure was stopped, and thereafter the pups and dam were kept in clean air. The pups were studied at intervals between the postnatal ages of 4 and 15 days (P4 and P15).

The pups in both groups were studied using a protocol that has been reported previously (Xia et al., 2008b). The experimenter did not know the treatment group of each animal at the time of the study. Each pup was anesthetized initially with 3–4% halothane. Once the animal was unconscious, urethane (1.0 mg/kg) and chloralose (20 mg/kg) were given by intraperitoneal injection. Halothane was then gradually withdrawn over the next 15–20 min as the non-volatile agents took effect. The level of anesthesia was monitored to achieve stable respiratory rates, but assure that each animal was unresponsive when the paw was pinched. A thermistor probe was inserted into the rectum to record body temperature, which was controlled initially at 35–36 °C (Schmidt et al., 1986) by means of a thermostatically regulated heating pad.

The animal was placed in the supine position, and hooked wire electromyogram (EMG) electrodes were positioned in the trunk musculature to record inspiratory activity. Single-filament (0.002-in. diameter) stainless steel Teflon-coated EMG wires (A-M Systems, Everett, WA) were placed in the body wall in the lowest part of the rib cage in the anterior axillary line, one on each side of the chest, using a 25-gauge needle as an introducer. A ground wire was inserted subcutaneously through the skin over the abdomen. The EMG signal, which represented both diaphragmatic and intercostal muscle activity, was amplified, moving time averaged by a laboratory computer using a 100-ms time constant, and displayed on a monitor along with body temperature. These signals were examined off-line to determine the respiratory responses to laryngeal stimulation and their influence by body temperature.

A midline anterior skin incision was made in the neck, and the cervical trachea was freed from adjacent tissues with the aid of an operating microscope (model OPMI, Zeiss, Germany). Care was taken to identify the SLNs and avoid them in the dissection. The trachea was opened with a transverse incision that exposed the lumen but left the posterior tracheal wall intact. This permitted the animal to breathe freely through the unintubated caudal segment of the trachea. The rostral segment of the trachea was cannulated with polyethylene tubing, heat tapered at the tip to about PE10 outside diameter (0.6 mm). The tapered tubing, marked at 1 mm intervals, was advanced until it was gently wedged into the trachea with its tip just caudal to the larynx and was secured in that position with a fine silk ligature. The ligature may have damaged the recurrent laryngeal nerves; we made no effort to assess laryngeal muscle function.

The animal was then tipped approximately 15° head-down so that water injected into the larynx would run out through the nose and mouth. After stable breathing was recorded for at least 10 min, 2–5- μ l injections of water were made into the rostral trachea and larynx by means of a 1-ml Hamilton syringe and a computer-controlled infusion pump (model SP100i, WPI, Sarasota, FL), starting at the beginning of inspiration. The volume of injected water was determined by animal size (slightly larger injection volumes were necessary in larger animals to generate reflex apnea consistently), and once chosen, the injected volume remained constant throughout the experiment. At least 5 min passed between sequential trials of the LCR. After three trials under baseline conditions, the animal was warmed to approximately 38 °C using a servo-controlled infrared heat lamp, and another three trials were made. Finally, body temperature was lowered back to 35–36 °C, and three further trials were performed. The entire experiment required approximately 1 h. In our previous study, the procedure was repeated in five animals after section of both SLNs to verify that the responses were mediated by these nerves, and time-control experiments were performed with 12 pups following the same protocol, but with no heating (Xia et al., 2008b).

Because the LCR is a complex mix of respiratory inhibition, of activities that clear the airway, such as swallowing, and of cardiovascular responses, we operationally defined the duration of each respiratory response as the period of respiratory disruption from the beginning of the stimulus until the onset of at least five regular breaths (van der Velde et al., 2003). We separately analyzed the duration of the longest apneic period occurring during each response.

Statistical analysis of the results was done by repeated measures analysis of variance, using the average response of each animal in each condition (baseline, hyperthermia and recovery). As baseline and recovery data did not differ significantly, these values were combined for linear regression analysis of the interactive effects of age and smoke exposure.

3. Results

The 6 pregnant rats used in the study (3 smoke-exposed and 3 controls) tolerated the conditions without apparent difficulty and delivered their pups (33 exposed and 29 control) on day 21 of gestation, as expected. As previously reported in similar studies (Carmines and Rajendran, 2008; Pendlebury et al., 2008; Tachi and Aoyama, 1983), the pups of the exposed mothers had lower body weight than that of the controls at birth (5.77 ± 0.15 g vs. 6.66 ± 0.12 g ($P < 0.0001$)) in the two litters of each group in which this was measured, and their growth over the first four postnatal days was slower as well (3.74 ± 0.17 g vs. 4.64 ± 0.25 g; $P < 0.005$).

Two examples of the effect of hyperthermia on the LCR are shown in Fig. 1. One set of data was obtained from a female P6 rat pup born to a mother exposed to clean air throughout pregnancy (left panels of Fig. 1), and the other from a female P6 rat pup born to a dam exposed to cigarette smoke during pregnancy (right panels). Hyperthermia prolonged the LCR in both animals, but the thermal prolongation of the LCR was much greater in the rat pup born to the dam exposed to smoke during pregnancy. Note that the hyperthermic response was reversible, and the LCR returned to control values in both animals once their body temperatures were allowed to fall to the normal range.

As documented in Table 1, the baseline and recovery respiratory frequency values did not differ from each other or between the exposed animals and the controls. Hyperthermia increased the respiratory frequency in both groups; the average frequency was slightly higher in the smoke-exposed group, but the difference from the control group was not statistically significant. The respiratory component of the LCR in the control pups, whether measured as the duration of the reflex or of the longest associated apnea, was significantly prolonged when body temperature was increased from 35.47 ± 0.02 to 38.25 ± 0.04 °C and then returned to baseline values when body temperature was restored to 35.48 ± 0.03 °C ($P < 0.05$). In previously reported time-control animals treated with this protocol, but without heating, there was no change in the duration of the LCR or of the longest apnea (Xia et al., 2008b), so the responses to laryngeal stimulation were stable during the time of the experiment. In the smoke-exposed animals, the baseline and recovery LCR and longest apnea durations were similar to those in the controls (Table 1). Hyperthermia prolonged the LCR duration significantly more in the smoke-exposed pups than in the controls ($P < 0.03$). Hyperthermia also prolonged the duration of the longest apnea in both groups (a main effect of hyperthermia; $P < 0.05$). The absolute apnea durations in each thermal condition were not different when comparing the exposure group to control rat pups, but the change between baseline and hyperthermic apnea durations was longer in the exposure group animals compared to the controls ($P = 0.05$). No differences in any of the variables were apparent between male and female animals.

Under baseline conditions, before the pups were made hyperthermic, both measures of the LCR were most prolonged in the youngest animals and diminished slightly, but significantly, with age ($P < 0.01$). This was true of both exposed and control animals, and there was no significant difference in the age-related baseline response pattern between the two treatment groups. Thus the LCR became shorter as postnatal age increased up to P15, but maternal smoke exposure alone had no effect on the LCR under baseline conditions and no effect on the age-related shortening of the LCR. However, as illustrated in Fig. 2, the thermal prolongation of the LCR was enhanced in young animals, and this enhancement was further exaggerated in the young pups of rats exposed to cigarette smoke during pregnancy. The slopes of the regression lines relating thermal prolongation of both measures of the LCR to age were significantly different from zero ($P < 0.01$), as in our earlier study (Xia et al., 2008b). Moreover, the regression slopes for thermal prolongation in relation to age for the

pups in the exposed group were significantly steeper than those for the unexposed animals ($P < 0.01$). Thus gestational smoke exposure of mother rats enhanced the hyperthermic prolongation of the LCR in the youngest pups, but this effect of exposure waned with age and was not apparent by day P15.

4. Discussion

The most important new finding of this study is that the hyperthermic enhancement of the LCR is significantly exaggerated in the pups of dams exposed to cigarette smoke during pregnancy (Table 1), and this effect of gestational smoke exposure is most prominent in the youngest animals (Fig. 2). The enhancement of laryngeal apnea by hyperthermia was first demonstrated by Haraguchi and associates, who found that the threshold for laryngeal adductor contraction during SLN stimulation was greatly reduced by hyperthermia in puppies, but much less so in adult dogs (Haraguchi et al., 1983). More recently, we have shown that the duration of the LCR—both the respiratory disruption and the associated apnea—following water injection into the laryngeal lumen is prolonged by hyperthermia in decerebrate neonatal piglets (Curran et al., 2005; Xia et al., 2008a; Xia et al., 2007; Xia et al., 2006) and anesthetized rat pups (Xia et al., 2008b). The enhancement of the reflex appears to depend on a temperature-sensitive mechanism in or near the nucleus of the solitary tract (Xia et al., 2006) and can be reversed by pharmacological blockade of GABA_A receptors in the same region (Xia et al., 2007). Many investigators feel that the LCR initiates apneas that may lead, in rare cases, to SIDS (Downing and Lee, 1975; Leiter and Böhm, 2007; Page et al., 1996; Thach, 2003; Thach, 2008). Prolonged apnea with attendant hypoxia may require successful autoresuscitation for recovery (Guntheroth and Kawabori, 1975; Leiter and Böhm, 2007; Thach, 2008), and failed autoresuscitation may be the final common pathway for infants who die of SIDS. Therefore, the demonstration that maternal smoke exposure in rats increases the duration of reflex apnea in the offspring may provide an important mechanistic link among the LCR, thermal stress, maternal cigarette smoking and the pathogenesis of SIDS.

The results of this study confirm our earlier report (Xia et al., 2008b) that the duration of the LCR, however measured, is enhanced by mild hyperthermia in young rat pups, as previously shown in piglets (Curran et al., 2005; Xia et al., 2008a; Xia et al., 2007; Xia et al., 2006), and that the enhancement is greatest in the youngest animals studied. In our previous study, with a smaller number of animals, the baseline LCR responses, before the imposition of hyperthermia, did not vary significantly with age. The present data set shows slight, but statistically significant decreases in baseline LCR and apnea durations with increasing age. The reason for the emergence of this finding is not clear: it may reflect the larger number of animals studied or a practice-related increase in the skill with which the experiments were performed. In any case, the age-related response pattern is consistent with previous reports that the LCR is age-related in other species (Abu-Shaweesh, 2004; Boggs and Bartlett, 1982; Lee et al., 1977; Storey and Johnson, 1975; Sutton et al., 1978).

This is the first study of which we are aware that demonstrates that maternal smoke exposure prolongs the LCR in the progeny of the mothers. It is important to emphasize that the effect we observed was obtained with rather modest levels of cigarette smoke exposure. These results raise the question: what component of cigarette smoke is responsible for the effects of maternal smoking on the LCR in neonatal rats? The answer to this question is uncertain, but nicotine must be considered a likely suspect. Nicotine administered to pregnant rats is taken up rapidly by the fetus via the placenta (Jauniaux and Burton, 2007). Moreover, nicotine is concentrated in milk (Dahlström et al., 1990), so some may have reached the pups through nursing in early postnatal life. Nicotine administered directly to neonatal lambs (Sundell et al., 2003) and piglets (Frøen et al., 2000) prolongs and

accentuates the LCR. In more mechanistic terms, stimulation of nicotinic acetylcholine receptors on presynaptic nerve terminals increases GABA release by brainstem neurons (Bertolino et al., 1997). Elevation of GABA or its agonists increases the density and activity of GABA_A receptors in intact brain (Sykes et al., 1984) and cultured cells (Pericic et al., 2003). Thus, as proposed by Luo and associates (Luo et al., 2004), prenatal (or early postnatal) nicotine exposure may lead to up-regulation of brainstem GABA release and/or the density of GABA_A receptors, enhancing the GABAergic inhibition of breathing elicited by the LCR and/or its exaggeration during hyperthermia. Moreover, we and others have already demonstrated that the thermal prolongation of the LCR in piglets depends on GABAergic neuro-transmission (Abu-Shaweesh et al., 2001; Böhm et al., 2007; Xia et al., 2007).

Several previous studies have shown that gestational exposure to cigarette smoke retards fetal growth, resulting in low birth weight in both rats and humans (Bailey and Byrom, 2007; Esposito et al., 2008; Gaworski et al., 2004; Pendlebury et al., 2008). Our data are consistent with this finding and also show some reduction in the rate of postnatal growth in pups of gestationally smoke-exposed dams. The component of cigarette smoke that is chiefly responsible for slowing fetal growth is uncertain. Carbon monoxide (CO) has this action when administered at similar doses on its own, without other cigarette smoke constituents (Carmines and Rajendran, 2008; Tachi and Aoyama, 1983). CO-induced hypoxia is probably responsible, as pregnant rats exposed to reduced oxygen pressures also produce pups with low birth weight (Gleed and Mortola, 1991). The slow rate of postnatal growth in the pups of dams exposed to smoke during pregnancy and in others similarly treated (Tachi and Aoyama, 1990) is probably not attributable directly to CO, which would be cleared by breathing clean air within a few hours of birth (Ayres et al., 1989; Benignus and Annau, 1994). Nicotine has also been shown to retard fetal growth. Cohen and associates (Cohen et al., 2005) found that wild type mice treated during pregnancy with nicotine produced significantly smaller offspring than animals given control infusions. Mutant mice lacking the $\alpha 2$ subunit of the nicotinic acetylcholine receptor also produced smaller pups than wild type controls, but nicotine treatment had no effect on fetal growth in the mutants, indicating that nicotine's effect on fetal growth in the wild type mice required intact receptors.

Several technical limitations must be acknowledged. The smoke exposure of our experimental animals did not accurately mimic that typically experienced by a cigarette smoking human mother or by a non-smoking mother exposed in a home or workplace where other people smoke. The concentration of smoke to which the rats were exposed was in the low range for smokers and in the high range for those exposed only to environmental cigarette smoke (Esposito et al., 2008). More importantly, perhaps, the timing was different from typical human exposures: the rats were exposed for only four hours per day, five days per week and were not exposed at all during the first three days of their 21-day pregnancies.

By necessity the experiments were done under light general anesthesia with agents that are likely to influence the LCR (Lee et al., 1977). This is a clear limitation, but is unlikely to account for the demonstrated effects of smoke exposure as the pups of both exposed and control mothers were treated with exactly the same protocol by an experimenter who did not know the treatment group of each pup. Further, the results are unlikely to be due to the inverse relationship between laryngeal reflex apnea and respiratory drive (Litmanovitz et al., 1994): respiratory frequency—the index of drive in these studies—was increased by hyperthermia in both treatment groups, but did not differ significantly between the two groups, and the trend toward higher frequency in the smoke-exposed group would actually favor a reduction in LCR duration rather than the observed increase.

A final technical problem is the unavoidable confounding of treatments with litters in the study design. This problem has received considerable attention (Holson and Pearce, 1992; Hughes, 1979), but has no fully satisfactory solution. Each of our litters from a smoke-exposed dam was studied concomitantly with a control litter, and in each case the basic findings that we attribute to maternal smoke exposure were apparent. Moreover, the variation of the results among litters within each treatment group was less than the variation between treatment groups. We performed a post-hoc analysis of variance in which litter was a nested variable within each treatment group (maternal smoke exposure versus control), and this analysis did not reveal any litter effect in either treatment group.

In summary, the study indicates that intermittent exposure of pregnant rats to cigarette smoke exaggerates the hyperthermic enhancement of the LCR in their pups for several days after delivery. This finding may be significant for the pathogenesis of SIDS in human infants. The risk of SIDS is increased in overheated environments, increased when infants are covered, especially the head, with insulating blankets and increased in infants whose mothers have smoked during pregnancy (Anderson and Cook, 1997; Blair et al., 1996; Blair et al., 2008; Fleming et al., 1996; Guntheroth and Spiers, 2001; Mitchell et al., 1992; Stanton, 1984). Thus, multiple risk factors for SIDS may conspire to prolong reflex apneas in neonates at risk for SIDS. Of the many constituents of cigarette smoke, nicotine seems most likely to be responsible in view of its effects on GABAergic processes (Luo et al., 2004), its effects on the LCR when administered directly to neonatal lambs (Sundell et al., 2003) and piglets (Frøen et al., 2000), and the GABAergic influences on the LCR and its enhancement by hyperthermia (Abu-Shaweesh et al., 2001; Böhm et al., 2007; Xia et al., 2007).

Acknowledgments

This research was supported in part by Grant P01-HD36379 from the National Institute of Child Health and Human Development and by grants to M. A. C.-G. and D. B. from the Flight Attendants Medical Research Institute.

References

- Abu-Shaweesh JM. Maturation of respiratory reflex responses in the fetus and neonate. *Semin Neonatol.* 2004; 9:169–180. [PubMed: 15050210]
- Abu-Shaweesh JM, Dreshaj IA, Haxhiu MA, Martin RJ. Central GABAergic mechanisms are involved in apnea induced by SLN stimulation in piglets. *J Appl Physiol.* 2001; 90:1570–1576. [PubMed: 11247962]
- Anderson HR, Cook DG. Passive smoking and sudden infant death syndrome: review of the epidemiological evidence. *Thorax.* 1997; 52:1003–1009. [PubMed: 9487351]
- Anderson ME, Johnson DC, Batal HA. Sudden infant death syndrome and prenatal maternal smoking: rising attributed risk in the back to sleep era. *BMC Med.* 2005; 11:3–4.
- Ayres PH, Mosberg AT, Burger GT, Hayes AW, Sagartz JW, Coggins CR. Nose-only exposure of rats to carbon monoxide. *Inhal Toxicol.* 1989; 1:349–363.
- Bailey BA, Byrom AR. Factors predicting birth weight in a low-risk sample: the role of modifiable pregnancy health behaviors. *Matern Child Health J.* 2007; 11:173–179. [PubMed: 17091398]
- Benignus VA, Annau Z. Carboxyhemoglobin formation due to carbon monoxide exposure in rats. *Toxicol Appl Pharmacol.* 1994; 128:151–157. [PubMed: 8079348]
- Bertolino M, Keller KJ, Vicini S, Gillis RA. Nicotinic receptor mediates spontaneous GABA release in the rat dorsal motor nucleus of the vagus. *Neuroscience.* 1997; 79:671–681. [PubMed: 9219932]
- Blair PS, Fleming PJ, Bensley D, Smith I, Bacon C, Taylor E, Berry J, Golding G, Tripp J. Smoking and the sudden infant death syndrome: results from 1993–5 case-control study for confidential inquiry into still births and deaths in infancy. *BMJ.* 1996; 313:195–198. [PubMed: 8696194]
- Blair PS, Mitchell EA, Heckstall-Smith EMA, Fleming PJ. Head covering—a major modifiable risk factor for Sudden Infant Death Syndrome: a systematic review. *Arch Dis Child.* 2008

- Boggs DF, Bartlett D Jr. Chemical specificity of a laryngeal apneic reflex in puppies. *J Appl Physiol.* 1982; 53:455–462. [PubMed: 7118665]
- Böhm I, Xia L, Leiter JC, Bartlett D Jr. GABAergic processes mediate thermal prolongation of laryngeal reflex apnea in decerebrate piglets. *Respir Physiol Neurobiol.* 2007; 156:229–233. [PubMed: 17137847]
- Carmines EL, Rajendran N. Evidence for carbon monoxide as the major factor contributing to lower fetal weights in rats exposed to cigarette smoke. *Toxicol Sci.* 2008; 102:383–391. [PubMed: 18203688]
- Cohen G, Roux JC, Grailhe R, Malcolm G, Changeux JP, Lagercrantz H. Perinatal exposure to nicotine causes deficits associated with a loss of nicotinic receptor function. *Proc Natl Acad Sci.* 2005; 102:3817–3821. [PubMed: 15738419]
- Curran AK, Xia L, Leiter JC, Bartlett D Jr. Elevated body temperature enhances the laryngeal chemoreflex in decerebrate piglets. *J Appl Physiol.* 2005; 98:780–786. [PubMed: 15542573]
- Dahlström A, Lundell B, Curvall M, Thapper L. Nicotine and cotinine concentrations in the nursing mother and her infant. *Acta Paediatr Scand.* 1990; 79:142–147. [PubMed: 2321475]
- Downing SE, Lee JC. Laryngeal chemosensitivity: a possible mechanism of sudden infant death. *Pediatrics.* 1975; 55:640–649. [PubMed: 236536]
- Espósito ER, Horn KH, Greene RM, Pisano MM. An animal model of cigarette smoke-induced *in utero* growth retardation. *Toxicology.* 2008; 246:193–202. [PubMed: 18316152]
- Fleming PJ, Blair PS, Bacon C, Bensley D, Smith I, Taylor EW, Berry J, Golding J, Tripp J. Environment of infants during sleep and risk of the sudden infant death syndrome: results of 1993–5 case-control study for confidential inquiry into stillbirths and deaths in infancy. *BMJ.* 1996; 313:191–195. [PubMed: 8696193]
- Frøen JF, Akre H, Stray-Pedersen B, Saugstad OD. Adverse effects of nicotine and interleukin-1 β on autoresuscitation after apnea in piglets: implications for Sudden Infant Death Syndrome. *Pediatrics.* 2000; 105:E52. [PubMed: 10742373]
- Gaworski CL, Carmines EL, Faqi AS, Rajendran N. *In utero* and lactation exposure in rats to 1r4f reference cigarette mainstream smoke: effect on prenatal and postnatal growth. *Toxicol Sci.* 2004; 79:157–169. [PubMed: 14976347]
- Gleed RD, Mortola JP. Ventilation in newborn rats after gestation at simulated high altitude. *J Appl Physiol.* 1991; 70:1146–1151. [PubMed: 2032980]
- Guntheroth WG, Kawabori I. Hypoxic apnea and gasping. *J Clin Invest.* 1975; 56:1371–1377. [PubMed: 811688]
- Guntheroth WG, Spiers PS. Thermal stress in Sudden Infant Death: is there an ambiguity with the rebreathing hypothesis? *Pediatrics.* 2001; 107:693–698. [PubMed: 11335746]
- Haraguchi S, Fung RQ, Sasaki R. Effect of hyperthermia on the laryngeal closure reflex. Implications in the Sudden Infant Death Syndrome. *Ann Otol Rhinol Laryngol.* 1983; 92:24–28. [PubMed: 6824275]
- Heron MP. Deaths: leading causes for 2004. *Stat Rep.* 2007; 56:1–95.
- Holson RR, Pearce B. Principles and pitfalls in the analysis of prenatal treatment effects in multiparous species. *Neurotoxicol Teratol.* 1992; 14:221–228. [PubMed: 1635542]
- Hughes CW. Outcome of early experience studies as affected by between-litter variance. *J Nutr.* 1979; 109:642–645. [PubMed: 430265]
- Jauniaux E, Burton GJ. Morphological and biological effects of maternal exposure to tobacco smoke on the feto-placental unit. *Early Hum Dev.* 2007; 83:699–706. [PubMed: 17900829]
- Lee JC, Stoll BJ, Downing SE. Properties of the laryngeal chemoreflex in neonatal piglets. *Am J Physiol.* 1977; 233:R30–R36. [PubMed: 18025]
- Leiter JC, Böhm I. Mechanisms of pathogenesis in the Sudden Infant Death Syndrome (SIDS). *Respir Physiol Neurobiol.* 2007; 159:127–138. [PubMed: 17644048]
- Litmanovitz I, Dreshaj I, Miller MJ, Haxhiu MA, Martin RJ. Central chemosensitivity affects respiratory muscle responses to laryngeal stimulation in the piglet. *J Appl Physiol.* 1994; 76:403–408. [PubMed: 8175537]

- Luo Z, Costy-Bennett S, Fregosi RF. Prenatal nicotine exposure increases the strength of GABAA receptor-mediated inhibition of respiratory rhythm in neonates. *J Physiol (Lond)*. 2004; 561.2:387–393. [PubMed: 15513949]
- Mitchell EA, Ford RPK, Stewart AK, Taylor BJ, Becroft DMO, Thompson JMD, Scragg R, Hassall MB, Barry DMJ, Allen EM, et al. Smoking and the Sudden Infant Death Syndrome. *Pediatrics*. 1993; 91:893–896. [PubMed: 8474808]
- Mitchell EA, Taylor BJ, Ford RP, Stewart AW, Becroft DM, Thompson JM, Scragg R, Hassall IB, Barry DM, Allen EM. Four modifiable and other major risk factors for cot death: the New Zealand study. *J Paediatr Child Health*. 1992; 28 (Suppl 1):S3–S8. [PubMed: 1524879]
- Page M, Jeffery HE, Post EJ, Wood AKW. Simulated pharyngeal reflux can lead to life-threatening apnea if swallowing and arousal are depressed. *J Sudden Infant Death Syndr Infant Mortal*. 1996; 1:281–294.
- Paterson DS, Trachtenberg FL, Thompson EG, Belliveau RA, Beggs AH, Darnall RA, Chadwick AE, Krous HF, Kinney HC. Multiple serotonergic brainstem abnormalities in the sudden infant death syndrome. *JAMA*. 2006; 296:2124–2132. [PubMed: 17077377]
- Pendlebury JD, Wilson RJA, Bano S, Lumb KJ, Schneider JM, Hasan SU. Respiratory control in neonatal rats exposed to prenatal cigarette smoke. *Am J Respir Crit Care Med*. 2008; 177:1255–1261. [PubMed: 18310476]
- Pericic D, Strac DS, Jembrek MJ, Rajcan I. Prolonged exposure to gamma-aminobutyric acid up-regulated stably expressed recombinant alpha 1 beta 2 gamma 2 s GABAA receptors. *Eur J Pharmacol*. 2003; 482:117–125. [PubMed: 14660012]
- Pickens DL, Schefft G, Thach BT. Prolonged apnea associated with upper airway protective reflexes in apnea of prematurity. *Am Rev Respir Dis*. 1988; 137:113–118. [PubMed: 3337450]
- Schmidt I, Barone A, Carlisle HJ. Diurnal cycle of core temperature in huddling, week-old rat pups. *Physiol Behav*. 1986; 37:105–109. [PubMed: 3737706]
- St Hilaire M, Samson N, Nsegbe E, Duvareille C, Moreau-Bussière F, Micheau P, Lebon J, Praud JP. Postnatal maturation of laryngeal chemoreflexes in the preterm lamb. *J Appl Physiol*. 2007; 102:1429–1438. [PubMed: 17170207]
- Stanton AN. Overheating and cot death. *Lancet*. 1984; 2:1199–1201. [PubMed: 6150244]
- Storey AT, Johnson P. Laryngeal water receptors initiating apnea in the lamb. *Exp Neurol*. 1975; 47:42–55. [PubMed: 1123009]
- Sundell HW, Karmo H, Milerad J. Impaired cardiorespiratory recovery after laryngeal stimulation in nicotine-exposed young lambs. *Pediatr Res*. 2003; 53:104–112. [PubMed: 12508088]
- Sutton D, Taylor EM, Lindeman RC. Prolonged apnea in infant monkeys resulting from stimulation of superior laryngeal nerve. *Pediatrics*. 1978; 61:519–527. [PubMed: 96414]
- Sykes C, Prestwich S, Horton R. Chronic administration of the GABA-transaminase inhibitor ethanolamine O-sulfate leads to up-regulation of GABA binding sites. *Biochem Pharmacol*. 1984; 33:387–393. [PubMed: 6322803]
- Tachi N, Aoyama M. Effect of cigarette smoke and carbon monoxide inhalation by gravid rats on the conceptus weight. *Bull Environ Contamin Toxicol*. 1983; 31:85–92.
- Tachi N, Aoyama M. Postnatal growth in rats prenatally exposed to cigarette smoke or carbon monoxide. *Bull Environ Contamin Toxicol*. 1990; 45:641–648.
- Thach BT. The brainstem and vulnerability to sudden infant death syndrome. *Neurology*. 2003; 61:1170–1171. [PubMed: 14610114]
- Thach BT. Some aspects of clinical relevance in the maturation of respiratory control in infants. *J Appl Physiol*. 2008; 104:1828–1834. [PubMed: 18420716]
- van der Velde L, Curran A, Filiano JJ, Darnall RA, Bartlett D Jr, Leiter JC. Prolongation of the laryngeal chemoreflex after inhibition of the rostroventral medulla in piglets: a role in SIDS? *J Appl Physiol*. 2003; 94:1883–1895. [PubMed: 12679348]
- Xia L, Bartlett D Jr, Leiter JC. An adenosine A_{2A} antagonist injected in the NST reverses thermal prolongation of the LCR in decerebrate piglets. *Respir Physiol Neurobiol*. 2008a; 164:358–365. [PubMed: 18775519]

- Xia L, Damon T, Niblock MM, Bartlett D Jr, Leiter JC. Unilateral micro-dialysis of gabazine in the dorsal medulla reverses thermal prolongation of the laryngeal chemoreflex in decerebrate piglets. *J Appl Physiol.* 2007; 103:1864–1872. [PubMed: 17823299]
- Xia L, Damon TA, Leiter JC, Bartlett D Jr. Focal warming of the nucleus of the solitary tract prolongs the laryngeal chemoreflex in decerebrate piglets. *J Appl Physiol.* 2006; 102:54–62. [PubMed: 16959905]
- Xia L, Leiter JC, Bartlett D Jr. Laryngeal apnea in rat pups: effects of age and body temperature. *J Appl Physiol.* 2008b; 104:269–274. [PubMed: 17962578]
- Yu M, Pinkerton KE, Witschi H. Short-term exposure to aged and diluted sidestream cigarette smoke enhances ozone-induced lung injury in B6C3F1 mice. *Toxicol Sci.* 2002; 65:99–106. [PubMed: 11752689]

Fig. 1.

Examples of the LCR from female P6 rat pups during baseline conditions (top panels), during mild hyperthermia (middle panels) and during a final, recovery period (bottom panels). The data displayed on the left were obtained from a rat pup born to a dam exposed to clean air while pregnant, and the data displayed on the right were obtained from a rat pup born to a dam exposed to cigarette smoke during pregnancy. The downward arrows indicate the time at which small volumes of water were injected into the larynx, and the body temperature (BT) is listed to the right of each example. Note the prolonged apnea and respiratory disruption during hyperthermia compared to normothermic conditions, and also note that maternal exposure to cigarette smoke markedly prolonged the LCR in the rat pup shown on the right.

Fig. 2.

Prolongation of the LCR (upper panel) and the longest associated apnea (lower panel) by hyperthermia as a function of age in pups of dams exposed during gestation to cigarette smoke (●) and controls (○). Each point indicates the ratio of the average of hyperthermic tests to the average of control and recovery tests for one animal. The regression lines for exposed (solid lines) and control animals (dashed lines) are significantly different in each panel, indicating that the hyperthermic prolongation of the LCR and the longest associated apnea are both exaggerated in the younger animals by maternal exposure to cigarette smoke during pregnancy.

Table 1

Average body temperature, respiratory frequency, LCR duration and longest apnea duration in control and smoke-exposed rat pups.

	Baseline	Hyperthermia	Recovery
Control (<i>n</i> = 29)			
Body temp. (°C)	35.47 ± 0.02	38.25 ± 0.04 [†]	35.48 ± 0.03ns
f (breaths/min)	75.3 ± 4.5	82.1 ± 4.9 [†]	66.7 ± 5.2ns
LCR (s)	4.00 ± 0.73	6.42 ± 1.38 [*]	3.78 ± 0.63ns
Longest apnea (s)	2.81 ± 0.69	3.50 ± 0.88 [†]	2.10 ± 0.22ns
Smoke-exposed (<i>n</i> = 33)			
Body temp. (°C)	35.50 ± 0.02	38.16 ± 0.04 [†]	35.43 ± 0.03ns
f (breaths/min)	78.0 ± 5.3	91.6 ± 5.3 [†]	73.5 ± 4.3ns
LCR (s)	3.64 ± 0.42	8.94 ± 1.28 ^{*,**}	2.89 ± 0.31ns
Longest apnea (s)	2.39 ± 0.29	3.95 ± 0.54 ^{†,***}	2.24 ± 0.23ns

Values are means ± SE; *n* = number of animals; ns = not significantly different from baseline value.

^{*} Significantly different from baseline value within each smoking condition (*P* < 0.05).

^{**} Indicates that the difference between hyperthermic and baseline values in the exposed animals was significantly different from the corresponding difference in the control animals (*P* < 0.05).

[†] A significant main effect of hyperthermia (*P* < 0.05).