THE UNIVERSITY OF CALGARY

Effect of Perinatal Exposure to Cocaine on Protective Responses that Newborn and Older Rats Exhibit during Exposure to Hypoxia

by

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A THESIS

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ABSTRACT

Failure to recover from apnea has been suggested to play a role in sudden infant death. Furthermore, perinatal exposure to cocaine is a risk factor for sudden infant death. I, therefore, carried out experiments to investigate the influence of perinatal exposure to cocaine on protective responses that newborn and older rats exhibit during exposure to hypoxia as may occur during prolonged apnea. Pregnant rats received either cocaine HCL (20, 50, or 100 mg/kg/day) or control solution continuously from day 6 or 7 of gestation via an osmotic minipump implanted subcutaneously. On postnatal days 1-2, 5-6, and 10-11, pups were exposed either to a single period of hypoxia (97% N_2 & 3% CO_2) and the time to last gasp determined, or they were exposed repeatedly to hypoxia and their ability to autoresuscitate from primary apnea determined. Perinatal exposure to cocaine did not alter the time to last gasp. It did, however, impair the ability of rat pups to autoresuscitate from primary apnea during repeated exposures to hypoxia in an age- and dose- dependent manner. Thus, perinatal exposure to cocaine may place infants at risk for sudden infant death by impairing protective responses to hypoxia as may occur during prolonged apnea.

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1.0 INTRODUCTION

1.1 Pharmacological Effects and Metabolism of Cocaine

Classified as a central nervous system stimulant, cocaine is a lipophilic tertiary amine, with a chemical structure that is comprised of an ester of benzoic acid and the complex alcohol 2-carbomethoxy, 3-hydroxy-tropane (or methylecgonine). Cocaine can be hydrolyzed non-enzymatically to the active water-soluble metabolite benzoylecgonine and to ecgonine methyl ester by liver and plasma cholinesterases. Norcocaine is another metabolite of the oxidation of cocaine by mixed functional oxidases in the liver. Cocaine itself is excreted via the kidney into urine and via bile into the gastrointestinal tract at 1-5% of an administrated dose; 85-90% of the administrated dose is excreted via the kidney into urine and the rest via bile into the gastrointestinal tract. But cocaine itself only accounts for 1-5%, and the rest is mainly its metabolites.

Cocaine has many pharmacological effects. It can affect nerve function directly in at least two different ways: blockade of the re-uptake of certain neurotransmitters and alteration of voltage-gated sodium channels (1). By blocking the presynaptic re-uptake of dopamine in the central nervous system, cocaine produces a neurochemical magnification of the pleasure response and creates a heightened sense of power, euphoria, and sexual excitement. The metabolites of cocaine have a higher stability and a longer half-life than cocaine and can complex Ca^{2+} that affects calcium-regulated events like neurotransmitter release (2). Cocaine also stimulates dopamine synthesis and

causes an upregulation of postsynaptic dopamine receptors. With chronic use, cocaine depletes dopamine in the central nervous system, which is thought to cause depressive symptoms. Cocaine blocks the re-uptake of norepinephrine at the presynaptic terminals, increases norepinephrine synthesis, and upregulates norepinephrine receptors on the postsynaptic nerves.

Evidence has also been provided that cocaine may induce changes in human fetal neurotransmitter systems. These changes among infants with *in utero* cocaine exposure were suggested by a study showing that blood levels of the norepinephrine precursor dihydroxyphenylalanine were higher in cocaine-exposed than in unexposed newborns (3). Among the cocaine-exposed newborns, high norepinephrine concentrations in blood were associated with poor responsivity to auditory and visual stimuli. Needlman et al. (4) measured neurotransmitter precursors and metabolites in the cerebral spinal fluid of 10 cocaine-exposed and 21 unexposed neonates undergoing spinal taps for a variety of clinical indications and showed that cocaine-exposed infants had significantly lower levels of cerebrospinal fluid homovanillic acid, the principal metabolite of dopamine when compared with unexposed infants.

Slotkin et al. have provided evidence that cocaine produces neurobehavioral damage in the fetus and neonate both through its ischemic actions and through direct effects mediated by the drug within the developing brain. In their studies, newborn and older rats (1, 7, 14 and 21 days old) were given cocaine (30 mg/kg) acutely and the turnover of norepinephrine and dopamine, a measure of

synaptic activity, was evaluated in vivo in three different brain regions known to be affected by cocaine (i.e., forebrain, midbrain and brainstem). For norepinephrine, cocaine suppressed transmitter turnover in the immediate postnatal period in all regions, reaching a maximal effect within the first 2 postnatal weeks; at subsequent ages, the inhibitory actions were no longer evident. For dopamine, an inhibitory effect also appeared during the first postnatal week, but by 14 to 21 days the effect was replaced by the excitatory response that is characteristic of mature brain; effects on dopamine turnover were restricted to the forebrain. They speculated that the inhibitory effects of cocaine on the immature brain could not be attributed to localized actions at the nerve terminal itself (blockade of re-uptake, autoreceptor activation, local anesthesia), but instead may represent reductions in nerve impulse activity (5). They also found that acute cocaine pretreatment of rats induces the activity of ornithine decarboxylase (ODC) and increases protein synthesis during hypoxia, a characteristic known to reflect heat shock protein formation, a harbinger of cell injury and death (6).

1.2 Cocaine Abuse and Its Distribution

Cocaine is typically administered by human abusers in one of two forms: the alkaloidal (free base) form with the street name of 'crack' cocaine, and the hydrochloride (HCl) powder. Crack cocaine is suitable for smoking, but the HCl form is administered by the nasal or intravenous route (7). Human cocaine abusers can be categorized into two groups: the intermittent recreational user

and the chronic continuous 'binging' abuser. Both types of abusers typically engage in a binge pattern of drug consumption but are distinguishable by the duration of the binge. The 'binge' pattern of drug consumption is characterized by the frequent readministration of the drug so that a sustained plasma level is attained over a prolonged period. These binges can last for hours or days, and are followed by a triphasic withdrawal syndrome (7, 8, 9).

It is estimated that 10% of pregnant women in the United States use cocaine (10). Although the pharmacokinetics of cocaine show high variability with the route and temporal pattern of administration, it is clear that cocaine and several of its active metabolites penetrate the fetal-placental barrier without metabolic conversion and accumulate in significant concentrations in both the fetal plasma, brain, and other tissues (11-14). In addition, cocaine crosses readily into the breast milk, with the levels in breast milk being 7 times that observed in maternal plasma (15, 16). Several researchers have also described that pregnancy alters the pharmacokinetic properties of cocaine (11, 17). Spear et al. (12) have measured rat fetal and maternal brain and plasma levels of cocaine and benzoylecgonine following chronic subcutaneous administration of 10, 20, and 40 mg/kg/day cocaine HCl from days 8 to 20 of the gestational period. They reported dose-related increases in plasma and brain levels of cocaine in dams and fetuses. However, fetal concentrations of cocaine in the brain and plasma were approximately 2-3 fold less than those of dams, suggesting that the placenta may partially restrict passage of cocaine into the

fetal circulation. Shah et al. (11) observed similar findings in pregnant mice who received acute intraperitoneal injections of cocaine. Although there are numerous pharmacokinetic mechanisms (e.g., physicochemical properties such as protein binding, maternal versus fetal plasma pH, molecular weight of cocaine, etc.) which could act on the fetal-placental barrier to partially account for the restricted passage of cocaine across the placenta, cocaine may in fact be directly acting to decrease its own access to the fetus by causing vasoconstriction in the placenta (11). Spear et al. (12) also reported greater concentrations of benzoylecgonine in fetal brain than was observed in the maternal brain of the rat and it was proposed that this might be a result of the immaturity or limited integrity of the blood-brain barrier in the fetus.

Shah et al. (11) demonstrated that pregnancy alters the pharmacokinetics of cocaine in mice. Tritiated levo-cocaine was administrated with an acute introperitoneal injection to pregnant and nonpregnant mice, and accumulation of the drug at 15 minutes post-injection (time of peak concentration in all tissues) was observed to be highest in the uterus followed by the placenta > spleen > kidney > fat > liver > lung > brain > spinal cord > heart > muscle > eye > plasma of the pregnant mice. In fact, the uteri of the pregnant mice accumulated a mean concentration that was approximately four times higher than the nonpregnant mice. Pregnant mice also had higher cocaine concentrations in the brain, spinal cord, and heart than non-pregnant cohorts. Shah proposed that the variation in distribution of cocaine could be a result of altered hormonal

balance in pregnancy that may cause increased tissue accumulation and/or decreased breakdown of the drug. From their study, they also reported that excretion of cocaine and its metabolites was higher in the urine of non-pregnant mice than their pregnant cohorts within the first hour post-injection. This was likely a reflection of the observed increased tissue:plasma accumulation, which generally slows excretion of the drug, and/or because the activity of plasma esterases is lower in the pregnant female.

1.3 Cocaine Abuse and SIDS

Over the last decade, a number of epidemiological studies have suggested a strong link between in utero substance abuse and the occurrence of sudden infant death (18-21). The sudden infant death syndrome (SIDS), was redefined in 1989 by a National Institute of Child Health and Human Development panel of experts as: "the sudden death of an infant under one year of age which remains unexplained after a thorough case investigation, including performance of a complete autopsy, examination of the death scene, and review of the clinical history" (22). Research over the past 25 years has emphasized that not all SIDS victims are entirely normal prior to death (23, 24). SIDS is thought to occur during sleep or during the transition from sleep to wakefulness, and population studies have shown a typical pattern of age at death throughout the world. The majority of deaths occur between 4 and 20 weeks, with a peak incidence in the third month. This time frame of SIDS coincides with rapid growth of the brain, as well as dramatic changes in cardiovascular and

respiratory control. Maturation of sleep-states also occurs which includes a decline in active sleep, and development of a circadian variation in the sleep-wake cycle during this period of time.

Many life-threatening events such as cardiac arrhythmia, obstructive apnea, and hypoglycemia have been the focus of previous SIDS research (25). Whatever the mechanism, it now seems obvious that there is no single cause of SIDS. Those attributing causes to obstructive apnea seem to be the most plausible among the hypothesis for the cause of SIDS. Postmortem studies of SIDS victims have revealed relatively consistent findings of chronic or recurrent hypoxia including increased extramedullary erythropoisis (26); increased periadrenal brown fat cells (27, 28); astroglial proliferation in the brainstem (29-33); elevated vitreous humor hypoxanthine levels (34) and intrathoracic petechiae hemorrhages distributed on heart, lungs and thymus (35, 36). These latter intrathoracic petechiae are prominent and characteristic as diagnostic of suffocation in the majority of SIDS victims and its special distribution suggests that obstructive apnea occurs as breathing against a closed airway can produce marked increases in intrathoracic negative pressure and microvascular rupture (37).

Because clinical data gathered over the last two decades suggest that a large percentage of infants in North America have been exposed to cocaine throughout gestation, and that the rate of SIDS in these infants is higher than that of the unexposed population, further studies have been performed in an

attempt to determine both the short-term and long-term consequences of *in utero* cocaine exposure (38-44). Structural and physiologic deficits in human neonates have been associated with *in utero* exposure to cocaine. Relative to cohort-matched control, Rosen et al. (18) reported that prenatal cocaine produces several cardiovascular abnormalities including congenital malformations, eletrocardiographic abnormalities, high-grade ventricular ectopia and cardiorespiratory arrest. Several cardiorespiratory abnormalities have also been reported (45-47). For example, full-term infants exposed to cocaine exhibited significantly longer apneas and increased incidences of bradycardia compared with full-term controls (40). Such cocaine-exposed infants have shown smaller decrements in carbon dioxide tension in response to hypoxia (47). Suguihara et al. (48) observed that newborn piglets exposed to cocaine *in utero* have a depressed ventilatory response to hypoxia. Although the underlying physiological mechanisms of prenatal cocaine exposure on the ventilatory response in the neonate remain unclear, a number of possible factors may be involved. Cocaine's effects on respiration may be related to the drugs alterations on total body oxygen consumption, teratogenic effects during the development of central respiratory control centers, alterations of dopamine levels which in turn affect central dopaminergic pathways involved in respiratory control, defective peripheral respiratory control secondary to increased catecholamine levels, the presence of active metabolites of cocaine, or even the symptoms which are characteristic of cocaine's withdrawal syndrome, as

well as a number of other possible factors. There is also evidence that acute exposure to cocaine causes adverse effects on the cardiovascular system. Woods et al. (49) administered three different doses of cocaine by intravenous bolus during late gestation in the pregnant ewe and subsequently measured the cardiovascular effects in the ewe and fetus separately, as well as blood gases to determine fetal oxygenation. They observed a significant and dose-dependent increase in maternal mean arterial pressure during the first 15 minutes after cocaine injection, and a significant and dose-dependent decrease in total uterine blood flow. Their study also showed significant reductions of oxygen content of the fetus, and fetal heart rate increased significantly at 10 to 15 minutes post-injection. Fetal mean arterial pressure also increased significantly from baseline values during the first five minutes post-injection. Since it is known that certain stresses such as hypoxic challenge in the neonate induce catecholamine release, it is likely that cocaine's effect on the blockade of re-uptake of catecholamines is exacerbated by hypoxia (50). These cardiovascular malformations and cardiorespiratory events are likely to negatively affect the prognosis of the cocaine-exposed neonate and may increase its risk for sudden death.

From the above, it is apparent that a relationship exists between prenatal cocaine exposure and disrupted postnatal cardiorespiratory control. If perinatal exposure to cocaine also impairs the protective responses that newborn rats exhibit during exposure to hypoxia, as may occur during prolonged apnea, an

adverse outcome (i.e., death) may occur. In humans, spontaneous recovery from obstructive sleep apnea can occur early as a result of arousal from sleep or later as a result of hypoxic gasping known as "autoresuscitation" (51, 52). A failure of these protective mechanisms during obstructive apnea will lead to severe hypoxia and death. The arousal response from sleep is important since it is a potent stimulus for reestablishment of upper airway patency and to breathing during obstructive apnea which results from the loss of upper airway muscle tone during sleep (53, 54). Arousal also permits the initiation of an appropriate behavioral response such as head turning which is particularly important for resolution of obstructive apnea secondary to sleeping in the prone position. Some infants at risk for SIDS have an abnormal arousal response to respiratory stimuli (55, 56).

Experiments done by Fewell et al. on young lambs (57-60) as well as the experiments of others on newborn lambs (61), newborn calves (62), adult dogs (63, 64) and adult cats have provided evidence that a number of cardiorespiratory stimuli are capable of causing arousal from sleep. These include an acute increase in blood pressure, upper airway obstruction, rapidly developing hypoxemia alone, and rapidly developing hypercapnia alone. Active sleep can delay the occurrence of arousal in response to these stimuli compared to quiet sleep. They have also found that repeated upper airway obstruction (65) or rapidly developing hypoxemia alone (66), over 21 to 34 hours (~100 epochs of sleep), produces an arousal response decrement.

These observations provide evidence that a life sustaining mechanism (i.e., arousal from sleep) becomes diminished to, rather than sensitized to, repeated exposure to a life-threatening stimulus (e.g., upper airway obstruction). The crucial factor is whether or not arousal from sleep and resumption of tidal ventilation occurs before hypoxic cerebral depression since hypoxic cerebral depression will lead to death unless gasping produces autoresuscitation. The respiratory response of both newborn (67, 68) and adult (69) animals to progressive hypoxemia typically passes through 4 stages: hyperpnea, primary apnea, gasping, and terminal apnea. Severe hypoxemia ultimately results in cerebral cortical depression as evidenced by electroencephalographic depression and "decerebrate seizures" (70, 71). During primary apnea, an infant can easily be resuscitated by medical personnel, or "autoresuscitated" by gasping if an adequate circulation has been maintained. The ability of the myocardium to function during hypoxia is important since the increased oxygen by gasping has to be transported via the circulation to vital centers such as the heart and brain. It has been shown that the persistence of a stable circulation during hypoxia has direct relation to the pre-hypoxia glycogen concentration in the cardiac ventricles (72, 73). Guntheroth (74) suggests that in the older infant, the resistance to hypoxia is less, reflecting the diminished stores of cardiac glycogen and, therefore, limited substrate for anaerobic metabolism. When the gasp occurs in the older infant or adult, it occurs with inadequate circulation and the oxygen is not transported to the vital structures.

1.4 Aim and Hypothesis

It follows from the foregoing discussion that any impairment of the protective responses to hypoxia produced by cocaine could have very sinister effects (i.e., "autoresuscitation failure" and death). My experiments were carried out to investigate the effect of perinatal exposure to cocaine on time to last gasp and ability to autoresuscitate during hypoxia in three different age groups of newborn and older rats. I tested the hypothesis that perinatal exposure to cocaine impairs the protective responses that newborn and older rats exhibit during exposure to hypoxia as may occur during episodes of prolonged apnea.

2.0 MATERIALS AND METHOD

2.1 Animals

Experiments were carried out on 177, 1 to 2, 5 to 6 and 10 to 11 day old Sprague-Dawley rats. Each pup, born by spontaneous vaginal delivery, was housed in plastic cages in the Animal Resource Center with its mother at an ambient temperature of ~22°C, with a 12:12 hour light / dark cycle. Litter size ranges from 6 to 15 pups.

2.2 Ethical Considerations

All surgical and experimental procedures were carried out in accordance with the "Guide to the Care and Use of Experimental Animals" provided by the Canadian Council on Animal Care, and with the approval of the Animal Care Committee of the University of Calgary.

2.3 Drugs and Dosage

Cocaine HCl (BDH Inc., Toronto, ON) was dissolved in sterile isotonic saline. Doses of 0, 20, 50 and 100 mg/kg/day were based on a mean term pregnant weight of 330 grams. Doses of 20 or 50 mg/kg/day produce maternal rat serum levels of cocaine that are comparable to those measured in human beings described as a moderate to high-dose range (12).

2.4 Minipump Preparation and Surgery

Osmotic minipumps (model 2ML4 Alza Corporation Palo Alto, CA) were filled with either 2 mL of 110 mg/mL, 2 mL of 275 mg/mL, 2 mL of 550 mg/mL cocaine HCL or 2 mL of sterile saline before their implantation. Surgery was

performed on gestational day 6 or 7. Each pregnant rat was anaesthetized by inhalation of halothane (~2.0% for induction and maintenance) in oxygen and was placed in a prone position. A small incision was made over the scapulae and a subcutaneous pocket was formed with a hemostat between the two scapulae of the rat. A filled osmotic minipump was then inserted into the pocket with the delivery port facing toward the trunk for continuous infusion of cocaine or vehicle. The pumps deliver solution at a rate of 2.5 μL/h for a 28 days period. The incision was closed with sutures, and prophylactic antibiotic treatment applied (Gentacin).

2.5 Experimental Protocols

2.5.1 Time to Last Gasp Experiments

The first experiment was performed to determine the time to last gasp during a single exposure to hypoxia. Experiments were carried out on 94 rat pups (cocaine 0 mg/kg/day, 1-2 day old n=6, 5-6 day old n=11, 10-11 day old n=8; cocaine 20 mg/kg/day, 1-2 day old n=9, 5-6 day old n=6, 10-11 day old n=10; cocaine 50 mg/kg/day, 1-2 day old n=7, 5-6 day old n=8, 10-11 day old n=7; cocaine 100 mg/kg/day, 1-2 day old n=8, 5-6 day old n=7, 10-11 day old n=7). For an experiment, each pup was removed from its lactating mother and siblings and weighed. Immediately afterward, two ECG surface electrodes were attached: one located between the scapulae, and the other located on the right side, just proximal to the hindleg. A 35-40 mm mercury strain gauge (DM Davis Inc. New York, NY) was then placed around the circumference of the

neonate just distal to the front legs to record respiration. Then the pup was placed into a metabolic chamber regulated at 37°C into which flowed room air at a rate of one liter per minute. At the end of a 30-minute stabilization period, the gas which flowed into the chamber was changed from room air to 97% N_2 and 3% CO_2 at a flow rate of 5 L/min until the gas concentrations in the chamber stabilized; then the flow rate was reduced and maintained at one liter per minute.

2.5.2 Autoresuscitation Experiments

The second experiment was performed to assess the ability of the rat pups to autoresuscitate from primary apnea during repeated episodes of hypoxia. A total of 83 rat pups were used in these experiments (cocaine 0 mg/kg/day, 1-2 day old n=4, 5-6 day old n=6, 10-11 day old n=7; cocaine 20 mg/kg/day, 1-2 day old n=6, 5-6 day old n=11, 10-11 day old n=8; cocaine 50 mg/kg/day, 1-2 day old n=5, 5-6 day old n=8, 10-11 day old n=8; cocaine 100 mg/kg/day, 1-2 day old n=5, 5-6 day old n=8, 10-11 day old n=7). For an experiment, each pup was removed from its lactating mother and siblings, weighed, and placed into a metabolic chamber regulated at 37°C into which flowed room air at a rate of 1 L/min. Two ECG electrodes and a strain gauge were attached as described above. At the end of a 30-min stabilization period, the gas that flowed into the metabolic chamber was changed from room air to 97% N_2 and 3% CO_2 until primary apnea occurred. Primary apnea was characterized as the cessation of airflow and no respiratory wave on the tracing. The gas was then changed

back to room air, and the ability of the pup to autoresuscitate from primary apnea by gasping was determined. This procedure was repeated at 5-min intervals until death occurred. Again, when the gas mixture was changed, the flow rate was increased until the gas concentrations in the chamber had stabilized; the flow rate was then lowered to 1 L/min.

2.5.3 Plasma Levels of Cocaine in Rat Dams and Pups

On gestation day 10-11, 15-16, and 21-22, blood samples were obtained by cardiopuncture on those pregnant rat dams at three different doses and kept in grey-top tubes from coagulating. After centrifugation, the upper layer of plasma was obtained and kept in freezer waiting for the measurement. On postnatal day 2, 6 and 11, we decapitated those rat pups at their mothers' dose of 0, 50 and 100 mg/kg/day to get the blood samples. The rest procedures were the same as those on mothers. A gas chromatograph/mass spectrometer was used to detect the plasma level of cocaine in department of Toxicology. The limitation of detection was 20 ng/mL.

2.6 Experimental Apparatus

The metabolic chamber used in our experiments consists of a double-walled plexiglass cylinder (30 cm long-internal diameter 6 cm) into which flowed room air or 97% N_2 and 3% CO_2. Chamber ambient temperature was controlled to 37.0±0.1°C by circulating water from a temperature controlled bath (Neslab-Endocal refrigerated Circulating Bath RTE-8DD) through the space between the

walls. This ambient temperature was selected because it is within the thermoneutral zone of rats less than two weeks of age (75).

2.7 Experimental Measurements and Calculations

During an experiment the electrocardiogram, respiratory movements and chamber oxygen or carbon dioxide levels were recorded on a Model 7 polygraph (Grass Instrument Company) at a paper speed of 10 mm/sec. The electrocardiogram was recorded from multistranded stainless steel wire electrodes (AS 633 Cooner Wire Company) sewn across the chest wall that were connected to a Model 7HIP5 High Impedance Probe coupled to a Model 7P5 Wide Band EEG A.C. Preamplifier (Grass Instrument Company). Respiratory movements were recorded from a mercury in silicone rubber strain gauge (D.M. Davis, Incorporated) placed around the pups' chest which was connected to bridge amplifier (Biomedical technical Support Center, University of Calgary) which was coupled to a Model 7P03 Adapter Panel (Grass Instrument Company). Chamber oxygen or carbon dioxide levels were measured using an Applied Electrochemistry Oxygen Analyzer or Carbon Dioxide Analyzer.

2.8 Analysis of Results

Control heart rates and respiratory rates per minute were measured from the polygraph during the control period of the experiments.

2.8.1 Time to Last Gasp Experiments

Time to last gasp and the total number of gasps were determined from the polygraph tracings. Heart rate and the number of gasps were also measured at each minute interval following the time from the induction of hypoxia.

2.8.2 Autoresusciation Experiments

The number of successful autoresuscitations was determined from the polygraph tracings. Autoresuscitation was deemed to occur when heart rate and respiratory rate returned to >60% of control levels within five minutes.

2.9 Statistical Analysis

Statistical analysis was carried out using a one-factor ANOVA followed by a Newman-Keul's comparison test of multiple means to determine if perinatal exposure to cocaine affected the time to last gasp, the total number of gasps or the number of successful autoresuscitations. All results were reported as means+one standard deviation, and $p<0.05$ was considered to be of statistical significance.

3.0 RESULTS

3.1 Cocaine and Body Weight at Different Postnatal Ages

The influence of perinatal exposure to cocaine on postnatal body weight is shown in figure 3.1. Perinatal exposure to cocaine did not produce any consistant effects on postnatal body weight.

3.2 Cocaine and Control Heart Rate at Different Postnatal Ages

The influence of perinatal exposure to cocaine on control heart rate during early postnatal development is illustrated in figure 3.2. Perinatal exposure to cocaine did not alter control heart rate during normoxia except in the 1-2 day old pups at the highest cocaine dose.

3.3 Cocaine and Control Respiratory Rate at Different Postnatal Ages

The lack of influence of perinatal exposure to cocaine on control respiratory rate during early postnatal development is illustrated by figure 3.3.

3.4 Time to Last Gasp (TLG) Experiments

Perinatal exposure to cocaine did not alter the time to last gasp or the total number of gasps during a single period of hypoxia (figures 3.4.1 and 3.4.2).

Exposure to a single period of hypoxia resulted in a reproducible respiratory response that was not altered by perinatal exposure to cocaine (figure 3.4.3). Initially there was a period of hyperpnea and arousal which preceded primary apnea (a); primary apnea was followed by a period of rapid gasping (b); this period of rapid gasping was followed by a period of slower gasping of one to three gasps per minute (c); finally there was a period of rapid gasping which

eventually gave way to terminal apnea and death (d). Figures 3.4.4A to 3.4.6D illustrate the lack of effect of perinatal exposure to cocaine on gasping and heart rate during hypoxia in 1-2, 5-6, and 10-11 day old rat pups.

3.5 Autoresuscitation Experiments

Perinatal exposure to cocaine produced an age- and dose- specific effect on the number of successful autoresuscitations as shown in figure 3.5.1. Perinatal exposure to cocaine impaired autoresuscitation from primary apnea during repeated exposures to hypoxia but only in the youngest animals at the highest dose of cocaine. Before autoresuscitation failure, all successful autoresuscitations exhibited the same cardiorespiratory pattern as illustrated in figure 3.5.2. Initially there was a period of hyperpnea (a) and arousal (b) that preceded primary apnea and bradycardia (c); the onset of gasping (d) was followed by an increase in heart rate (e) and then restoration of a normal respiratory pattern (f). In control and cocaine groups, autoresuscitation failure was associated with cardiac arrhythmia that preceded the cessation of gasping (figure 3.5.3).

3.6 Plasma Level of Cocaine in Rat Dams and Pups

Plasma levels of cocaine in dams at three different ages at four different doses were shown in figure 3.6. The level of cocaine increased with doses and decreased as gestation advanced. Plasma levels of cocaine in rat pups at three different postnatal ages at three different doses were shown in table 1.

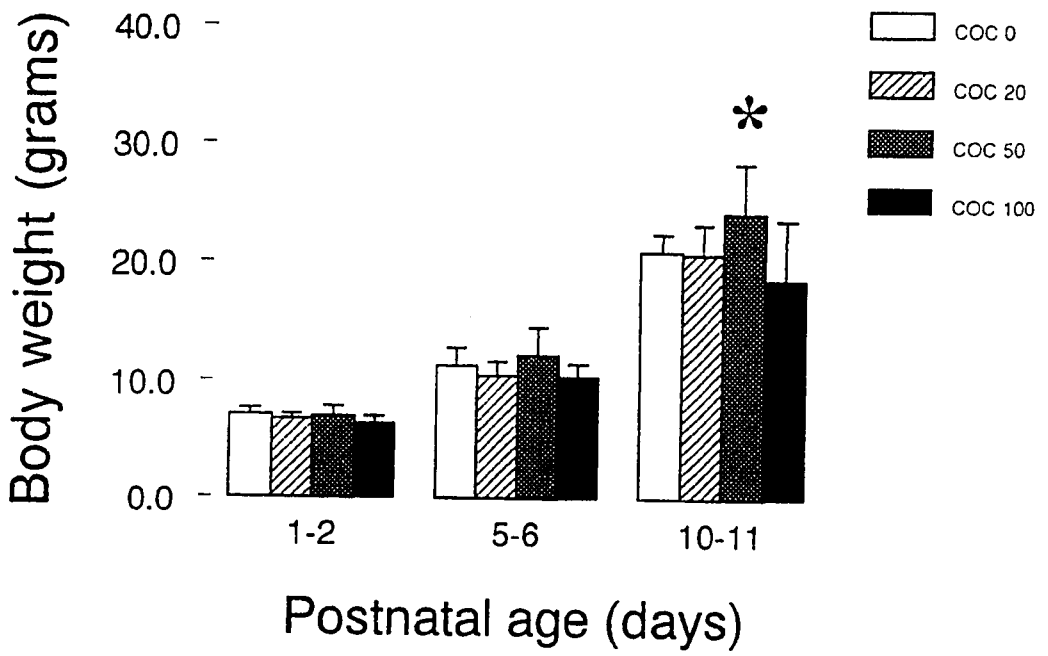

Figure 3.1 Influence of perinatal exposure to cocaine on body weight of 177, 1-2 day, 5-6 day and 10-11 day old rat pups. * $p<0.05$ vs. 10-11 day old controls.

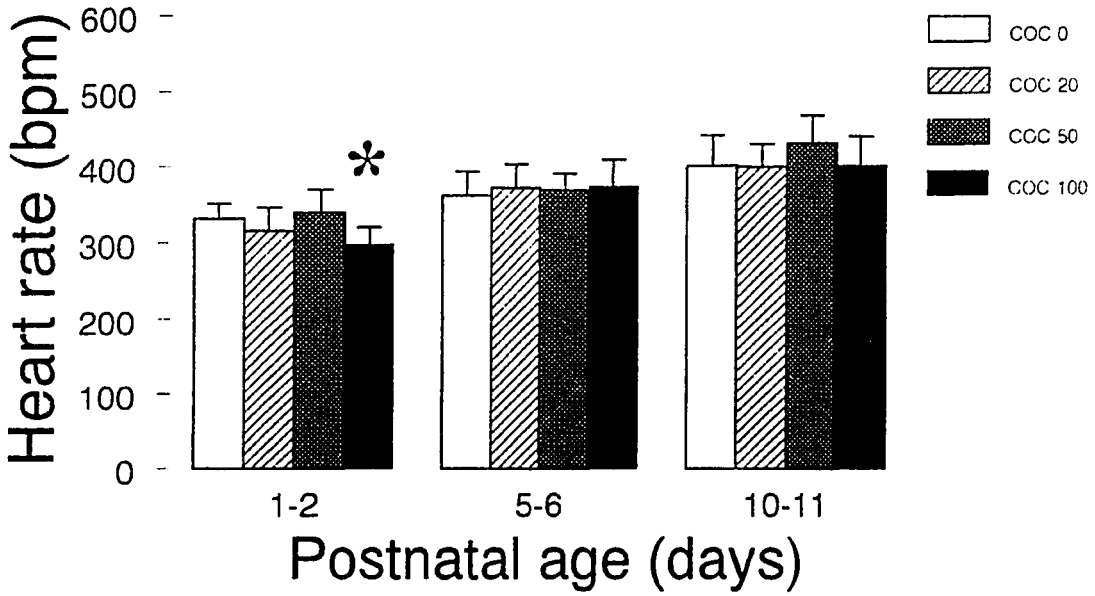

Figure 3.2 Influence of perinatal exposure to cocaine on control heart rate of 177, 1-2 day. 5-6 day and 10-11 day old rat pups. * $p<0.05$ vs. 1-2 day old controls.

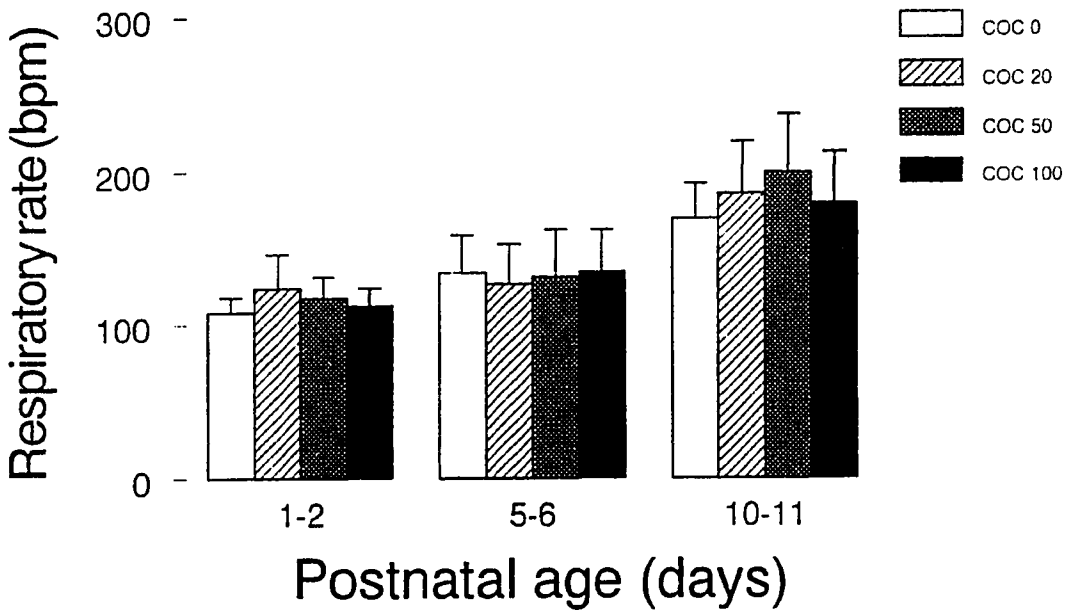

Figure 3.3 The lack of influence of perinatal exposure to cocaine on control respiratory rate of 177, 1-2 day, 5-6 day and 10-11 day old rat pups.

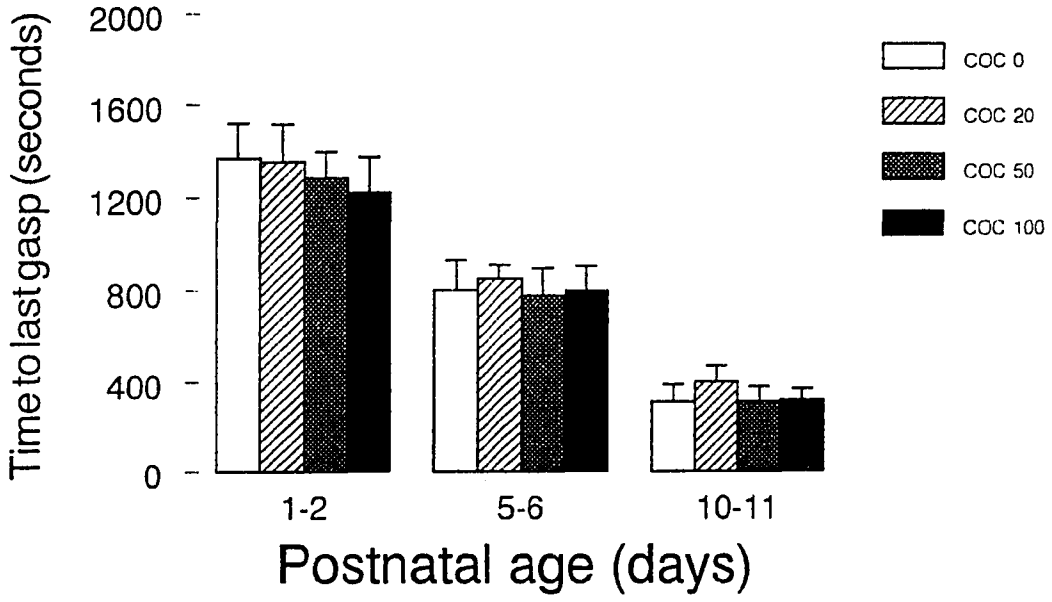

Figure 3.4.1 Lack of influence of perinatal exposure to cocaine on the time to last gasp during a single period of hypoxia in 94, 1-2 day, 5-6 day and 10-11 day old rat pups.

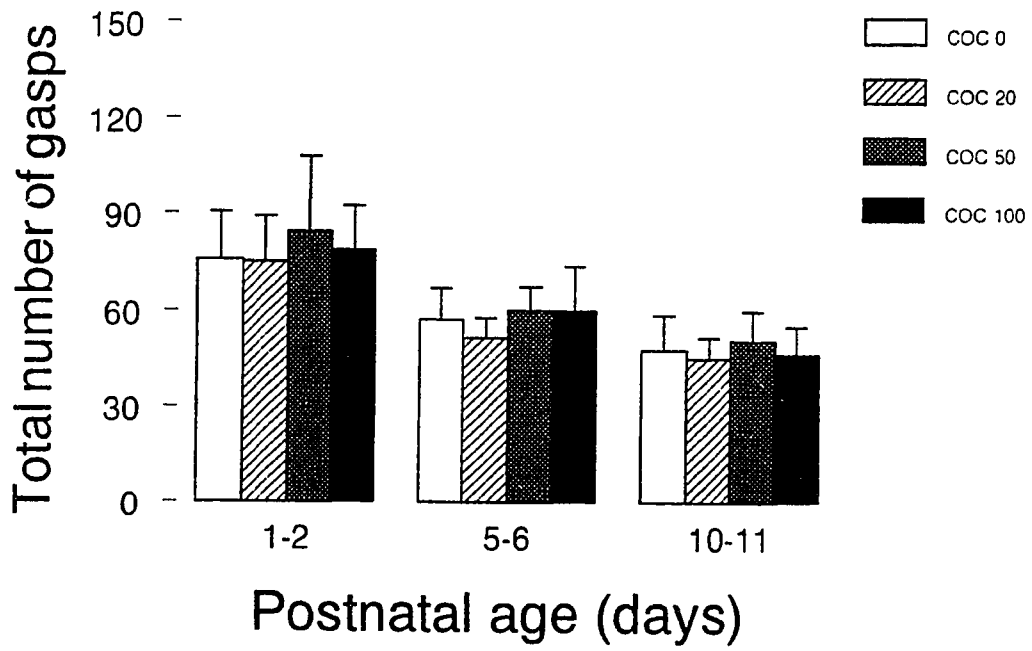

Figure 3.4.2 Lack of influence of perinatal exposure to cocaine on the total number of gasps during a single period of hypoxia.

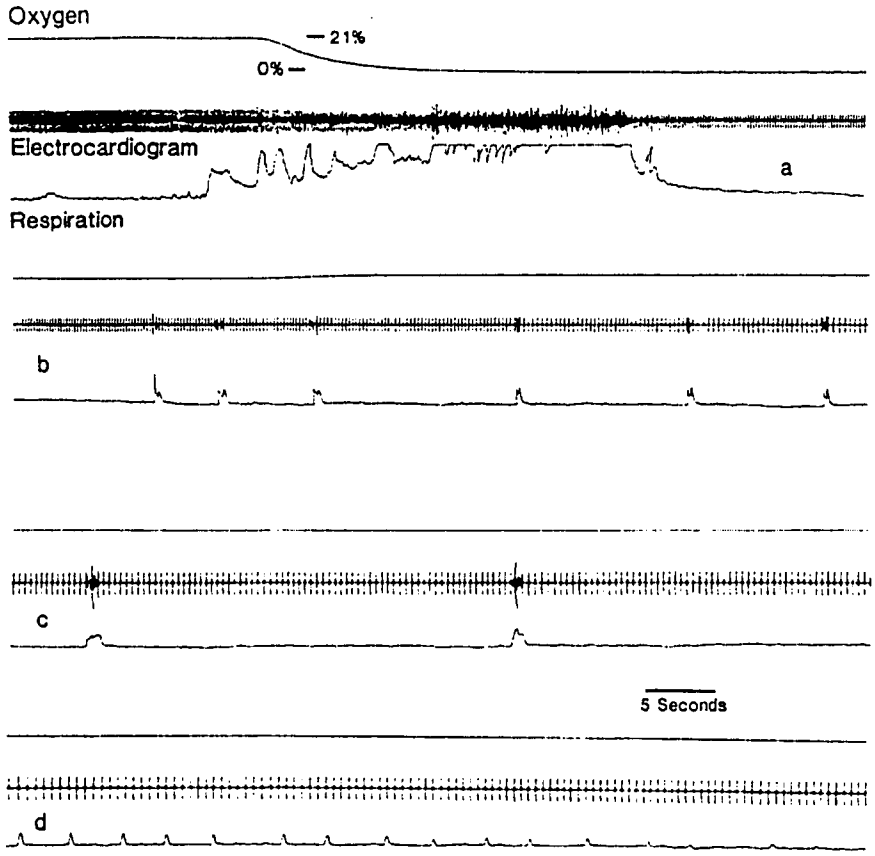

Figure 3.4.3 Four segments of a continuous polygraph tracing showing the respiratory response to hypoxia of a 5-day old rat pup that was exposed to vehicle during the perinatal period. Variables shown are chamber oxygen concentration, electrocardiogram and respiratory pattern. Exposure to a single period of hypoxia resulted in a reproducible respiratory response that was not altered by perinatal exposure to cocaine. Initially there was a period of hyperpnea and arousal which preceded primary apnea (a); primary apnea was followed by a period of rapid gasping (b); this period of rapid gasping was followed by a period of slower gasping of one to three gasps per minute (c), finally there was a period of rapid gasping which eventually gave way to terminal apnea and death (d).

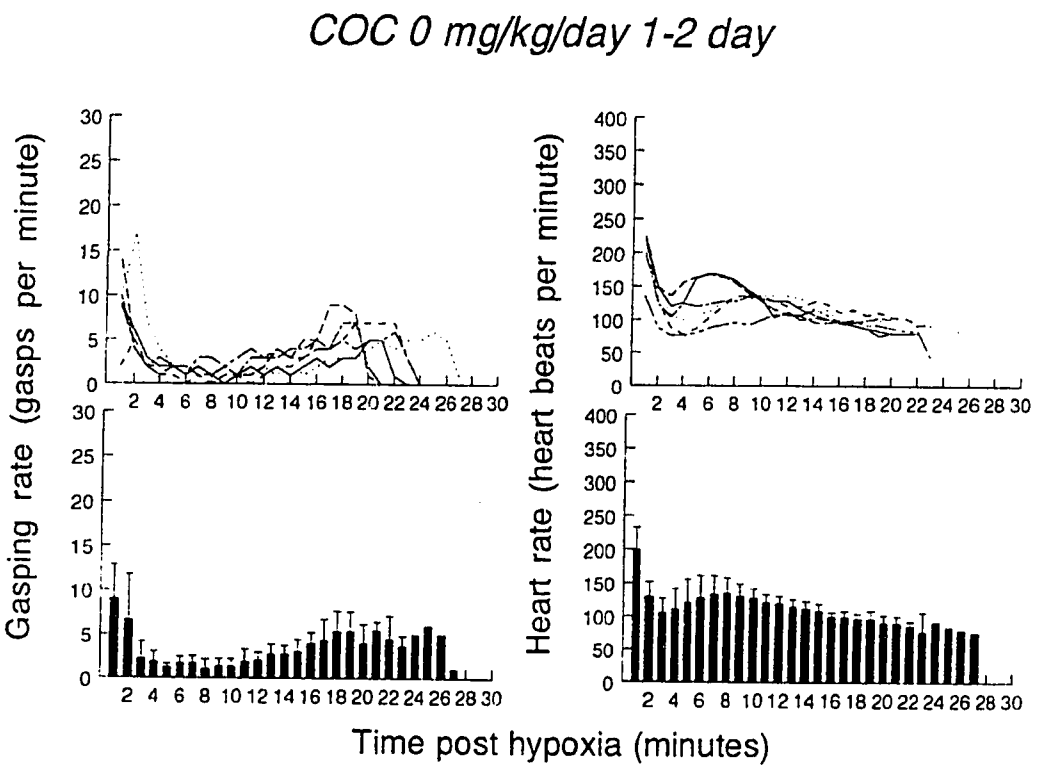

Figure 3.4.4A Gasping rate and heart rate of 1-2 day old pups during a single period of hypoxia; these 6 pups were exposed to saline during the perinatal period. The graph shows raw data and means + SD.

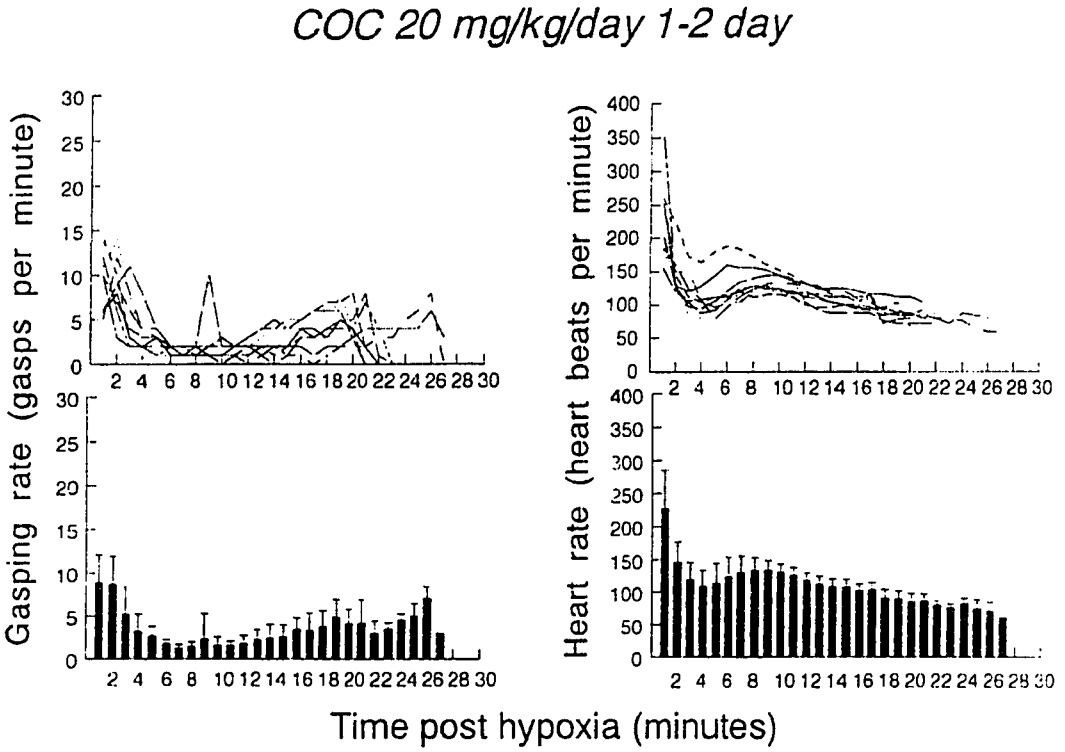

Figure 3.4.4B Gasping rate and heart rate of 1-2 day old pups during a single period of hypoxia: these 9 pups were exposed to 20 mg/kg/day of cocaine during the perinatal period. The graph shows raw data and means + SD.

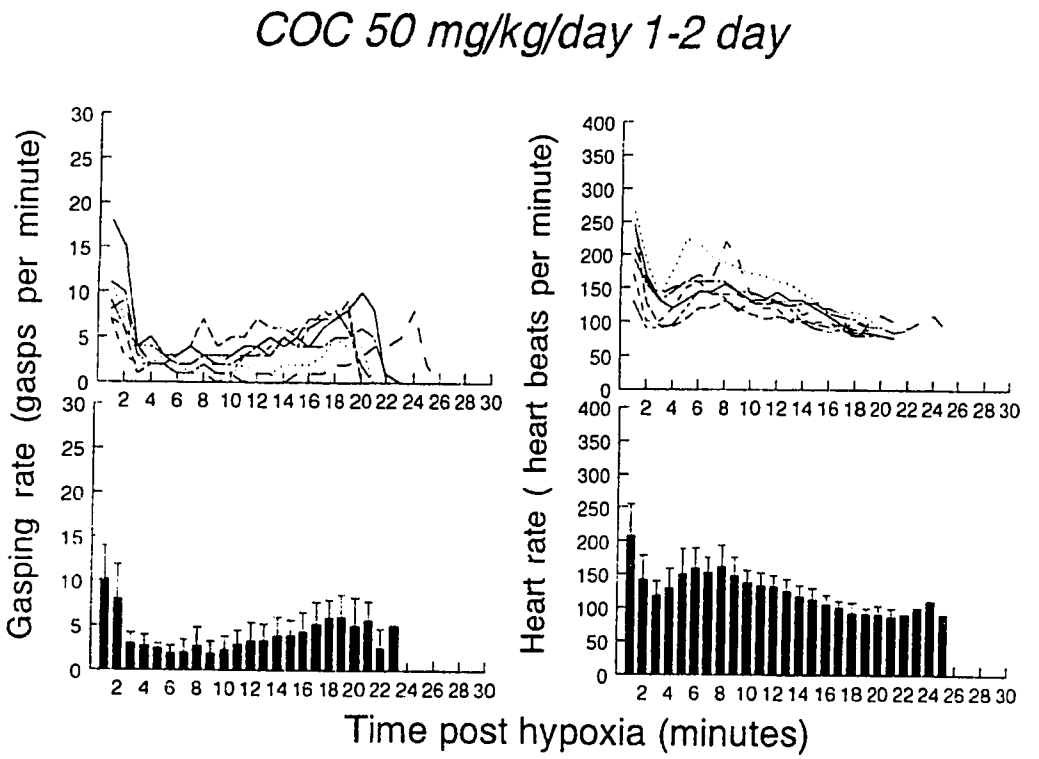

Figure 3.4.4C Gasping rate and heart rate of 1-2 day old pups during a single period of hypoxia; these 7 pups were exposed to 50 mg/kg/day of cocaine during the perinatal period. The graph shows raw data and means + SD.

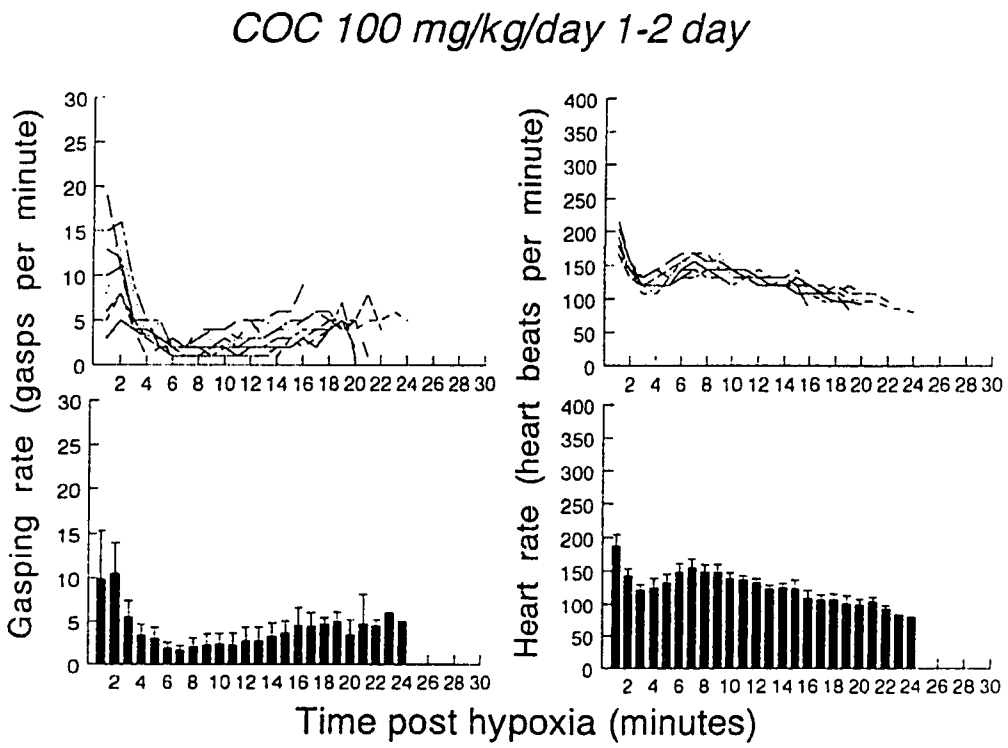

Figure 3.4.4D Gasping rate and heart rate of 1-2 day old pups during a single period of hypoxia; these 8 pups were exposed to 100 mg/kg/day of cocaine during the perinatal period. The graph shows raw data and means + SD.

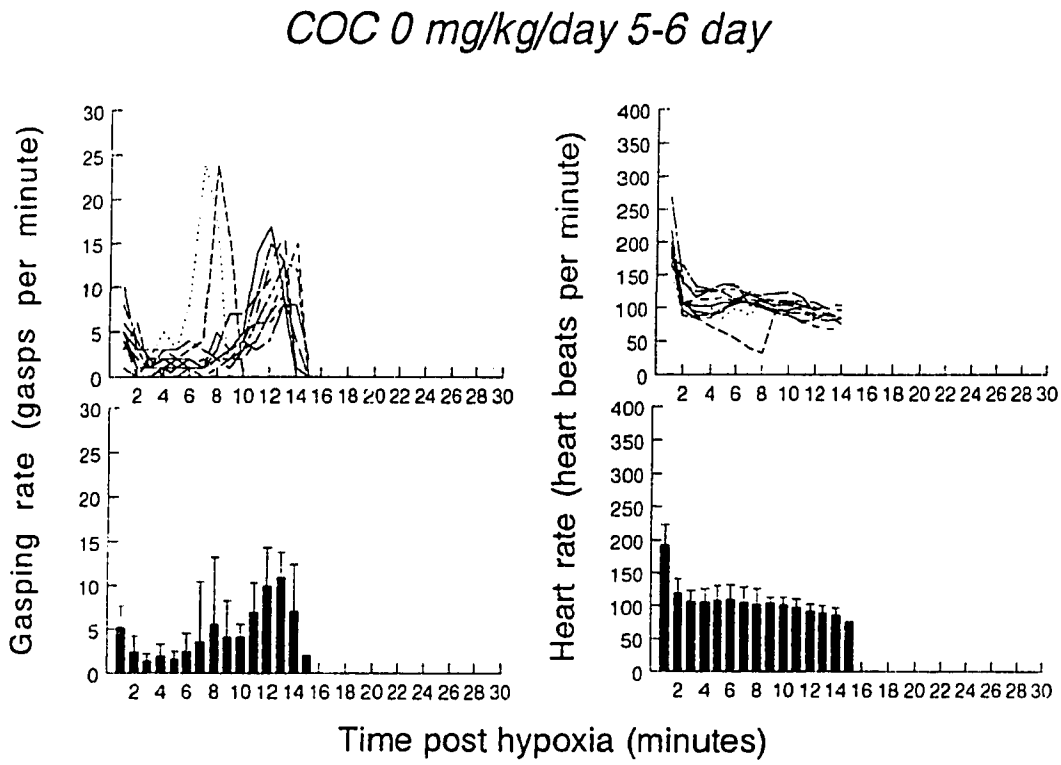

Figure 3.4.5A Gasping rate and heart rate of 5-6 day old pups during a single period of hypoxia; these 11 pups were exposed to saline during the perinatal period. The graph shows raw data and means + SD.

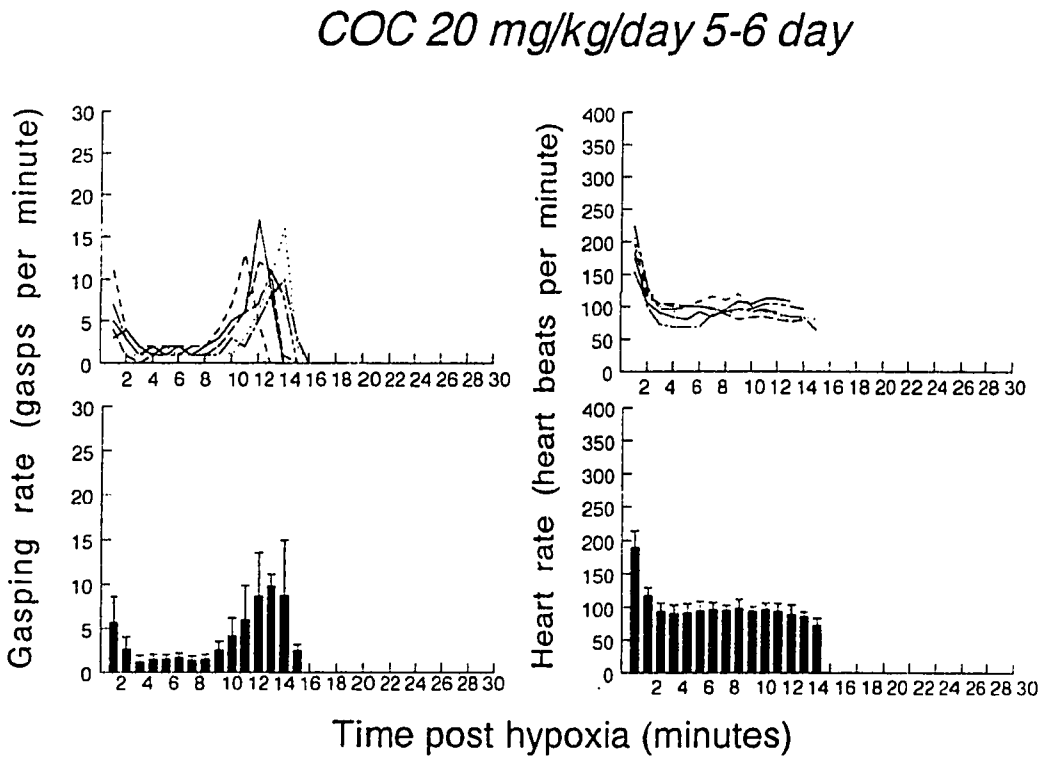

Figure 3.4.5B Gasping rate and heart rate of 5-6 day old pups during a single period of hypoxia; these 6 pups were exposed to 20 mg/kg/day of cocaine during the perinatal period. The graph shows raw data and means + SD.

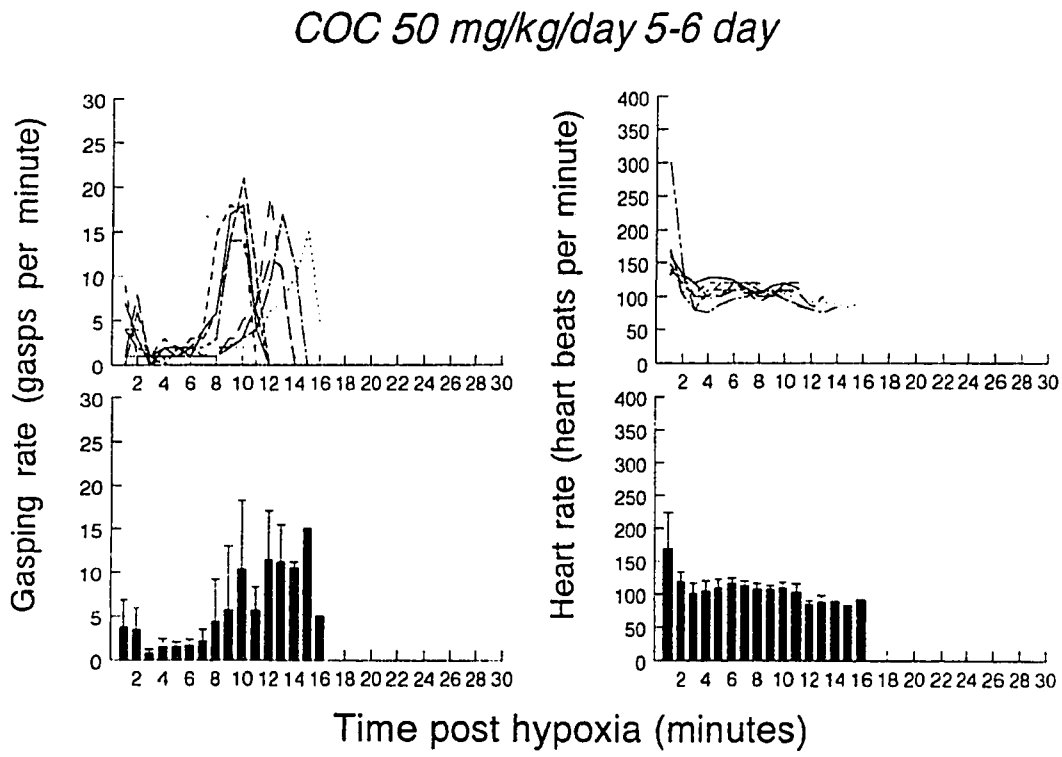

Figure 3.4.5C Gasping rate and heart rate of 5-6 day old pups during a single period of hypoxia; these 8 pups were exposed to 50 mg/kg/day of cocaine during the perinatal period. The graph shows raw data and means + SD.

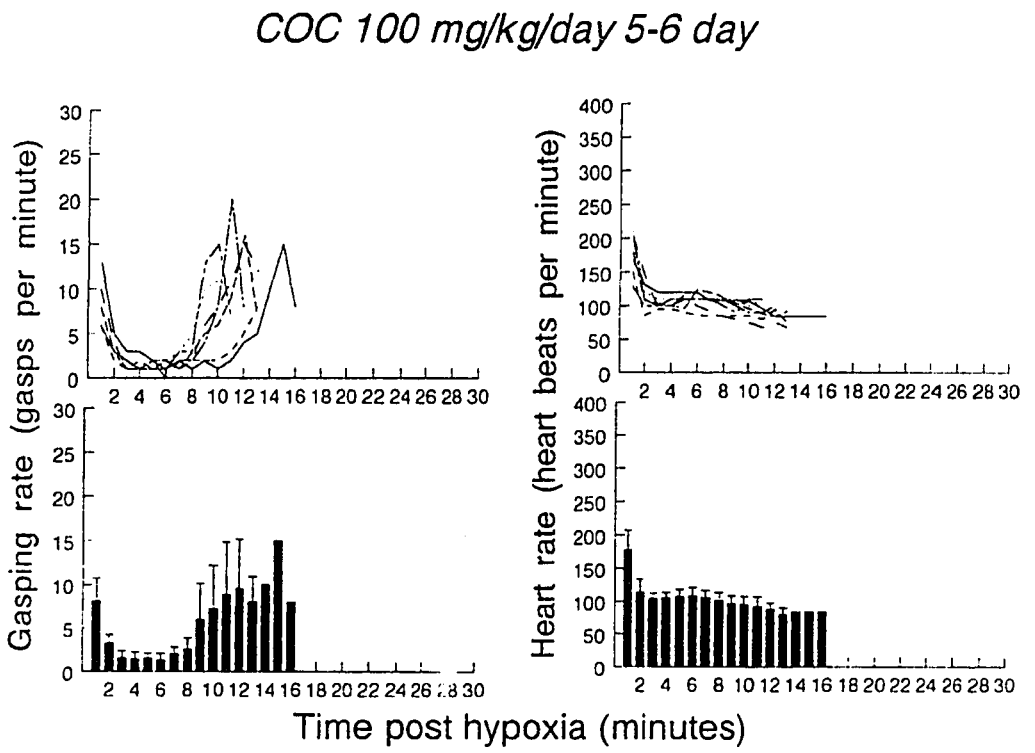

Figure 3.4.5D Gasping rate and heart rate of 5-6 day old pups during a single period of hypoxia; these 7 pups were exposed to 100 mg/kg/day of cocaine during the perinatal period. The graph shows raw data and means + SD.

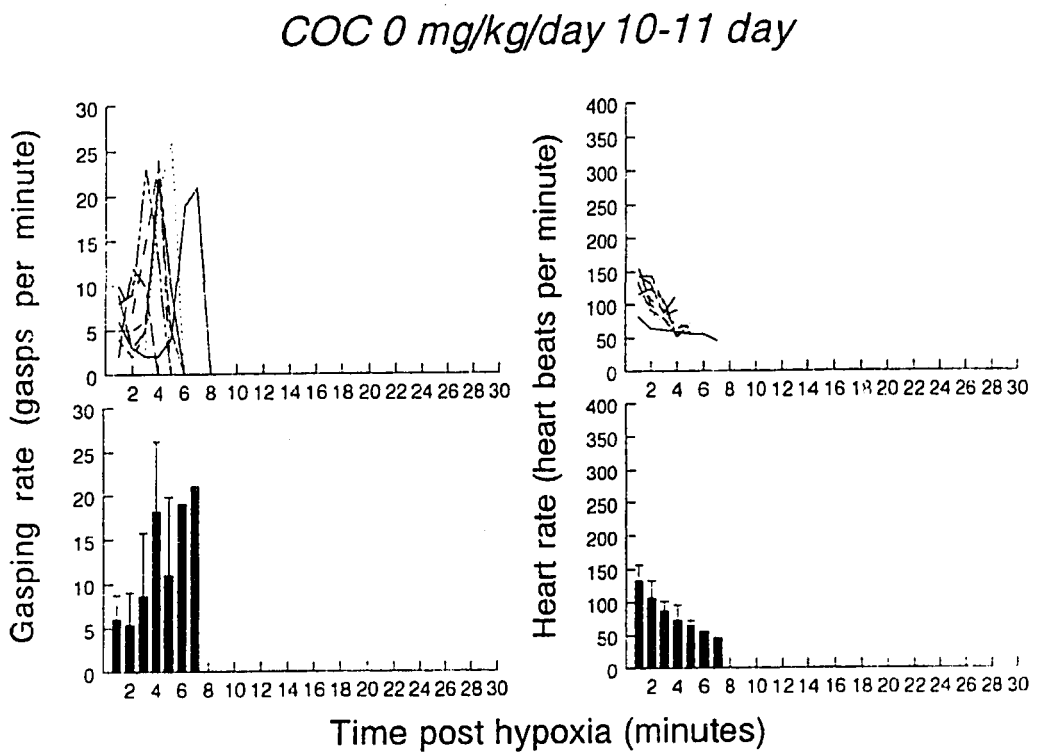

Figure 3.4.6A Gasping rate and heart rate of 10-11 day old pups during a single period of hypoxia; these 8 pups were exposed to saline during the perinatal period. The graph shows raw data and means + SD.

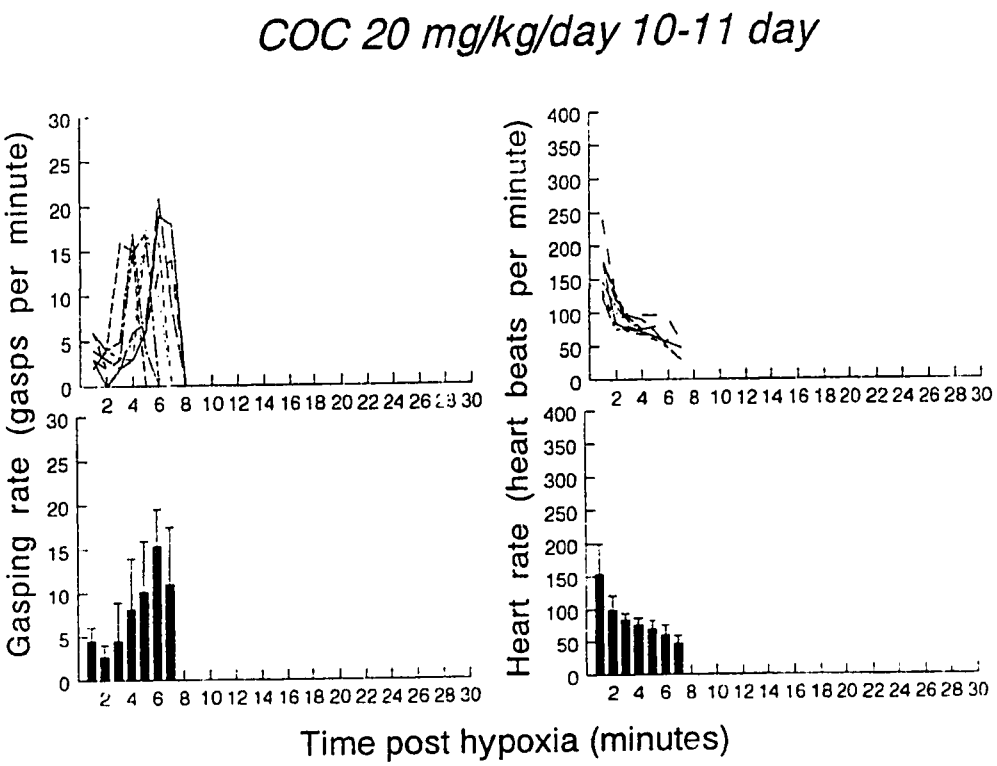

Figure 3.4.6B Gasping rate and heart rate of 10-11 day old pups during a single period of hypoxia; these 10 pups were exposed to 20 mg/kg/day of cocaine during the perinatal period. The graph shows raw data and means + SD.

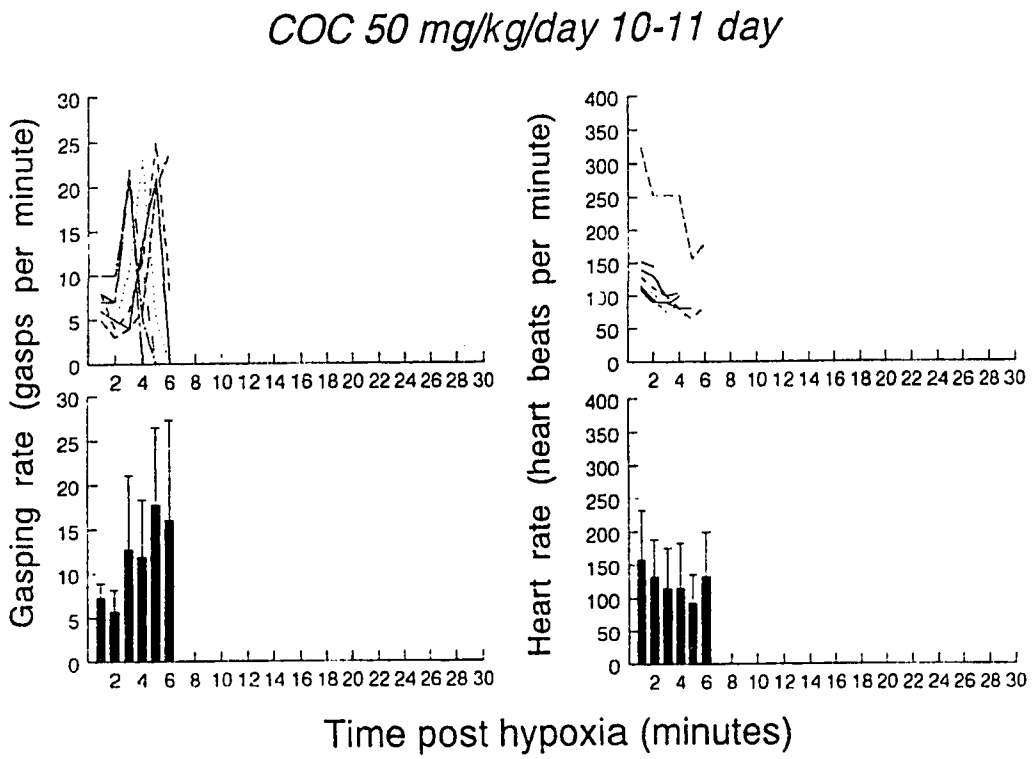

Figure 3.4.6C Gasping rate and heart rate of 10-11 day old pups during a single period of hypoxia; these 7 pups were exposed to 50 mg/kg/day of cocaine during the perinatal period. The graph shows raw data and means + SD

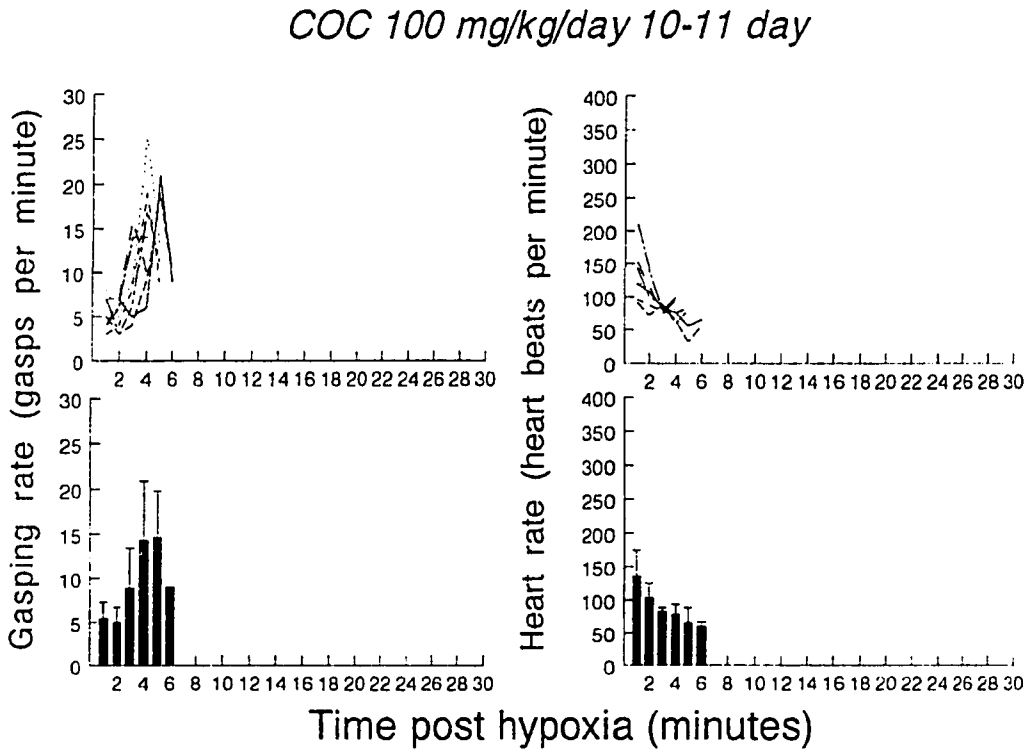

Figure 3.4.6D Gasping rate and heart rate of 10-11 day old pups during a single period of hypoxia; these 7 pups were exposed to 100 mg/kg/day of cocaine during the perinatal period. The graph shows raw data and means + SD.

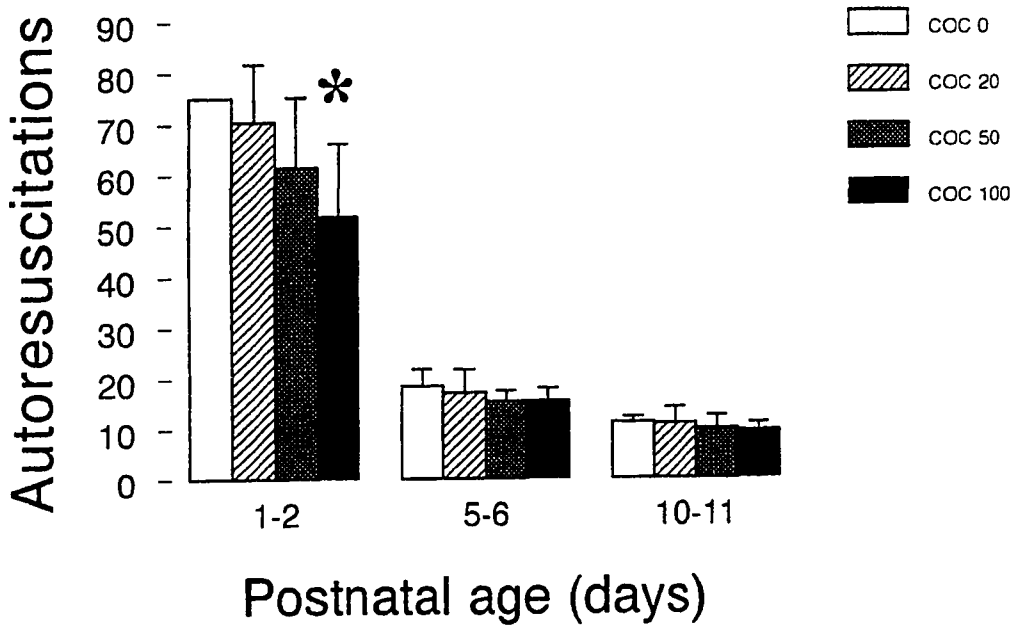

Figure 3.5.1 Influence of perinatal exposure to cocaine on successful autoresuscitations from primary apnea during repeated exposures to hypoxia. *$p<0.05$ vs. 1-2 day old control.

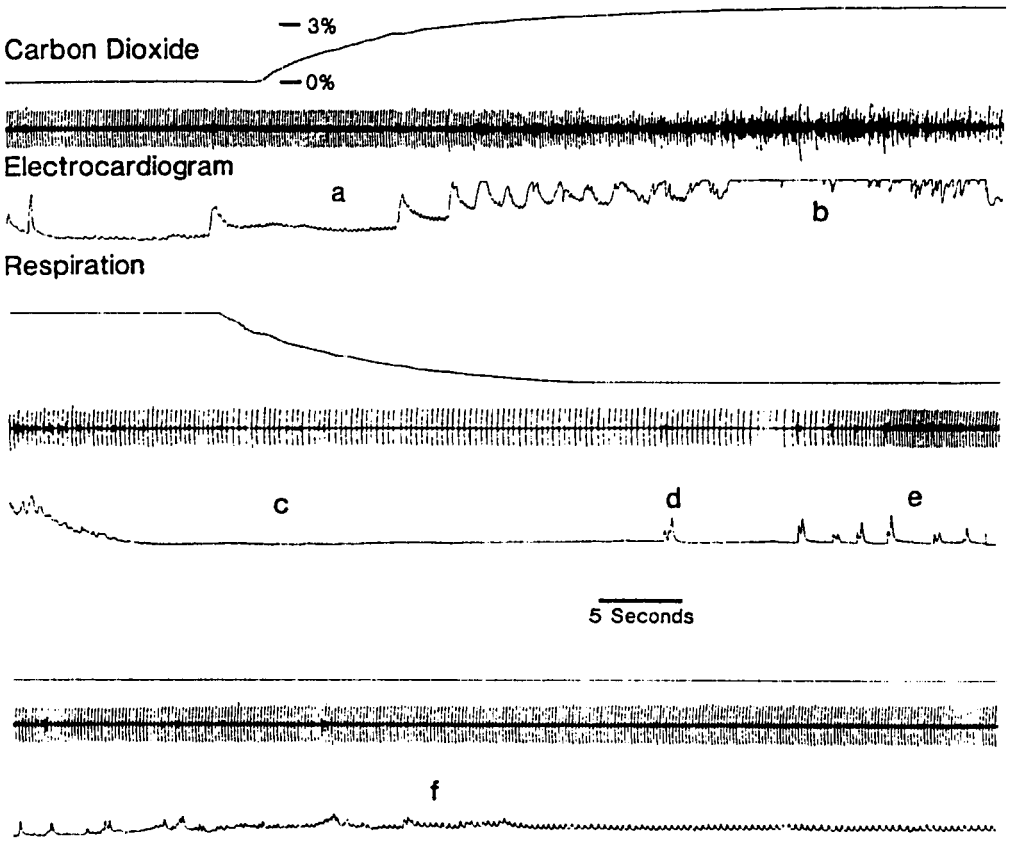

Figure 3.5.2 Continuous polygraph tracing showing a successful autoresuscitation from primary apnea in a 5 day-old rat that was exposed to saline during the perinatal period. During exposure to hypoxia there was an initial period of hyperpnea (a) and arousal (b) which preceded primary apnea and bradycardia (c), the onset of gasping (d) was followed by an increase in heart (e) and restoration of a normal respiratory pattern (f).

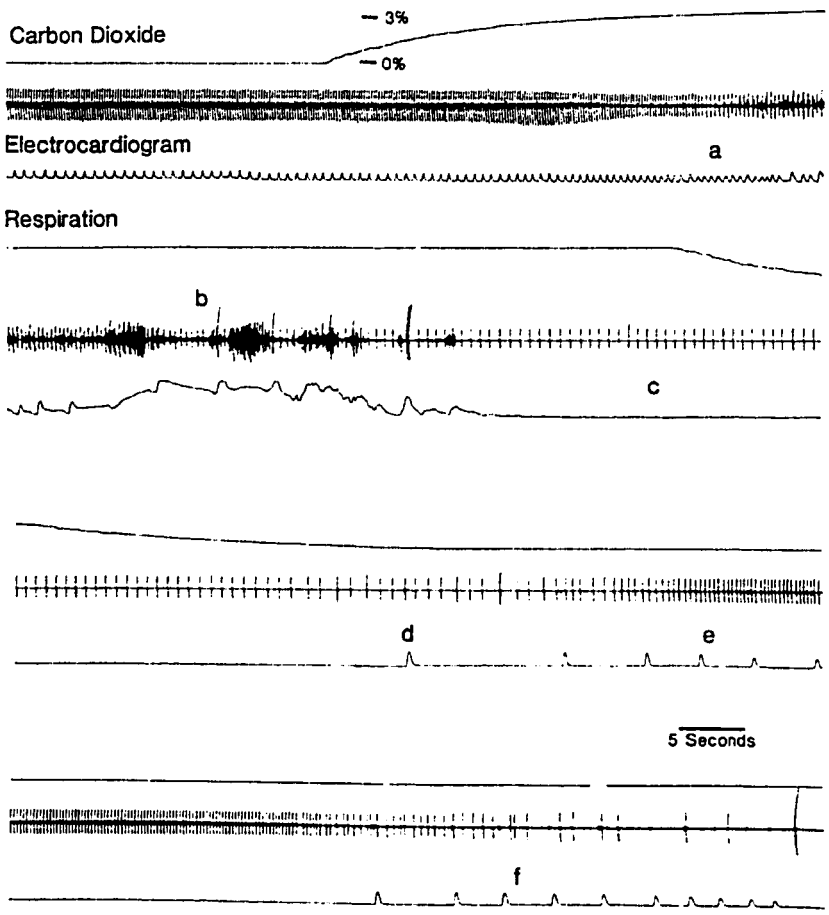

Figure 3.5.3 Continuous polygraph tracing showing autoresuscitation failure from primary apnea of a 5 day old rat pup who was exposed to saline during the perinatal period. During exposure to hypoxia there was an initial period of hyperpnea (a) and arousal (b) which preceded primary apnea and bradycardia (c), the onset of gasping (d) was followed by an increase in heart rate (e) and then the occurrence of cardiac arrhythmia (f) which preceded the cessation of gasping.

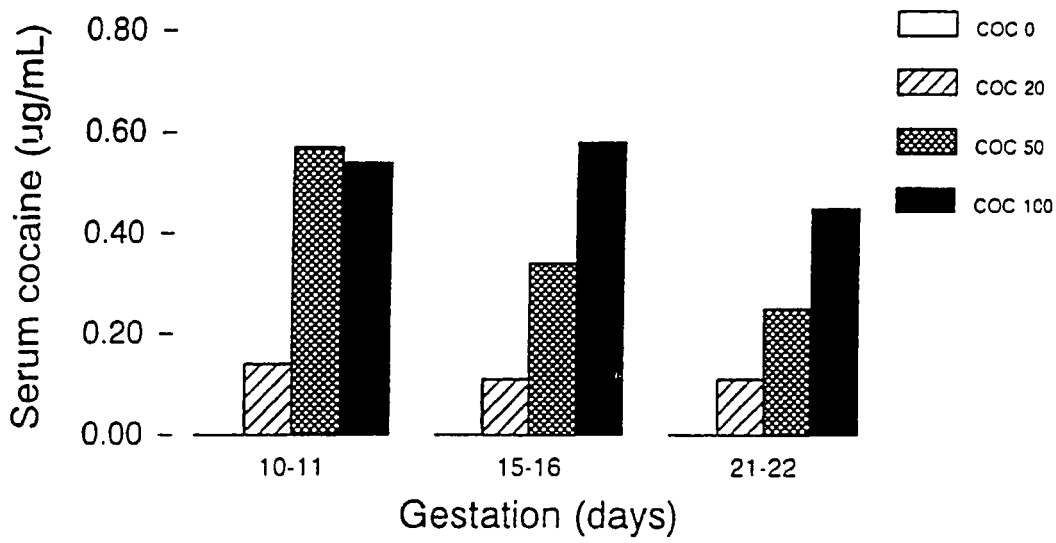

Figure 3.6 Plasma level of cocaine in rats at three different gestation ages at four different doses. The serum cocaine increased with doses and decreased with gestation.

Dose of Cocaine on Mothers (mg/kg/day)	Plasma Level of Cocaine in Rat Pups (ng/mL)		
	2 postnatal day old	6 postnatal day old	11 postnatal day old
0	<20	<20	<20
50	<20	<20	<20
100	30	30	45

Table 1. Plasma level of cocaine in three different postnatal age groups of rat pups at three different doses. At the doses of 0 and 50 mg/kg/day, the plasma levels were <20 ng/mL in all groups. At the dose of 100 mg/kg/day, the plasma levels were approximately constant throughout three different postnatal ages.

4.0 DISCUSSION

My experiments provide new information about factors that influence the newborn's ability to survive hypoxia as may occur during prolonged or repeated apnea. A novel finding in my study was that although perinatal exposure to cocaine did not shorten the time to last gasp during a single hypoxic exposure, it did impair the ability of rat pups to autoresuscitate from primary apnea during repeated exposures to hypoxia in an age- and dose- specific manner. In all control pups that were exposed to saline during the perinatal period, autoresuscitation failure appeared to result from cardiac arrhythmia (atrio-ventricular dissociation) that followed early cardiac resuscitation; the atrio-ventricular dissociation and ultimate loss of ventricular depolarization preceded the cessation of gasping. Cocaine did not alter this sequence of events leading to autoresuscitation failure. Thus, my data provide evidence that perinatal exposure to cocaine impairs the ability of newborn rats to autoresuscitate from primary apnea during repeated exposures to hypoxia but it doesn't alter the mechanism of autoresuscitation failure.

Exposure to a single period of hypoxia resulted in a similar respiratory response in rat pups that received cocaine as well as in control rat pups that received saline during the perinatal period. The respiratory response consisted of hyperpnea, primary apnea, gasping and terminal apnea. In all animals, the gasping phase of the respiratory response was triphasic in nature, characterized by: (1) an initial period of rapid gasping (phase I) following

primary apnea, (2) this period of rapid gasping was followed by a period of slower gasping (phase II) and finally, (3) a period of rapid gasping (phase III) which eventually waned and gave way to terminal apnea and death. This triphasic gasping pattern is similar to that observed in rats by Gozal et al. (76).

Although it is well known in rats that maturity at birth (77), postnatal age (78, 79, 80) and body temperature (78, 79) influence the time to last gasp following exposure to a single period of hypoxia, little is known of the actual mechanisms that influence the pattern or duration of gasping. Gozal et al. (76) have suggested that the triphasic nature of gasping in response to hypoxia could result either from the existence of one single gasping center or from multiple neural sites that are crucial for gasp generation. In the first instance, the lateral tegmental field of the medulla (i.e., the putative gasping center) could contain several populations of neurons with differing hypoxic thresholds. In the second instance, multiple neuronal gasping loci outside the lateral tegmental field would exist and each of these loci would have different hypoxic thresholds. Regardless of the mechanism for the triphasic gasping pattern, my experiments show that perinatal exposure to cocaine does not alter the gasping or heart rate pattern or the time to last gasp during a single hypoxic exposure.

Peiper, Steven, and Thach (52, 81) have emphasized the importance of gasping in "self-resuscitation" or "autoresuscitation" during apnea in human infants and that repeated episodes of apnea may lead to autoresuscitation failure and death. The process of recovery from hypoxia by gasping was first

termed "self-resuscitation" in 1969 by Adolph (77) and then "autoresuscitation" in 1975 by Guntheroth (69). Cardiorespiratory events that occur during successful autoresuscitation from hypoxic apnea in mice have recently been defined by Gershan et al. (82). These consist of three sequential stages: 1) gasping with marked bradycardia, 2) cardiac resuscitation with a rapid increase in heart rate to greater than 60% of baseline, and 3) respiratory resuscitation with an increase in respiratory rate to greater than 60% of baseline. A similar sequence of events during successful autoresuscitation happened in my experiments. Likewise, I found, as did Gershan, Jacobi and Thach (83), that repeated exposure to hypoxia led to autoresuscitation failure which was associated with cardiac arrhythmia (i.e., A-V dissociation) that preceded cessation of gasping in normal animals. Failed autoresuscitation might result from either failure of oxygen transport from the lung to the heart or failure of heart to respond to oxygenation with increased rate and cardiac output. Failed transport of oxygen from the lung to the heart can induce sinus bradycardia because of the absence of oxygen supply to the pacemaker of atrium. Hypoxia can also alter cardiac conducting mechanisms resulting in atria-ventricular block. Accumulation of endogenous adenosine during hypoxia may be involved in the production of heart block (84, 85).

The ability to survive hypoxia depends on the maintenance of brain stem function, as well as maintenance of cardiac function and blood pressure. In my experiments perinatal exposure to cocaine impaired the ability of newborn rat

pups to autoresuscitate following repeated exposures to hypoxia, but it did not alter the sequence of events leading to autoresuscitation failure. Although my experiments were not designed to investigate the mechanism of the changes in the physiology of this protective response following perinatal administration of cocaine, there are a number of possibilities. Cocaine is a neuroteratogen which easily crosses the rodent placenta and accumulates in fetal blood and brain (11, 12). Slotkin et al. (86) have shown that fetal rat exposure to cocaine is associated with increased perinatal cardiac risk. Cocaine inhibits re-uptake of norepinephrine released by peripheral sympathetic neurons, leading to intense vasoconstriction of rat hemoendothelial type of placenta and resultant fetal ischemia/hypoxia, factors that are etiological for fetal brain and heart damage (87). Increased intracranial pressure in combination with hypoxia associated with the birth process leads to a profound surge of catecholamine release in rats at delivery (50). Studies (86) indicate that the high levels of progesterone associated with pregnancy sensitize both the maternal and fetal rat myocardium to catecholamine-related actions of cocaine.

Early in postnatal life, the rat adrenal chromaffin cells possess a developmentally regulated oxygen-sensing mechanism – similar to that of carotid body type I cells – which are responsible for the non-neurogenic mediated catecholamine release which is essential to survival in the fetus and neonate. It plays a vital role in modulating cardiovascular, respiratory and metabolic response to hypoxia and any interference either with catecholamine

release or with catecholamine actions at adrenergic targets results in loss of the ability of the neonatal rat to survive hypoxia or other stressors (50). At about five to six days of postnatal life, this non-neurogenic mechanism for catecholamine release is replaced by a neurogenic mechanism when the splanchnic nerve innervates the adrenal medulla. It is possible that perinatal exposure to cocaine impairs "non-neurogenic" mediated catecholamine release significantly in one to two day old rat pups at the highest dose and this impaired their ability to autoresuscitate during repeated exposures to hypoxia. This postulate requires further investigation.

Slotkin et al. (88) also stated that the local anesthetic actions of cocaine could induce the alterations of rat central nervous system development, (i.e., interfere with cell excitation, an important signal in replication and differentiation). In addition, cocaine may have actions on developing cells, independent of ischemia or cell excitation – by directly inhibiting cell replication within the developing central nervous system of the neonatal rat (88). As the central nervous system develops gradually and completely with age, the adverse effect of cocaine would be possibly counteracted and compensated compared to rats just after birth. Thus, perturbations of fetal rat brain cell development may also be a factor contributing to the adverse effect of cocaine on the ability to autoresuscitate successfully.

The plasma levels of cocaine in dams increased with doses and decreased along with gestation. It is possibly because of the increase of maternal body

weights during this period. The levels of cocaine in rat pups at the doses of 0 and 50 mg/kg/day were below the limit of detection at three postnatal ages and around 35 ng/mL at the dose of 100 mg/kg/day. That the plasma cocaine accumulated through breast milk in these rat pups at those two low doses might be metabolized completely may account for it. The accumulating rate of plasma cocaine in rat pups from milk at the highest dose exceeded the rate of metabolism so that certain amount of cocaine remained in that group. In addition, since the limit of detection of plasma cocaine was around 20 ng/mL, it is also possible that there was certain amount of cocaine in plasma in the rats at the two low doses that could not be detected. Therefore, the effect of cocaine on autoresuscitation was most likely to be attributed to the prenatal influence of cocaine on fetuses.

Perinatal exposure to cocaine decreased the control heart rate in 1-2 day old pups but only at the highest dose. As postulated above, the plasma level of cocaine in pups after birth kept constant within every dose group. Therefore, it is possible that some prenatal adverse effects of cocaine at high dose played a role in it. They might induce intense vasoconstriction with a resulting increase in blood pressure that remained existence after birth. The decrease in heart rate may have resulted from activation of the arterial baroreceptors. Vagal innervation of the heart is functional in the first postnatal day in this species (89).

The results of my experiments provide insight into how maternal cocaine abuse may place offspring at an increased risk of SIDS. As previously discussed, an inability to recover from prolonged sleep apnea has long been postulated as a factor in SIDS (50, 90) and that recovery from sleep apnea is thought to occur early as a result of arousal from sleep or later as a result of hypoxic gasping when it is known as "autoresuscitation" (50). Given the results of previous human studies (40) that maternal abuse with cocaine perturbed the maturation of respiratory control and impaired the respiratory function of the infants resulting in disruption of postnatal respiration, and my results that perinatal exposure to cocaine impairs the ability of rat pups to autoresuscitate during repeated exposure to hypoxia, I speculate that maternal abuse with cocaine places infants who have apnea at increased risk for severe hypoxia and death. The underlying phenomenon may be an impairment of protective responses that terminate apnea and restore normal tidal ventilation.

5.0 CONCLUSIONS

My experiments show that perinatal exposure to cocaine impairs the ability of newborn rats to autoresuscitate. This, however, occurs only at the highest dose of cocaine. Perinatal exposure to cocaine doesn't shorten the time to last gasp.

Thus, my data support the hypothesis that perinatal exposure to cocaine impairs the protective responses that newborn rats exhibit during exposure to hypoxia as may occur during episodes of sleep apnea. But my data don't support the hypothesis that perinatal exposure to cocaine impairs the protective responses of older rats.

BIBLIOGRAPHY

1. **Olson GD, Weil JA**: *In utero* cocaine exposure: effect on neonatal breathing in guinea pigs. *J Pharmacol Exp Ther 1992; 261:420-428*
2. **Misra AL, Mule SJ**: Calcium-binding property of cocaine and some of its active metabolites-formation of molecular complexes. *Res Commun Chem Pathol Pharmacol 1975; 11:663-666*
3. **Mirochnick M, Meyer J, Cole J, Herren T, Zuckerman B**: Circulating catecholamine concentrations in cocaine-exposed neonates: a pilot study. *Pediatrics 1991; 88:481-485*
4. **Needlman R, Zuckerman BS, Anderson G, Mirochnick M, Cohen DJ**: CSF monoamine precursors and metabolites in human neonates following in utero cocaine exposure. *Pediatrics 1993; 92:55-60*
5. **Seidler FJ, Temple SW, McCook EC, Slotkin TA**: Cocaine inhibits central noradrenergic and dopaminergic activity during the critical developmental period in which catecholamines influence cell development. *Brain Res Dev Brain Res 1995; 85:48-53*
6. **Spraggins YR, Seidler FJ, Slotkin TA**: Cocaine exacerbates hypoxia-induced cell damage in the development brain: effects on ornithine decarboxylase activity and protein synthesis. *Biol Neonate 1994; 66:254-266*

7. **O'Brien CP:** Drug addiction and drug abuse. In: Goodman and Gilman's The Pharmacological Basis of Therapeutics. 9th edition. *Pergamon Press. 1996; pp 557-578*

8. **Joyner C, King G, Lee TH, Ellinwood EH**: Technique for the continuous infusion of high doses of cocaine by osmotic minipump. *Pharmacol Biochem Behav 1993; 44:971-973*

9. **King GR, Joyner C, Ellinwood EH**: Continuous or intermittent cocaine administration: effects of flupenthixol treatment during withdrawal. *Pharmacol Biochem Behav 1994; 49:883-889*

10. **Zuckerman B, Frank DA, Hingson R, Amaro H, Levenson SM, Kayne H, Parker S, Vinci R, Aboagye K, Fried LE**: Effects of maternal marijuana and cocaine use on fetal growth. *N Engl J Med 1989; 320:762-728*

11. **Shah NS, May DA, Yates JD**: Disposition of levo-[^{3}H] cocaine in pregnant and nonpregnant mice. *Toxicol Appl Pharmacol 1980; 53:279-284*

12. **Spear LP, Frambes NA, Kirstein CL**: Fetal and maternal brain and plasma levels of cocaine and benzoylecgonine following chronic subcutaneous administration of cocaine during gestation in rats. *Psychopharm 1989; 97:427-431*

13. **Robinson SE, Enters KE, Jackson GF, Chinchilli VM, Maher JR, McDowell KP, Allen HM, Guo H**: Maternal and fetal brain and plasma levels of cocaine and benzoylecgonine after acute or chronic maternal

intravenous administration of cocaine. *J Pharmacol Exp Ther 1994; 271:1234-1239*

14. **Dow-Edwards, Fico TA, Osman M, Gamagaris Z, Hutchings DE**: Comparison of oral and subcutaneous routes of cocaine administration on behaviour, plasma drug concentration, and toxicity in female rats. *Pharmacol Biochem Behav 1989; 33:167-173*

15. **Tudehope DI**: Perinatal cocaine intoxication: a precaution. *Med J Aust 1989; 150:290-291*

16. **Wiggins RC, Rolsten C, Ruiz B, Davis CM**: Pharmacokinetics of cocaine: basic studies of route, dosage, pregnancy and lactation. *Neurotoxicology 1989; 10:367-382*

17. **DeVane CL, Simpkins JW, Miller RL, Braun SB**: Tissue distribution of cocaine in the pregnant rat. *Life Sci 1989; 45:1271-1276*

18. **Rosen TS, Johnson HL**: Drug-addicted mothers, their infants, and SIDS. *Ann N Y Acad Sci 1988; 533:89-95*

19. **Ward SL, Schuetz S, Kirshna V, Wingert W, Wachsman L, Keens TG**: Abnormal sleeping ventilatory pattern of infants of substance-abusing mothers. *Am J Dis Ch 1986; 140:1015-1020*

20. **Durand DJ, Espinoza AM, Nickerson BG**: Association between prenatal cocaine exposure and sudden infant death syndrome. *J Pediatr 1990; 117:909-911*

21. **Ward SL, Keens TG**: Prenatal substance abuse. *Clin Perinatol 1992; 19:849-860*

22. **Willinger M, James LS, Catz C**: Defining the sudden infant death syndrome (SIDS): Deliberations of an expert panel converted by the National Institute of Child Health and Human Development. *Pediatr Pathol 1991; 11:677-684*

23. **Valdes-Dapena M**: Sudden infant death syndrome: Overview of recent research developments from a pediatric pathologist's perspective. *Pediatrician 1988; 15:222-230*

24. **Hoffman HJ, Damus K, Hillman L, Krongrad E**: Risk factors for SIDS. Resutls of the National Institute of Child Health and Human Development SIDS Cooperative Epidemiological Study. *Ann N Y Acad Sci 1988; 533:13-30*

25. **Kinney HC, Filiano JJ, Harper RM**: The neuropathology of the sudden infant death syndrome. A review. *J Neuropathol Exp Neurol 1992; 51:115-126*

26. **Gilbert-Barness EF, Kenison K, Giulian G, Chandra S**: Extramedullary hematopoiesis in the liver in sudden infant death syndrome. *Arch Pathol Lab Med 1991; 115:226-229*

27. **Naeye RL**: Hypoxemia and the sudden infant death syndrome. *Science 1974; 186:837-838*

28. **Valdes-Dapena MA, Gillane MM, Catherman R**: Brown fat retention in sudden infant death syndrome. *Arch Pathol Lab Med 1976;100;547-549*

29. **Naeye RL**: Brain-stem and adrenal abnormalities in the sudden infant death syndrome. *Am J Clin Pathol 1976; 66:526-530*

30. **Kinney HC, Burger PC, Harrell FE Jr, Hudson RP Jr**: 'Reactive gliosis' in the medulla oblongata of victims of sudden infant death syndrome. *Pediatrics 1983; 72:181-187*

31. **Quattrochi JJ, McBride PT, Yates AJ**: Brainstem immaturity in sudden infant death syndrome: a quantitative rapid Golgi study of dendritic spines in 95 infants. *Brain Res 1985;325:39-48*

32. **Takashima S, Becker LE**: Developmental abnormalities of medullar "respiratory centers" in sudden infant death syndrome. *Exp Neurol 1985; 90:580-587*

33. **Takashima S, Armstrong D, Becker LE, Bryan C**: Cerebral hypoperfusion in the sudden infant death syndrome? Brain stem gliosis and vasculature. *Ann Neurol 1978;4:257-262*

34. **Rognum TO, Saugstad OD**: Hypoxanthine levels in vitreous humor: evidence of hypoxia in most infants who died of sudden infant death syndrome. *Pediatrics 1991; 87:306-310*

35. **Krous HF, Jordan J**: A necropsy study of distribution of petechiae in nonsudden infant death syndrome. *Arch Pathol Lab Med 1984; 108:75-76*

36. **Beckwith JB**: The sudden infant death syndrome. *Curr Probl Pediatr 1973; 3:1-36*

37. **Abu-Osba YK, Mathew OP, Thach BT**: An animal model for airway sensory deprivation producing obstructive apnea with postmortem findings of sudden infant death syndrome. *Pediatrics 1981; 68:796-801*

38. **Durand DJ, Espinoza AM, Nickerson BG**: Association between prenatal cocaine exposure and sudden infant death syndrome. *J Pediatr 1990; 117:909-911*

39. **Bauchner H, Zuckerman B**: Cocaine, sudden infant death syndrome, and home monitoring. *J Pediatr 1990; 117:904-906*

40. **Silvestri JM, Long JM, Weese-Mayer DE, Barkov GA**: Effect of prenatal cocaine on respiration, heart rate, and sudden infant death syndrome. *Pediatr Pulmonol 1991; 11:328-334*

41. **Lutiger B, Graham K, Eiarson TR, Koren G**: Relationship between gestational cocaine use and pregnancy outcome: a meta-analysis. *Teratol 1991; 44:405-414*

42. **Ward SL, Keens TG**: Prenatal substance abuse. *Clin Perinatol 1992; 19:849-860*

43. **Chen C, Duara S, Silva NG, Tan S, Bandsttra ES, Gerhardt T, Banciari E**: Clinical and laboratory observations: respiratory instability in neonates with in utero exposure to cocaine. *J Pediatr 1991; 119:111-113*

44. **Doberczak TM, Shanzer S, Senie RT, Kandall SR**: Neonatal neurologic and electroencephalographic effects of intrauterine cocaine exposure. *J Pediatr 1988; 113:354-358*

45. **McCann EM, Lewis K**: Control of breathing in babies of marcotic and cocaine-abusing mothers. *Early Hum Dev 1991; 27:175-186*

46. **Seidler FL, Slotkin TA**: Prenatal cocaine and cell development in rat brain regions: effects on ornithine decarboxylase and macromolecules. *Brain Res Bull 1993; 30:91-99*

47. **Ward SL, Bautista DB, Woo MS, Chang M, Schuetz S, Wachsman L, Sehgal S and Bean X**: Responses to hypoxia and hypercapnia in infants of substance-abusing mothers. *J Pediatr 1992; 121:704-709*

48. **Suguihara C, Hehre D, Huang J, Devia C, Bancalari E**: Decreased ventilatory responses to hypoxia in sedated newborn piglets prenatally exposed to cocaine. *J Pediatr 1996; 128:389-395*

49. **Woods JR, Plessinger MA, Clark KE**: Effects of cocaine on uterine blood flow and fetal oxygenation. *JAMA 1987; 257:957-961*

50. **Slotkin TA, Seidler FJ**: Adrenomedullary catecholamine release in the fetus and newborn: secretory mechanisms and their role in stress and survival. *J Dev Physiol 1988; 10:1-16*

51. **Guntheroth WG**: Arrhythmia, apnea or arousal? In: *Sudden Infant Death Syndrome.* Tildon WT, Rolder LM,Steinschneider A (eds):. *London, Academic Press, 1983, pp253-269*

52. **Thach BT**: The role of pharyngeal airway obstruction in prolonging infantile apneic spells, In: *Sudden Infant Death Syndrome.* Tildon JT, Roeder LM, Steinschneider A (eds) *New York, Academic Press, 1983, pp279-292*

53. **Sauerland EK, Harper RM**: The human tongue during sleep: electromyographic activity of the genioglossus muscle. *Exp Neurol 1976; 51:160-170*

54. **Remmers JE, deGroot WJ, Sauerland EK, Anch AM**: Pathogenesis of upper airway occlusion during sleep. *J Appl Physiol 1978; 44:931-938*

55. **McCulloch K, Brouilette RT, Guzzetta AJ, Hunt CE**: Arousal responses in near-miss sudden infant death syndrome and in normal infants. *J Pediatr 1982; 10:911-917*

56. **van der Hal AL, Rodriguez AM, Sargent CW, Platzker AC, Keens TG**: Hypoxic and hypercapneic arousal responses and prediction of subsequent apnea of infancy. *Pediatrics 1985; 75:848-854*

57. **Fewell JE, Johnson P**: Acute increases in blood pressure cause arousal from sleep in lambs. *Brain Res 1984; 311:259-265*

58. **Baker SB, Fewell JE**: Effects of hyperoxia on the arousal response to upper airway obstruction in lambs. *Pediatr Res 1987; 21:116-120*

59. **Fewell JE, Baker SB**: Arousal from sleep during rapidly developing hypoxemia in lambs. *Pediatr Res 1987; 22:471-477*

60. **Fewell JE, Baker SB**: Arousal and cardiopulmonary response to hyperoxic hypercapnia in lambs. *J Dev Physiol 1989; 12:21-26*

61. **Henderson-Smart DJ, Read DJ**: Ventilatory responses to hypoxemia during sleep in the newborn. *J Dev Physiol 1979; 1:195-208*

62. **Jeffrey HE, Read DJ**: Ventilatory responses of newborn calves to progressive hypoxia in quiet and active sleep. *J Appl Physiol 1980; 48:892-895*

63. **Phillipson EA, Sullivan CE, Read DJ, Murphy E, Kozar LF**: Ventilatory and waking responses to hypoxia in sleeping dogs. *J Appl Physiol 1978; 44:512-520*

64. **Phillipson EA, Kozar LF, Rebuck AS, Murphy E, Kozar LF**: Ventilatory and waking responses to CO_2 in sleeping dogs. *Am Rev Res Dis 1977; 115:251-259*

65. **Fewell JE, Williams BJ, Szabo JS, Taylor BJ**: Influence of repeated upper airway obstruction on the arousal and cardiopulmonary response to upper airway obstruction in lambs. *Pediatr Res 1988; 23:191-195*

66. **Fewell JE, Konduri GG**: Influence of repeated exposure to rapidly developing hypoxemia on the arousal and cardiopulmonary response to rapidly developing hypoxemia in lambs. *J Dev Physiol 1989; 11:77-82*

67. **Davis JA**: The effect of anoxia in newborn rabbits. *J Physiol 1961; 155:56P*

68. **Godfrey S**: Respiratory and cardiovascular changes during asphyxia and resuscitation of fetal and newborn rabbits. *Q J Exp Physiol 1968; 53:97-118*

69. **Guntheroth WG, Kawabori I, Breazeale D, McGough G**: Hypoxic apnea and gasping. *J Clin Invest 1975; 56:1371-1377*

70. **Dell P, Hugelin A, Bonvallet M**: Effects of hypoxia on the reticular and cortical diffuse systems, in Gastaut H, Meyer JS (eds): *Cerebral anoxia and the electroencephalogram. Springfield, Thomas, 1961, pp 46*

71. **Ward AA**: Decerebrate rigidity. *J Neurophysiol 1947; 10:89-103*

72. **Dawes GS, Mott JC, Shelly HJ**: The importance of cardiac glycogen for the maintenance of life in fetal lambs and newborn animals during anoxia. *J Physiol 1959; 146:516-538*

73. **Shelly HJ**: Blood sugars and tissue carbohydrates in fetal and newborn lambs and rhesus monkeys. *J Physiol 1960; 153:527-552*

74. **Guntheroth WG**: The Sudden Infant Death Syndrome (Crib Death). *Am Heart J 1977; 93:784-793*

75. **Taylor PM**: Oxygen consumption in new-born rats. *J Physiol 1960; 154:153-168*

76. **Gozal D, Torres JE, Gozal YM, Nuckton TJ**: Characterization and developmental aspects of anoxia-induced gasping in the rat. *Biol Neonate 1996; 70:280-288*

77. **Adolph EF**: Regulations during survival without oxygen in infant mammals. *Respir Physiol 1969; 7: 356-368*

78. **Stafford A, Weatherall J**: The survival of young rats in nitrogen. *J Physiol 1960; 153:457-472*

79. **Adolph EF**: Tolerance to cold and anoxia in infant rats. *Am J Physiol 1948; 155:366-377*

80. **DeHaan RL, Field J**: Anoxic endurance of cardiac and respiratory function in the adult and infant rat. *Am J Physiol 1959; 197: 445-448*

81. **Peiper A**: Chapter 8. *Cerebral Function in Infancy and Childhood. New York: Consultants Bureau, 1963, p373*

82. **Gershan WM, Jacobi MS, Thach BT**: Matuation of cardiorespiratory interactions in spontaneous recovery from hypoxic apnea (autoresuscitation). *Pediatr Res 1990; 28: 87-93*

83. **Gershan WM, Jacobi MS, Thach BT**: Mechanisms underlying induced autoresuscitation failure in BALB/c and SWR mice. *Appl Physiol 1992; 72:677-685*

84. **Selardinelli L, Belloni FL, Rubio R, Berne RM**: Atrioventricular conduction disturbances during hypoxia: possible role of adenosine in rabbit and guinea pig heart. *Circ Res 1980; 47:684-691*

85. **Berne RM**: Adenosine: an important physiological regulation. *News Physiol Sci 1986; 1:163-167*

86. **Slotkin TA, Johnson DJ, Seidler FJ**: Acute effects of cocaine on ornithine decarboxylase activity in fetal and neonatal rat heart: evidence for cardiotoxicity. *Biol Neonate 1993; 63:290-296*

87. **Anderson-Brown T, Slotkin TA, Seidler FJ**: Cocaine acutely inhibits DNA synthesis in developing rat brain regions: Evidence for direct actions. *Brain Res 1990; 537:197-202*

88. **Seidler FJ, Slotkin TA**: Prenatal cocaine and cell development in rat brain regions: effects on ornithine decarboxylase and macromolecules. *Brain Res Bull 1993; 30:91-99*

89. **Mills E**: Time course for development of vagal inhibition of the heart in neonatal rats. *Life Sci 1978; 23:2717-2720*

90. **Hunt CE**: The cardiorespiratory control hypothesis for sudden infant death syndrome. *Clin. Perinatol 1992; 19:757-771*