THE UNIVERSITY OF CALGARY

Influence of Core Temperature on the Time to Last Gasp and Autoresuscitation from Primary Apnea during Hypoxic Exposure in Newborn Rats

by

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ABSTRACT

Failure to "autoresuscitate" by hypoxic gasping during sleep apnea has been suggested to play a role in sudden infant death. Furthermore, hyperthermia brought about by a contribution of fever, excessive wrapping, or excessive environmental heating has been shown to be associated with an increased risk of sudden infant death. The present experiments were carried out on newborn rat pups to investigate the influence of alterations in core temperature - over a range measured in the nest under normal physiological conditions (approximately 33 - 40 degrees Celsius) - on the time to last gasp during a single hypoxic exposure, and on the ability to autoresuscitate during repeated exposures to hypoxia. My data provide evidence that an increase in core temperature brought about by environmental heating impairs the ability of newborn rats to "autoresuscitate" from primary apnea during a single or repeated exposure to hypoxia, as may occur in some human infants during sleep apnea.

ACKNOWLEDGEMENTS

I would like to thank my supervisor, Dr. J. E. Fewell, for his unwaivering support throughout the duration of my studies in the Cardiovascular/Respiratory graduate program. Secondly, I would like to thank Dr. F. G. Smith, who presented an opportunity to me, and in so doing, proceeded to "right" a "wrong". Thanks again for the support, opportunities, and a smooth transition through start to finish.

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1.0 INTRODUCTION

1.1 The Cardiorespiratory Control Hypothesis for Sudden Infant Death Syndrome (SIDS)

Sudden Infant Death Syndrome (SIDS) is a leading cause of death of infants between the ages of 1 month and 1 year in North America, with the peak incidence occuring between 2 and 4 months of age.[1,2] Approximately, 250-400 Canadian infants die yearly from SIDS, representing a rate of reported infant deaths at 0.7 to 1.0 death(s) per 1,000 live births.[3] Although the etiology of SIDS is currently unknown, it is generally accepted that the fatal event is a result of deficient or compromised cardiac and/or respiratory function. Over the last several decades, researchers have generated a number of different hypotheses to attempt to explain the pathogenesis of SIDS, but at the present time, no single hypothesis has been universally accepted.[2,4-9] These hypotheses have been simply inadequate in accounting for all of the possible factors and interactions which may be occurring at different periods of gestation and/or in the very early neonatal period. Despite these difficulties, however, the most compelling and comprehensive hypothesis that exists to date, is the cardiorespiratory control hypothesis for SIDS.[4] It proposes a brainstem-related abnormality in conjunction with one or more other abnormalities in cardiorespiratory control, which ultimately lead to a prolonged and fatal apneic episode. It has also been proposed that a deficit in arousal and/or the gasping reflex during an episode of hypoxic apnea is the final event in the sequelae which results in a SIDS death.[4,7-8]

Since apneic episodes are known to be a common phenomenon in the human neonate, there is a growing interest in this area.[8] Both arousal and gasping have been studied extensively in several species, and researchers have established that these respiratory responses serve as vital protective mechanisms during a sleep apneic episode.[10-15] Currently, it is known that arousal is the initial response to an apneic episode during sleep, and is a potent stimulus for breathing.[7,10-11] However, in the event that the arousal response fails, a second protective mechanism exists, the gasping reflex, which may restore respiration if adequate oxygen is available in the neonate's environment.[7,12-15] This latter occurrence is known as self-resuscitation, or autoresuscitation. In the laboratory, the rodent has been frequently used to study the effects of hypoxic challenge on: (1) the time to the last gasp which is considered an arbitrary index of survival time, and (2) the ability of the animal to autoresuscitate.[12-16] The cardiorespiratory responses that accompany hypoxic challenge are as follows: initially one observes an excitement phase characterized by an increase in heart rate, myogenic activity, and hyperpnea; followed promptly by the primary apnea phase which can be as short as several seconds or as long as several minutes; and finally, the onset of gasping. If adequate oxygen is not available to the animal during the primary apnea or early gasping stage, death is the inevitable outcome barring medical intervention.[13]

1.2 The newborn's ability to withstand anoxia

The remarkable ability of the mammalian foetus and neonate to withstand birth

asphyxia or anoxia has long since been recognized. Researchers have observed that this marked resistance falls within days of parturition, and over time shows no difference from the adults level of ability to resist anoxia. In the late 1940's, to early 1960's, a number of experiments were performed to study this phenomenon.[17-23] Researchers investigated how long newborn rabbits, dogs, guinea pigs, and rats could survive while inhaling nitrogen, rather than air.[20-24] The established end point was typically a measure of the time from induction of anoxia, until observation of the final respiratory movement or last gasp. A steady decline of the time to last gasp during hypoxic challenge with an increase in postpartum age was observed. For example, Glass et al. demonstrated that in rabbits born at full term, 31 days (or 1st day of postnatal age), respiratory movement continues for an average of 34 minutes; at 32 days (2nd day), 31 minutes; at 33 days (3rd), 27 minutes; with a continuous fall until finally at 18 days postnatal, the adult level of resistance was recorded at approximately 1.5 minutes.[23]

The marked difference in the time to last gasp between the adult and newborn was consistently observed both intra- and interspecies. Adult animals succumbed at an average of 1.5-3.0 minutes after induction of anoxia, which contrasted sharply with the newborn: rats, 50 minutes; cats, 25 minutes; dogs, 23 minutes; and guinea pigs, 7 minutes.[21,23]

Apart from the age factor, it was also found that the time to last gasp of the neonate during induced anoxia varied significantly between species, as demonstrated in the rat versus the guinea pig, or 50 and 7 minutes,

respectively. It was consistently observed that newborn animals who were relatively immature at birth such as the rat, dog, or cat, were able to resist anoxia to a much greater extent than the more mature species (e.g., guinea pig, monkey).[23,24] Maturation stages were defined as the level of structural differentiation of the species at birth. For example, the newborn guinea pig shows advanced muscular co-ordination, presence of bodily hair, a higher level of nervous system development, and the ability to feed independently. In contrast, the dog and rat exhibit deficient muscular-coordination, limited locomotion, closed eyelids, integumentary development characterized by scant growth of hair, and dependence on maternal feeding (suckling).[23] Hence, it was evident that the time to last gasp was closely correlated to the stage of development of the species at parturition, which led researchers to speculate[23,47] that the maturation level of the species at birth played a crucial role in survival capacity during anoxia. From the results of these earlier studies, researchers postulated that a shift from the aerobic to the anaerobic metabolic pathway which serves to maintain ATP production during anoxia, played a critical role in the animals capacity to survive the event.[18-21,25] Anaerobic metabolism is dependent on carbohydrate stores in the tissues, and glycogen is the major storage form of carbohydrate in animals. Consequently, it was hypothesized that larger glycogen stores in major organs such as the heart, brain, or liver, as is found in the neonate compared with the adult animal of many species, would permit a sustained production of energy during anoxia, and hence, a longer survival period or higher time to last gasp in the neonate.[18-21,25,26]

In addition to postnatal age and maturation level at birth, it was clear that a

number of other factors demonstrated a correlation with increased survival time of the newborn mammal during anoxia. Several investigators demonstrated that the time to last gasp during anoxia varied inversely with ambient-induced changes in internal, or core body temperature.[20,24,27] Given that all metabolic processes are slower at lower core temperatures as a result of suppressed biochemical functions, or decreased rates of reaction, it was believed that survival time was indirectly affected through this mechanism.[20] The latter concept adheres to the Q10 effect which states that within the physiological temperature range, most reaction rates vary approximately as an exponential function of absolute temperature: increasing temperature by 10 degrees Kelvin increases the reaction rate by a factor of 2-3.[28] Since the homeothermic adult mammal regulates its internal body temperature within a very narrow range near 37 degrees Celsius, it follows that the Q10 effect would be minimal. This effect would, however, be particularly relevant in young mammals who may be less able than the adult to thermoregulate their core body temperature, and as a consequence, may experience a more pronounced shift in body temperature with a given change in ambient temperature. Any decrease in ambient temperature would then show a comcomitant decrease in the body temperature of the newborn, and an increase in survival time or time to last gasp during a period of lower metabolic demand. For example, Adolph[27] investigated the tolerance to cold and anoxia in the newborn rat in a core temperature range of approximately 0 to 40 degrees Celsius, and observed that newborn rats survived in nitrogen for two hours at 10 degrees Celsius, but for shorter times at lower or higher temperatures. It was inferred that below 10 degrees Celsius, the destruction of enzymatic processes was likely occuring, and therefore this

clearly constituted temperatures outside of the newborn rat's physiological temperature range during exposure to anoxia. At temperatures of 10 degrees and above, a strong inverse correlation with survival time was demonstrated, and therefore metabolic processes within this range were likely dominated by the Q10 effect.

Although, it is very clear that the observed correlations from the earlier studies do not imply a causative relationship between time to last gasp and temperature, metabolic rate, species or maturational differences, these results are still very much the basis of present investigations, and many researchers believe that these factors may be linked to the underlying pathophysiology of SIDS.[12-15]

1.3 Body Temperature and SIDS

Over the last decade, a number of epidemiological studies have been performed to attempt to determine potential risk factors for SIDS.[29-35] Many of these investigations have involved case-control studies using either near-miss infants and/or siblings of SIDS infants, both of which have been reported to be at a higher risk for SIDS than an infant in the general population.[29] The near-miss infant is often referred to as an ALTE, or infant who has experienced an apparent life-threatening event (A-L-T-E), in which the infant has previously been found in a lifeless condition requiring resuscitation. An ALTE infant may show certain signs and symptoms of risk factors for SIDS either before and/or after the apparent life-threatening event, such as profuse sweating, increased episodes of apnea, repeated upper airway obstructions during sleep,

cardiovascular or other respiratory abnormalities, etc... A growing body of evidence in the literature suggests a possible link between hyperthermia, and the occurence of a SIDS event.[29-31,35,36] Hyperthermia of the infant may be due to one or more of the following factors: high environmental temperature; overdressing; or fever resulting from an infective illness. Several studies have reported observations by the parents upon discovery of their dead infant, in which they described the infant as "sweating excessively", or feeling "abnormally" warm to the touch.[31,33,35] In a case study performed by Stanton[35], the environmental conditions to which the infants were exposed just prior to death were examined individually, and retrospectively, as well as any clinical evidence available from examination of the dead infant upon presentation to the hospital. It was reported that: 19/34 (56%) babies were unusually hot or sweating profusely when found dead; out of 15 of these infants who had their rectal temperatures recorded, 6 (40%) were above 37 degrees Celsius; 24/34 (71%) babies were excessively clothed or wrapped; and 17/34 (50%) had evidence of terminal infective illness. The results from this study also suggested that 94% of cases examined had presented with one or more of the specified risk factors. Other epidemiological findings include a consistent and strong association between SIDS and low environmental temperature such that an excess of SIDS cases occur during the winter months.[30] It has been proposed that parents would more frequently overdress the infant during these periods of colder temperatures. An association between prone sleep and an increased risk for SIDS has been reported for several decades, and it is believed that prone sleep could contribute to conditions predisposing to hyperthermia by increasing the infant's body area in contact with the underbedding, resulting in impaired heat loss.[29,36] In addition, relationships between: thermoregulation

and control of respiration; autonomic dysfunction and ambient temperature variation; sleep state and thermoregulation; incidence of obstructive sleep apnea and heavy sweating, have all been reported and investigators have hypothesized possible links to the underlying pathophysiology of SIDS.[32,37-40] Interestingly, it has been suggested that certain pharmacological agents such as phenothiazines, a type of anticholinergic drug, used in the treatment of colic may increase an infants risk for SIDS.[41,42] In addition to their therapeutic advantage in the treatment of colic, the anticholinergic effects of phenothiazines act to depress the sweat mechanisms through the inhibition of sympathetic transmission, and as a result, may predispose the infant to hyperthermia. It should be recognized that the majority of these epidemiological studies have been based on anecdotal evidence and therefore their internal validity is questionable. Consequently, there is presently a neccessity for carefully controlled experimental studies to investigate these reported risk factors for SIDS.

In accordance with data from the above epidemiological studies, it has been suggested that an important link may exist between variation in ambient temperature, an immature thermoregulatory response in the neonate, and the occurrence of SIDS.[36] This evidence also corresponds with the data from earlier studies (see above) that found inverse correlations between ambient temperature and time to last gasp in a number of species. A higher core body temperature induced by an elevation in ambient temperature was consistently found to be associated with a reduction in time to last gasp. These researchers have proposed that this observation was at least in part the result of

alterations in metabolism secondary to changes in temperature. Attempts to extrapolate these results to the human situation is not without its complications. For example, the poikilothermic characteristics of the newborn rat which have been described by several investigators are not present in the human infant, whom under normal physiological conditions is able tc regulate its body temperature within a narrow range despite relatively large fluctuations in ambient temperature.[27,43-45] It is conceivable that underdeveloped sweat mechanisms which have been previously observed in the human neonate, coupled with various external stressors such as prone sleeping position, overdressing of the infant, high ambient temperature, and a number of other possible factors, could act to overwhelm the infants thermoregulatory control and consequently predispose the infant to hyperthermia. Under these circumstances, the Q10 effect would predominate, causing a significant rise in metabolic rate and a rapid exhaustion of substrate reserves critical to ATP production. In the event of hypoxic challenge, or an episode of sleep apnea, these effects could possibly create sufficient conditions for a lethal event in the young mammal or human infant, and may in fact have significant relevance to the pathogenesis of SIDS. Although there is a physiological basis for a causal relationship between abnormal respiratory patterns such as an impaired arousal response or gasping reflex, and alterations in metabolism of the neonate secondary to ambient-induced changes in core body temperature; research in this area is still lacking. Clearly, studies of this nature could potentially provide valuable information in the etiology and prevention of further SIDS victims.

1.4 Aim and Hypothesis

The majority of the earlier studies designed to investigate the time to the last gasp in newborn mammals were performed either with a lack of consideration for ambient-induced changes in core body temperature of the animal under study, or at a very broad ambient temperature range (0-40 degrees Celsius) corresponding to core body temperatures far beyond the normal physiological range observed within the nest.[16-24,27] The ability to extrapolate these data to the clinical situation was therefore limited. Secondly, the influence of core body temperature on the ability to autoresuscitate during hypoxic challenge has not been adequately investigated to date. The more recent studies in rodents investigating the time to last gasp, and ability to autoresuscitate have mainly been focused on age-related differences, strain or species differences, and characterizing phases of the gasping response to anoxia, but few studies have been performed to investigate the influence of external or environmental stressors on these vital respiratory responses during hypoxic challenge.[12-15,46]

Based on the preceding information, I therefore focused on one of a number of possible environmental stressors: core temperature variation as influenced by alterations in ambient temperature, which has also been implicated as a risk factor for SIDS. The purpose of this study was to determine if the independent variable: ambient temperature-induced changes in core body temperature in a range consistent with normal physiological temperatures in the nest (at approximately 33-40 degrees Celsius), influence the dependent variables: (1) the time to the last gasp during a single hypoxic exposure, and/or (2) the

ability to autoresuscitate during repeated hypoxic exposures, in the 5-6 day old rat pup. I tested the hypothesis that an increase in core temperature, brought about by environmental heating, impairs the ability of newborn rats to autoresuscitate from primary apnea during a single or repeated exposure to hypoxia, as may occur in some human infants during sleep apnea.

2.0 MATERIALS AND METHODS

2.1 Animals

Fifty-three, five to six day old Sprague-Dawley pregnant rats (received from Charles River Breeding Laboratories) were studied. Each pup, born by spontaneous vaginal delivery, was housed separately in plastic cages at 22± 1 degrees Celsius in a light-dark cycle with lights on from 0700 to 1900 and with continuous access to food (Lab Diet 5001) and tap water. The gestational period for these rats ranges between 21-22 days. Following parturition, the rat pups remained with their mother and siblings until commencement of the experiments. Although the pups were housed at a temperature below the thermoneutral zone (~35 degrees Celsius) of newborn rats, we have previously observed in our laboratory that these pups are able to select their ambient temperature between experiments by huddling with their mother or siblings, which suggests that they are engaging in a type of behavioural thermoregulation.

2.2 Ethical Considerations

All 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 Experimental Protocol

2.3.1 Time to Last Gasp Experiments

The first experiment was performed to determine the time to the last gasp of five to six day old rat pups at five different core temperatures (Tc) during a single exposure to hypoxia. Experiments were carried out on 35 rat pups (age five to six days) with seven rat pups at each of the five selected ambient temperatures. On the day of an experiment, the dam and rat pups were brought to the laboratory and left in their cage until observation. For each experiment, one rat pup to be studied a single time, was selected randomly from the litter, and subjected to weighing and sex determination. Immediately afterward, two ECG electrodes, each created from one metre of biomed wire (AS 633, Cooner Wire Company) were sewn into the skin: one located between the scalpulae, and the other located on the right side, just proximal to the hindleg. The wire was stripped with a pair of wire strippers at the point of contact with the skin, and lubricated with a sterile lubricating jelly (Taro Pharmaceuticals) to ensure an adequate electrical signal. A 40 mm mercury strain gage (DM Davis Incorporated) was placed around the chest of the pup to record respiration, and a thermocouple inserted one cm into the rectum and secured with Vetbond tissue adhesive (3M Animal Care Products). The rat pup was then placed in the metabolic chamber regulated to either of the five preselected ambient temperatures (33, 35, 37, 38 or 40 degrees Celsius), and left to stabilize for 30 minutes. Ambient temperatures were chosen randomly for each time to last gasp experiment. Throughout the stabilization period, the chamber was flushed with room air at approximately one L/min. At 30 minutes, control

ECG, respiratory rate, and temperature were recorded. The chamber was then flushed with a 97% nitrogen-3% carbon dioxide mixture (time zero), until gas concentrations in the chamber were observed to have stabilized, at which time the flow rate was reduced and maintained at one L/min for the remaining experimental period. The polygraph recording (see description of apparatus below) was continued until five minutes after the final gasp was observed. Rectal temperature was recorded at two minute intervals from the onset of induction of hypoxia.

The range of ambient temperatures (i.e., temperatures between 33 and 40 degrees Celsius) was selected based on previous data collected in our laboratory, where we measured mean rectal temperatures of the rat pups in their nest in several different orientations: huddled with their mother and siblings; huddled with their siblings only; or huddled alone in the nest. Becaused young rat pups are known to exhibit poikilothermic characteristics[27,43], this ambient temperature range represented an approximation of the core temperature range measured in the nest under normal physiological conditions.

2.3.2 Autoresuscitation Experiments

The second experiment was performed to assess the ability of the rat pups to autoresuscitate at three different Tc's during repeated episodes of hypoxia. The animal preparation and set-up for these experiments was identical to the time to last gasp experiments, but the experimental protocol varied slightly from that of the time to last gasp. A total of 18 animals were used in these

experiments with six animals at each of the three preselected ambient temperatures of 33, 37, and 40 degrees Celsius. Subsequent to the recording of control respiration, ECG, and temperature, the chamber was flushed with 97% nitrogen - 3% carbon dioxide and respiration closely monitored on the polygraph for evidence of primary apnea. When primary apnea was observed, the chamber was flushed with room air, and the animal given a five minute window to autoresuscitate (from the time of induction of anoxia). This procedure was repeated at five minute intervals until the animal was unable to autoresuscitate. Temperature readings were recorded at 25 minute intervals from the time of the initial induction of anoxia.

2.4 Apparatus

The metabolic chamber consisted of a double-walled Plexiglas cylinder (8 X 2.5 cm). Chamber ambient temperature was controlled by circulating water from a temperature-controlled bath (Model 1147, VWR Scientific) through the space between the walls. ECG, respiration, and oxygen concentration in the chamber were monitored on a Grass polygraph (model 7E, Grass Instruments Company), and the polygraph run at 10 mm/sec throughout the experiment. The concentration of oxygen in the chamber was measured using an oxygen analyzer (model S-3A, AMETEK). Temperature was recorded using Iso-Thermex software (Columbus Instruments International Corporation).

2.5 Analysis of results

2.5.1 Time to Last Gasp Experiments

Control temperature readings were taken to be the true Tc of the animal throughout the experiment. Control respiratory rates (RR_O) and control heart rate (HR_O) per minute were measured from the polygraph during conditions of normoxia. HR and the number of gasps per minute were also measured at each minute interval following the time from the induction of anoxia. Time to last gasp was determined from the time of induction of anoxia to the final gasp. The total number of gasps over the period of hypoxic exposure was also determined.

2.5.2 Autoresuscitation Experiments

The initial control temperature readings were taken to be the true Tc of the animal throughout the experiment. RR_O and HR_O per minute were measured from the polygraph at every 15 minute interval (i.e., at each odd numbered hypoxic exposure) under normoxic conditions. The number of successful autoresuscitations (SA) was also measured from the polygraph. In previous studies investigating autoresuscitation from hypoxic apnea in mice, the investigators typically deemed SA to occur when heart rate and respiratory rate returned to >60% of control levels within five minutes.[12,14] This standard was also used in our experiments.

2.6 Statistics

Mean values for the measured and calculated variables were determined for each experiment. Statistical analysis was carried out using the Pearson Product Moment Correlation (r) coefficient to determine the degree of association between core temperature and chamber ambient temperature, and core temperature and the time to the last gasp. A one-factor ANOVA for repeated measures followed by a Newman-Keul's multiple comparison test was applied to determine if variation in core temperature affected the time to the last gasp, or the number of successful autoresuscitations. This test was also applied to determine if core temperature variation influenced control heart rate, control respiratory rate, or the total number of gasps during a single hypoxic exposure. All results are reported as means $\pm$ one standard deviation, and $p<0.05$ was considered to be of statistical significance. All components of the analysis were carried out using Sigmastat v2.0 (SPSS Inc., Chicago, IL) on an IBM compatible computer.

3.0 RESULTS

3.1 Chamber Ambient Temperature and Core Temperature

Core temperature correlated strongly with chamber ambient temperature as illustrated in figure 3.1 ($r=0.93$; $p<0.001$).

3.2 Time to Last Gasp (TLG) Experiments

Chamber ambient temperatures (Ta) and the corresponding mean core temperatures (Tc) are shown in Table 3.1. Alterations in Tc did not significantly alter mean control respiratory rate (Table 3.2). Mean control heart rate at 39 degrees Celsius was statistically significant from mean control heart rate at all other Tc's ($p<0.05$; Table 3.2). An increase in core temperature significantly decreased the time to the last gasp. A Newman-Keuls multiple comparison test indicated that mean TLG's at a given Tc were statistically significant compared with mean TLG's at all other Tc's with the exception of 35 vs. 36 degrees Celsius ($p<0.05$; Table 3.2). Core temperature showed a strong inverse correlation with the time to the last gasp as illustrated in figure 3.2 ($r = -0.80$; $p<0.001$). There were no significant effects of Tc variation on the mean total number of gasps during the hypoxic exposure (Table 3.2).

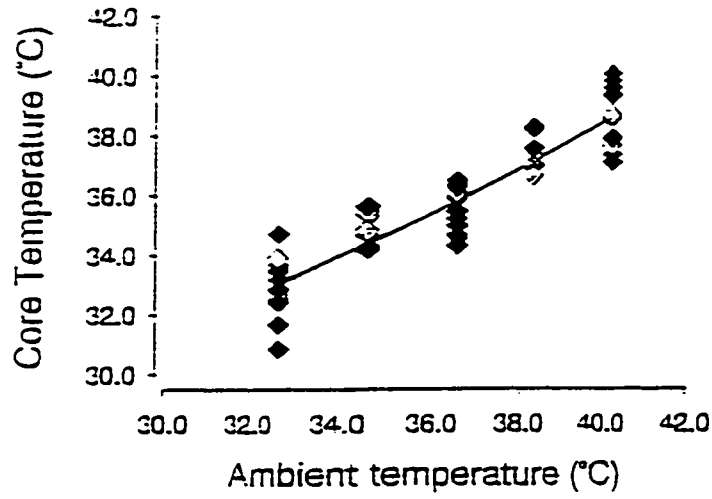

Figure 3.1 Relation between core temperature and chamber ambient temperature of 53, five to six day old rat pups. Rectal temperatures of the pups were measured after a 30 minute stabilization period in a metabolic chamber regulated to either 33, 35, 37, 38, or 40 degrees Celsius. Individual values and regression line are plotted; Pearson Product Moment Correlation Coefficient (r) = 0.93; $p<0.001$.

TABLE 3.1 Mean core temperatures (Tc) of rat pups in a metabolic chamber regulated to different ambient temperatures (Ta)

Temperature (degrees Celsius)	
Ta	Tc[a]
33	33±1
35	35±1
37	36±1
38	37±1
40	39±1

[a]Values are means ± SD for 7 rat pups at each given Ta.

TABLE 3.2 Effect of mean core temperature (Tc) on control respiratory rate (RRo), control heart rate (HRo), TLG, and total number of gasps (Gasp #) during a single exposure to hypoxia

Tc	RRo[a]	HRo	TLG	Gasp[a]
(degrees Celsius)	(breaths/min)	(beats/min)	(seconds)	(#)
33±1	139±33	401±35	*1108±212	56±12
35±1	133±12	407±19	**752±185	55±8
36±1	123±13	386±33	**761±183	56±10
37±1	126±15	405±45	*591±44	45±6
39±1	146±18	*448±27	*425±64	48±8

Values are means ± SD for 7 rat pups at each Tc.

[a]There were no statistically significant differences between Tc groups for these variables. *$p<0.05$, compared with all other Tc groups. **$p<0.05$, compared with Tc groups - 33, 37, and 39 degrees Celsius.

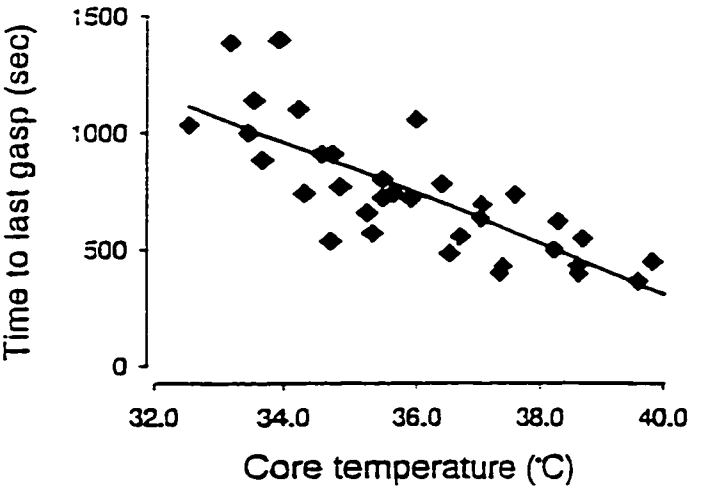

Figure 3.2 Relation between time to the last gasp and core temperature of 35, five to six day old rat pups. Pups were placed in a metabolic chamber regulated to either 33, 35, 37, 38, or 40 degrees Celsius, and exposed to a single period of hypoxia (97%N_2 & 3% CO_2), and the time to last gasp determined. Individual values and regression line are plotted; Pearson Product Moment Correlation Coefficient (r) = -0.80; $p<0.001$.

Exposure to a single period of hypoxia resulted in a similar respiratory response in all five groups of animals as illustrated in figure 3.3 (example shown is a pup at chamber ambient temperature of 37 degrees Celsius) with the exception of the gasping rate pattern. Initially there was a period of hyperpnea and arousal which preceded primary apnea (a); primary apnea was followed by a period of rapid gasping that lasted one to two minutes (b); this period of rapid gasping was followed by a period of slower gasping of one to three gasps per minute that decreased with an increase in temperature, lasting only seconds at a core temperature of 39 degrees Celsius, whereas at 33 degrees Celsius, this period lasted up to nine minutes (c - temperature effect not shown - see figures 3.4A-C); finally there was a period of rapid gasping which eventually waned and gave way to terminal apnea and death (d). Figures 3.4A, 3.4B, and 3.4C illustrate the relation between core temperature and the duration of the slow gasping period, at chamber ambient temperatures of 33, 37, and 40 degrees Celsius (35, and 38 degrees Celsius not shown), respectively. In all animals, gasping ceased before the appearance of arrhythmias or an isoelectric pattern on the electrocardiogram. Exposure to a single period of hypoxia at the different core temperatures demonstrated a temperature dependent effect on heart rate pattern as illustrated in figures 3.5A, 3.5B, and 3.5C corresponding to chamber ambient temperatures of 33, 37, and 40 degrees Celsius (35, and 38 degrees Celsius not shown), respectively. Heart rate was higher at a core temperature of 39 degrees Celsius during hypoxic exposure in comparison to heart rate at 33 degrees Celsius, showing a general tendency to increase with an increase in core temperature. In addition, heart rate fell markedly in all groups during the transition from the initial period of rapid gasping to the period of slower gasping.

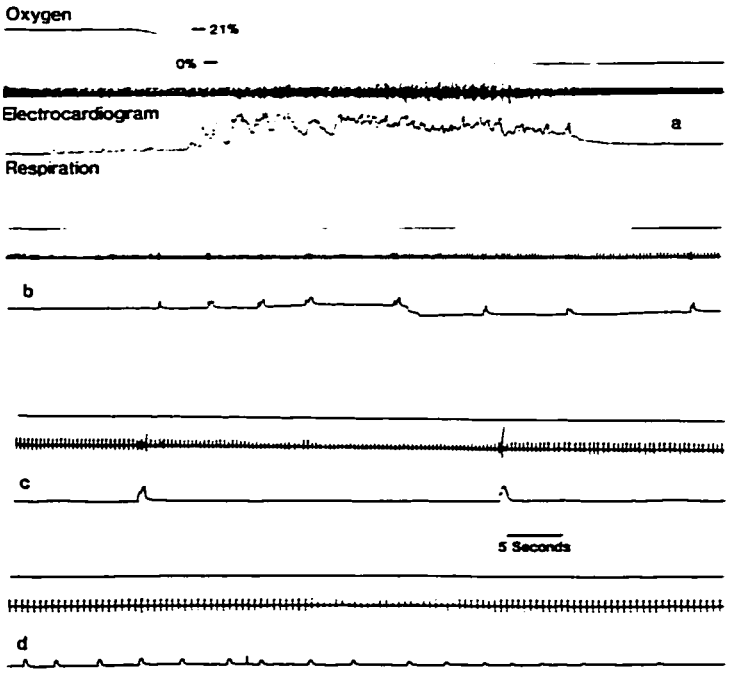

Figure 3.3 Four segments of a continuous polygraph tracing showing the respiratory response of a 5-day old rat pup to a single period of hypoxia at a chamber ambient temperature of 37 degrees Celsius. Variables shown are chamber carbon dioxide concentration, electrocardiogram and respiratory pattern. Exposure to a single period of hypoxia resulted in a similar respiratory response at the five different core temperatures with the exception of the gasping rate pattern (not shown). Initially there was a period of hyperpnea and arousal which preceded primary apnea (a); primary apnea was followed by a period of rapid gasping that lasted one to two minutes (b); this period of rapid gasping was followed by a period of slower gasping of one to three gasps per minute that showed a tendency to decrease with an increase in core temperature (c), (temperature dependence not shown - see figures 3.4A-C); finally there was a period of rapid gasping which eventually waned and gave way to terminal apnea and death (d).

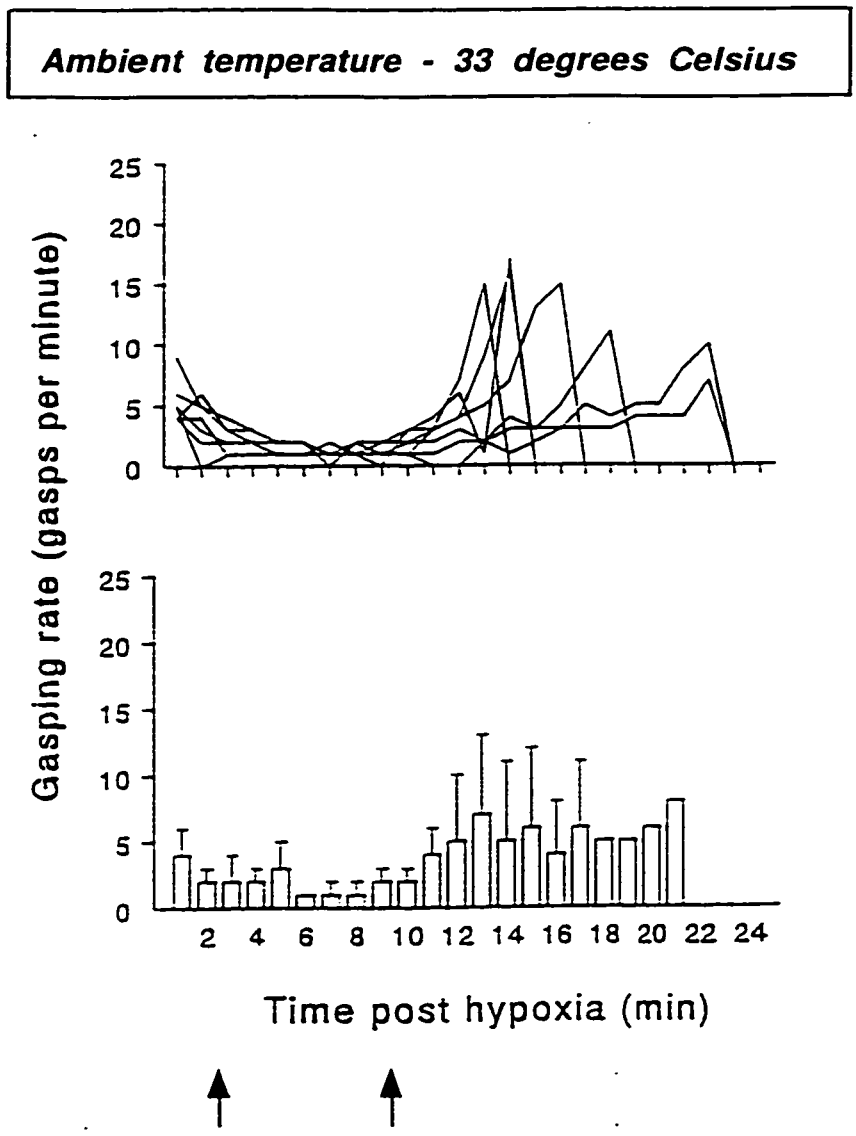

Figure 3.4A The effect of variation in core temperature on the gasping rate response to a single period of hypoxia. As core temperature increased (see also figures 3.4B & 3.4C), the period of slower gasping (arrows indicate relative length of period) showed a tendency to decrease. The graph shows raw data and means plus 1 standard deviation. n = 7

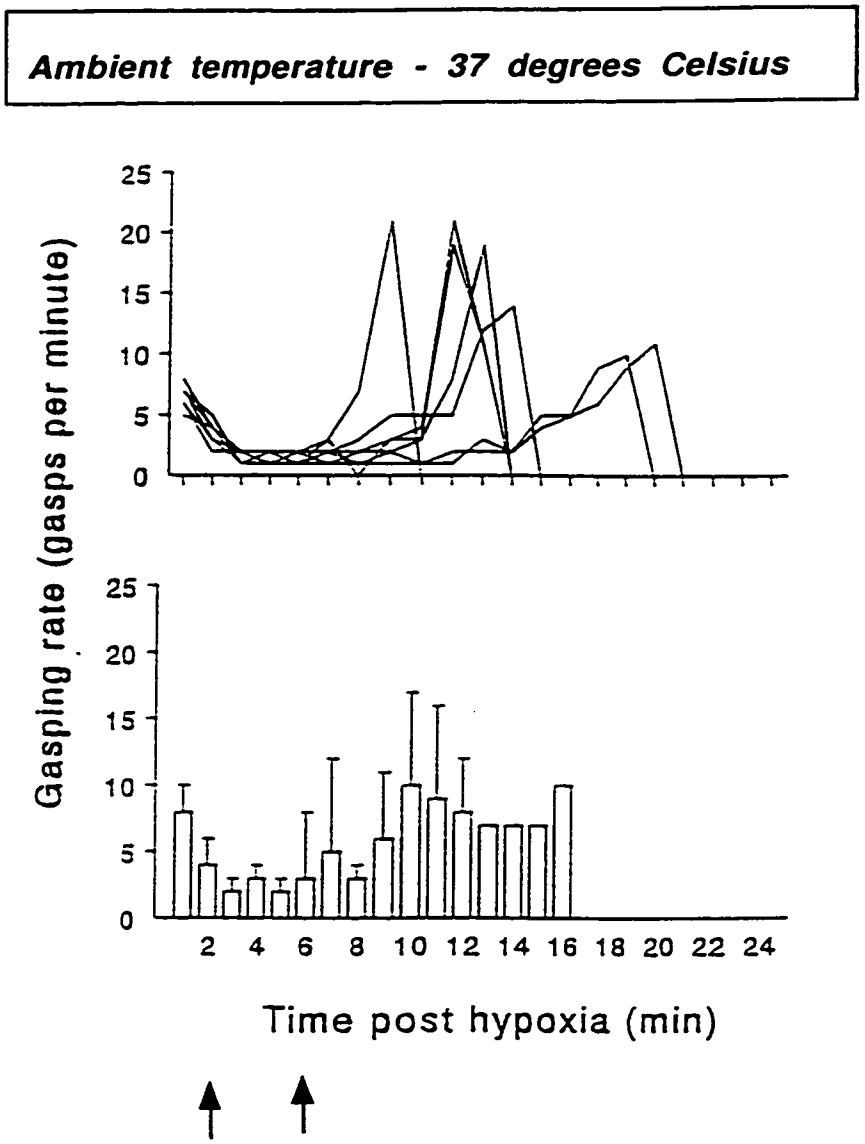

Figure 3.5B The effect of variation in core temperature on the gasping rate response to a single period of hypoxia. As core temperature increased (see also figures 3.4A & 3.4C), the period of slower gasping (arrows indicate relative length of period) showed a tendency to decrease. The graph shows raw data and means plus 1 standard deviation. n = 7

Ambient temperature - 40 degrees Celsius

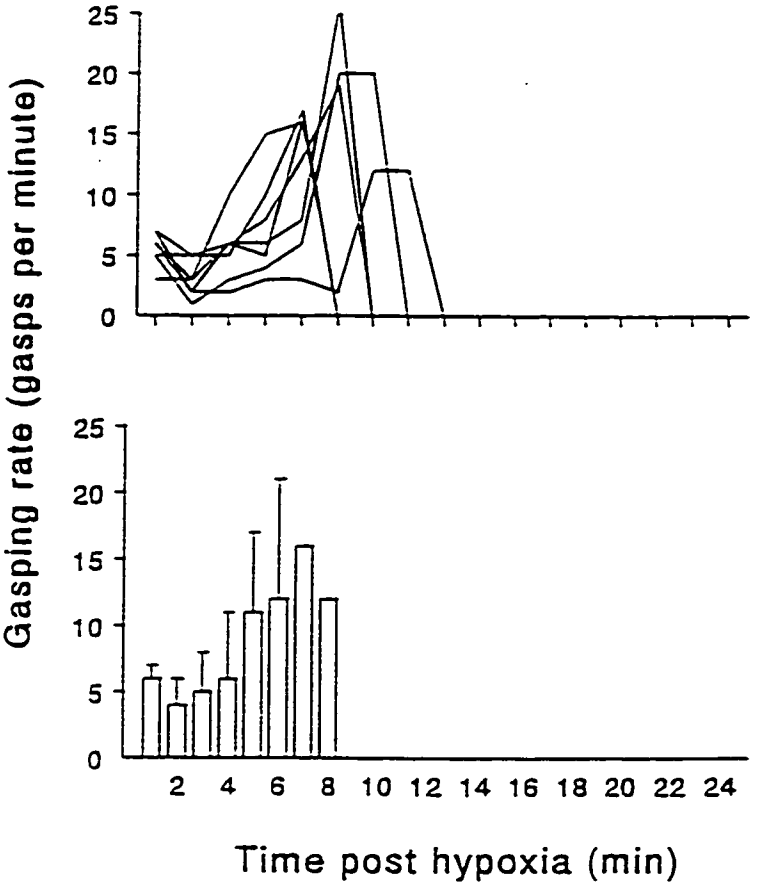

Figure 3.6C The effect of variation in core temperature on the gasping rate response to a single period of hypoxia. As core temperature increased (see also figures 3.4A & 3.4B), the period of slower gasping (arrows indicate relative length of period) showed a tendency to decrease. The graphs shows raw data and means plus 1 standard deviation. n = 7

Ambient temperature - 33 degrees Celsius

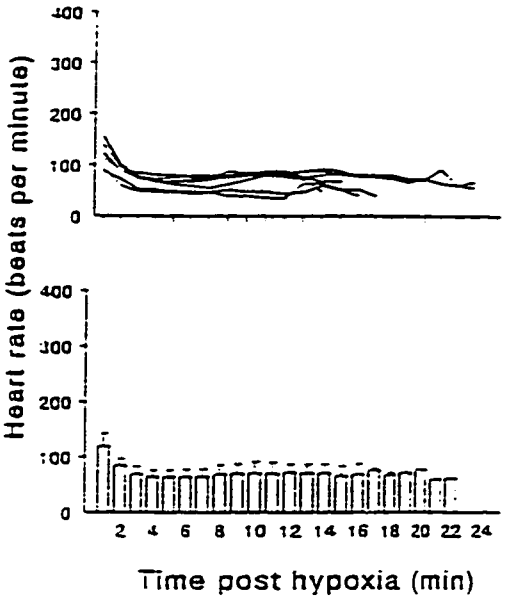

Figure 3.5A The effect of variation in core temperature on the heart rate response to a single period of hypoxia. As core temperature increased (see also figures 3.4B & 3.4C), the heart rate showed a tendency to increase. A marked heart rate deceleration can be seen at each temperature corresponding to the transition (arrow) from the initial period of rapid gasping to the period of slower gasping. The graph shows raw data and means plus 1 standard deviation. n = 7

Ambient temperature - 37 degrees Celsius

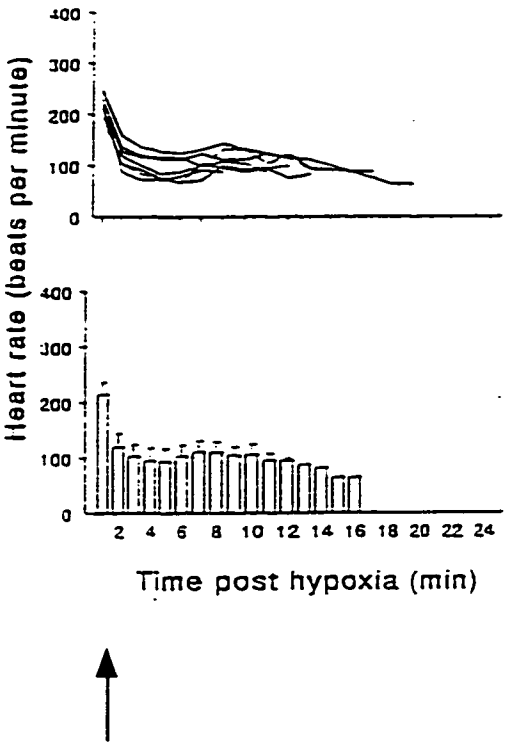

Figure 3.5B The effect of variation in core temperature on the heart rate response to a single period of hypoxia. As core temperature increased (see also figures 3.4A & 3.4C), the heart rate showed a tendency to increase. A marked heart rate deceleration can be seen at each temperature corresponding to the transition (arrow) from the initial period of rapid gasping to the period of slower gasping. The graph shows raw data and means plus 1 standard deviation. n = 7

Ambient temperature - 40 degrees Celsius

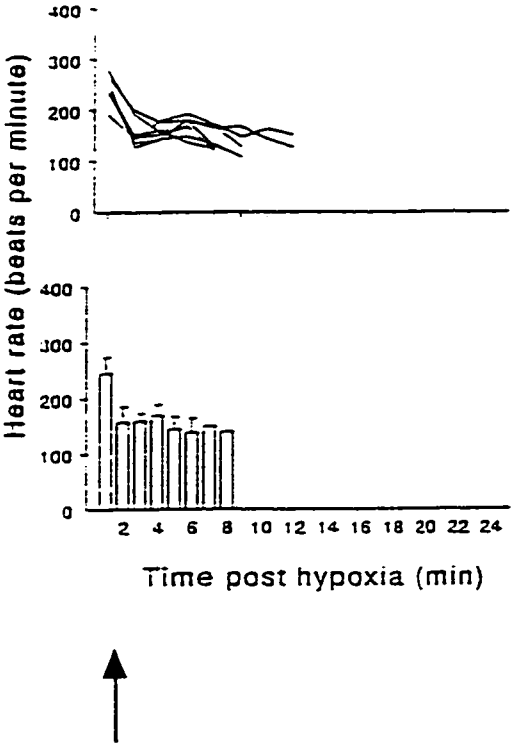

Figure 3.5C The effect of variation in core temperature on the heart rate response to a single period of hypoxia. As core temperature increased (see also figures 3.4A & 3.4B), the heart rate showed a tendency to increase. A marked heart rate deceleration can be seen at each temperature corresponding to the transition (arrow) from the initial period of rapid gasping to the period of slower gasping. The graph shows raw data and means plus 1 standard deviation. n = 7

3.3 Autoresuscitation Experiments

Chamber ambient temperatures (Ta) and the corresponding mean core temperatures (Tc) are shown in Table 3.3. An increase in core temperature impaired the ability of the rat pups to autoresuscitate from primary apnea (Table 3.3). The mean number of successful autoresuscitations was statistically significant between Tc's 38 and 32 degrees Celsius ($p<0.05$). Before autoresuscitation failure, all successful autoresuscitations exhibited the same cardiorespiratory pattern as illustrated in figure 3.6. Initially there was a period of hyperpnea and arousal (a) which preceded primary apnea and bradycardia (b), at which time the chamber was flushed with room air; the onset of gasping (c) was followed by an increase in heart rate and gasping rate (d) and then restoration of a normal heart rate and respiratory pattern (e). The mechanism of autoresuscitation failure, however, appeared to be different in a significant number of the pups at a core temperature of 38 degrees Celsius, in comparison to the other two core temperatures. In all of the pups with core temperatures of 32, and 35 degrees Celsius, autoresuscitation failure was associated with cardiac arrhythmia (i.e., A-V dissociation) that preceded the cessation of gasping (figure 3.7). In three of the six pups at a core temperature of 38 degrees Celsius, however, autoresuscitation failure was associated with the cessation of gasping that preceded cardiac arrhythmia on the electrocardiogram.

TABLE 3.3 Effect of mean core temperature (Tc) on the number of successful autoresuscitations (SA#) during repeated exposures to hypoxia

Temperature (degrees Celsius)		SA[b]
Ta[a]	Tc[b]	(#)
33	32±1	*18±4
37	35±1	15±2
40	38±1	*11±4

[a]Values represent chamber ambient temperature.

[b]Values are means ± SD for 6 rat pups within each Tc group. *$p<0.05$, comparing SA# at Tc's: 38 vs. 32 degrees Celsius.

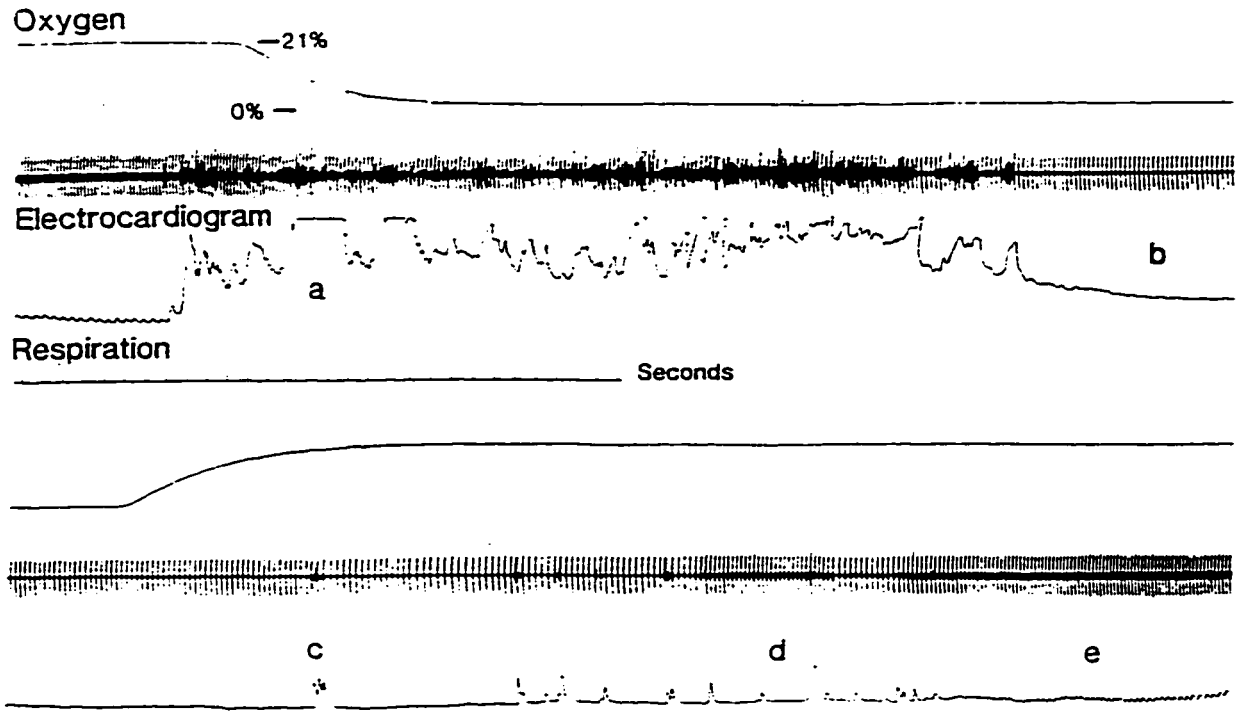

Figure 3.6 Continuous polygraph tracing showing a successful autoresuscitation from primary apnea in a rat pup at a chamber ambient temperature of 37 degrees Celsius. During exposure to hypoxia there was an initial period of hyperpnea and arousal (a) which preceded primary apnea and bradycardia (b), at which time the chamber was flushed with air; the onset of gasping (c) was followed by an increase in heart rate and gasping rate (d) and then restoration of a normal heart rate and respiratory pattern (e).

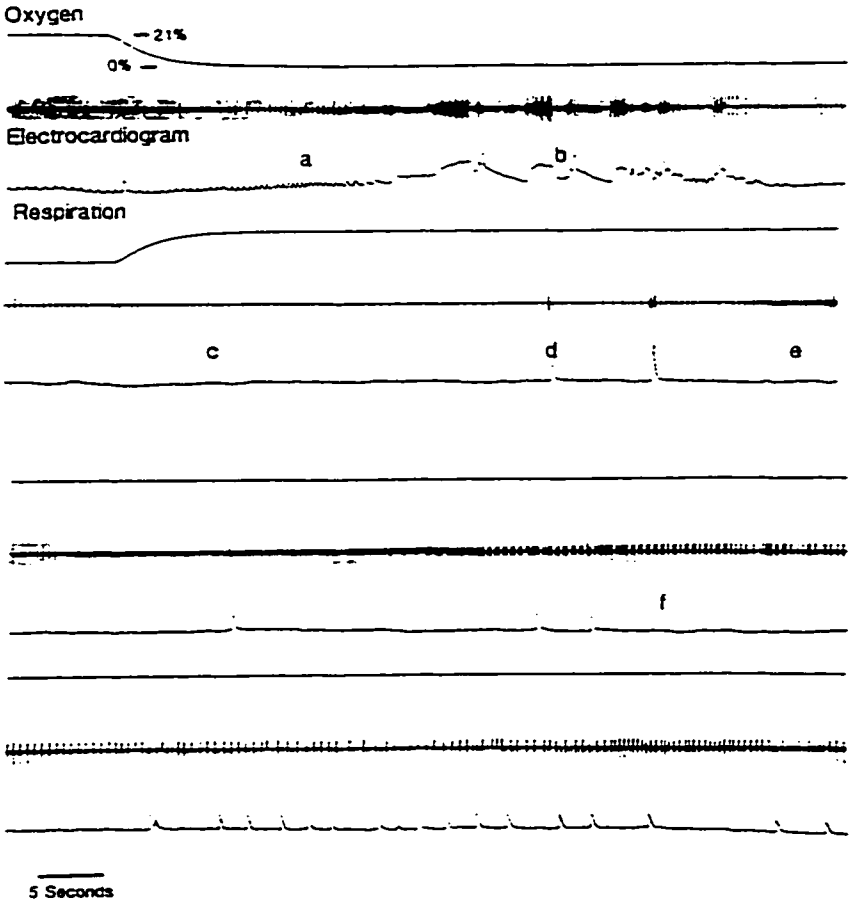

Figure 3.7 Continuous polygraph tracing showing autoresuscitation failure following repeated exposure to hypoxia in a rat pup at a chamber ambient temperature of 37 degrees Celsius. During exposure to hypoxia there was an initial period of hyperpnea (a) and arousal (b), which preceded primary apnea and bradycardia (c), at which time the chamber was flushed with air; the onset of gasping (d) was followed by an increase in heart rate (e) and then the occurence of cardiac arrhythmia (f) which preceded cessation of gasping. In all of the pups at core temperatures of 32 and 35 degrees Celsius, autoresuscitation failure was associated with cardiac arrhythmia (i.e., A-V dissociation) that preceded the cessation of gasping.

4.0 DISCUSSION

My experiments provide new information about factors that influence the newborn's ability to survive hypoxia as may occur during an episode of sleep apnea. My experiments show that an increase in core temperature reduced the time to last gasp following a single hypoxic exposure. A novel finding in this experiment was that although the gasping rate pattern was altered by changes in core temperature, the inverse relationship between core temperature and time to the last gasp was independent of the total number of gasps during the hypoxic exposure. A second novel finding in my study was that an increase in core temperature impairs the ability of rat pups to autoresuscitate from primary apnea during repeated exposure to hypoxia. Furthermore, my results suggest that an increase in core temperature may have influenced the mechanism of autoresuscitation failure. In all rat pups at core temperatures of 32 or 35 degrees Celsius, autoresuscitation failure followed cardiac arrhythmia (i.e., A-V dissociation) that preceded the cessation of gasping. In three of the six rat pups at a core temperature of 38 degrees Celsius, however, autoresuscitation failure followed the cessation of gasping that preceded cardiac arrhythmia. Thus, my data provide evidence that an increase in core temperature not only impairs the ability of newborn rats to autoresuscitate from primary apnea during repeated exposure to hypoxia, but that it may alter the mechanism of autoresuscitation failure.

Exposure to a single period of hypoxia resulted in a similar respiratory response in rat pups at all five core temperatures. 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) that lasted one to two minutes following cessation of primary apnea, (2) this period of rapid gasping was followed by a period of slower gasping (phase II) in which the duration was inversely related to core temperature, and finally, (3) a period of rapid gasping (phase III) which eventually waned and gave way to terminal apnea and death. A recent developmental study by Gozal *et al.* [46], has also shown that the anoxia-induced gasping response in rat pups <25 days old is triphasic in nature and displays a similar gasping rate pattern during each phase corresponding to those observed in our study. In addition to describing the gasping rate during each phase, Gozal *et al.* [46] went one step further by characterizing particular features of a single gasp. It was noted that the "Type I" gasp, characterized by an expiratory excursion preceding an inspiratory effort and finally by a second expiration, was more prevalent in phase I of the gasping response of the neonate. Type II gasps consisted of an initial inspiratory effort followed by a small expiratory component, and appeared only at the final stages of phase II; being more prevalent during phase III. These investigators have proposed that the initial expiratory component of the Type I gasp may serve a particular purpose in the early stages of hypoxia by acting to clear an obstructed airway. In the absence of oxygen, the expiratory component undergoes functional loss at a later stage of anoxia, giving way to Type II gasps exclusively, which may in effect be serving as an intrinsic energy conservation measure. It is well known that maturity at birth[47], and postnatal age[17,23,27], are inversely correlated with the time to last gasp in a number of species. Previous studies have also shown an inverse correlation between body temperature[17,22,27] and

the time to last gasp as reported in this study. These investigators have attributed gasping duration, in part, to alterations in metabolism, and glycogen tissue reserves. In my experiments then, the marked temperature effect on the duration of phase II of the gasping response may be related to the metabolic rate of the rat pup. More specifically, it would be expected that the metabolism of the rat pup would increase with temperatures above its thermoneutral zone which may ultimately be causally related to the observed decrease in gasping duration. Gozal *et al.* [46], also reported a marked inverse relationship between age and duration of the three phases. Interestingly, their results also suggested a stronger age-dependent effect on phase II duration in comparison to phase I or III duration. A further observation by Gozal and colleagues[46] demonstrated that a pronounced deceleration in heart rate occurs from phase I to phase II. My study has also shown a deceleration in heart rate at the onset of phase II, however, a temperature dependent effect on heart rate during hypoxia was also evident. Swann, Christian, and Hamilton[16] have previously attributed the bradycardic response to the animals ability to achieve energy efficiency during anoxia. In my study, it is conceivable that the higher heart rate in relation to a higher core temperature was detrimental in the pups ability to conserve energy during anoxia, and ultimately contributed to the observed reduction in the time to last gasp.

A seemingly contradictory finding in my study has shown that the total number of gasps was independent of core temperature. Thus, the total number of gasps throughout an episode of hypoxia would seem to be inconsistent with the possibility that an intrinsic metabolic response to conserve energy during

hypoxia is a key factor in gasping duration.

A number of other possibilities to explain my findings exist. It has been suggested[38] that hyperthermia may lead to CNS damage, the most notable being loss of sensitivity of the respiratory chemoreceptors. Alternatively, high core temperature may act directly to depress the "gasping center", which has been localized to the lateral tegmental field of the medulla in the rat[48], and is believed to be distinct from the region responsible for eupnoea.

Nevertheless, it would appear that phase II of the gasping response is a critical phase, and that certain factors or environmental stressors may have a larger influence on the animals ability to sustain its gasping efforts during this period. Although, my experiments were clearly not designed to assess the effects of temperature on the particular type I and type II gasps, described by Gozal and his colleagues[46] during each phase of the gasping response, this type of study could provide valuable information in our understanding of how temperature, as well as other environmental stressors, affect the pattern of gasping elicited by hypoxic apnea in rat pups.

Peiper[49], Stevens[50], and Thach[7] 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[47] and then "autoresuscitation" in 1975 by Guntheroth[51]. Gershan, Jacobi and Thach[12] have recently defined the

cardiorespiratory events that occur during successful autoresuscitation from hypoxia apnea in mice. These consisted 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% baseline. I observed a similar sequence of events during successful autoresuscitation in my rat pups. Likewise, I found, as did Gershan, Jacobi and Thach[15], 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 many of my animals. A novel finding in my current experiments was that an increase in core temperature not only impaired the ability of rat pups to autoresuscitate following repeated exposure to hypoxia, but that in a significant number of pups, it altered the sequence of events leading to autoresuscitation; that is, autoresuscitation failure was associated with cessation of gasping that preceded cardiac arrhythmia. Although my experiments were not designed to investigate the mechanism of the changes in the physiology of this protective response associated with core temperature variation, the mechanisms are likely similar to those described above. An impaired ability to autoresuscitate from primary apnea during repeated episodes of hypoxia at higher core temperatures may be attributed to central depression in the region critical for sustainment of gasping, chemoreceptor dysfunction, or may be the result of alterations in metabolism. However, it seems likely that the mechanism can be ascribed in part to a combination of these factors.

The results of my experiments provide insight into how hyperthermia 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 possible factor in SIDS[8,52,53] 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".[7,8] Given that environmental thermal changes have been associated with a significant increase in apnea incidence and duration in premature infants[54,55], as well as in experimental animals[56], and that a number of epidemiological studies have implicated hyperthermia as a risk factor for SIDS[31,57,58,59], I speculate that an increase in core temperature places infants who have apnea from whatever cause at an increased risk for severe hypoxia and death because of an impairment of protective responses that terminate apnea and restore normal tidal volume.

5.0 CONCLUSIONS

My experiments show that core temperature influences the time to last gasp following a single hypoxic exposure and the ability of newborn rat pups to autoresuscitate following repeated hypoxic exposure.

Thus, my data support the hypothesis that an increase in core temperature brought about by environmental heating may impair the ability of some infants to "autoresuscitate" during single or repeated exposures to hypoxia, as may occur during sleep apnea.

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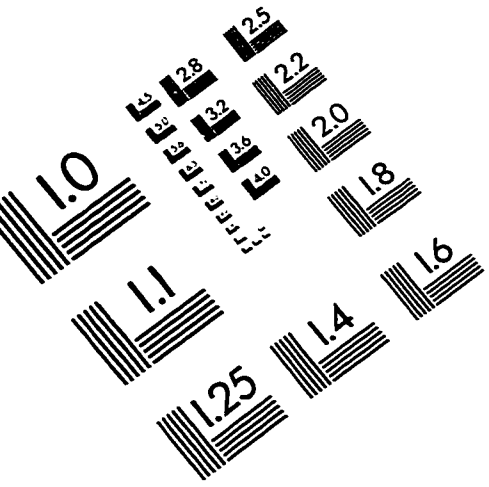
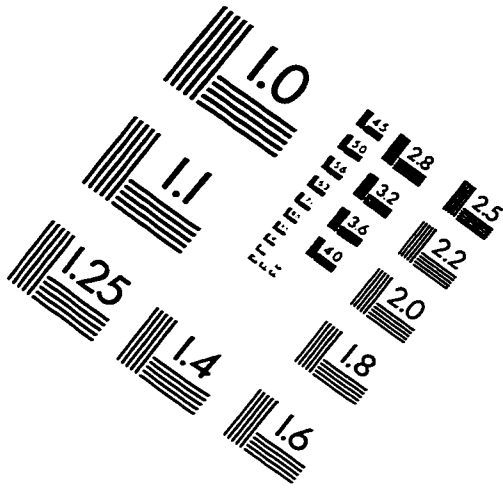
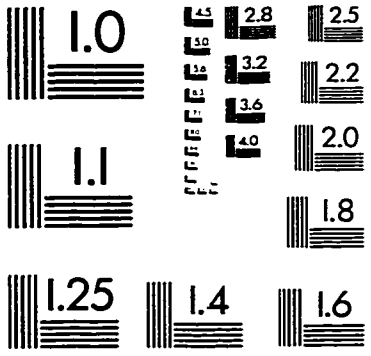
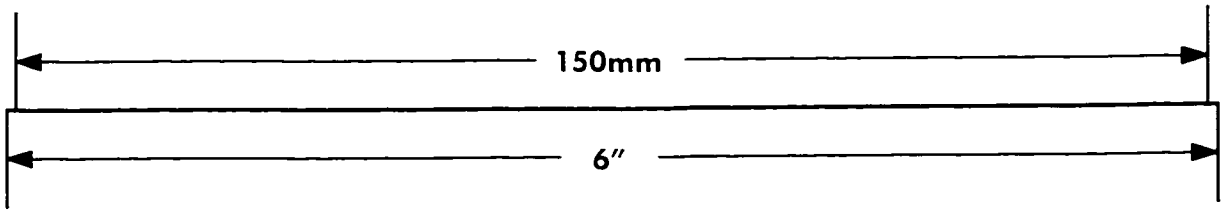
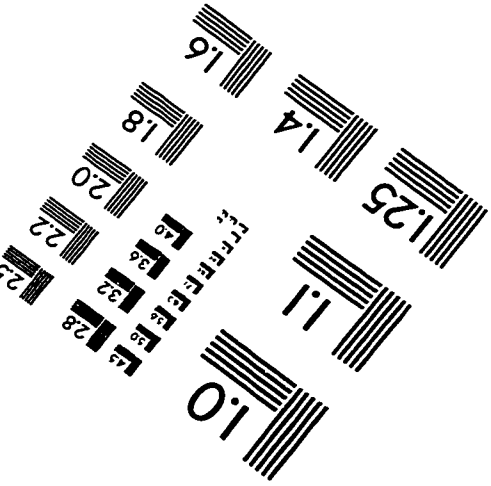
IMAGE EVALUATION TEST TARGET (QA-3)

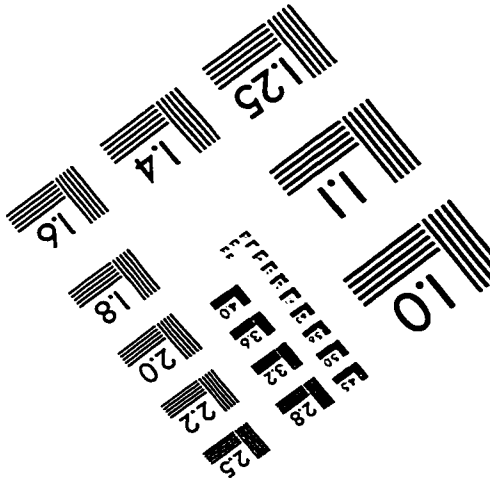












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