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The role of the cerebral cortex in the thermoregulatory responses to acute, moderate hypoxemia in young male rats

by

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Abstract

In rats, acute hypoxemia produces a regulated decrease in core temperature (Tc), the mechanism of which is unknown. Considering the cerebral cortex has been shown to influence thermoregulation, these experiments were carried out to determine if functional decortication produced by cortical spreading depression (CSD) would alter the thermoregulatory responses to hypoxemia. Tc and oxygen consumption were measured in chronically instrumented adult male rats (n=29) studied in a metabolic chamber (27 or 18°C) during normoxemia and during acute hypoxemia (10% O_2) in the presence and absence of CSD. CSD was produced by the local application of 25% KCl to the parietal cortex. CSD did not significantly alter thermoregulation during normoxemia, nor the Tc or oxygen consumption responses during acute hypoxemia. Our data do not support the hypothesis that the cerebral cortex plays a role in mediating the thermoregulatory responses to acute hypoxemia, at or below the thermoneutral zone for the rat.

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1. Introduction

1.1 Hypoxemia

All organisms, with the exception of anaerobic bacteria, require oxygen throughout their lives. Oxygen is required in the energy production processes that are necessary for organ and tissue function and growth, and thus sustaining the life of the organism. Mammals have developed a respiratory system such that oxygen passes into the body in an efficient, automatic fashion. The circulatory system then provides a carrier for oxygen, delivering it to the organs and tissues. In normal, healthy organisms, oxygen supply to the tissues may become reduced despite adequate perfusion. This is known as hypoxia. When oxygen supply becomes limited and is not restored, a decrease in the partial pressure of oxygen in the blood occurs. This decrease in the partial pressure of oxygen in the blood is known as hypoxemia.

The distinction between hypoxia and hypoxemia becomes important when discerning both the cause and effects of a limited oxygen supply to the tissues. For example, there are different mechanisms which can cause a decrease in oxygen supply to the tissues. One example is anemic hypoxia in which total hemoglobin concentrations decrease, or when hemoglobin

becomes malformed and thus reduces oxygen carrying capacity. In these cases, the partial pressure of oxygen is not reduced, but the capacity to carry the oxygen to the tissues is limited.

Hypoxemia, on the other hand, results from a limitation of the amount of oxygen getting to the blood, usually resulting from a decrease in the percent of oxygen inhaled (which can be controlled during an experiment). This results in a decrease in the partial pressure of oxygen in the blood. I will be focusing on the physiological effects of hypoxemia, in particular, with respect to the rat. These effects vary according to the severity and duration of the hypoxic exposure, maturity level, effects of anesthesia, and, of course, species differences. I will be focusing on the physiological effects of acute moderate hypoxemia in adult mammals, especially rats, noting important differences in maturity levels where appropriate. Effects of anesthesia are confounded by species differences and type of anesthetic, therefore, when possible only conscious animal preparations will be discussed. Thus, unless otherwise stated I will be referring to acute, moderate hypoxemia in adult conscious mammals.

1.1.1 Ventilatory responses

As mentioned, the physiologic effects of hypoxemia on humans and animals are numerous and are complicated by species differences and maturity levels.

Most of what is known about hypoxemia is restricted to the cardiorespiratory responses during and after such exposures. In 1995, a review of the central and peripheral effects of hypoxemia was outlined by Bisgard and Neubauer, describing in detail the ventilatory responses (6). Initially, acute hypoxia produces a rapid increase in ventilation which is not sustained but slows during the first thirty minutes of the exposure. This phenomenon is now known as "biphasic ventilatory response" and was first observed by Cross et al. in 1952 (30). Although this biphasic response was originally thought to be specific to newborns, investigators have demonstrated this to be incorrect (83,121). Also, most recently in humans, Easton et al. (43), demonstrated that this response is present (although somewhat modified) in adults as well.

In most mammals, including humans, the peripheral chemoreceptors, the carotid and aortic bodies, are considered the only source for reflex ventilatiory stimulation in response to hypoxemia (6). Furthermore, Fitzgerald and Lahiri (46), showed that hypoxia produced by a reduction in arterial oxygen levels causes an increase in ventilation by an intense stimulation of the carotid chemoreceptors and relatively little or no stimulation of ventilation via the aortic chemoreceptors. Prior to the demonstration that the biphasic response is present in adult species, it was thought that newborns were unable to maintain the hyperpnea associated with the reflex stimulation of the chemoreceptors. It is now recognized that hyperventilation wanes during an

hypoxic exposure, and this is the normal respiratory response to hypoxemia. The mechanism of this waning of hyperventilation will be discussed below, in conjunction with the cardiovascular component of the response to hypoxemia.

1.1.2 Cardiovascular responses

The cardiovascular component of hypoxemia is greatly affected by species differences. Depending on the animal model, cardiovascular parameters such as cardiac output can either increase, decrease, or not change significantly. The same can be said for blood pressure, peripheral vascular resistance, as well as heart rate. In the rat, hypoxemia induces pulmonary hypertension, which has recently been shown to be prevented by an endothelin-A receptor antagonist (141). The mechanism of peripheral vasodilation associated with hypoxemia in the rat has yet to be fully elucidated. Certain general patterns have been observed, however, as there has been considerable interest in the cardiovascular responses during hypoxemia.

It has generally been assumed that the cardiovascular and respiratory responses to hypoxemia are coupled. For example, in the dog, it was concluded that the primary vascular response evoked by stimulation of the carotid body chemoreceptors by hypoxic blood is peripheral vasoconstriction. Also concluded was that in the spontaneously breathing dog, this response may be wholly or partially masked by events occurring secondarily as a result

of the concomitant reflex stimulation of breathing (34). The same study reported that the primary cardiac response to hypoxia is bradycardia, shown in artificially ventilated dogs. They postulated that the slowing of the heart is due to a reflex increase in vagal tone, and a decrease in the activation of sympathetic fibers to the heart .

In rats, the cardiovascular effects of hypoxemia have been studied in detail, usually along side respiratory responses. The pattern of responses have been described as: an initial tachycardia (and hyperventilation), followed by a secondary bradycardia accompanied by a fall in arterial pressure, along with a widespread vasodilation being most pronounced in skeletal muscle and in the brain (114,115,137). Similar responses have been observed in the neonate of both small and large mammals (33,57,58). Certain steps have been taken to discern the mechanisms involved in the secondary bradycardia and hypoventilation associated with hypoxemia.

It has been shown that adenosine is released by brain tissue within a few seconds of the onset of hypoxia (171) and that adenosine is also released by other tissues including the heart, muscle and kidney (4,142). Furthermore it has been shown that adenosine can induce bradycardia by an action on the sinoatrial node, vasodilation in skeletal muscle, and vasoconstriction in the kidney (29,99).

Since it had previously been shown that the adenosine antagonist, theophylline, inhibits the secondary hypoventilation induced by hypoxemia in neonates (33), it seemed reasonable to expect adenosine to be involved the in the cardiovascular as well as the respiratory responses to hypoxemia, considering they are closely coupled. Indeed, both theophylline and 8-phenyltheophylline abolished the tendency for the hypoxia-induced tachycardia (as well as the secondary muscle vasodilation) to wane during a 5 minute period of hypoxic breathing (137). It has since been shown that adenosine is largely responsible for the secondary fall in ventilation via its action on central respiratory neurones (162). The same authors propose that adenosine contributes to the fall in arterial pressure by inducing vasodilation in skeletal muscles and possibly by inducing bradycardia via an action on the heart (162). It was proposed that the cardiovascular and respiratory components of the responses induced by systemic hypoxia can become interdependent in a positive feedback manner. This was hypothesized because it was demonstrated that adenosine antagonists are more effective in decreasing the hypoxia-induced fall in arterial pressure if they also blocked the secondary fall in ventilation, and even more so if the secondary bradycardia was also blocked (137,162). In other words, the secondary fall in ventilation may exacerbate the fall in the arterial partial pressure of oxygen (Pa_{O2}), so potentiating the bradycardia and peripheral vasodilation caused by

hypoxia and inducing a further fall in arterial pressure. In a study using adenosine antagonists, Thomas and Marshall (163) compared the effects of a 10 minute exposure to 8% oxygen in spontaneously breathing and artificially ventilated rats (gas mixtures were altered in the latter group to result in the same arterial partial pressures of oxygen and carbon dioxide to those resulting from the 10 minute hypoxic exposure in the spontaneously breathing rats). They concluded that the respiratory and cardiovascular responses evoked by systemic hypoxia are interdependent in the following ways:

(i) the hypocapnia that arises from the initial hyperventilatory response to peripheral chemoreceptor stimulation makes a substantial contribution to an initial tachycardia, but attenuates the initial cerebral vasodilatation;
(ii) alleviation of the hypocapnia concomitant with the secondary reduction in ventilation contributes to the secondary fall in heart rate and facilitates the cerebral vasodilatation; and;
(iii) the secondary fall in heart rate together with the vasodilatation induced in skeletal muscles and other peripheral tissues lead to a progressive fall in arterial pressure, which is ultimately responsible for a secondary fall in cerebral blood flow and a fall in oxygen delivery to the brain.

(Thomas and Marshall, J. Physiol. 480: 627-636, 1994)

1.1.3 Thermoregulatory responses

While the majority of research has focused on cardiorespiratory responses such as those previously discussed, until more recently, little attention has been placed on the thermoregulatory responses during hypoxemia. There is an important physiological difference between cardiorespiratory and

thermoregulatory responses during hypoxia. The former serve to attenuate reductions in oxygen supply, while the latter serve to decrease the oxygen demand to the tissues.

An important consequence of hypoxemia is a decrease in core temperature (Tc). This hypothermic response to a decreased fraction of inspired oxygen has been observed in a variety of species (31,56,109,131) and has been documented extensively in the rat (41,70,127,152). A decrease in metabolic rate is also known to accompany the decrease in core temperature during hypoxemia. This hypoxic hypometabolism depends on the resting metabolism at the time of the hypoxic insult (7,41,51,54,83,117,131-133,146).

The decrease in core temperature (and metabolism) during hypoxemia may be a practical solution to the problem of matching oxygen supply to oxygen demand during periods of limited oxygen availability, since Tc has a marked effect on metabolic rate via the "Q_{10} effect" (101,147). By reducing the amount of oxygen required for basal metabolism the animal is, in effect, reducing its susceptibility to cell and tissue damage or death. More impressively, the decrease in Tc associated with hypoxia has been shown in numerous animal studies to be correlated with an increased survival rate for the animal in question (2,82,122-127) which further emphasizes the importance of this response. Experimental evidence for the protective effects of hypothermia

include the Busto et al. (20) study which showed that neuronal damage to the brain in hypoxic rats is clearly decreased by lowering the brain temperature during and/or immediately after ischemia. Also, Carlsson et al. (24) concluded that hypothermia exerts a pronounced protective effect on the brain during hypoxic hypoxia, and involves two mechanisms. First, since hypothermia shifts the oxyhemoglobin-dissociation curve towards the left (i.e. since oxygen combined in whole blood is dissociated more readily at higher temperatures (14)), and therefore prevents or minimizes a rightward shift due to acidosis, the decrease in Tc maintains a high total oxygen content in arterial blood at a given arterial oxygen concentration. Second, by reducing cerebral oxygen consumption, and thereby presumably also cellular energy requirements, hypothermia exerts a protective effect at the cellular level. Furthermore, in 1969, Dunn and Miller showed a potential clinical importance of inducing hypothermia in asphyxiated human neonates resulting in an impressive 11% mortality compared to an international average of 44% (39).

The mechanism of hypothermia in mammals is not completely understood and is due in part to the difficulty in determining if reduced body temperature is a cause or effect of reduced metabolic rate. An important step has been taken by a number of investigators in determining the type of thermoregulatory response which occurs during hypoxemia. Before expanding on the type of thermoregulatory response that occurs during hypoxemia, an overview of

thermoregulation will be given, which will detail the types of thermoregulatory responses as well as different thermoregulatory states. I will then be able to discuss the thermoregulatory response to hypoxemia and the literature which supports or refutes the current views.

1.2 Thermoregulation

1.2.1 Homeothermy

Thermoregulation in mammals can be distinguished from lower vertebrates and invertebrates in that mammals actively maintain their body temperatures close to 37°C independent of changes in ambient temperature. This concept of regulating body temperature at a fixed level despite fluctuations in ambient temperature is known as homeothermy. Homeotherms have developed a multi-input system whereby core temperature, skin temperature and brain temperature (sensed by peripheral and deep body thermoreceptors) are integrated within the central nervous system (CNS), particularly the anterior hypothalamus, and operate to maintain body temperature within a set-point of approximately 37°C. Through evolution, this stability has become dictated by the enzymatic reactions in homeotherms, whose optimal activity occurs at approximately 37°C. Thus, when the brain "senses" a deviation from its set-

point temperature, effectors are activated, via efferent pathways, thus returning body temperature to set-point level. There are numerous thermoregulatory effectors which regulate the processes of heat production, heat conservation, and heat dissipation.

1.2.2 Thermoregulatory effectors

The thermoregulatory effectors of the mammal can be divided into two limbs, designed to maximize or minimize heat loss or heat conservation. There are both autonomic and behavioral effectors available to the mammal (compared to a lizard, for example, which only has behavioral means to alter its body temperature).

1.2.2.1 Autonomic thermoregulatory effectors

Firstly, control of peripheral vasomotor tone allows for fast reflex responses to small increases or decreases in body temperature. This is based on the fact that when peripheral blood vessels dilate, more surface area is exposed, allowing for convective heat loss. Conversely, if the animal is trying to minimize heat loss, vasoconstriction of the peripheral vascular bed minimizes this form of heat loss. The degree of vasomotor tone is under adrenergic control of the sympathetic nervous system (102) with α_1- or α_2-stimulation resulting in a pressor response (73), and β_2 stimulation causing a decrease in

blood pressure (15). Adjustment of vasomotor tone is the primary way in which heat balance is maintained in mammals. In the rat, several physiological and physical properties make the tail crucial in the dissipation of body heat. In particular, the tail lacks fur which accentuates heat loss, it is highly vascularized permitting a high rate of blood flow during heat stress, and it has a relatively high surface area:volume ratio that further aids in heat exchange (64,145). However, when there is a more rigorous challenge to body temperature, there are numerous alternatives to maintain thermoregulatory balance, where each species has developed various mechanisms to counteract thermal challenges.

In furred animals, piloerection provides one alternative of reducing heat loss. When the fur is raised, this increases the distance between the skin (where heat is lost) and the outside air, effectively forming a barrier against convective heat loss.

Evaporative water loss comes into play when the animal is trying to lower body temperature. This method of heat loss depends on the species in question. For those animals with sweat glands, an increase in body temperature results in water release, resulting in evaporative water loss, thus cooling the animal. For those animals that do not have functional sweat

glands (i.e. dogs) grooming and panting become the primary method of evaporative heat loss.

When body temperature is decreased, and vasoconstriction and piloerection are not sufficient to maintain body temperature, there are two forms of thermogenesis mammals use which increase heat generation, thus increasing body temperature. These are shivering and nonshivering thermogenesis. Nonshivering thermogenesis (NST) is used primarily by neonates, but is present and functional in many adult mammals as well, the rat being a primary example (1,84,97,163). NST is provided by a special type of adipose tissue, called brown adipose tissue, where due to uncoupling proteins in the mitochondria, an exothermic reaction occurs, thus heat is released into the surrounding tissue. During cold exposure, NST becomes activated, and blood flow to brown adipose tissue increases (49). As well, continued exposure to cold ambient temperatures accentuates the development of brown adipose tissue (50). Brown adipose tissue is primarily found in the intrascapular region, but is also found in other anatomical regions, including cervical, pericardial, intercostal, and perirenal areas (64). Activation of brown adipose tissue is controlled by the sympathetic nervous system and is adrenergic in nature. Specifically, norepinephrine release from sympathetic nerve terminals activates β-adrenergic receptors located on brown adipocytes to cause an increase in both oxygen consumption (48,128) and local tissue temperature

(143,148). Evidence for this is has been provided by numerous studies which have shown that adrenergic β-blockers such as propanolol, abolish brown adipose tissue activation (3,77). In addition, there is mounting evidence suggesting that α_1-adrenoceptors may play a synergistic role with the β-receptors in mediating nonshivering thermogenesis (48,128,164).

When NST is not sufficient for heat generation or when brown adipose tissue has regressed in adult animals, shivering thermogenesis (ST) takes over as the primary mechanism of heat production, and is controlled by the somatomotor nervous system (3). Thus it is more rare to see a neonate shiver when body temperature is decreased, whereas in adults, shivering represents the primary method of heat production. The rat is somewhat of an exception, since NST through brown adipose tissue continues throughout the lifespan of the rat. Threshold temperatures for the initiation of ST have not been fully established, although it is generally accepted that ambient temperatures below approximately 20°C initiate ST in the rat (64,76,157).

1.2.2.2 Behavioral Thermoregulation

Behavioral thermogenesis is the least costly method of regulating body temperature, and may or may not have autonomic accompaniment. When heat gain is greater than heat loss (i.e. the animal has an elevated body

temperature) the animal will seek lower ambient temperatures, and will sprawl out to increase the surface area available for convective heat loss. The animal will also lower its activity level, providing a means of decreasing metabolic rate. Conversely, when the animal needs to increase body temperature, it will seek out warmer ambient temperatures, increase its activity, or huddle to decrease skin surface area exposure, thus decreasing convective heat loss.

1.2.3 Set-point

The activation of thermoregulatory effectors occurs when body temperature deviates from what is called the set-point temperature. Set-point has become an important concept in determining thermoregulatory responses, and is defined as the value of a regulated variable which a healthy organism tends to stabilize by the process of regulation (66,94). Therefore, this means that set-point is not is not fixed, but can be altered by a variety of internal and external factors. For example, the intrinsic temperature of the preoptic area of the anterior hypothalamus (POAH), the activity level of the peripheral and deep body thermoreceptors, exercise, fever, and sleep/wake status can all contribute to alterations in set-point (66,72,80).

1.2.4 Thermoneutral zone

Another important concept in thermoregulation is the thermoneutral zone (TNZ). This concept brings together the interaction between ambient

temperature and metabolic rate of homeothermic animals. Specifically, the TNZ is defined as the range of ambient temperatures where metabolic rate is minimal and body temperature is maintained primarily though modulation of peripheral vasomotor tone and the control of dry or sensible heat loss (66,94). With behavioral thermoregulation in mind, Bianca (5), defines the TNZ as the range of ambient temperatures in which an animal neither combats cold by raising heat production, nor heat by evaporative heat loss, and in which behavioral thermoregulation is normally absent. There are two critical temperatures associated with the TNZ. The ambient temperature above which a resting animal recruits evaporative mechanisms for thermoregulation, thus increasing metabolic rate, is termed the upper critical temperature (UCT) (10,64,94). Conversely, the ambient temperature below which metabolism is elevated above minimal levels is defined as the lower critical temperature (LCT) (64,94). Thus, metabolic heat production via non-shivering or shivering thermogenesis must increase in order to maintain a balance between heat loss and heat production.

Due to the utilization of thermal gradients in the study of behavioral thermoregulation, an alternative definition of the TNZ has arisen. Specifically, when placed in a thermal gradient, animals tend to choose an ambient temperature associated with their minimal metabolic rate. This has been

demonstrated in the rat (60,65,67,68), mouse (61), golden hamster (69), and the guinea pig (62).

1.2.5 Forced versus regulated thermoregulatory responses

A distinction has been made between two types of thermoregulatory responses and depends on the interaction between body temperature and set-point. According to Gordon, body temperature can change if one or a combination of two principal mechanisms are operative: (a) an alteration in the apparent "set-point" temperature, resulting in a regulated shift, or (b) an excessive environmental or endogenous heat load, or heat sink, overwhelming the animal's capacity to thermoregulate, and resulting in a forced shift in temperature (59,64). In the first case, the change in temperature is generated with a coordinated set of physiological responses whereby the preferred operating core temperature has shifted in a regulated or controlled manner. In the second case, body temperature is changing against the animal's preferred level. The idea of regulated versus forced responses has resulted in a thermoregulatory paradigm which consists of five thermoregulatory states. These five thermoregulatory states have been reviewed in detail by Gordon, (66), and are normothermia, regulated hypothermia, regulated hyperthermia, forced hypothermia, and forced hyperthermia. The animal has both autonomic (i.e. vasomotor tone, evaporation, piloerection, shivering and nonshivering thermogenesis) and

behavioral thermoregulatory effectors to utilize. More specifically, in the first state, normothermia, body temperature is maintained primarily through vasomotor tone while the other autonomic responses remain quiescent. During regulated hyperthermia, such as fever, an agent affects the CNS resulting in an elevation of set-point. Thus the animal behaves as if it were cold, reducing skin blood flow and evaporative heat loss, while increasing metabolic rate. If given the opportunity (i.e. the animal is unrestrained and not under the effects of anesthesia) the animal will proceed to warmer temperatures, thus behaviorally effecting body temperature. Similar responses would be seen in an animal under forced hypothermic conditions. However, instead of an altered set-point, body temperature is reduced below set-point by an environmental (i.e. an ice-bath) or drug related reduction in metabolism without affecting the CNS. Conversely, regulated hypothermia and forced hyperthermia are analogous in that similar behavioral and autonomic responses are employed. For example, the animal behaves as though it were hot and activates heat dissipating responses to effectively lower its body temperature. The difference is that in regulated hypothermia there is a reduction in set-point, whereas in forced hyperthermia the animal's thermoregulatory effectors are overwhelmed and the animal is forced to try to reduce its own body temperature.

1.3 Regulated Hypothermia

1.3.1 Rationale

Considering the above discussion, the challenge to physiologists was to determine if the observed decrease in core temperature associated with hypoxemia was regulated or forced. The rationale for defining the hypothermia would therefore be based on the autonomic and behavioral manifestations of the decrease in core temperature. Therefore, if the hypothermia is forced, one or more of the following would occur in an effort to raise core temperature back to set point: an increase in peripheral vasomotor tone, a decrease in evaporative heat loss, NST would be activated and the animal may shiver, and the animal would seek out warmer ambient temperatures, or huddle, resulting in an increase in metabolic rate. On the other hand, if the hypothermia was regulated in nature, the animal would effectively try to reduce its Tc to the lower set point, presumably caused by the decrease in partial pressure of oxygen in the blood. Thus, one or a combination of the following would occur: skin blood flow would increase, evaporative water loss would increase, and the animal would seek lower ambient temperatures, or sprawl, with a concomitant decrease in metabolism. Overall, therefore, what

had to be determined was whether or not there is a resetting of the thermoregulatory set-point during hypoxemia.

1.3.2 Evidence

Some circumstantial evidence for set-point alteration was provided by Hicks and Wood in 1985 using iguanas in a shuttle box apparatus (82). They found that exposure to a hypoxic gas mixture in a thermogradient resulted in the animals lowering their selected (preferred) ambient temperature. This behavioral reduction of body temperature, thus lowering metabolic demand, provides an effective, if not life-saving, adaptation to hypoxemia. Further experimentation demonstrated that the metabolic responses of hypoxic Wistar rats are affected by changes in ambient temperature, providing further support of an hypoxia-induced shift of thermoregulatory set-point (41). Not only was there a significant reduction in metabolic rate at temperatures below the normoxic lower critical temperature, but both the upper and lower critical temperatures bounding the thermoneutral zone were lower during hypoxia. Because of some difficulty in interpreting the data from the previous study (body temperature actually declined slightly in the hypoxic thermoneutral zone despite a general independence of oxygen consumption over that range of ambient temperatures), Dupre and Owen (40) set out to definitively determine whether or not hypoxic hypothermia is a regulated response. Although their

original hypothesis, that hypoxic rats would select lower ambient temperatures, was not supported by their data, the rats did maintain a lower body temperature than when normoxic. The hypoxic rats moved to new temperatures when appropriate to maintain body temperature constant at the new lower body temperature. Thus, taken together, their data support the concept that body temperature is regulated at a lower level during hypoxia. Gordon and Fogelson (70) furthered this evidence by showing that a variety of species including rats, hamsters, and mice, decrease their preferred ambient temperature during hypoxic exposures. Most recently, data have been provided by Clark and Fewell (28), who have shown that the decrease in body-core temperature during hypoxemia in newborn and older guinea pigs is a regulated rather than a forced phenomenon, since selected ambient temperature decreased (especially in the newborn animals) during hypoxemia compared to when the guinea pigs were breathing normal air.

Gordon (59) defines regulated hypothermia as a significant decrease in body temperature where there is a decrease in activity of heat generating/conserving effectors and an increase in heat dissipating effectors. The decrease in core temperature in rats exposed to hypoxic conditions is thought to be the result of the animal behaviorally enhancing the autonomic downward shift of the thermoregulatory set-point. The regulated hypothermia associated with hypoxemia is thus an example of rheostasis (135), or

regulation around a shifted set-point, rather than a failure of homeostasis (22). However, the mechanism of the regulated decrease in body temperature during hypoxemia is still not known.

1.3.3 Mechanisms - possible mediators

Numerous possibilities have been proposed as mediators of the regulated hypothermia that occurs during acute exposure to hypoxia. Examples include arginine vasopressin (AVP) (47), adenosine (103,167), histamine (158,168), endogenous opioid peptide(s) (120), and alpha-MSH (9). However, AVP has been refuted as a possible mediator, since Brattleboro rats, which are deficient in central AVP, when held at a constant ambient temperature, still show a regulated decrease in core temperature during hypoxemia (27). Although it remains possible that their results may have been different if the animals were allowed to behaviorally thermoregulate, the study has provided evidence that, at least in rats, AVP is not involved in mediating the hypothermic response to hypoxemia. Thus far, the other possible mediators have not been ruled out, but no relevant evidence has been put forth to either accept or refute these proposals.

An interesting possibility has been suggested by Tamaki and Nakayama (160). They propose that there is a direct effect of hypoxia on the thermosensitive neurons in the preoptic areas of the anterior hypothalamus

(POAH), and showed that under hypoxic conditions the warm-sensitive neurons in the POAH increase their activity thus stimulating effectors responsible for heat dissipation. If this were the case, there would not exist any mediators, central or otherwise, of the regulated hypothermia and the decreased oxygen concentration in the blood would act directly on the neurons responsible for heat loss.

1.3.4 Mechanisms - possible role of the cerebral cortex

Recent work has provided evidence that the cerebral cortex plays a role in both autonomic function (25), and thermoregulation (37,87,129,130,153), but whether this role is tonically active or active only during a perturbation remains unknown. The majority of work thus far has concentrated on the interaction of the cortex with the thermosensitive neurons in the POAH, and evidence for direct corticohypothalamic projections has been provided (25,90). However it is important to keep in mind that there are other areas in the brain which contain thermosensitive neurons (e.g. the medulla oblongata, the brain stem) and these may interact with the cortex as well (86,92,110,111).

Before going into relevant research on the role of the cerebral cortex in thermoregulation, it is important to review the technique of cortical spreading

depression (CSD), as both psychologists and physiologists have demonstrated it to be invaluable in determining the involvement of the cortex in numerous applications. Advantages over other "decortication" techniques, such as lesions or surgical ablations, make CSD an effective tool for the study of functional brain anatomy. Ideally, a functional ablation technique should be fully reversible, its effects must be limited to the ablated structures, and it must be accompanied by clear cut electrophysiological signs indicating its extent and duration (16). As most of these requirements are met by CSD, the technique was introduced into behavioral experiments by Bures and Buresova (16).

Leao, in 1944, first described spreading depression when he observed a reaction in the cerebral cortex of rabbits elicited by strong electrical and/or mechanical stimuli. These stimuli resulted in a local decrease in both spontaneous and evoked EEG activity in the stimulated region, which spread over the entire cortex, with a velocity of approximately 3mm/min (108). The functional ablation effects of CSD are based on the blockade of neural activity elicited by the moving wave of depolarization (16). Whereas single CSD waves are desirable in some investigations, more prolonged functional ablation is required in many behavioral (and physiological) applications. This can be provided by continuous exposure to depolarizing chemicals (such as K^+). For example, repeated waves of CSD can be evoked by making a

trephine opening in the skull and applying a piece of filter paper soaked in 25% KCl (16,17). Reversibility of this "decortication" occurs when the KCl is removed and the dura is gently rinsed with saline. Since it is not always convenient to implant electrodes into the brain (to electrophysiologically monitor the waves of depolarization) during an experiment, behavioral tests have been developed to check for the presence or absence of CSD. These tests are based on innate reactions representing the highest level of coordination of posture (16). See Materials and Methods for a more detailed discussion of spreading depression.

A group in Japan, including T. Hori and M. Shibata, published a series of reports in the early 1980's investigating the role of the cerebral cortex and its interaction with the thermosensitive neurons in the POAH, with respect to both behavioral and autonomic thermoregulation (87,88,150-153). They investigated the effect of CSD on the thermosensitive neurons in the POAH since their early work indicated an ipsilateral corticohypothalamic interaction with respect to behavioral and autonomic thermoregulation (87). Support for this theory was provided when they showed that during CSD, warm-sensitive neurons were seen to decrease in activity while cold-sensitive neural activity was augmented (87). Later it was shown that the sulcal prefrontal cortex was the most critical area in the cerebral cortex for altered activity of the thermosensitive neurons in the POAH (88,151).

More recently, the role of the cerebral cortex has been studied with respect to fever production (also a regulated thermoregulatory state) in rats. Monda and Pittman reported that CSD attenuates prostaglandin E_1 (PGE_1) and endotoxin fevers in conscious, male Sprague-Dawley rats (130). Their results are in accordance with the observations of DeLuca et al (37) who found that increases in thermogenesis and metabolic rate produced by overeating or lateral hypothalamic lesions failed to occur in decorticated rats. This same study also demonstrated that propanolol (a β-adrenergic receptor antagonist) impaired the increase in oxygen consumption to the same extent as did decortication. Therefore, to further study the role of sympathetic outflow to brown fat, Monda et al measured sympathetic nerve activity to intrascapular brown adipose tissue during PGE_1 fevers (at higher doses than the earlier study) and examined the effects of CSD (129). Their results were in accordance with Monda and Pittman's study in that CSD attenuated the increase in body temperature associated with intracerebroventricular (i.c.v.) PGE_1 administration. Furthermore, they were able to show that CSD reduces core temperature through reduced neuronal activation of brown adipose tissue as a means of reducing oxygen consumption. Thus during PGE_1 fever, the cerebral cortex appears to be involved in nonshivering thermogenesis through the control of sympathetic activity.

1.4 Hypothesis

The aforementioned investigations led to the hypothesis that the cerebral cortex plays a role in the regulated decrease in core temperature that accompanies acute moderate hypoxemia. This hypothesis was tested by determining the thermoregulatory responses (core temperature and oxygen consumption) to acute hypoxemia in rats, while at or below thermoneutrality, both when the cortex was intact and during functional decortication. Functional decortication was produced by the local application of KCl to the dura overlying the cerebral hemispheres, thus initiating long lasting cortical spreading depression. Cardiorespiratory variables (respiratory rate and heart rate) were also measured.

2. Materials and Methods

2.1 Cortical Spreading Depression

The use of CSD to induce functional decortication is an integral part of my experimental protocol. Because of this and since this technique has been used extensively in the study of functional brain anatomy, particularly in behavioral psychological studies, and in physiology, I will review the details of this phenomenon. Referring to CSD as a phenomenon is appropriate since the precise mechanisms governing the initiation, propagation, and duration of CSD remain unknown. Furthermore, application of this phenomenon is not limited to the cerebral cortex, but can also be initiated in other regions as well with varying degrees of susceptibility. Bures et al (19), and Czeh and Somjen (32) report that susceptibility to spreading depression decreases in the following order: hippocampus, neocortex, subcortical nuclei, brainstem gray matter, cerebellar cortex, and spinal cord. Therefore, although it may be more appropriate to refer to "spreading depression" (SD), the two terms are often used interchangeably. In the following discussion, therefore, when referring to specific cortical applications, CSD will be used. If referring to general aspects of the phenomenon, I will use SD.

As previously mentioned, there are certain advantages CSD has over other ablation techniques. Ideally, a functional ablation technique should be fully reversible, the effects limited to the ablated structures, and it must be accompanied by clear cut physiological signs indicating its extent and duration. Drawbacks of surgical ablations include irreversibility, shock, edema, interference with circulation, retrograde degeneration, and glial scars (18). Lesions have drawbacks in that they are irreversible, and the extent of the ablation produced by the lesion is not always consistent, which can therefore result in erroneous judgement in data interpretation.

CSD therefore, has at least one obvious advantage over lesions and surgical ablations in that it is fully reversible. Single waves of CSD can be elicited repeatedly in the same animal with out complications, provided a suitable recovery time is allowed to lapse. However, when multiple waves are elicited (usually by topical application of 25% KCl), Bures (17) warns that focal damage (due to the osmotic imbalance that results from prolonged CSD) to the dura will occur. This will make initiation of further CSD's more difficult, and furthermore, makes the reversibility of CSD incomplete. Thus, Bures recommends that repeated application of CSD is not suitable for chronic experimentation (16,17).

Electrophysiological monitoring of CSD can be relatively simple, and can be done in a variety of ways. The usual technique involves implantation of D.C. (direct current) micro-electrodes, with a ground electrode placed on a bone to serve as a reference. This serves to monitor the biphasic negative-positive slow potential change (SPC) from the cortex, which spreads throughout the hemispheres (93). Together with the observation of decreased EEG activity, monitoring the SPCs with micro-electrodes has become the usual method of detecting the presence of CSD (or SD). Other techniques have been developed in the last ten years including a non-invasive technique that may result in clinical application of monitoring brain activity during seizure and migraine (both of which CSD has been implicated in) (44,85,104).

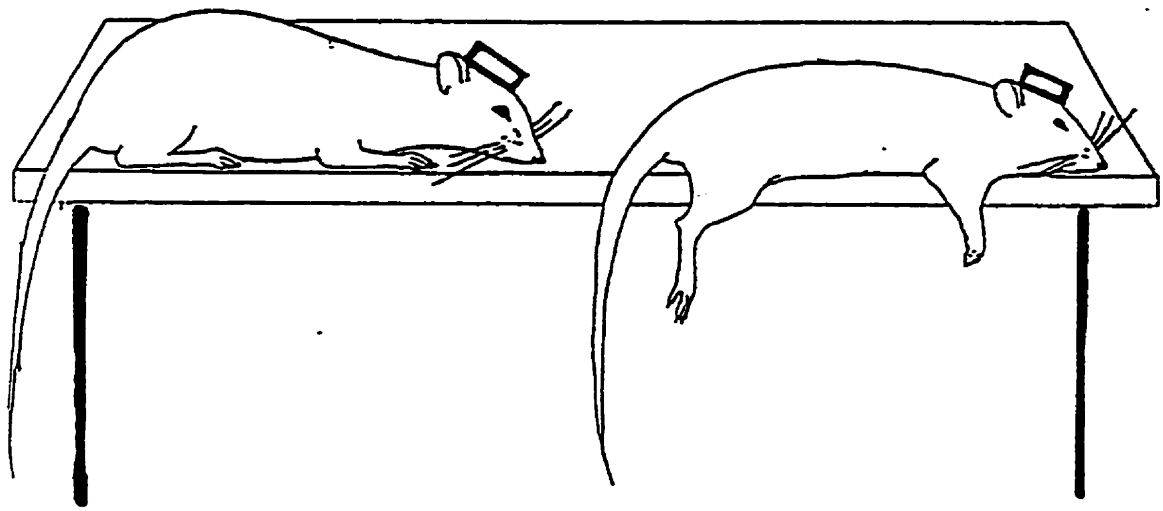
As in this study, it may be desired to study conscious, freely moving rats, making direct electrophysiological monitoring inconvenient. As an alternative, a number of neurological and behavioral tests have been developed to check for the presence or absence of CSD. This is possible since the development of behavioral signs are consistent criteria for the establishment of the presence of CSD (93). These direct behavioral tests consist of innate cortical postural reflexes which anticipate the consequences of displacement of the body. For my experiments two tests were chosen to check for the presence or absence of CSD, and are described as behavioral placing reactions (16). The

first test, the tactile placing reaction, consists of the rat being gently restrained by hand at the edge of a table. When the cortex is intact, and the fore- and hindlimbs are moved to hang over the edge of the table, they are immediately lifted back onto the table. Presence of CSD is confirmed when the limbs remain hanging off the edge of the table (Figure 2.1). The second test, the visual placing reaction, consists of the rat being gently held up by the scruff of the neck. A steel rod is then brought toward the rat at about head level. When the cortex is functional the rat will immediately attempt to grasp the rod; when non-functional, the forelimbs will remain hanging and no attempts are made to grasp the rod (Figure 2.2). Although it has been suggested that the behavioral effects of CSD paradoxically outlast electrographic indices (23), further research has revealed that the duration of the prolonged SPC corresponds well to the duration of the behavioral effects of SD (93).

Figure Legend

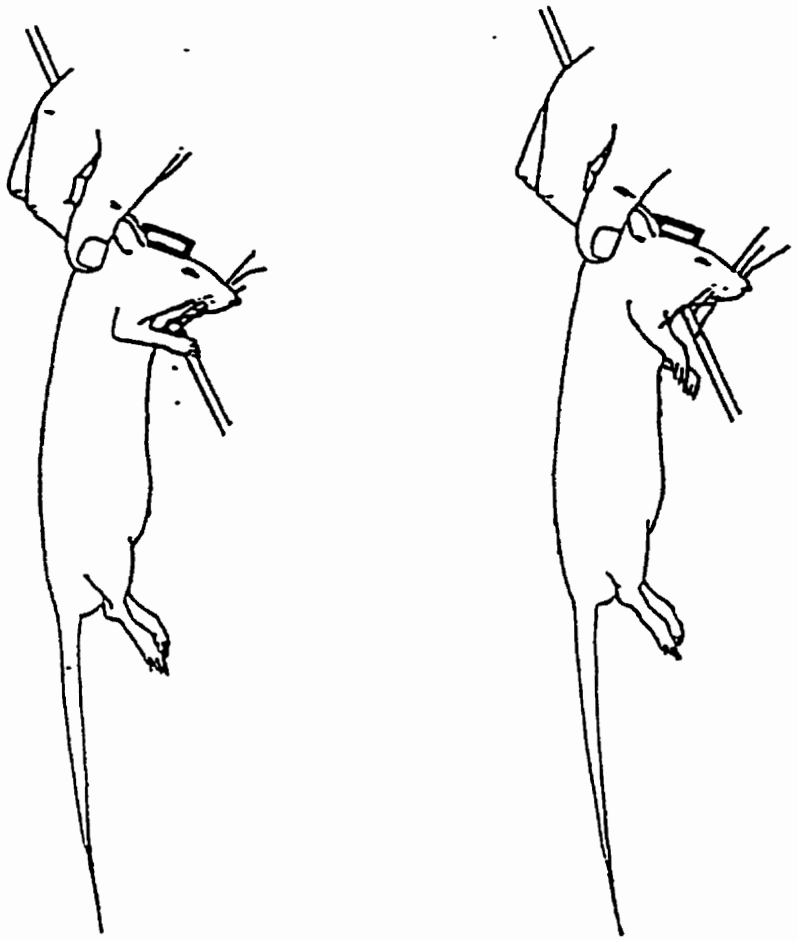
Figure 2.1. Tactile placing reaction in the rat. This figure shows a drawing of a rat showing positive (left) and negative (right) tactile placing reactions.

Figure 2.2. Visual placing reaction in the rat. This figure shows a drawing of a rat showing positive (left) and negative (right) visual placing reactions.



Intact cortex Functional decortication

Figure 2.1. Tactile placing reaction in the rat



Intact cortex

Functional decortication

Figure 2.2. Visual placing reaction in the rat.

As far as the extent of CSD being limited to the "ablated structure", spreading depression, when initiated in the cerebral cortex, usually halts at the border of white matter, and does not penetrate glial scar tissue (155,159). It has been shown, however, that CSD may traverse into subcortical structures. This is an obvious disadvantage or problem in behavioral or physiological studies. Jakobartl and Huston report that in conscious Sprague-Dawley rats, CSD is transmitted to the caudate nucleus, presumably through the amygdala, in approximately 4% of cases (96). These results differ from those reported by Fifkova and Skya who showed a 60% rate of cortico-striatal transmission on anesthetized Wistar rats (55). In addition, DeLuca et al report a 34% incidence of transmission to the caudate nucleus in Druckrey rats (38). Anatomical differences between different strains of rats has been proposed to account for the variability of cortico-striatal transmission of spreading depression (96).

Although the exact mechanism of CSD remains unknown, there have been numerous observations that are consistently associated with CSD. There are also a number of hypotheses that have been put forth in attempts to discern mechanisms, with hopes of furthering the study of this phenomenon. As previously mentioned, application of depolarizing chemicals (e.g. K+) to the cortex of rats induces so-called "spreading depression" which is described as

a slowly spreading wave of neural depolarization and depression of EEG activity lasting 2-3 minutes, traversing the cortical hemisphere at a rate of about 3mm/minute (108).

There are a variety of methods that are effective in producing SD other than application of KCl solutions. Other "depolarizing chemicals" that will initiate SD include glutamate (13,45) aspartate (18), $ACTH_{1-24}$ (95), enkephalins (156), quisqualate, kainate, and NMDA (107), and vasopressin (but only in the hippocampus, not the neocortex) (91). Other methods include strong electric stimuli, and mechanical stimuli, such as a needle prick (18,108).

The functional ablation effects of CSD are based on the blockade of neural activity elicited by the moving wave of depolarization (16). The speed at which the waves of CSD traverse the cortex was originally assumed to more or less equal in all circumstances, i.e. about 3 mm/minute. However, it has been shown that speed of propagation varies in each animal, and a range of 1.5 to 7.5 mm/minute has been reported (119).

Chemical and morphological analyses of cortical cells after CSD has been induced has shown that there is a drastic redistribution of ions resulting in swelling of the dendrites and contraction of the extracellular space. This is thought to be due to an increase in the sodium ion permeability of the plasma

membrane, as it has been shown that inside the cells there is a net accumulation of Na^+, Cl^-, Ca^{2+}, with a concomitant H_20 flux into the cell (155). Extracellularly, there is a net accumulation of K^+ , H^+, lactic acid, and arachidonic acid (75,106). This redistribution of ions is thought to be the mechanism behind the loss of neuronal membrane resistance associated with SD (155), and is consistent with the nearly total depolarization of neurons.

The main wave of SD is accompanied by the dilation of blood vessels and locally increased blood flow, followed by long-lasting vasoconstriction (44,104,119). It is also possible that the local increases in potassium ion concentration during SD contribute to the altered hemodynamics of the cortex (169). Increased oxygen consumption and accumulation of lactic acid (18,119), along with a depletion of creatine phosphate, glucose, glycogen, and a reduction in protein synthesis also occur during SD, and during recovery from SD. Lauritzen et al (106) suggests that the increased rate of metabolism is primarily due to activation of translocator-coupled ATP-ases pumping ions between intracellular and extracellular compartments. Chesler and Kraig (26) report that neuronal cytoplasm and interstitial fluid become markedly acidotic, whereas glial cells become alkaline.

Although SD tends to spread from its initiation site as a concentric wave of depolarization in gray matter, there is a spontaneous activity accompanying

the wave (155). More specifically, some cells fire a brief burst of activity at the start of depolarization. As the wave proceeds the cells become unexcitable. Thus membrane potential shifts slowly at first, but then accelerates and becomes maximally depolarized in 0.5 to 4.0 seconds. After a minute or two, the cells will repolarize (in the presence of metabolic substrates) and may become hyperpolarized for a few minutes. Following repolarization it takes several minutes before neuronal excitability and synaptic transmission return to normal (155). Thus there is a "refractory state" associated with SD, where further bouts of SD cannot be initiated. This state persists beyond recovery of synaptic transmission and is presumably due to the drastic metabolic requirements that are associated with SD (18,140).

Although the exact mechanism behind the initiation, propagation, and duration of CSD remains unknown, two main hypotheses have been suggested. Both of these hypotheses have been furthered to include the experimental evidence that has been collected in recent years. The first hypothesis, known as the neurohumoral or potassium hypothesis, was first reported by Grafstein in 1956 (71). This hypothesis suggests that the stimulation of cortical gray matter initiates a cascade reaction, whereby K^+ builds up extracellularly, depolarizing the neurons proximal to the initiation site and then propagating in a positive feedback manner, resulting in total neuron depolarization. That potassium is the sole ion necessary for propagation and initiation of SD has

been refuted on a number of grounds. Most importantly, it has been shown that the spontaneous activity of the neurons at the leading edge of a spreading depression wave precedes the rise in extracellular K^+ concentration (78,154).

The second hypothesis was first suggested by Van Harreveld in 1959 (165), and is known primarily as the glutamate hypothesis. This was originally suggested because application of glutamate antagonists altered (but did not prevent) the SD process, and that it was later shown that glutamate elicits SD in much lower concentrations than does potassium (106,166). Although both potassium and glutamate ions appear to play important roles in the evolution of spreading depression, neither has been definitively shown to be solely responsible for it. However, recent work suggests that high extracellular glutamate is not necessary for propagation of SD, but that high extracellular potassium is (138,139).

There is compelling pharmacological evidence that SD is linked to N-methyl-D-aspartate (NMDA) receptor ionophore complexes, which are known to be highly permeable to calcium ions (118). NMDA receptor blockade completely inhibits SD propagation, and reduces the sensitivity to the triggering stimulus (74,81,105,113). It has also been shown that SD propagation is dependent on extracellular calcium ions (18,98).

Further research has shown that gap junction function is necessary for propagation of SD which was first suggested by Somjen et al (155). It has since been shown that in isolated chicken retina (an accessible source of gray matter used extensively in SD research), that intracellular coupling through gap junctions is required for SD (136).

2.2 Rats and Surgical Preparation

2.2.1 Rats

Young male rats (between five and six weeks of age) of the Sprague-Dawley strain (Charles River Breeding Laboratories; St. Constant, Quebec) were used for the experiments and weighed between 151 and 175 grams upon arrival. The rats were housed in individual transparent plastic cages in the medical vivarium. They were housed at an ambient temperature of 22 ± 1°C, with a relative humidity of about 50%, in a light-dark cycle with lights on from 0700h to 1900h. Food (rat chow, Lab Diet 5001; St. Louis, Missouri) and tap water were provided ad libitum.

Sprague-Dawley rats were chosen because they have been used extensively in our laboratory, as well as in others which study hypoxemia and thermoregulation. This allows for direct comparisons between the data collected in these experiments to those in our laboratory, as well as to the data in the literature. Because we were interested in the thermoregulatory responses to hypoxemia, male rats were chosen since they do not undergo the day to day variations in body temperature that is associated with the estrous cycle in female rats (64,66).

The size of rat was basically dictated by the literature on CSD which states that young rats of about 200 grams are best suited for CSD induction (17). Although larger rats have been used (up to about 350 grams), the smaller ones permit easier handling (see below).

2.2.2 Handling

It was necessary to verify the presence or absence of CSD during the experiments in a reliable and reproducible manner. Since we were interested in studying freely moving conscious rats, electroencephalographic determination of the extent and duration of CSD was inconvenient. Thus, direct behavioral tests which consist of innate cortical postural reflexes anticipating the consequences of displacement of the body were employed. The advantage of innate testing is that they can be performed in naïve animals without complicated apparatus. However, most tests can not be easily quantified, therefore the experience of the experimenter is extremely important for correct evaluation of the observations. Furthermore, since it has been shown that not only with repeated handling do laboratory rats become rather tame and easy to manage, but neurological and behavioral testing is easier and more reliable in animals which have been systematically handled for several days preceding the testing (17). For this reason a systematic

handling protocol was developed and adhered to for each rat prior to surgical preparation.

Basically each animal was handled at least once a day for the five days preceding surgery. On the first day, handling began my simply touching and petting the rat. Once they were used to being touched they would gently be picked up. The rats were always picked up in the same manner: the forefinger and thumb firmly holding the rat behind the forelimbs, and then raising the rat vertically out of its cage. They would be picked up three to five times, and then returned to their room in the vivarium. On day two the animals would be petted again and then picked up, vertically out of their cages at first, and then they would be picked up and placed on the table next to the cages. They were placed on the table three times, and then returned to their cage. On days three to five the same protocol would be followed. The handling would begin as before, rats were petted first, and then lifted out onto the table. The rats were then manipulated with both hands on the table beside their cage. Sometimes the rats would become nervous and try to escape when both hands were used to manipulate the rat. Therefore, if a rat was not fully comfortable with this procedure, it would be handled twice each day until it was determined that the rat was sufficiently tamed. The usual pattern was for a rat to be tame and easy to handle by the fourth time it was handled. It was important for the rats to be comfortable with being held, and turned around on

a table with both hands, not only for easy and reliable interpretation of the behavioral tests, but to be able to easily restrain the rat when applying the KCl or normal saline to the dura during the experiments.

2.2.3 Surgery

On the day of surgery the rats were brought into the lab and weighed. The rats were anesthetized by an intraperitoneal (I.P.) injection of sodium pentobarbital (Somnotol, MTC Pharmaceuticals; Cambridge, Ontario) at a dose of either 60 or 65 mg/kg. Sterile 26 ½ gauge needles were used with a 1 cc syringe. Volume of anesthetic to be injected was determined by multiplying the weight of the animal (in kg) by the desired dose (60 or 65 mg/kg) and then dividing the product by the concentration of the sodium pentobarbital solution (65 mg/mL). Therefore for a 180 gram rat the volume of anesthetic at a dose of 60mg/kg would be determined as follows:

$$\frac{(0.180\text{kg}) \times (60\text{mg/kg})}{(65\text{ mg/mL})} = 0.17\text{ mL}$$

The dose of anesthetic was reduced to 60 mg/kg in the latter half of the experiments since numerous animals were not recovering after surgery (or would die during surgery; n=10). If after 20 to 30 minutes the rats had not reached the surgical plane of anesthesia (as determined by paw withdrawal reflex when the foot was pinched), a volume of anesthetic was further injected

to achieve a dose of 65 mg/kg. Occasionally the rats would not reach the level of surgical anesthesia. If this happened, they would be left for one or two days and then tried again. If on the second attempt anesthesia was not achieved, they were not used (n=4).

Once a rat was determined to be ready for surgery, it was placed on the surgical table. The abdomen, the top of the head and a small area under the right forelimb were shaved, and swabbed with savlon for disinfection purposes. The rat was then securely placed in a nose holder. Under aseptic conditions, the dorsum of the skull was exposed by a median incision, about 2 cm in length, thus exposing each of the parietal bones. Connective tissue (fascia) and periosteum were cleared with the scalpel, and haemostasis achieved by cauterizing any areas that were bleeding, including the skin flaps. Holes were then made, 4 mm in diameter, over both parieto-occipital cortices with a hand held trephine as described by Bures et al. (17). About two thirds of the hole was drilled through the bone, and then the circular piece of bone would be gently lifted off the parietal bone with tweezers. Throughout this step and for the rest of the surgery, care was taken to not damage the underlying dura matter. If the dura or the cortex were damaged, the rat would not be used further (n=4). A polyvinyl chloride cylinder (PVC) (15mm in diameter, 10mm in height and with a screw cap) with a small flange was then placed within the skin margins and secured with one or two sutures (4.0 silk

cutting). Care was taken to ensure the cap was placed directly over the two trephine holes. A piece of parafilm, followed by a saline soaked piece of filter paper (41 Ashless, Whatman; England) were placed on top of the trephine holes to protect the dura from drying and cooling. The cap was then replaced, the animal taken out of the nose holder, and laid on its back. A paramedian laparotomy was done (about 2 cm in length), and a free-floating battery-operated biotelemetry device (PhysioTel TA10ETA-F20, Data Sciences International; St. Paul, Minnesota) was inserted into the abdominal cavity for later measurement of body temperature, heart rate and activity level. The electrocardiogram lead of the transmitter was tunneled under the skin and positioned around the thorax, under the right forelimb, and secured with one or two sutures. The muscles of the abdominal cavity were then sutured (4.0 silk taper) and the area sprayed with Gentocin (Schering Canada; Point-Claire, Quebec). The skin was then closed with wound clips, and the incision area was sprayed with New-Skin (Medtech Labs,Inc.; Jackson, WY). The animals were put back into their cages, placed on a heating pad and monitored until they woke up, which usually took between one and three hours. They were then returned to the vivarium.

The following day a postoperative check was done. This consisted of restraining the rat, swabbing the area around the trephine holes, and replacing the parafilm and filter paper pledgets. This helped to ensure that no

connective tissue had built up over the holes before experimentation began. The experiments would commence the next day.

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 Experimental Protocol:

2.3.1 Control-1 : Establishment of core temperature stability

Before being placed in the experimental chamber (ambient temperature = 27°C, or 18°C) each rat was weighed and tested for the presence of bilateral tactile and visual placing reactions of its fore- and hindlimbs on both sides. This ensured that no postoperative damage had occurred and the cortex was subsequently deemed functionally intact (17).

Each rat was placed into the experimental chamber during normoxemia (21% O_2) for one hour. Measurements of oxygen consumption (VO_2), core temperature (Tc), heart rate (hr), respiratory frequency (f), and activity were then made at two minute intervals, providing control data for the animal. A suitable control period consisted of five consecutive two minute readings in which the core temperature did not vary by more than 0.2°C. These 5, 2 minute readings were averaged to give a control value for each variable and will be herein designated as "control-1" or C-1. This control period allowed us to be sure that the core temperature of the animal was stable before commencement of the experiments.

2.3.2 Normoxemia experiments

A group of fifteen male rats (n=7, 27°C; n=8, 18°C; with an average weight of 214g) were prepared as described above. Each rat was subjected to the following protocol on two successive days; the order of which was randomized to avoid any sequence effects. One experiment was carried out during "functional decortication" produced by cortical spreading depression, and one experiment was carried out with the rat's cerebral cortex intact.

2.3.2.1 Cortex intact - Saline Experiments

Once control data were collected, the rat was taken out of the chamber, the parafilm and filter paper were removed, the exposed skull region and dura rinsed with normal saline, and then thoroughly dried. A second pledget of filter paper soaked in normal saline was then placed in the cylinder covering the exposed hemispheres. The rat was placed back into the chamber for a period of ten minutes. The rat was then taken out of the chamber and tested for the presence of the visual and tactile placing reactions. If the placing reactions were positive (placing reactions were always positive after application of normal saline), the rats were placed back into the chamber and a thirty minute period was allowed to pass before the experimental period began. This adjustment period was necessary since preliminary experiments showed that core temperature tended to increase following manipulation and handling of

the rats. Measurements of core temperature, heart rate, oxygen consumption, respiratory frequency, and activity were taken every six minutes during the 30 minute adjustment period, and the last reading (at 30 minutes) was later used to compare the effects of the experimental period in statistical analyses. This second control value will be herein designated as "control-2" or C-2. In the case of the normoxemia experiments, the experimental period began immediately following the adjustment period; there were no changes made to the gas flowing into the chamber. Measurements (Tc, VO_2, hr, f, and activity) were continued every six minutes for the one hour experimental period. The rat was then finally taken out of the chamber, and placing reactions were again tested. Lastly, the filter papers were removed, the skull and dura rinsed with normal saline, a fresh parafilm sheet installed and covered with a filter paper soaked in normal saline. The cap was replaced and the animal returned to its home cage.

2.3.2.2 Functional Decortication - KCl Experiments

Each rat was treated in the same manner as above with the exception that after control data were taken, the pledgets of filter paper applied to the exposed dura were soaked in 25% KCl, instead of normal saline, to evoke cortical spreading depression (17). The experiment would proceed if after ten minutes the visual and tactile placing reactions were negative. However, if the

reactions were positive, a second piece of KCl soaked filter paper was applied, and six minutes were allowed to pass. This occurred on two occasions, and it was determined that spreading depression had not fully progressed because the filter papers had lifted off the dura into the cylinder well. Therefore, if, after a six minute period the reactions were negative, the experiment was allowed to proceed. At the end of the experiment, placing reactions were again tested. A negative result ensured that the cerebral cortex remained functionally decorticated for the entire duration of the experiment. In one experiment a rat was found to have an intact cortex at the end of the experimental period. Preliminary experiments revealed that the Sprague-Dawley rats would remain functionally decorticated for at least two hours after application of a filter paper soaked in 25% KCl. Therefore, it was presumed that the KCl filter paper lifted off the dura into the cap well during the experiment in which functional decortication did not last.

2.3.3 Hypoxemia experiments

A group of 14 rats (n=7, 27°C; n=7, 18°C; with an average weight of 216g) was implanted as earlier described. As before, each implanted rat was tested for the presence of bilateral tactile and visual placing reactions of its fore- and hind limbs, before being placed in the experimental chamber. After control data were collected, the rats were then subjected to the following

experimental protocols on two successive days; the order which was randomized to avoid sequence effects.

2.3.3.1 Cortex intact - Saline Experiments

Each rat was treated exactly the same as in the normoxemic saline experiments up to the point of the thirty minute adjustment period. At the end of the 30 minute adjustment period, the gas mixture flowing into the chamber was replaced with 10% oxygen, balance nitrogen. The flow rate of the gas mixture was increased to 4.0 L/min for 5 minutes to allow sufficient time for the normoxic gas to be completely replaced by the hypoxic mixture. After this 5 minutes, the flow rate was reduced back to 2 L/min. The experiment then proceeded as described above. As before, measurements of Tc, hr, f, VO_2, and activity were continued every six minutes during this one hour experimental period (of hypoxic hypoxia). The inhalation of 10% oxygen (balance nitrogen) results in acute moderate hypoxemia. At the end of this period, placing reactions were again tested. Finally, filter papers were removed, and a fresh parafilm sheet installed and covered with a filter paper soaked in normal saline. The cap was replaced and the animal returned to its home cage.

2.3.3.2 Functional Decortication - KCl Experiments

Each rat was treated in the same manner as described above with the exception that after the control period was established, the pledgets of filter paper placed on the dura were soaked in 25% KCl to evoke cortical spreading depression. As in the KCl - normoxemia experiments, placing reactions were tested for at the end of the experiment to ensure functional decortication lasted for the entire duration of the experiments.

2.3.4 Conclusion of experiments

At the conclusion of experiments (i.e. after two successive days of experimentation, one with the cortex intact, the other during functional decortication) the rats were euthanized in a CO_2 chamber, and the telemetry devices and PVC cylinders were retrieved for subsequent use.

2.4 Apparatus

2.4.1 Experimental chamber

The chamber that was used is a double-walled Perspex cylinder (27 cm. in length; 7.5 cm. in diameter) with a plastic grid along the bottom. The ambient temperature of the chamber (27 or 18°C during experimental protocols) was controlled by circulating water between the walls of the chamber with a constant temperature bath and pump (Endocal Refrigerated Circulating Bath RTE-8DD, Neslab; Newington, New Hampshire). Figure 2.3 shows a diagrammatic representation of the experimental set up.

2.4.2 Measurement core temperature, activity, heart rate and respiratory frequency

With a rat placed in the chamber, the latter was placed over platform antennae (PhysioTel CTR 86, Mini-Mitter Company; Sunriver, Oregon) which received the output frequency (Hz) from the biotelemetry device. This was interfaced with a peripheral processor (Dataquest III, Data Sciences International; St. Paul, Minnesota) which was connected to an IBM computer. The platform antennae were also interfaced with a grass recorder (Model 7E Polygraph, Grass Instruments Co.; Quincy, Mass.), and a heart rate signal

was thus recorded on paper at a speed of 25 mm/sec. After the experiments were complete the heart rate in beats per minute was calculated by visually counting 15 seconds of the trace and then multiplying that number by 4. Respiratory frequency was visually counted for 15 seconds. This value was then multiplied by four to determine a frequency in breaths per minute.

2.4.3 Measurement of oxygen consumption

Gas mixtures used (hypoxic: 9.95% oxygen, balance nitrogen; normoxic: medical air; compressed gases) were obtained from Praxair (Praxair Canada Inc.; Mississauga, Ontario). Flow into the chamber was controlled by flow regulators previously calibrated with a Stead-Wells Spirometer (P-1400, Warren E. Collins Inc.; Braintree, Mass.) An oxygen analyzer (Applied Electrochemistry Oxygen Analyzer S-3A/I, AMETEK; Pittsburgh, PA) was used to measure the fraction of oxygen entering and exiting the chamber at either end. From the difference in oxygen and constant flow rate (2 liters/minute) of selected gas mixtures, the volume of oxygen the animal consumes per unit time per kg body weight (STP) was computed.

Figure Legend: Figure 2.3

Experimental design

This figure shows the experimental design of each experiment. Each rat was placed in the metabolic chamber, into which flowed 10 or 21% oxygen at a flow rate of 2.0 L/min ('F' the figure represents the flow regulator). The metabolic chamber was placed over platform antennae which were interfaced with a grass recorder (for ECG recordings), and an IBM computer (for core temperature readings). Percent oxygen flowing out of the chamber was read from a previously calibrated oxygen analyzer. The ambient temperature of the metabolic chamber was controlled at either 27 or 18°C by a temperature controlled water bath (not shown).

- Groups: Normoxemic or Hypoxemic
- Experiments: Cortex Intact & Functional Decortication

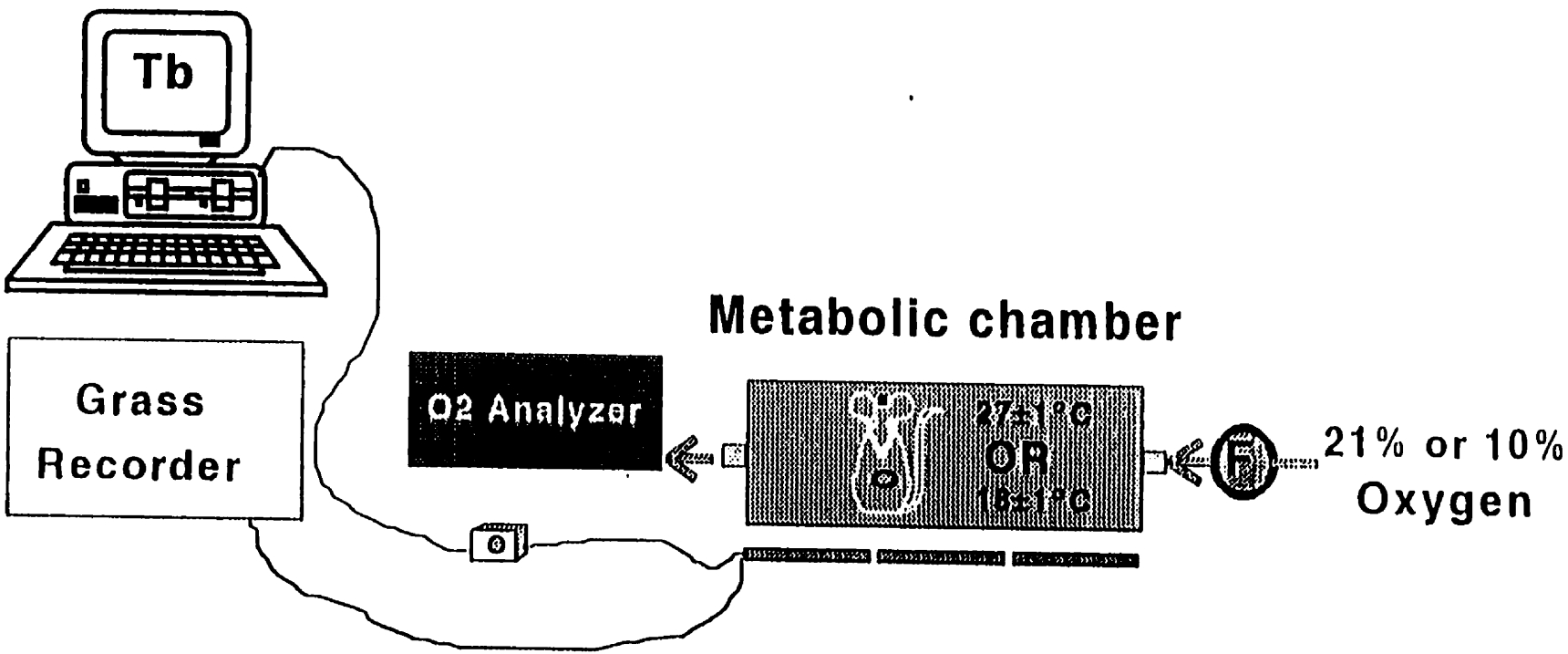


Figure 2.3 Experimental design

2.5 Statistical Analysis

Initial statistical analysis began by carrying out a four-factor analysis of variance (ANOVA) for repeated measures, to determine if there was a significant effect of period, state of cortex (cortex intact vs. functionally decorticated), gas mixture (normoxemia vs. hypoxemia), or ambient temperature (27°C vs. 18°C) (170). This was first done for each of the variables from the means of the absolute values recorded during each experiment. For this absolute value data set, further analysis was done. This consisted of carrying out Student-Newman-Keuls (SNK) multiple comparison tests to determine where in time the significant effects were found (as determined by the ANOVA) (170). As mentioned in "Materials and Methods", comparisons were made, not from the "Control-1" data, but from the last value recorded in the adjustment period before the gas mixture was changed, or C-2. This allowed a direct comparison of the effect that either saline or KCl treatments, or normoxemic or hypoxemic treatments had on the experimental period for each group of rats. To determine the effect that functional decortication had on baseline variables (normoxemia experiments), it was also necessary to compare the C-1 period to the C-2 value. If these two values were not found to be statistically significant from each other, an effect

of functional decortication on baseline variables could be ruled out. For this reason, C-1 values have been included in the graphs.

In order to determine the specific effect that decortication had on the thermoregulatory responses to hypoxemia, further statistical analysis was done. This consisted of determining the changes from Control-2, and graphs were constructed that show the differences in the mean values from Control-2, for each of the 10 experimental periods. A four-factor ANOVA for repeated measures was done to determine the standard error term for use in the SNK multiple comparison tests, and also to confirm significance that was found in the absolute value ANOVAs. SNK multiple comparison tests were then carried out to specifically determine if the state of cortex (cortex intact vs. functionally decorticate) affected both the baseline variables, or the responses to acute hypoxemia (170).

A final analysis was done for core temperature which consisted of creating "core temperature indices". These indices show the sum of the core temperature response over the hour of hypoxemia (or normoxemia). Thus a value is computed resulting in the change in core temperature per hour (°C/hr). Graphs were then constructed and show the change in core temperature (from C-2) per hour. A two way ANOVA was run for each of the

normoxemia and hypoxemia trials to determine if there was a significant effect of ambient temperature or state of cortex. This was followed by SNK tests to determine where the significant effects were found (170).

Results

Overall raw data for core temperature and oxygen consumption are presented in Appendix 1. These graphs show the responses of each animal in three dimensional ribbon plots. Thus it is possible to observe the individual differences, over time, for both core temperature and oxygen consumption.

To ensure that I am comparing the effect that decortication has on the response to hypoxemia, comparisons were made from C-2 for both absolute value and changes from "control" . This is possible since decortication was not seen to have an overall effect on any of the variables studied as determined from the ANOVAs (state of cortex: Tc, $p=0.568$; VO_2, $p=0.685$; f, $p=0.111$; hr, $p=0.522$). All graphs presented show means $\pm$ one standard deviation.

3.1 Baseline variables : effect of CSD

3.1.1 Thermoregulatory variables

As mentioned there was no significant effect of CSD on baseline Tc when C-1 and C-2 were compared with each other at either 18 or 27°C (Figure 3.1).

There was an overall significant effect of ambient temperature on core temperature ($p<0.001$).

Baseline VO_2 under normoxemic conditions was not significantly altered at 27°C. However, the SNK multiple comparison tests revealed 3 significant values when the rats were decorticated at 18°C. At periods E6, E8, and E9 (corresponding to times 36, 48, and 54 minutes in the experimental period) there was a significant decrease compared to C-2. Also, at 18°C, normoxemic VO_2 was increased significantly compared to normoxemic VO_2 at 27°C ($p=0.001$). If the C-1 values for the saline and KCl treated rats at 27°C are compared to the same values at 18°C, a 47% increase in VO_2 was found (Figure 3.2).

3.1.2 Cardiorespiratory variables

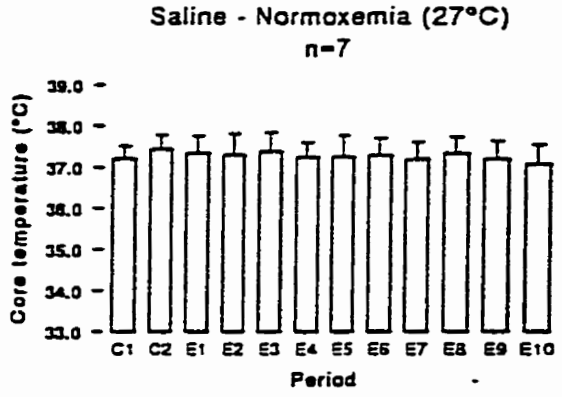
Baseline respiratory frequency was stable throughout the normoxemic experimental period and did not differ significantly from C-1 or C-2, nor was there a significant effect of KCl treatment. Respiratory frequency was elevated at 18°C compared to 27°C, but this difference was not found to be significant ($p=0.115$) (Figure 3.3).

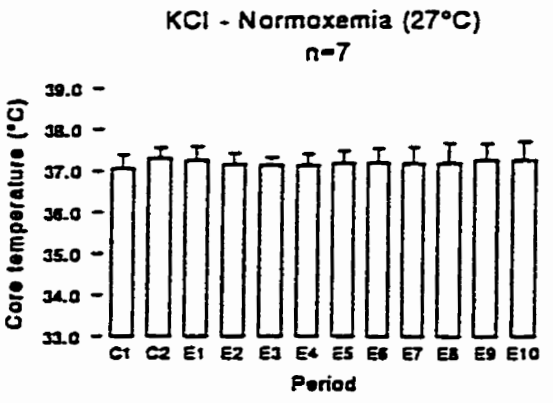
Heart rate under normoxemic conditions was seen to be stable at both ambient temperatures with no significant effect of KCl treatment (Figure 3.4). Baseline heart rate at 18°C was significantly greater than the heart rate at 27°C ($p<0.001$). When compared similarly as was done for VO_2, there was a 19% increase in heart rate at 18°C compared to the same values at 27°C.

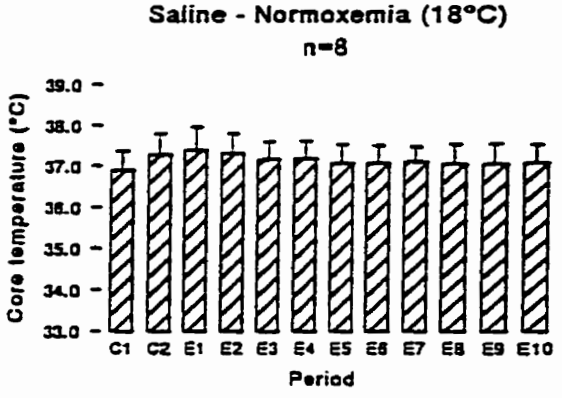
Figure Legend: Figures 3.1, 3.2, 3.3, 3.4.

Baseline variables: the effect of CSD.

Each graph shows: the mean responses of core temperature (3.1), oxygen consumption (3.2), respiratory frequency (3.3), and heart rate (3.4), for each normoxemia experiment. C-1 designates the 'control period' where core temperature remained stable over 5 consecutive two minute readings. C-2 is the last value recorded after 30 minutes were allowed to pass after handling of the rats (application of normal saline or KCl, and behavioral testing). E1 through E10 designate the 10 six minute periods of normoxemia. Asterisks designate significant differences in the experimental periods to the C-2 value. All bars represent means, plus one standard deviation.







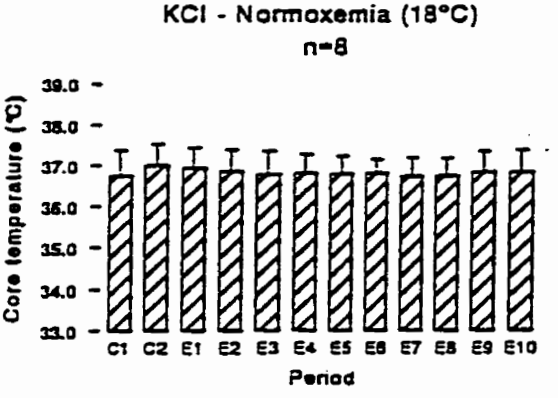
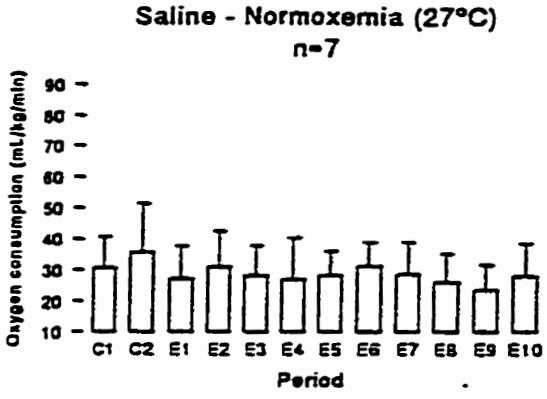
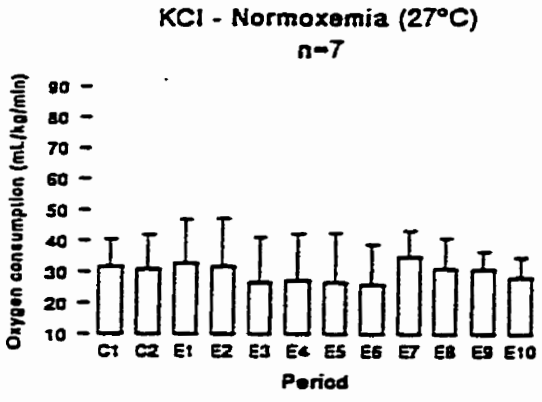
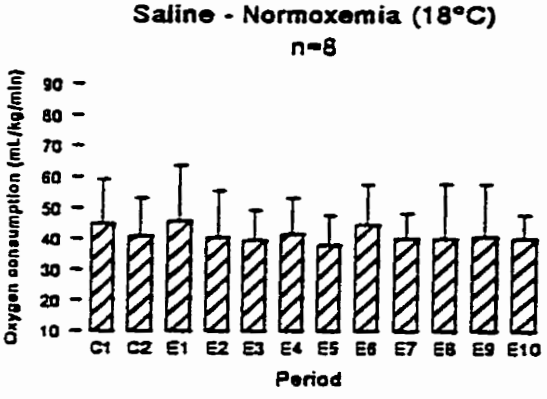


Figure 3.1.

Baseline Core Temperature







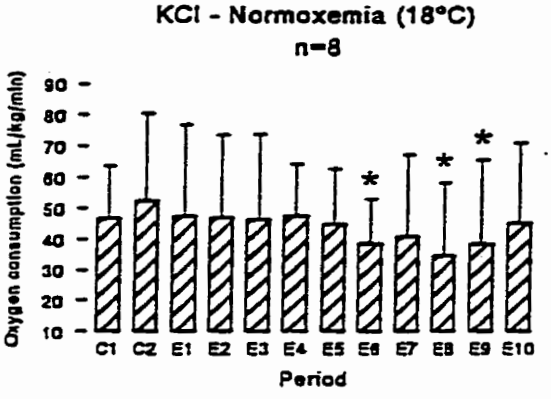
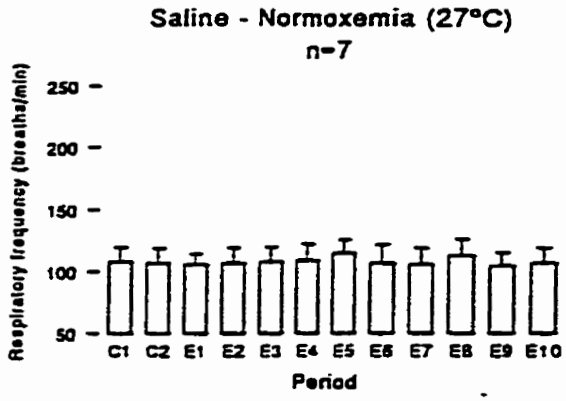
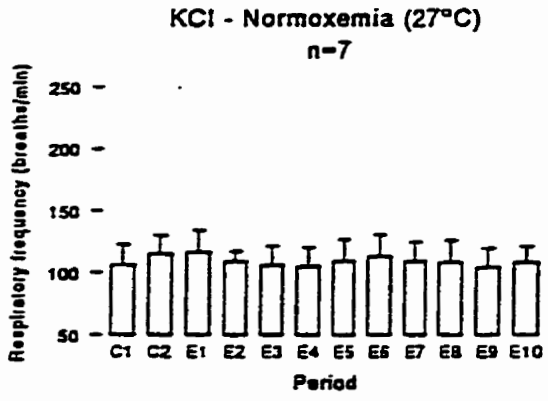
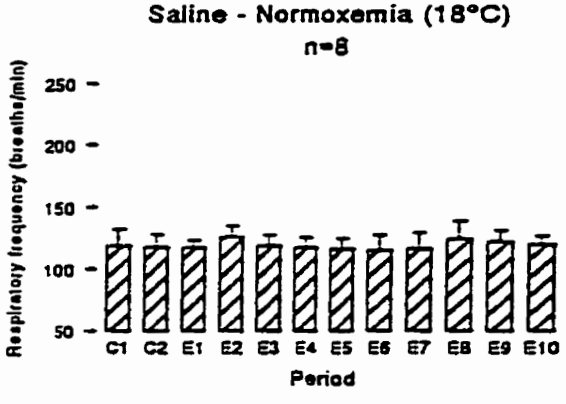


Figure 3.2.

Baseline Oxygen Consumption







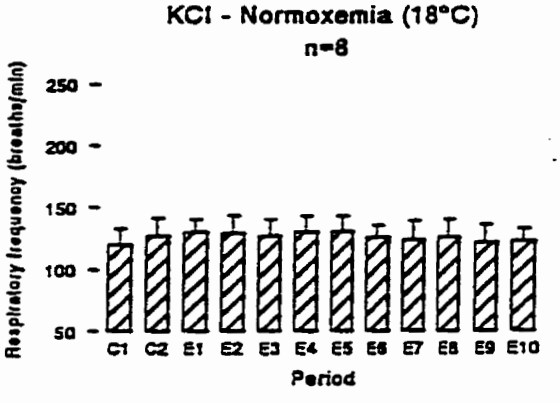
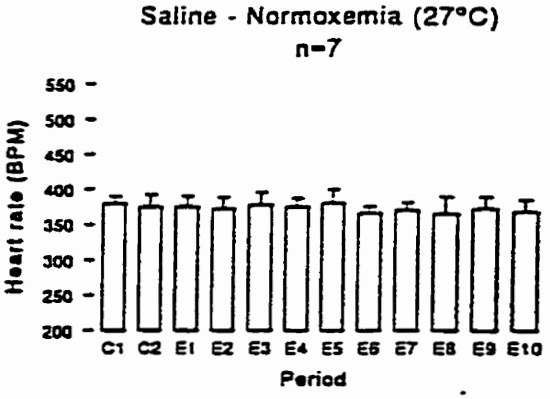
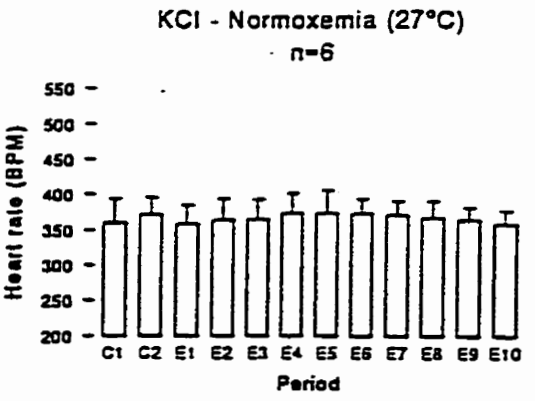
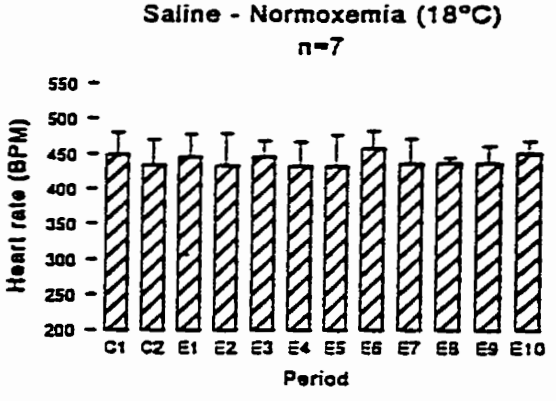


Figure 3.3.

Baseline Respiratory Frequency







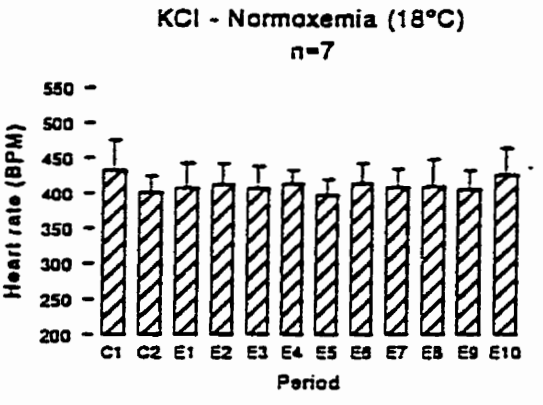


Figure 3.4.

Baseline Heart Rate

3.2.2 Responses to acute hypoxemia : effect of CSD

To further analyze the data, graphs were constructed to look at only the responses in the experimental periods (normoxemia and hypoxemia). These were made such that the effect of CSD could be directly compared to the cortex intact rats. This is important since the original hypothesis questioned whether or not the cerebral cortex is involved in the thermoregulatory responses to acute hypoxemia. As earlier discussed, the responses to hypoxemia have been compared to the C-2 values, or the last measurement taken before the gas mixture was changed from normal air to the hypoxic mixture. Thus the graphs presented show the difference in each of the 10 experimental periods to the C-2 value for each experimental parameter. Again, all graphs are presented as means ± one standard deviation.

Under normoxemic conditions, there was not found to be a significant effect of CSD on core temperature ($p=0.201$), respiratory frequency ($p=0.924$), and heart rate ($p=0.299$) (graphs not shown). The metabolic rate during CSD was however found to be significantly different from saline treated rats at 18°C. Although it was found that there was not an overall effect of CSD on $\dot{V}O_2$ ($p=0.924$), a significant effect of ambient temperature and state of cortex was found (state of cortex by ambient temperature, $p=0.030$). The SNK multiple

comparison test determined that the difference was found at the 36 minute point of the normoxemic experimental period. Overall, however, there was a large variation in the decorticated rats at 18°C.

Overall, there was not found to be a significant effect of the state of the cortex in any of the variables studied (core temperature, p=0.201; oxygen consumption, p=0.924; respiratory frequency, p=0.926; heart rate, p=0.287)

3.2.1 Thermoregulatory Variables

The decrease in Tc that occurred during acute hypoxemia was not affected by functional decortication at 27°C. There was, however, a significant effect of temperature, with the decrease in Tc being accentuated at 18°C. Also, it appears that by the end of the hypoxic exposure, the core temperature response had reached a plateau when the rats were studied at 27°C. On the other hand, when the rats were studied at 18°C, it appears that core temperature was continuing to decrease by the end of the experiment. Furthermore, when the rats were studied at 18°C, the decrease in core temperature was attenuated at various points in the hour of hypoxemic exposure. These significant attenuation's occurred at 18, 36, 54, and 60 minutes of the experimental period (E3, E6, E9, and E10) (Figure 3.5).

When the rats were studied at 27°C, metabolic rate during hypoxemia remained stable, with no significant difference between the cortex intact and the functionally decorticate animals. Although there was not a significant interaction between the gas mixture and the ambient temperature at which the animals were studied ($p=0.063$), VO_2 tended to be lower during acute hypoxemia when the rats were studied at 18°C, compared to when they were studied at 27°C. Overall, however, there was no significant effect of state of cortex and gas mixture ($p=0.888$), or between state of cortex, gas mixture and ambient temperature ($p=0.651$) (Figure 3.6).

3.2.2 Cardiorespiratory variables

When the rats were studied at 27°C, the respiratory frequency response to hypoxemia was accentuated at the beginning of the exposure (times 12, and 18 minutes, or at E2 and E3 in the graphs) when the rats were decorticated compared to when the cortex was intact. This observation was not consistent with the ambient temperature at which the animals were studied, although overall there was a significant interaction between gas mixture and the ambient temperature at which the rats were studied ($p=0.050$). There was

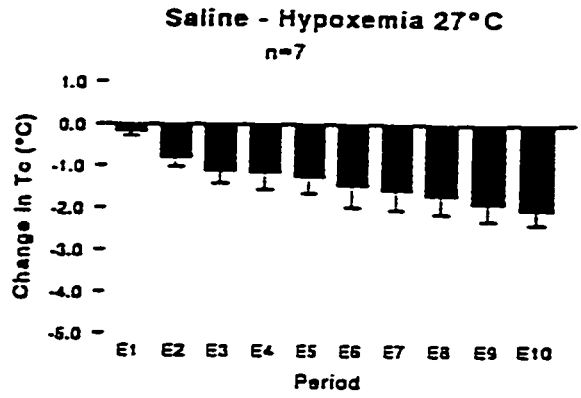
also a significant interaction between the state of cortex and the respiratory frequency over time (p=0.007) (Figure 3.7).

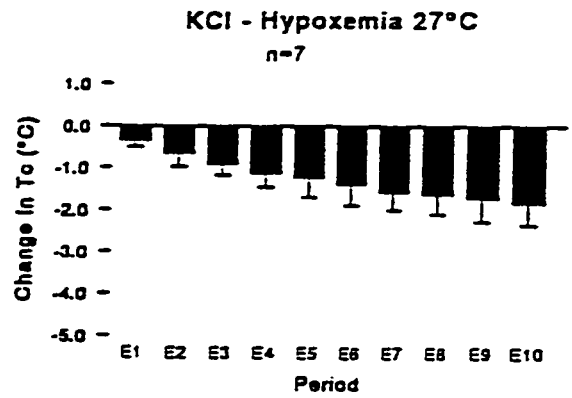
The decrease in heart rate which occurred during acute hypoxemia in this study was not significantly affected by the ambient temperature at which the animals were studied (ambient temperature, p=0.843; ambient temperature by gas mixture, p=0.281). There were, however, significant interactions between the state of cortex, the ambient temperature at which the rats were studied, and the time of exposure (p=0.006). Specifically, when the rats were functionally decorticated the decrease in heart rate was significantly attenuated at the beginning of the hypoxemic exposure. At 18°C, the attenuation in the heart rate during hypoxemia persisted for the first 24 minutes, while thereafter there were no significant difference between the cortex intact and the decorticated rats. In addition, after 6 minutes of acute hypoxemia the decorticated rats had a significant increase in heart rate from C-2, compared to when the rats cerebral cortex was intact. This transient increase in heart rate was consistent at both ambient temperatures (Figure 3.8).

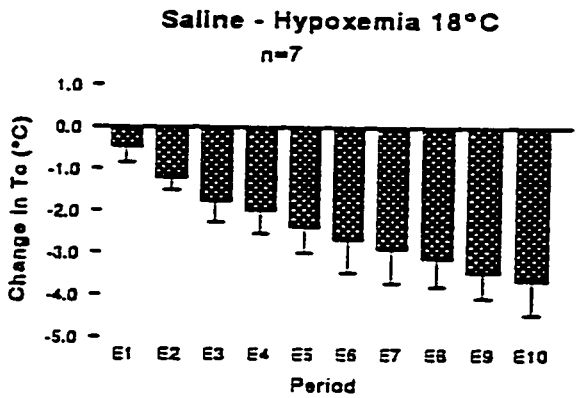
Figure Legend: Figures 3.5, 3.6, 3.7,3.8

The effect of CSD on the responses to acute hypoxemia.

In this set of graphs, the mean differences from C-2 for each of the 10 experimental periods is shown for core temperature (3.5), oxygen consumption (3.6), respiratory frequency (3.7), and heart rate (3.8). Asterisks indicate a significant difference from saline treated rats at the same ambient temperature. As before, each bar represents means plus one standard deviation.







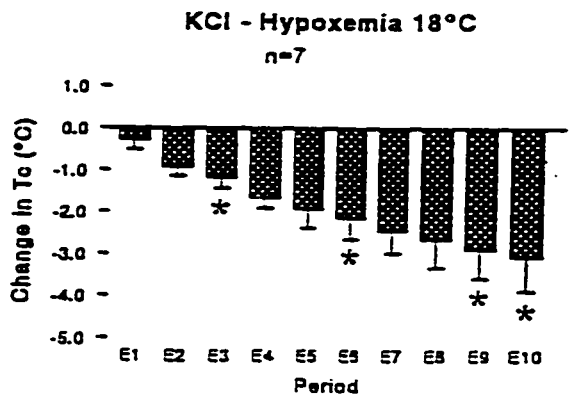
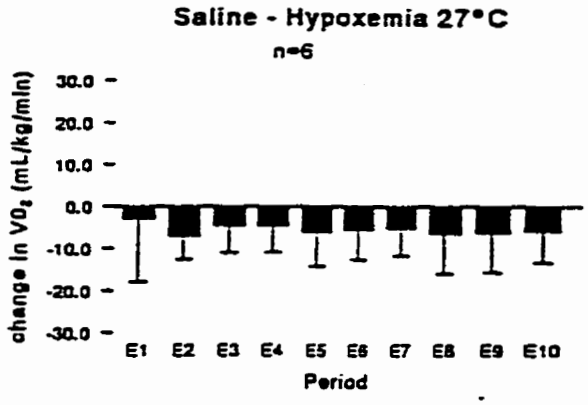
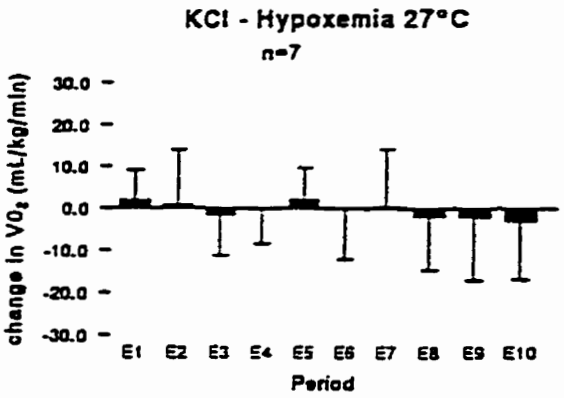
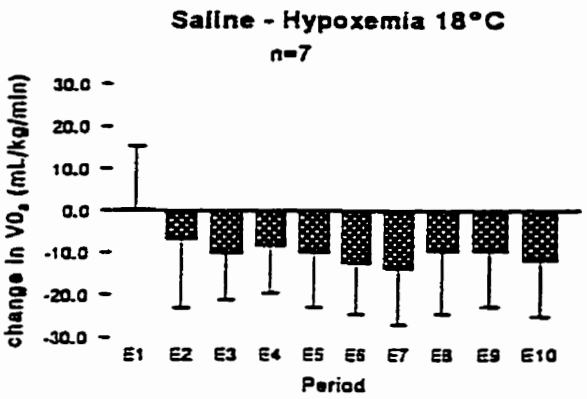


Figure 3.5.

Core temperature response to hypoxemia: effect of functional decortication







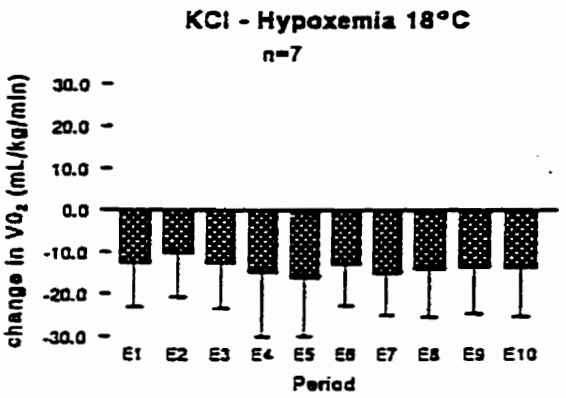
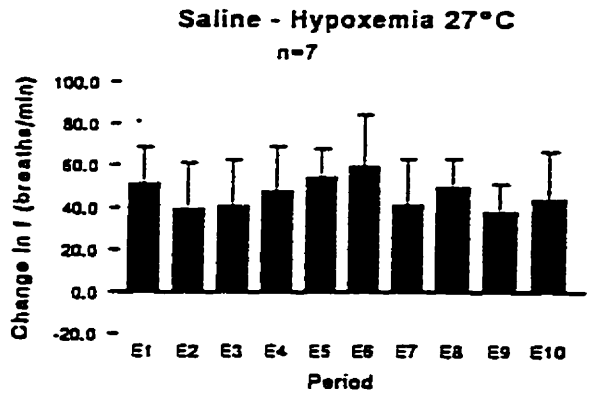
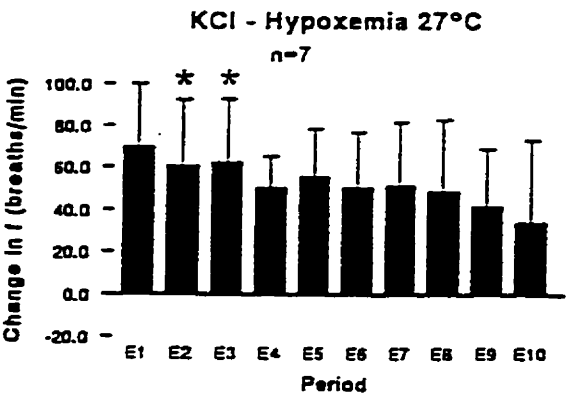
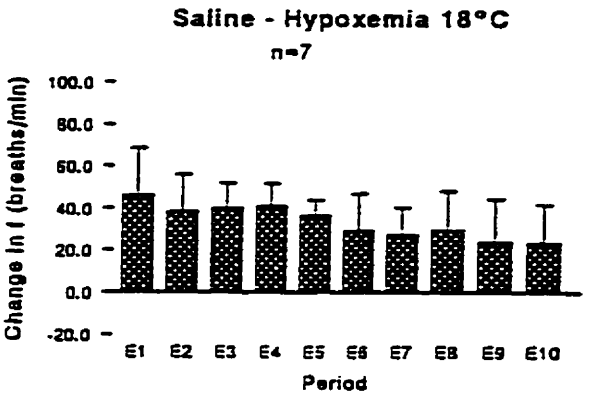


Figure 3.6.

Oxygen consumption response to hypoxemia:
effect of functional decortication







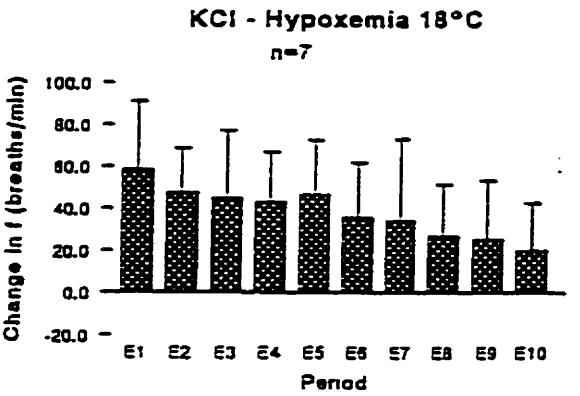
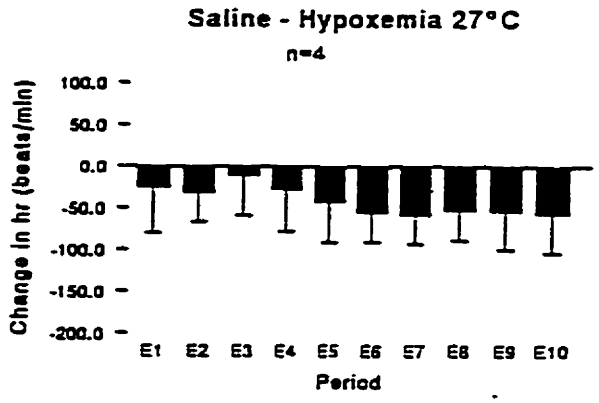
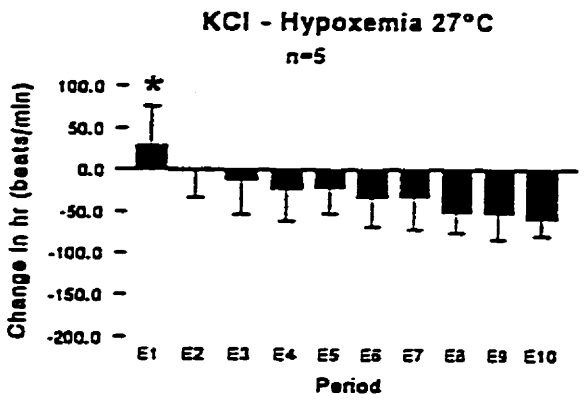
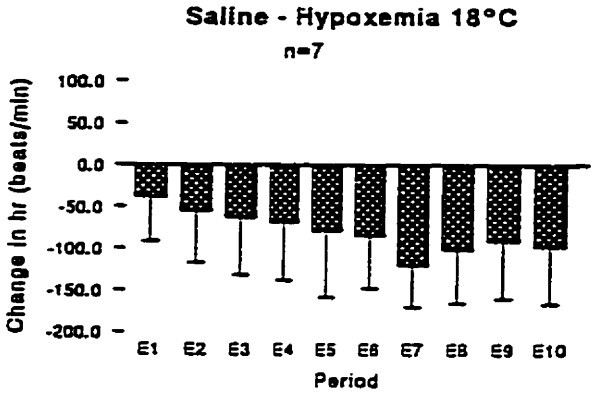


Figure 3.7.

Respiratory ferquency response to hypoxemia: effect of functional decortication







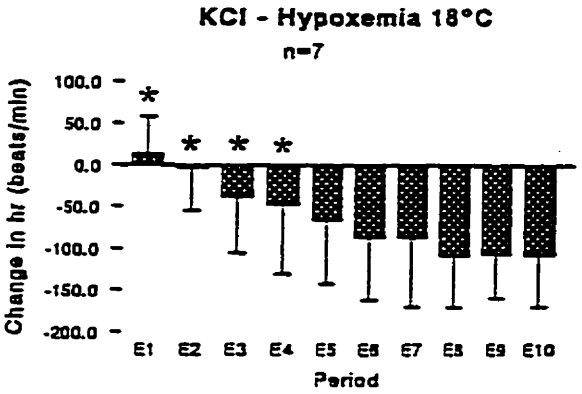


Figure 3.8.

Heart rate response to hypoxemia:
effect of functional decortication

3.2.3 Core temperature indices

When the cumulative change in core temperature over each experimental period is calculated, core temperature indices result (°C/hour) (Figure 3.9). During normoxemia, there were no significant effects of either ambient temperature ($p=0.794$), or state of cortex (0.992), nor was there a significant interaction between state of cortex and ambient temperature ($p=0.458$). During hypoxemia, the core temperature response to hypoxemia was accentuated when the rats were studied at 18°C compared to when they were studied at 27°C. This effect was significant ($p=0.001$). However, as with the normoxemia experiments, during hypoxemia, there were no significant effects of the state of the cortex ($p=0.067$), nor was there a significant interaction between state of cortex and ambient temperature ($p=0.507$).

Figure Legend: Figure 3.9

Core Temperature Indices

Each graphs represents the sum of the change in core temperature over the one hour experimental period (either normoxemia or hypoxemia) for both intact and functionally decorticate animals, at 27°C (top graph) and 18°C (bottom graph). Asterisks indicate significant differences between the responses at 27°C compared to when the rats were studied at 18°C.

N-NS: normoxemia - normal saline (intact)

N-KCl: normoxemia - KCl (functional decortication)

H-NS: hypoxemia - normal saline (intact)

H-KCl: hypoxemia - KCl (functional decortication)

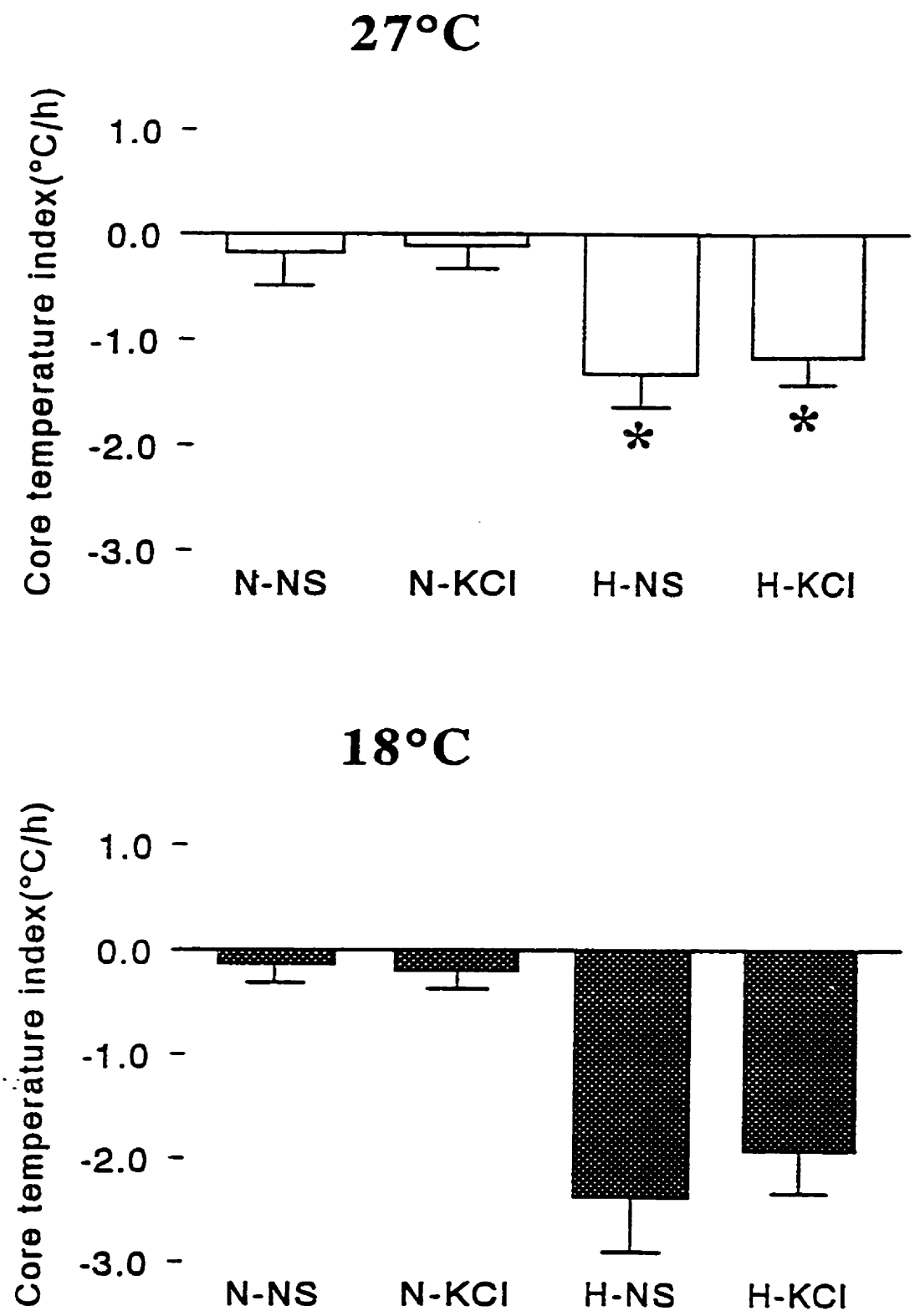


Figure 3.9. Core Temperature Indices

4. Discussion

4.1 General

These experiments provide new information about the regulated decrease in core temperature during acute, moderate hypoxemia in rats. The mechanism underlying this regulated hypothermia during hypoxemia is important in the study of various disease states and syndromes both in newborns and adults (i.e. respiratory distress syndrome, persistent pulmonary hypertension, SIDS). Elucidation of this mechanism has received much attention in recent years, although the majority of emphasis has been placed on cardiorespiratory responses. With the development of biotelemetry in recent years, the ease and accuracy of measuring core temperature has advanced the study of thermoregulation, in general, and with respect to hypoxemia.

It may be viewed that the thermoregulatory response to hypoxemia is a cumulative response which involves metabolic, cardiorespiratory, and behavioral mechanisms. It is therefore likely that not one mediator, or mechanism is solely responsible for the decrease in core temperature. Viewing this response to acute hypoxemia in such an integrative manner

should ultimately reveal the pathway that the decrease in core temperature takes during hypoxemia.

One such step has already been taken in that it has been conclusively shown that the decrease in core temperature during hypoxemia is a regulated rather than a forced thermoregulatory state (28,40,70,82). This fact intrinsically implies a centrally mediated mechanism, since set-point is considered to be altered in regulated thermoregulatory states (59,66). With this in mind, and considering that convincing data which support that the cerebral cortex modulates the regulated hyperthermia associated with central PGE_1 injection (129,130), it was reasonable to hypothesize that the cerebral cortex may play a role in another regulated thermoregulatory state, i.e. the regulated decrease in core temperature associated with acute hypoxemia. Furthermore, numerous studies have shown that ablation or stimulation of the cerebral cortex alters thermoregulation. Specifically, surgical decortication in cats, dogs, and monkeys, impaired the ability to thermoregulate when thermally challenged (35,144). Also. DeLuca et al (36) have shown that stimulation of the prefrontal, but not the parietal or occipital cortex increases metabolic rate (through stimulation of the sympathetic nervous system). More recently, with the advent of CSD in the study of behavioral and autonomic thermoregulation (as in the aforementioned fever studies), the cerebral cortex has been implicated in exerting a tonic role in thermoregulation (see below).

As mentioned in the Introduction, Shibata and Hori's groups observed that when CSD passed through the cerebral cortex of Wistar rats, the activities of the thermosensitive neurons located in the POAH were altered. Specifically, warm-sensitive neurons' activity was decreased and cold-sensitive neurons activity was augmented (87). Refinement of their methods and the use of single waves of CSD led the researchers to expand on their previous studies, and in 1983 they reported that behavioral thermoregulation was altered during CSD. Specifically, using rats that were operantly conditioned to behaviorally thermoregulate, they found that there was an inhibition of thermoregulatory cooling behavior and a facilitation of heating behavior when a single wave of CSD crossed into the prefrontal cortex (150,152). Later, when using a double CSD technique, they determined the sulcal prefrontal cortex (S-PFC) to be the most critical area for the altered activity of the POAH thermosensitive neurons (88,151). Since electrical stimulation of this area increased and decreased the firing rate of considerable numbers of POAH warm and cold-sensitive neurons, respectively, they concluded that the S-PFC exerts a tonic influence on the activity of these neurons and is thus involved in the central control of thermoregulation. This agrees with DeLuca's findings that stimulation of the prefrontal cortex increases metabolic rate.

Because most of their work was related to behavioral thermoregulation, in 1985 Shibata, Hori and Nagasaka shifted their attention to autonomic responses (153). They reasoned that frontal CSD would alter metabolic heat production much as it did for behavioral thermoregulatory responses. Their results demonstrated that a single wave of CSD elicited in the frontal cortex affected autonomic thermoregulation (increasing metabolic heat production) with a similar time course as behavioral thermoregulation. Taken together, Shibata and Hori's experiments have led to the conclusion that the frontal cortex, specifically the S-PFC, of the rat, exerts a tonic influence on thermoregulatory responses by its action on POAH thermosensitive neurons and thus has a role in central thermoregulation.

Evidence to support the tonic role of the cerebral cortex in thermoregulation has not been provided. In each of the studies where the cerebral cortex has been implicated in fever production, CSD did not alter baseline core temperature or oxygen consumption (37,129,130). Only when the rats were challenged did CSD have an effect on thermoregulatory responses (attenuation of core temperature and oxygen consumption during PGE_1 fever, or lateral hypothalamic lesioning). Therefore, these studies indicate that the thermoregulatory role of the of the cerebral cortex is phasically active.

Furthermore, in a study designed to determine the effect that CSD may have on the ventilatory responses to hypoxia and hypercapnia, Maskrey et al (116), revealed that during hypoxia and hypercapnia, decortication accentuated the respiratory responses in rats (unfortunately no body temperature values were included in the study). Since no ventilatory responses were altered when the rats breathed normal air, they concluded that an inhibitory influence of the cerebral cortex is not tonically active and that the absence of cortical inhibition only becomes obvious when the rat is challenged by experimental gas mixtures. They believed this would occur if decortication were to raise the background neuronal activity within the brainstem, thus effectively lowering the response threshold of neurons within the respiratory complex, making them more responsive to input from chemoreceptors. Their conclusion is supported by studies by Bures et al. (18) who reported that, following CSD, neurons in the reticular formation (which forms the "matrix" in which the respiratory centers are imbedded) show a net increase in activity.

4.2 Baseline thermoregulatory variables : effect of CSD

In the present study, CSD did not significantly affect baseline core temperature. This was true when the rats were studied at or below thermoneutrality for the Sprague-Dawley rat. That CSD did not alter baseline

core temperature is in keeping with the results shown in Sprague-Dawley rats (37,129,130). On the other hand, the present data differs from the studies of Shibata and Hori's groups who did observe an increase in body temperature (and metabolic heat production) during CSD, in Wistar rats (88,150,152,153). Reasons for this discrepancy are unclear. However, there are at least two possible differences which concern the methodologies of these experiments. First is the method of body temperature measurement. When body temperature is unchanged following CSD, temperature was measured by telemetry, thus effectively measuring "core" temperature. On the other hand, in the studies where an increase in body temperature was observed, temperature was measured by a thermistor inserted in the rectum, which represents an indirect measure of "core" temperature. Furthermore, this method of recording body temperature has been associated with inducing what is commonly referred to as stress-induced hyperthermia, and thus may have resulted in the activation of thermoregulatory effectors that was observed in these studies. Specifically, the handling of rats, and the insertion of an anal or colonic probe are regarded as "stressful" or "emotional" situations for the rat, which are directly associated with stress-induced hyperthermia (13,21). Stress-induced hyperthermia has been shown to be a regulated hyperthermia analogous to fever (11,12,100,112,149). Secondly, and more likely, it is possible that there exists a strain difference for rats in the response to CSD. The increase in core temperature and metabolism was

seen only in Wistar rats, whereas Sprague-Dawley rats have yet to show alteration of baseline variables during CSD. It has been shown that the incidence of neocortical-striatal transmission of spreading depression may vary across rat strains (96). It is unlikely that this type of transmission is responsible for the differences seen in the alteration of baseline variables during CSD, but emphasizes the fact that it is possible that CSD may affect the different rat strains in different ways.

As with core temperature, CSD was not seen to alter baseline oxygen consumption in the present experiment. This was true when the rats were studied at 27°C, and, as with core temperature, the results correspond to the aforementioned studies where metabolism either increased in the Wistar rats (42,88,153), or remained unchanged in the Sprague-Dawley rat studies (37,129,130). Each of these studies were conducted at similar ambient temperatures as this study, i.e. between 25 and 29°C. Again, strain differences are assumed to be the cause of these discrepancies. To my knowledge, the effect of CSD on metabolism has not been studied at ambient temperature below the thermoneutral zone. The results in the present study are however somewhat inconclusive. In the normoxemia experiments, VO_2 remained stable after the 30 minute adjustment period, as well as 30 minutes into the experimental period. There were, however, significant decreases in VO_2 after this point (only when comparing back to C-2, not from C-1, and thus

not comparing the effect that CSD has on baseline). In that a consistent pattern was not observed (i.e. significant decreases from C-2 and not C-1, at 36, 48, and 54 minutes), and there was not a concomitant decrease in core temperature, these decreases are not viewed as having a physiological significant effect of CSD on VO_2 at 18°C. Furthermore, statistical analysis did not reveal a significant effect of decortication on VO_2.

4.3 Baseline cardiorespiratory variables: effect of CSD

As with Maskrey et al's study (130), baseline respiratory rate was not altered by functional decortication. This was true at both ambient temperatures at which the animals were studied. Thus it can be said that at thermoneutral ambient temperatures, and at ambient temperature below thermoneutrality (Maskrey et al's study was conducted at 23±1°C) CSD does not alter baseline respiratory rate.

To my knowledge the effects of CSD have not been studied with respect to heart rate. In the present study, CSD did not alter baseline heart rate, as determined by the analysis of variance. Throughout the normoxemia experiments, heart rate remained stable both when the cortex was intact and during functional decortication when the rats were studied at 18 and 27°C.

Therefore, during normoxemia, the cerebral cortex is not involved in the control of heart rate.

4.4 Thermoregulatory responses to hypoxemia: effect of CSD

The decrease in Tc which occurred during acute hypoxemia produced by the inhalation of 10% oxygen is similar to that previously reported in rats (40,41,53,63), and in other species (30,51,56,70,79). As expected, at 18°C, the decrease in Tc was greater than at 27°C (within the thermoneutral zone of Sprague-Dawley rats, see below).

It has been demonstrated that the response to hypoxemia depends on resting metabolic rate (41,54,83,89,132,146). This is because at ambient temperatures below TNZ, metabolic rate is above basal levels since the rat must activate thermoregulatory effectors (i.e. peripheral vasoconstriction, piloerection, shivering and non-shivering thermogenesis) to maintain set-point body temperature. Thus, at ambient temperatures below TNZ, the thermoregulatory responses to challenges such as acute hypoxemia become accentuated (7,8,83).

The fact that CSD did not alter the Tc response to hypoxemia indicates that the cerebral cortex is not involved in the regulated hypothermia associated with

hypoxemia in rats. This is an important novel finding indicating that the cerebral cortex is not involved in thermoregulation when set-point is decreased (presumably due to the decrease in partial pressure of oxygen in the blood). Since hypoxia has been implicated in altering thermosensitive neurons firing rates (160) and that direct neuronal connections have been shown to be present between the cortex and the hypothalamus (90) and that CSD and electrical stimulation are known to alter firing rates of thermosensitive neurons in the POAH (87,88,151), it seemed reasonable that CSD may alter the thermoregulatory response to hypoxemia.

At 27°C, hypoxemia did not produce a decrease in VO_2, while VO_2 was decreased when the rats were studied at 18°C. This confirms previous investigations, that in both cats and rats, at thermoneutral temperatures, hypoxemia does not necessarily induce a decrease in oxygen consumption, but at ambient temperatures below thermoneutrality, oxygen consumption will decrease during hypoxemia (52,54,117,146). Further support for the finding that VO_2 responses depend on resting metabolic rate (as described by Hill in 1959) is that an accentuated decrease in VO_2 occurs during acute exposure to hypoxemia in newborns, who have naturally high metabolic demands to maintain body temperature and growth (30,89,132). The metabolic response to hypoxemia observed in this study was not altered by functional decortication. This indicates that, in rats, the cerebral cortex is not involved in

the metabolic response to acute hypoxemia when studied at or below thermoneutral ambient temperatures.

Thus, the hypothesis that the cortex is phasically involved in thermoregulation when Tc is "far from set-point" is not supported in the present study (129). Thus, although the cortex has been implicated in fever production (129,130), where Tc diverges from set-point, during hypoxemia, when Tc also diverges from set-point (although in the opposite direction), the cortex cannot be implicated.

The attenuation of the decrease in core temperature which was observed at various time points when the rats were functionally decorticated, and studied at 18°C, may indicate a possible role for the cortex under these circumstances. Statistically, however, there was no effect of KCl treatment, and thus it is difficult to determine the reasons behind this attenuation. Considering a consistent pattern of attenuation was not observed, and there was not an accompanying attenuation in the decrease in oxygen consumption during the same experiments, this attenuation is not considered to be of physiological significance. Furthermore, the core temperature indices (Figure 3.9) showed that there was not a significant effect of functional decortication at either temperature at which the rats were studied. This was true for both the normoxemia and the hypoxemia experiments.

Overall, therefore, this study supports the finding that the cerebral cortex may play a phasic, but not a tonic role in thermoregulation in the rat. The finding that CSD does not alter the core temperature and oxygen consumption responses to acute, moderate hypoxemia, disagrees with the original hypothesis. These results also disagree with the hypothesis that cortex is involved in thermoregulation when core temperature diverges from set-point (129). It remains possible that the cortex is involved when set point is increased, but not necessarily decreased, with respect to core temperature.

4.5 Cardiorespiratory responses to hypoxemia: effect of CSD

The finding that basal respiratory frequency was not significantly affected by decortication agrees with the findings of Tenney & Ou's work in cats (161) and Maskrey et al.'s work in rats (116). Like these studies, an accentuated respiratory frequency response to "experimental gas mixtures" (i.e. inhalation of 10% O_2) was found at 27°C when the rats were functionally decorticated. This was not the case when the rats were studied at 18°C. Our results indicate that during exposure to 10% oxygen, CSD did not significantly increase the respiratory frequency response compared to intact controls when the rats were studied at 18°C. Maskrey et al. found an accentuated ventilatory response

(increased minute ventilation) to breathing experimental gas mixtures, due to an exaggerated increase in frequency, principally when breathing CO_2 (21%O_2 & 4% CO_2 and 10%O_2 & 4%CO_2 versus 10%0_2 and 21%O_2). This study was conducted at an ambient temperature of 23±1°C. They postulate that decortication presumably removed an inhibitory effect of the cerebral cortex on the mechanism governing the frequency response to CO_2. Furthermore, since the ventilation of decorticate rats breathing normal air is no different than that of intact controls (as in our study), they speculate the absence of cortical inhibition only becomes obvious when the rat is challenged by experimental gas mixtures (especially changes in CO_2 concentrations); the inhibitory influence of the cerebral cortex is not tonically active. If this is the case, and that the neurones within the respiratory complex are more responsive to input from the chemoreceptors during functional decortication (due to an increase in background neuronal activity within the brainstem (18)) our data support this speculation. It is interesting that the respiratory frequency response to hypoxemia was not accentuated during CSD when the rats were studied at 18°C. The fact that cold exposure tends to attenuate the respiratory frequency response to acute hypoxemia (54,134,146) may override the absence of cortical inhibition, resulting in a similar increase in respiratory frequency to intact rats.

In this study, the decrease in heart rate during hypoxemia in cortex intact rats is in accordance with previously reported decreases in heart rate following

inhalation of 8 or 10% O_2 (114,115,137,163). These results in the rat, are comparable to the responses seen in neonates of both small and large mammalian species when hypoxemia is induced (33,58). Thomas and Marshall (163) report that during hypoxemia there is a transient tachycardia followed by a secondary bradycardia. Their results were obtained when studying rats breathing 8% O_2 for either 3, 5 or 10 minutes. The fall in heart rate became apparent after 5 minutes, and was further accentuated by 10 minutes of hypoxemia. This would explain why a significant tachycardia was not observed in our study, since measurements were not taken until 6 minutes into the hypoxic exposure.

Interestingly, CSD appears to modulate the bradycardia associated with acute, moderate hypoxemia in rats. At both 18 and 27°C, a significant increase in heart rate was observed after 6 minutes of hypoxemia compared to intact controls. As well, when the rats were studied at 18°C, the decrease in heart rate during hypoxemia was attenuated when the rats were functionally decorticated. Thus the cortex appears to play a role in the heart rate response to hypoxemia at thermoneutral temperatures as well as below TNZ.

As far as I know, the effect of cold exposure on the cardiovascular response to hypoxemia has not been studied. As well, although adenosine has been implicated as a mediator of this response, the mechanism of this secondary

bradycardia during hypoxemia has yet to be fully elucidated. Therefore it may be that there are central (i.e. cerebral) influences governing this response. This is not a new idea, as portions of the of the cerebral cortex (insular, medial prefrontal, and somatomotor and sensory areas) have been implicated in cardiovascular control during both physiological and pathophysiological states (i.e. hypoxemia) (25). This alteration, of cardiovascular control during CSD needs to be further studied, and may be important in elucidating the mechanisms governing both the cardiovascular responses to hypoxemia, and cardiovascular regulation in general.

4.6 Conclusions

The hypothesis that the cerebral cortex plays a role in the regulated decrease in core temperature during acute, moderate hypoxemia in young male rats was not supported by the data collected in this study. The thermoregulatory responses to hypoxemia when subjected to a cold ambient temperature were not significantly affected by functional decortication, indicating that the cortex is not involved in these responses regardless of ambient temperature. However, because the results obtained when the rats were studied at 18°C were not entirely consistent, it would be interesting to further study the role of the cortex in thermoregulation in the cold.

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6. Appendix

As mentioned in the results section, raw data for core temperature and oxygen consumption are presented here in the appendix.

Figure Legend:

Each figure shows the raw data for both saline and KCl treated rats, both during normoxemia (upper panels) and hypoxemia (lower panels). "Control" in the graphs represents the C-1 value (the average of 5 consecutive 2 minute readings in which core temperature did not vary by more than 0.2°C. The remaining time periods (from 36 to 90 minutes) are the periods following application of either saline or KCl, after the 30 minute adjustment period (including the last time point before the gas mixture was changed).

Figure 5.1: Core temperature at 27°C

Figure 5.2: Core temperature at 18°C

Figure 5.3: Oxygen consumption at 27°C

Figure 5.4 Oxygen consumption at 18°C

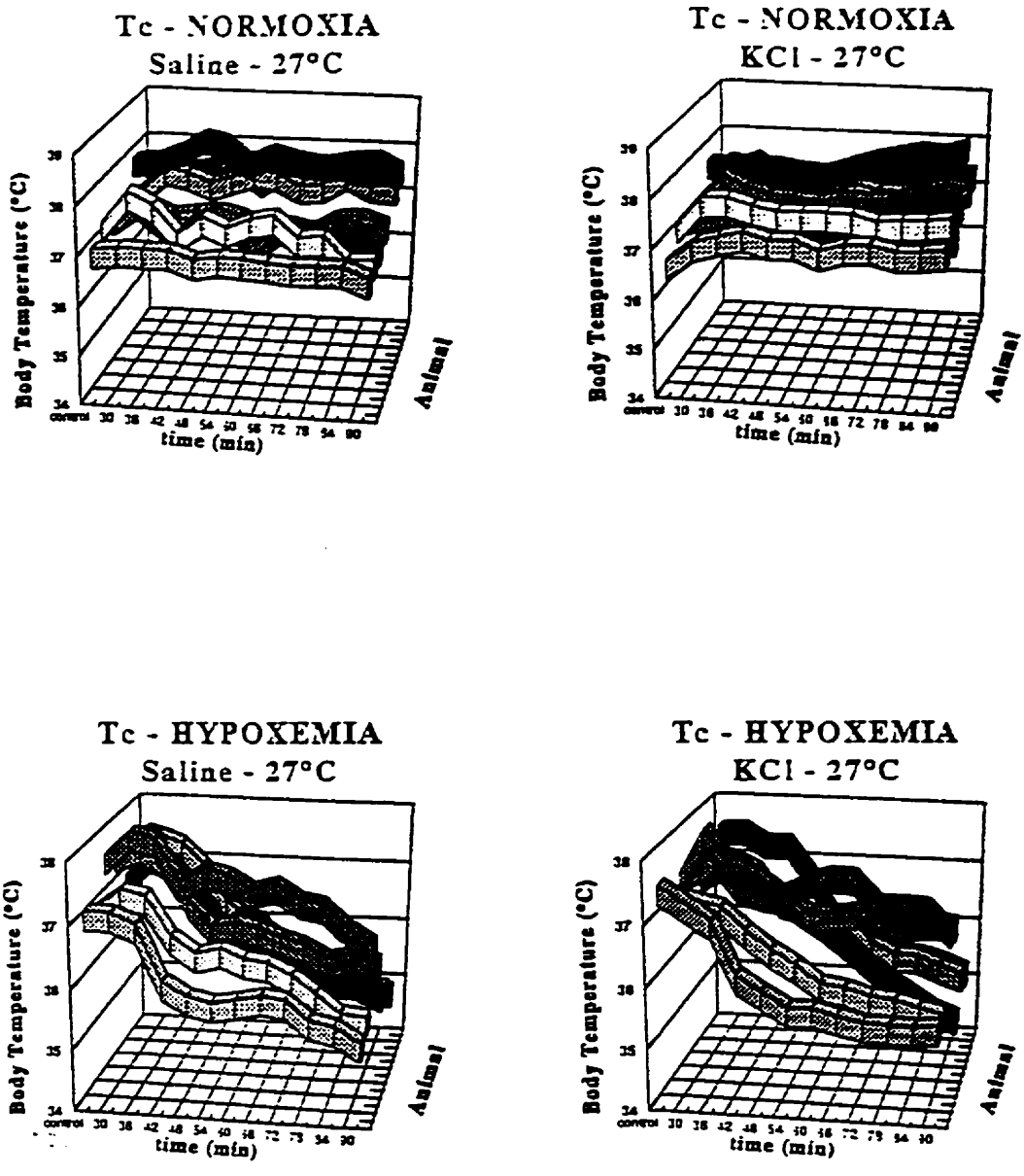


Figure 5.1. Core temperature (27°C)

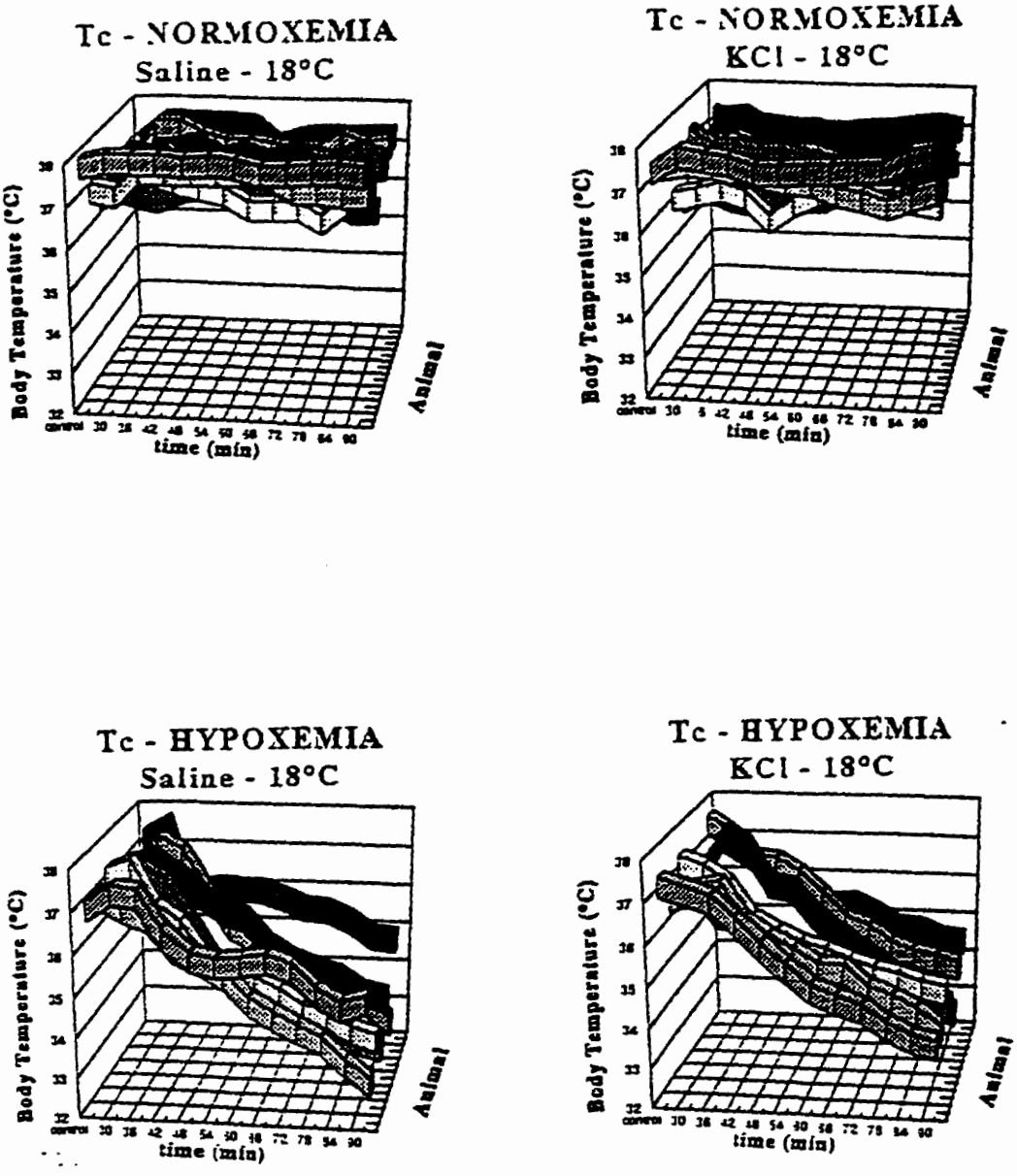


Figure 5.2. Core temperature (18°C)

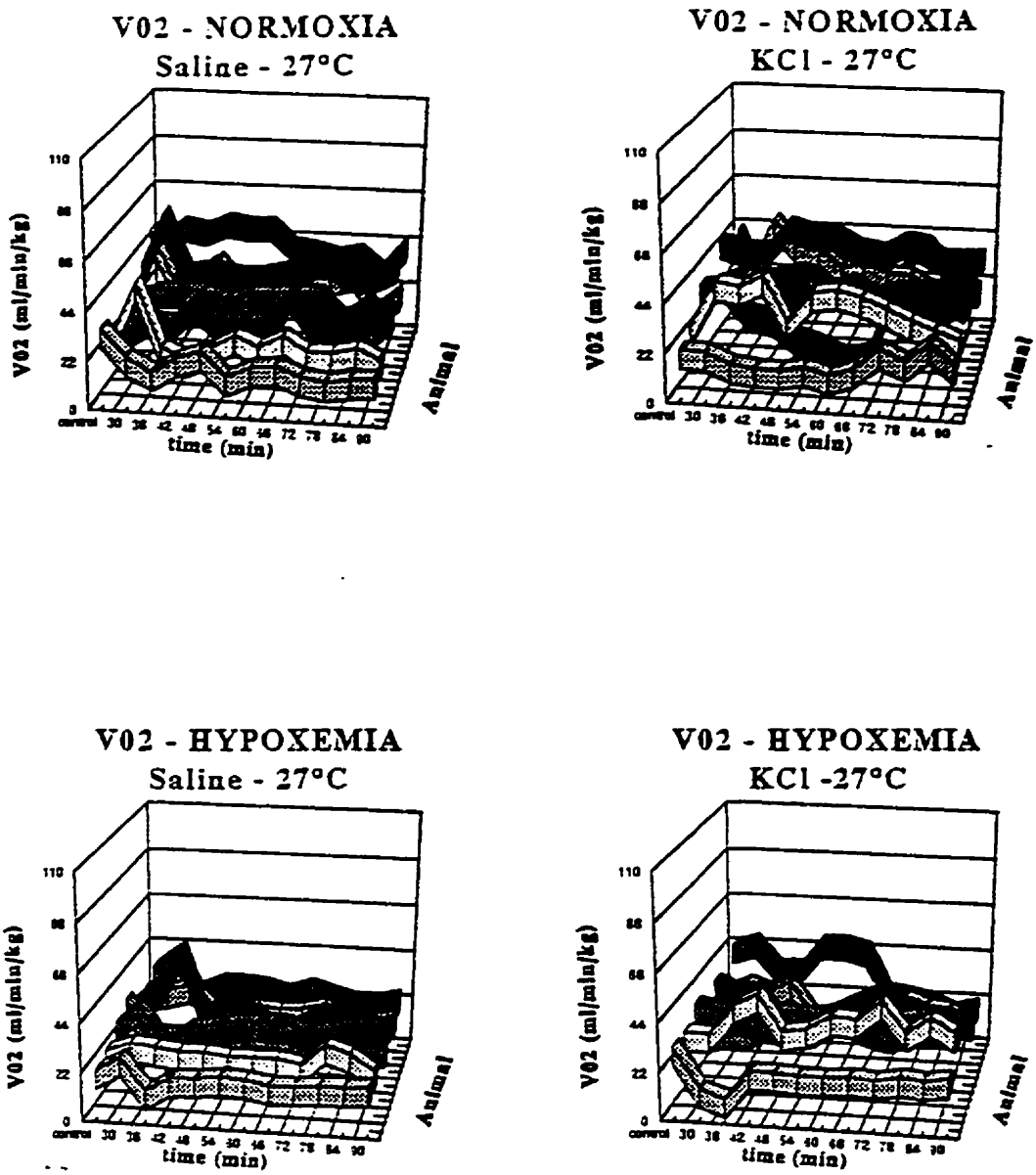
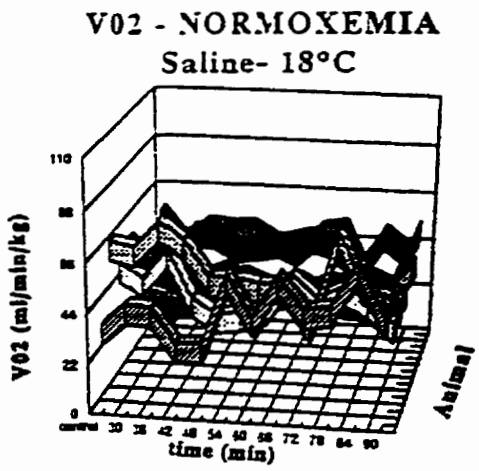
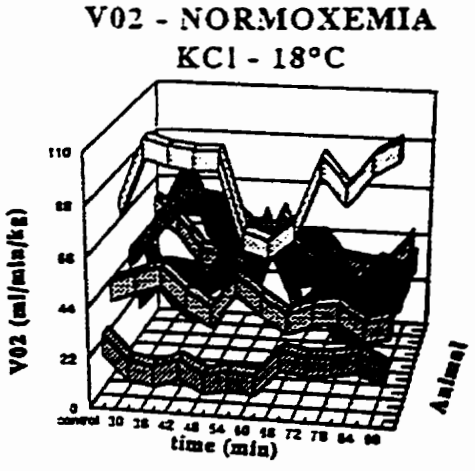
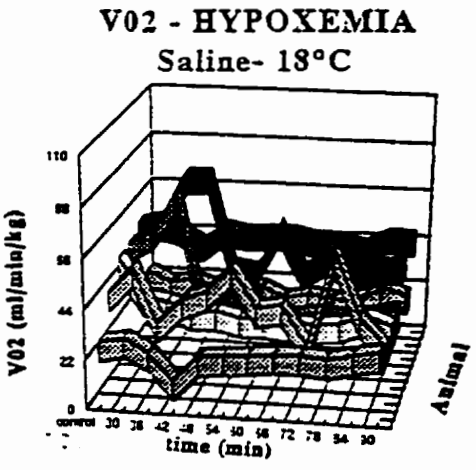


Figure 5.3. Oxygen consumption (27°C)







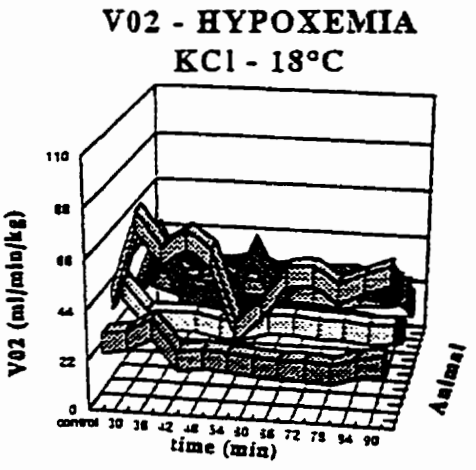
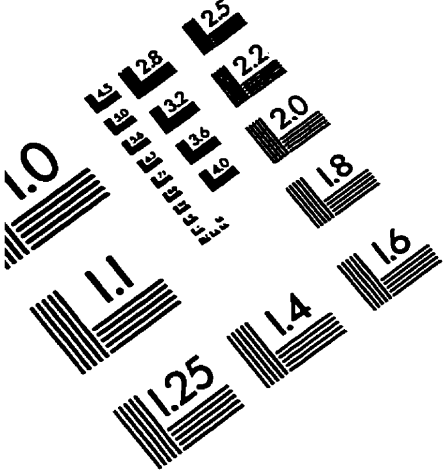
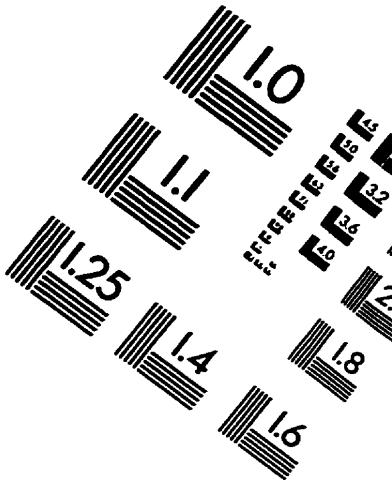
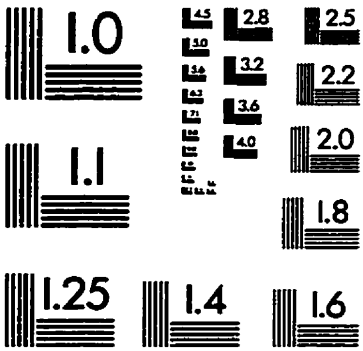


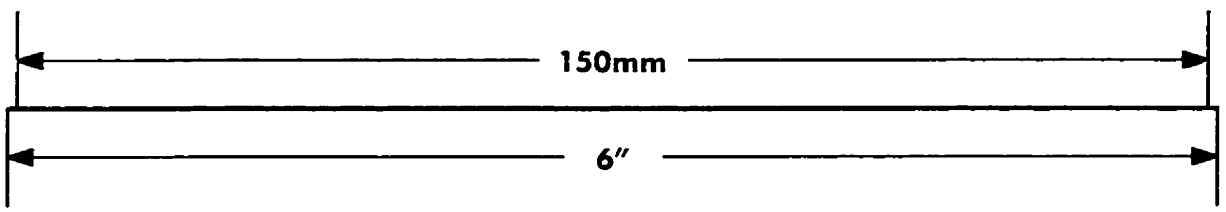
Figure 5.4. Oxygen consumption (18°C)

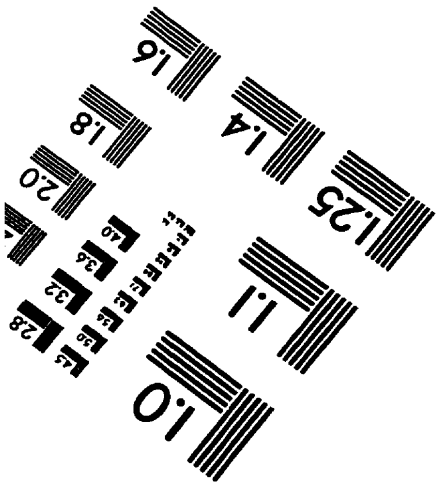
TEST TARGET (QA-3)

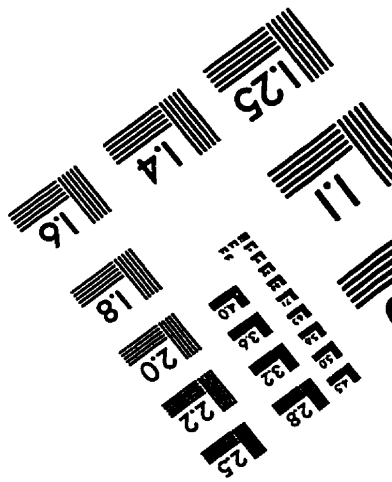












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