ASPECTS OF THE CONTROL OF BREATHING IN THE GOLDEN-MANTLED GROUND SQUIRREL

By

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ABSTRACT

Spermophilus lateralis, the golden-mantled ground squirrel, while euthermic exhibits a strong hypoxic ventilatory response, but a relatively blunted hypercapnic ventilatory response similar to other semi-fossorial mammals. Under resting conditions, carotid body chemoreceptors provide a tonic excitatory input to the frequency component of ventilation. Carotid body denervation (CBX) results in a 40% decrease in minute ventilation ($\dot{V}$). The overall ventilatory response to hypoxia is unaffected by CBX, although the ventilatory threshold is significantly shifted to lower levels of inspired O_2. CBX also has little effect on the overall response to hypercapnia. Thus, in S. lateralis, it appears that changes in the partial pressure of O_2 (P_{O2}) in the blood act centrally, rather than peripherally, to play a predominate role in ventilatory control.

Chronic exposure to hypoxia and hypercapnia (CHH, 17% O_2 and 4% CO_2) does not result in overall ventilatory acclimation, with minute ventilation being similar to control squirrels acutely exposed to hypoxic and hypercapnic conditions. In spite of this, CHH exposure does result in adjustments to respiration; frequency is decreased and tidal volume is elevated compared to control squirrels acutely

exposed to CHH conditions. Overall $\dot{V}$ sensitivities to both hypoxia and hypercapnia are not significantly altered by CHH exposure. It appears that acclimation to chronic hypoxic and hypercapnic conditions in S. lateralis may increase alveolar minute ventilation relative to total minute ventilation and thus minimize the changes in arterial PO_2 and P_{CO2} during hypoxic and hypercapnic exposure.

During entrance into hibernation, as metabolic rate and body temperature decline, concomitant decreases in ventilation occur. Two patterns of respiration occur during deep hibernation; a burst breathing pattern characterized by long non-ventilatory periods (T_{NVP}) separated by bursts of several breaths and a single breath pattern characterized by single breaths separated by a relatively short T_{NVP}.

In S. lateralis during hibernation at body temperatures between 6^{o} and $10^{o}C$, a burst breathing pattern prevails. At slightly lower body temperatures, less than $4^{o}C$, a single breath breathing pattern prevails. Both burst breathing and single breath breathing squirrels have similar overall levels of resting minute ventilation. Burst breathing squirrels exhibit a significant respiratory response to hypoxia (3% O_2) and when the decreases in metabolic rate during hibernation are taken into account (air convection requirement) their hypoxic sensitivity is similar to that in awake S. lateralis. In contrast, single

breath breathing squirrels do not respond to hypoxia at any level tested (down to 3% O_2). Both burst breathing and single breath breathing squirrels show large ventilatory repsonses to hypercapnia. In the burst breathing state hypercapnic sensitivity is significantly higher compared to the single breath breathing state, due to an augmented frequency response during burst breathing. In both groups of hibernating squirrels ventilation is increased during hypercapnia solely by decreases in the nonventilatory period. When ventilation is standardized for the decreases in metabolic rate during hibernation both burst breathing and single breath breathing S. laterlis exhibit a much higher hypercapnic sensitivity than that seen in awake S. lateralis. Carotid body denervation has little effect on ventilatory pattern generation or ventilatory sensitivities to hypoxia and hypercapnia in hibernating squirrels.

It appears that during hibernation in S. lateralis, ventilation is controlled primarily by changes in the partial pressure of CO_2 (P_{CO2}) in the blood acting centrally to stimulate ventilation. The burst breathing pattern is produced centrally, as are the respiratory responses to hypoxia and hypercapnia. Thus, central mechanisms involved with ventilatory control are extremely important in both the euthermic state and the hibernating state, but the chemical stimuli regulating ventilation appear to be fundamentally different in euthermic and hibernating S. lateralis.

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G'day.

INTRODUCTION

Mammalian hibernation is a seasonal phenomenon characterized by a profound decrease in body temperature and metabolic rate. In this way an animal can severely reduce its energy expenditure during periods of low food availability and inclement conditions (Wang, 1982). This reduction of energy expenditure is particularly important for small mammals. Small animals have a high surface area to volume ratio and, therefore, tend to lose heat more readily to the environment. As a result, they must expend more energy to maintain a high body temperature than do larger mammals. This becomes extremely difficult when food supply is limited. By entering hibernation and allowing its body temperature to drop to levels slightly above ambient temperature, a small mammal can reduce its energy expenditures by more than 80% over the winter (Wang, 1978).

Deep hibernation is defined by Lyman (1982) as a state of dormancy in which body temperature drops to a point near ambient temperature, often as low as 2^{o} to $5^{o}C$ and rewarming from hibernation requires only self-generated heat. This definition separates hibernation from other states where metabolic rate and body temperature are lowered, such as torpor and hypothermia. Torpor is a state of inactivity in which body temperature declines, but not usually below $15\text{-}20^{o}C$. Torpor includes the daily cycles

observed in some birds and small mammals, as well as seasonal torpor observed in small mammals such as chipmunks and larger mammals such as bears (Lyman, 1982). Hypothermia is a state of depressed metabolic rate and lowered body temperature which requires an outside or exogenous heat source for rewarming to normal levels.

During hibernation, metabolic rate falls to about 1% to 2% of euthermic levels (Hammel et al., 1968; Wang, 1979) and breathing frequency falls from about 40 to 60 breaths/minute to 1 to 5 breaths/minute (Biorck et al., 1956; Holloway and Heath, 1984; Landau and Dawe, 1958; Lyman, 1982; Walker et al., 1985). Even with such extreme physiological changes, hibernating animals are capable of responding to external stimuli and environmental changes, implying that hibernation is an actively controlled state (Lyman, 1982). For example, work done by Heller et al. (1974) shows that the thermoregulatory system not only functions during hibernation but works to maintain a "set" body temperature regardless of changes in ambient temperature.

As body temperature falls during entrance into hibernation, concomitant decreases in ventilation occur. Few studies have attempted to determine if the ventilatory system is actively controlled during hibernation and if the nature of the respiratory control system is similar to that of the awake euthermic animal.

Ventilation in fossorial mammals

The primary role of respiration is to meet the demands of metabolism by supplying oxygen (O_2) to the tissues and removing carbon dioxide (CO_2). The chemical control of respiration in mammals involves a feedback control system which adjusts ventilation to regulate blood and extracellular fluid partial pressures of O_2 (P_{O2}) and either CO_2(P_{CO2}) or pH (Cherniack and Longobardo, 1982; Feldman, 1986). In order to regulate these variables, animals must monitor these chemical stimuli with receptors which transduce and transmit relevant information to the CNS which, in turn, controls the respiratory movements that regulate PO_2, P_{CO2} and pH (Feldman, 1986). In mammals, the two major groups of receptors involved with the chemical regulation of breathing are found in the peripheral circulation at the site of the carotid sinus and the aortic arch (carotid body chemoreceptors and aortic body chemoreceptors) and in the central circulation at a yet undetermined location (central chemoreceptors). It is generally assumed that steady state ventilatory responses to decreases in PO_2 (hypoxia) are primarily mediated by carotid body chemoreceptor inputs to the CNS, while responses to increases in P_{CO2} (hypercapnia) are primarily controlled by central chemoreceptor inputs. In most mammals both hypoxia and hypercapnia result in increases in ventilation as the control system attempts to return blood gas tension to their appropriate levels.

Most hibernating animals inhabit burrows which serve as protection from predation and from climatic extremes. Since gas exchange between the burrow and the atmosphere is slow, burrow conditions are often low in O_2 concentration (hypoxic) and high in CO_2 concentration (hypercapnic) (Withers, 1978). The levels of hypoxia and hypercapnia attained depend on a number of factors such as the number of occupants in the burrow, soil porosity, soil moisture and burrow geometry (Arieli, 1979; Hayward, 1966; Maclean, 1981; Withers, 1978). Literature values for burrow gas composition range from 20% to 8% for O_2 concentrations (Hayward, 1966; Williams and Rausch, 1973) and from 0% to 13% for CO_2 concentrations (Williams and Rausch, 1973). Although there is a wide range of reported compositions for burrow gases, average literature values are about 17% to 18% for O_2 concentration and 3% to 4% for CO_2 concentration (Baudinette, 1974; Darden, 1972; Hayward, 1966; McNab, 1966; Studier and Proctor, 1971; Williams and Rausch, 1973). Burrow gases in the nests of non-hibernating and hibernating fossorial animals during the summer period appear to be similar. Several studies reporting the composition of burrow gases during hibernation suggest that metabolic rate is so reduced that there is no depletion of O_2 and no build-up of CO_2 except during arousal and periods of euthermia (Kuhen, 1986; Williams and Rausch, 1973). Thus, during the hibernating period, exposure to hypoxic and hypercapnic conditions is sporatic. In contrast, during the summer

months semi-fossorial hibernators spend 65 to 75% of daylight hours and 100% of night hours below ground in the burrow and are, therefore, exposed chronically to hypoxic and hypercapnic conditions. (Scheck and Fleharty, 1980).

Fossorial mammals show certain respiratory adaptations which are believed to be a response to chronic exposure to the environmental conditions of the burrow (Boggs et al., 1982). Fossorial and semi-fossorial species typically have a higher O_2 carrying capacity in the blood than do non-fossorial species. Generally high values for hematocrit (Hct), hemoglobin concentration and red blood cell counts have been reported for these mammals (Ar et al., 1977; Baudinette, 1974; Boggs et al., 1982; Chapman and Bennett, 1975). In addition, a left-shift in the O_2-hemoglobin dissociation curve means that blood becomes fully saturated at lower O_2 partial pressures, such as those present under burrow conditions (Bartels et al., 1969; Baudinette, 1974; Boggs et al., 1982). These adaptations suggest that fossorial mammals may be more tolerant to at least moderate levels of hypoxia.

During resting conditions both metabolic rate and minute ventilation ($\dot{V}$) are reduced compared to non-hibernators of approximately the same size. McNab (1966) and Hudson and Deavers (1973) reported that metabolic rates in several fossorial species were 20% to 60% lower than

expected on the basis of body weight. In addition, ventilatory responses to hypoxia and hypercapnia appear to be slightly altered in fossorial species compared to non-fossorial species (Arieli and Ar, 1979; Boggs and Kilgore, 1983; Boggs et al., 1984; Faleschini and Whitten, 1974; Holloway and Heath, 1984; Schlenker, 1985; Walker et al., 1985).

A high tolerance to hypercapnia is typical of almost all fossorial mammals and birds (Boggs and Kilgore, 1983; Chapin, 1954; Holloway and Heath, 1984; Schlenker, 1985; Walker et al., 1985). The threshold for a ventilatory response to inspired CO_2 appears to be elevated compared to non-fossorial mammals, while CO_2 sensitivity is reduced. Darden (1972) suggested that the observed decrease in respiratory sensitivity to CO_2 may result from an adjustment in the sensitivity of the peripheral and central chemoreceptors to arterial CO_2 tensions or an adjustment in the central integration of inputs from chemoreceptors. The change in CO_2 sensitivity may also reflect differences in the ability of the blood to buffer changes in CO_2 and hydrogen ions (H^+).

Few studies have examined the hypoxic sensitivity of fossorial mammals. These studies indicate that burrow-dwelling animals are as sensitive or more sensitive to hypoxia than are non-fossorial animals (Boggs and Kilgore;

1983; Boggs et al., 1984; Holloway and Heath, 1984; McArthur, 1986; Walker et al., 1985). Thus, whereas a reduced ventilatory sensitivity to hypercapnia is a common characteristic of burrow-dwelling mammals a reduced ventilatory sensitivity to hypoxia is not.

It has been suggested that ventilation in awake hibernators is controlled primarily by arterial O_2 tensions monitored by the peripheral chemoreceptors (Leitner and Malan, 1973). The adjustments which allow for a decreased CO_2 sensitivity in burrow-dwelling animals is unknown. These adjustments may be a result of long term exposure to hypoxic and hypercapnic conditions during development or they may be genetically determined. Mice and rats raised both pre- and post-natally under conditions of chronic hypercapnia (6% CO_2) or chronic normocapnia (0% CO_2) show no difference in their CO_2 sensitivity. This result suggests that CO_2 sensitivity is genetically determined rather than developmentally determined (Birchard et al., 1984). In spite of this evidence, chronic exposure to air may alter respiratory responses to CO_2 and O_2 in fossorial species. These adjustments may lead to an overestimation of the actual hypercapnic response curve of these animals. In order to ensure that significant modifications do not occur after long term air exposure it is necessary to compare ventilatory sensitivities of animals chronically exposed to air and chronically exposed to hypoxic and hypercapnic conditions.

Respiratory patterns during hibernation

Studies of deep hibernation reveal the occurrence of two distinct ventilatory patterns. In some species, such as the marmot and the Columbian ground squirrel, the respiratory pattern consists of single breaths interrupted by breath-hold periods of 1-2 minutes in length (Endres and Taylor, 1930; Malan et al., 1973). In other species episodes or bursts of rapid, continuous breathing are separated by long variable periods of breath holding. This burst breathing pattern, often referred to as Cheyne-Stokes Respiration (CSR), has been observed in hibernating hedgehogs, dormice, woodchucks, marmots, golden hamsters, golden-mantled ground squirrels and bats (for review see Malan, 1982).

Although there is no direct evidence to explain the occurrence of the different patterns, for the most part the differences in breathing pattern have been assumed to be species specific. Several respiratory studies involving hibernating species known to show burst breathing, however, have reported the occurrence of a single breath pattern. Kristofferson and Sovio (1964) reported that the burst breathing pattern in the hibernating hedgehog could be converted into a single breath pattern by decreasing ambient temperature (down to $-5^{o}C$) or by handling the animal. Pajunen (1974) noted that in a hibernating dormouse,

disturbance caused a disruption of the burst breathing pattern, as did decreases in ambient temperature (Pajunen, 1984). Hammel et al., (1968), observed a burst breathing pattern in the golden-mantled ground squirrel as long as body temperature, specifically hypothalamic temperature, ranged from 5.5°C to 10°C. If hypothalamic temperature dropped below 5.5°C in response to decreases in ambient temperature, breathing took on a single breath pattern.

These observations suggest that ventilatory patterns may be temperature dependent during hibernation and may alter as a function of a disturbed versus an undisturbed state. Within a single species each pattern could represent different levels of ventilatory control and ventilatory sensitivity to chemical stimuli.

The role of peripheral chemoreceptors during hibernation

To date, studies on ventilatory control during hibernation have been limited by the difficulties in obtaining ventilatory measures without disturbing the animal. Most studies report only changes in pattern and frequency during hypoxia and hypercapnia and are therefore, not quantitative. In addition, most studies of ventilatory responses to chemical stimuli during hibernation have been concerned with responses to CO_2.

CO_2 elicits a fairly strong respiratory response during hibernation. The threshold for eliciting a ventilatory response is variable, falling between 1% and 4% CO_2 (Biorch et al., 1956; Endres and Taylor, 1930; Lyman, 1951, McArthur, 1986). At levels between 5% and 7% inspired CO_2, periodic breathing becomes continuous (McArthur, 1986; Tahti, 1975) and long periods of severe hypercapnia often result in arousal from hibernation (Tahti, 1975). Most studies report a strong frequency response to hypercapnia during hibernation (Endres and Taylor, 1930; Lyman, 1951; Tahti, 1975). Since, in most non-hibernating animals, hypercapnia is known to have a strong effect on V_T, it is likely that studies using only frequency as an indication of ventilatory sensitivity tend to underestimate the total CO_2 response. McArthur (1986) measured changes in both tidal volume, frequency and minute ventilation in response to hypercapnia and found increases of over 500% in minute ventilation in response to severe hypercapnia (7% CO_2). In addition, McArthur (1986) found a substantial increase in V_T during hypercapnic exposure.

As early as the nineteenth century the extreme resistance of hibernators to hypoxia and anoxia was recognized (Hall, 1836). At least some of this hypoxic tolerance during hibernation is thought to result from decreases in metabolic rate and from changes in the oxygen-hemoglobin (HbO_2) dissociation curve of the blood.

Adjustments to the curve result from a combination of body temperature changes, a slight respiratory acidosis (Bohr effect), and a reduced 2,3 diphosphoglycerate concentration in the red blood cells (Endres, 1930; Musacchia and Volkert, 1971). These alterations result in a very steep, left-shifted HbO_2 dissociation curve, such that the blood remains completely saturated down to levels of about 14-25 Torr (Endres, 1930; Musacchia and Volkert 1971). Thus, hibernating animals can potentially tolerate extremely low levels of inspired O_2 without having any desaturation of the blood.

Most studies concerning hypoxic tolerance reveal that hibernating animals show little or no hypoxic sensitivity (Biorch et al., 1956; McArthur, 1986). It appears that in some species inspired O_2 can be reduced to levels which cause central ventilatory depression and death (1% to 5% O_2) before any observed ventilatory stimulation occurs (Biorch et al., 1956; McArthur, 1986). The lack of a ventilatory response even during severe hypoxia has caused most previous investigators to conclude that changes in O_2 play little or no role in ventilatory control during hibernation (Biorch et al., 1956; Steffen and Reidesel, 1982; Tahti, 1975; Tahti et al., 1981). In contrast, Tahti (1975) observed a ventilatory response to hypoxia at 16% O_2 in the hedgehog. As O_2 levels decreased, breathing frequency increased until, at 3% O_2, breathing became

continuous. Additionally, McArthur (1986) noticed increases in minute ventilation with 3% inspired O_2 in Spermophilus lateralis.

It is possible that the extreme hypoxic tolerance seen in hibernation is due, in part, to adjustments in the sensory input from the peripheral chemoreceptors or the central integration of the peripheral inputs. The role that carotid body chemoreceptors play in ventilatory control during hibernation is uncertain.

Available evidence suggests that peripheral chemoreceptors may play an important role in the generation of periodic breathing in euthermic mammals. Lahiri et al. (1983) described a strong positive correlation between high peripheral chemoreceptor sensitivity and the advent of burst breathing (CSR) in humans at high altitude. In addition, studies on euthermic cats indicate that intact peripheral chemoreceptors are a prerequisite to the induction of periodic breathing (Cherniak et al., 1979). It is not known if the respiratory models for the production of periodic breathing can be applied to periodic breathing in euthermic mammals during hibernation or if intact peripheral chemoreceptors are a prerequisite for the occurrence of burst breathing.

It is evident that there is information available

on the ventilatory patterns and ventilatory sensitivities to hypoxia and hypercapnia in both fossorial mammals and hibernating mammals. Few studies have, however, attempted to examine the mechanisms of ventilatory control in these mammals. Furthermore, few studies have examined the relationship between ventilatory control in an awake fossorial mammal and a hibernating fossorial mammal.

Given the preceeding discussion the purpose of this study was threefold:

(a) to determine if chronic exposure to hypoxic and hypercapnic conditions, similar to those experienced in a burrow environment affects ventilatory responses to hypoxia and hypercapnia in awake golden-mantled ground squirrels;

(b) to describe the contribution of carotid body chemoreceptors to the respiratory responses of awake and hibernating golden-mantled ground squirrels and to the production of a burst breathing pattern in the hibernating animal; and

(c) to correlate the two reported respiratory patterns seen during hibernation to body and ambient temperature and to determine if the two patterns represent different levels of ventilatory control in a single species.

MATERIALS & METHODS

All studies were carried out on female golden-mantled ground squirrels (Spermophilus lateralis) obtained from Redding, California. Squirrels were trapped by a supplier in the spring of 1984 and 1985 and were shipped by air to U.B.C. The age of the ground squirrels ranged from first year juveniles to adults. Squirrels were housed either individually or in pairs in plexiglass cages (45 cm x 25 cm x 20 cm) with wire mesh lids. Cages were filled with wood shavings and cotton wool for nesting material and were cleaned weekly during the summer and monthly during the winter. Purina Lab Chow and water were provided ad libitum during both the summer and the winter. The squirrels' diet was supplemented with fresh fruit and vegetables during the summer. From May to October (summer, active season) the ground squirrels were housed in an environmental chamber maintained at an ambient temperature of 20.0 $\pm$ 0.5^{o}C and a photoperiod of 12 hours light and 12 hours dark (12L:12D).

One half of the squirrels used in these experiments were housed in room air, while the other half were housed under chronic hypoxic and hypercapnic conditions (CHH animals).

Animals chronically exposed to 17-18% O_2 and 3-4%

CO_2 were housed in pairs in a large airtight plexiglass chamber mimicking natural burrow conditions (see introduction). During the summer months, when the squirrels were active, these chronic gas levels were established by sealing the chamber until the fractional concentration of O_2 (F_{O2}) dropped and the fractional concentration of CO_2 (F_{CO2}) increased to the desired level. This usually occurred within 2 to 3 hours. These chronic gas levels were then maintained by a slow, steady flow of air through the plexiglass chamber. Chamber gases were monitored daily throughout the summer with Beckman O_2 (OM-11) and CO_2 (LB-2) gas analysers calibrated daily with room air and pre-mixed 5% and 10% CO_2 (Radiometer GMA 2 precision gas mixing pump). Air flow rates through the chronic chamber were adjusted to hold the chamber gas composition at the desired level. The chronic chamber was opened no more than twice per week except during periods of data collection.

During the hibernation period (November to May), when the metabolic rates of the ground squirrels were extremely low, appropriate levels of F_{O2} and F_{CO2} were obtained by mixing air, N_2 and CO_2 with calibrated flow meters. Chamber gas composition was monitored every second day and the N_2, CO_2 and air flows were adjusted as required. CHH animals were not studied while in hibernation, but were maintained under chronic hypoxic and hypercapnic conditions throughout the winter. A minimum of 2 months of continuous

exposure to the hypoxic and hypercapnic conditions was required for an experimental animal to be considered as having been chronically exposed to these conditions. Since all awake, euthermic studies were carried out in the summer of 1985, the majority of the ground squirrels in this experimental group had been exposed to chronic conditions for over 10 months prior to being used in experiments.

SURGERY

Carotid Sinus Denervations

In one half of both air exposed squirrels (n=8) and CHH squirrels (n=8) the carotid sinus nerves were sectioned bilaterally (CBX squirrels). The carotid sinus nerve innervates the carotid body chemoreceptors which are located at the bifurcation of the common carotid artery.

All surgery was performed on ground squirrels during the active phase of their yearly cycle. In the fall of 1984 carotid sinus denervations were performed on 8 control animals and 8 CHH animals and three additional operations were performed the subsequent summer in order to replace ground squirrels which did not survive the entire season.

Ground squirrels were anesthetized with sodium pentobarbital (Somnotol, MTC pharmacuticals, Mississauga, Canada) delivered intraperitoneally (6.5 mg/100 g). Once deep reflexes were abolished, but prior to any surgery a control test of the ventilatory responses of each animal to several respiratory stimuli was performed.

Ventilatory flow was measured with a pneumotachograph mask unit. A face mask was made using the end of a plastic syringe lined with plasticine. To minimize dead space the mask was molded by imprinting the snout of a dead ground squirrel in the plasticine and covering this with expoxy. A small plexiglass pneumotachograph was attached to the end of the mask. Total dead space for the unit was .15 ml. The pneumotachograph-mask unit was placed on the snout of the anesthetized ground squirrel and held secure with adhesive tape. The pneumotachograph was connected to a differential pressure transducer (Validyne model DP 103-18, Northridge, California) in order to measure air flow changes across the pneumotachograph membrane. The signal was amplified to record ventilatory pattern and frequency (Gould transducer amplifier model 13-4615-50 or Gould D.C. amplifier model 13-4615-10). Calibrations of the pneumotachograph were performed after each experiment by pumping known volumes of air across the pneumotachograph via the face mask. Twice a year the pneumotachograph was checked for linearity by pumping a large range of volumes across the membrane.

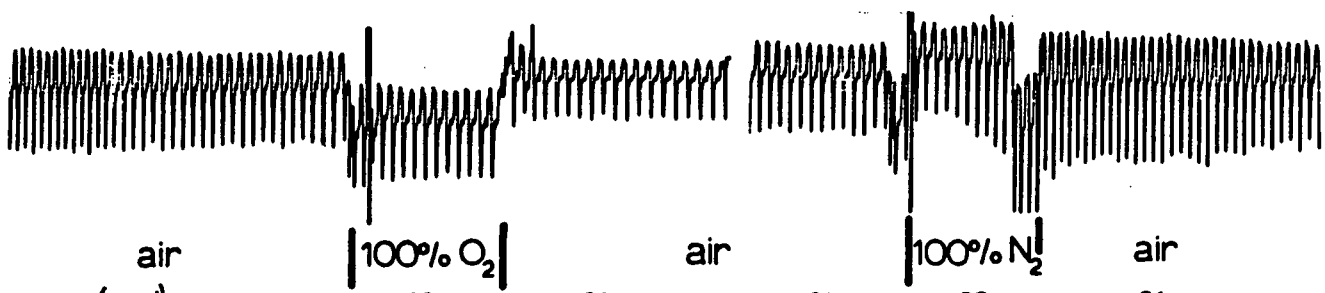
Once stable respiration was recorded the inspired gas was quickly changed to either 100% O_2 (hyperoxia) or 100% N_2 (anoxia). The acute changes in ventilatory frequency and air flow during 5-10 breaths of each gas were recorded from the anesthetized squirrel (Figure 1). Responses to hyperoxia and anoxia were fast, usually occuring within 4-5 breaths, but the magnitude of the responses varied between animals possibly due to differences in the level of anesthesia. In general, intact squirrels showed an increase in ventilatory frequency and an increase in the height of ventilatory deflection in response to anoxia and a decrease in ventilatory frequency in response to hyperoxia (Figure 1).

After measuring the ventilatory responses to anoxia and hyperoxia, the squirrel was prepared for surgery. Fur was shaved from the upper chest and neck region, and the skin was sprayed with antiseptic. A midventral incision, approximately 2 cm long, was made from the base of the jaw to the sternum. On one side of the trachea the common carotid artery, the carotid bifurcation and the base of the internal and external carotid arteries were located and exposed. The common carotid artery was ligated with surgical silk and, with the use of a dissecting microscope the carotid sinus nerve was isolated and cut as close as possible to the glossopharyngeal nerve. The carotid sinus nerve was then traced back to where it innervated the

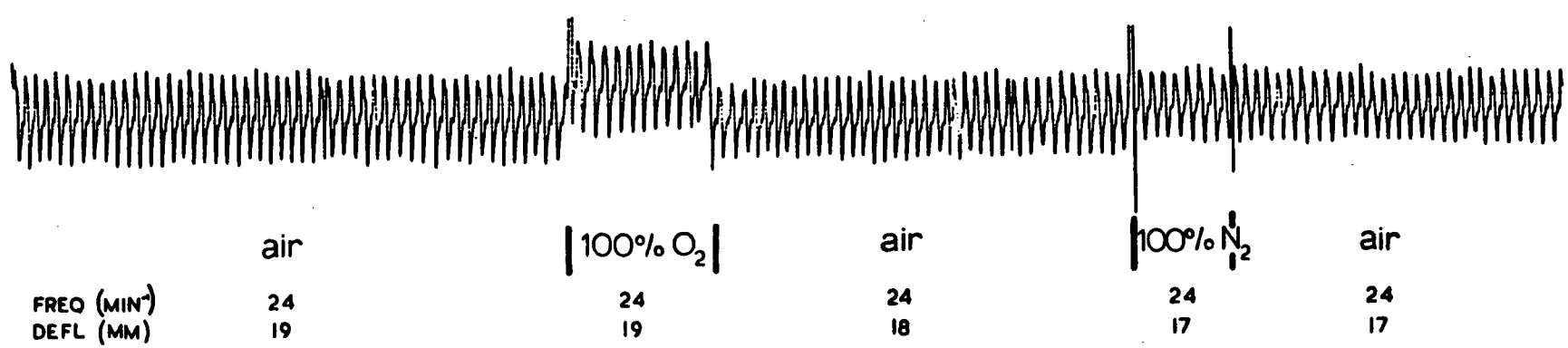
Figure 1. The effects of inhalation of 5 to 10 breaths of 100% O_2 (hyperoxia) and 100% N_2 (anoxia) on respiratory frequency and depth both before carotid body denervation and after carotid body denervation in S. lateralis.

CAROTID BODY DENERVATION

before CBX



after CBX



carotid sinus and cut again. The ligature was then removed from the carotid artery, and any bleeding in the area was stopped by applying pressure. The same procedure was then repeated on the controlateral carotid sinus nerve.

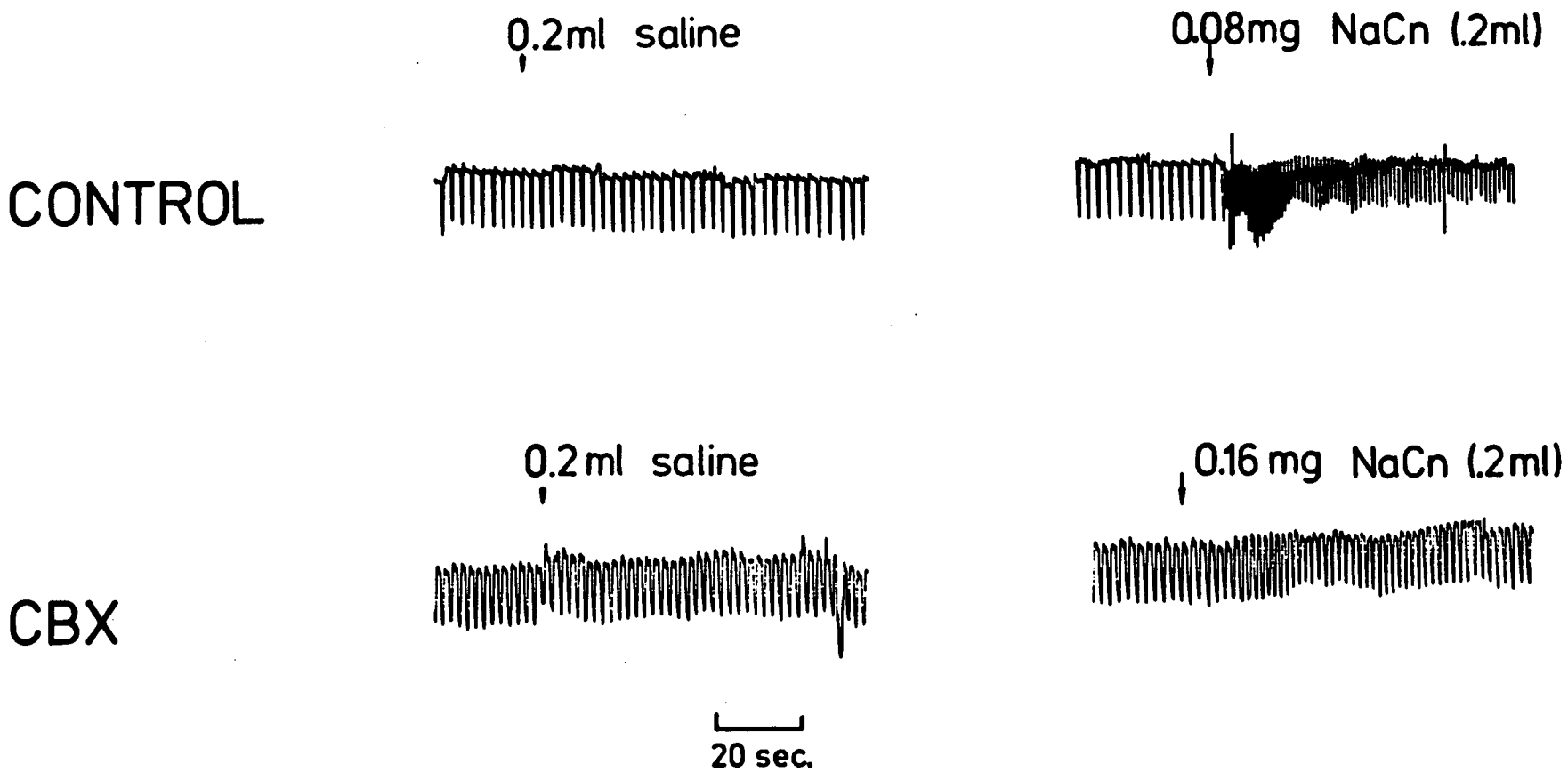
After removal of both carotid sinus nerves the ground squirrel was fitted with the pneumotachograph mask unit and the ventilatory responses to hyperoxia and anoxia were measured once again. If the ground squirrel showed no immediate response (within 5-10 breaths) to either anoxia or hyperoxia the denervation was considered successful (Figure 1) and the incision sewn closed. If the responses to anoxia and hyperoxia were not completely abolished another attempt was made to isolate and section the carotid sinus nerves. If carotid sinus denervation was not complete following this second attempt, the animal was not used in any experiments. Animals were allowed to recover from anesthesia and given Penbritin-250 (Ampicillin sodium, USP-Ayerst; 2.5mg/animal) intramuscularly.

These animals were allowed to recover for at least 2 months before being used in any experiments or being induced into hibernation. Ten months after surgery the ventilatory responses to anoxia and hyperoxia were measured once more, as previously described, on CBX squirrels. All CBX animals showed a reduced or absent ventilatory response to anoxia and hyperoxia as compared to intact ground squirrels.

At the completion of all experiments sodium cyanide (NaCn) tests were carried out on surviving CBX control squirrels (n=4). Sodium cyanide, in low concentrations, binds specifically to arterial chemoreceptors, displacing oxygen and acting as an acute hypoxic stimulus. Since NaCn is quickly metabolized, its actions are transient at low concentrations. In the absence of arterial chemoreceptors, particularly carotid body chemoreceptors, there should be no ventilatory response to NaCn (Bouverot et al., 1973) or a slight ventilatory response, mediated by the aortic chemoreceptors (Lahiri et al., 1981). Thus, in the CBX squirrels, there should be little or no response to NaCn.

Ground squirrels were anesthetized as previously described and the inner, right hind leg shaved and sprayed with antiseptic. A 1-2 cm incision was made and the femoral vein isolated and ligated. The vein was cannulated using P.E. 20 tubing. Breathing frequency was measured using either a pneumotachograph-mask unit, as previously described or a strain-gauge. The strain-gauge was attached to the sternum of the anesthetized animal and measured respiratory deflection of the thorax. The strain-gauge method gave an indication of both respiratory frequency and depth of respiration. A control injection of 0.2 ml saline into the femoral vein was used to ensure that pressure or temperature changes caused by drug injection did not affect ventilation (Figure 2). Injections of 0.08 mg NaCn (in 0.2 ml saline)

Figure 2. The effects on respiratory frequency and depth of intravenous injections of 0.2 ml saline and 0.08 mg NaCn or 0.16 mg NaCn (0.2 ml saline) in intact S. lateralis and carotid body denervated (CBX) S. lateralis respectively.


0.2ml saline
0.08mg NaCn (.2ml)
CONTROL
0.2 ml saline
0.16 mg NaCn (.2ml)
CBX
20 sec.

in control animals and 0.16 mg NaCn (in 0.2 ml saline) in CBX animals were then made and the ventilatory responses of the animals recorded. In all control animals an immediate and pronounced frequency response was elicited by the NaCn injection (Figure 2). In contrast, none of the CBX animals exhibited a ventilatory response to NaCn injections. It was assumed, from these results, that all the CBX animals remained denervated throughout the entire study.

Body Temperature Implants

Prior to hibernation, all experimental squirrels were chronically implanted with electrodes for the measurement of core body temperature (T_b). The ground squirrels were anesthetized as previously described. Once deep reflexes were abolished, fur was shaved from the abdomen and the skin sprayed with antiseptic. An incision was made through the skin and then through the body wall. A thermistor (Fenwall Electronics, Massachusetts, U.S.A.) coated with epoxy and paraffin wax was placed in the abdominal cavity and the inner incision closed. A double flanged plexiglass button containing connector pins attached to the thermistor was sewn into the outer incision allowing the outer skin to be closed. Post-operatively the ground squirrel was given Penbritin intramuscularly as previously described. The thermistor signal was checked on a digital display monitor. Calibrations of each thermistor

were performed, prior to surgery, in a water bath over a range of approximately 1°C to 40°C.

SUMMER PROTOCOL

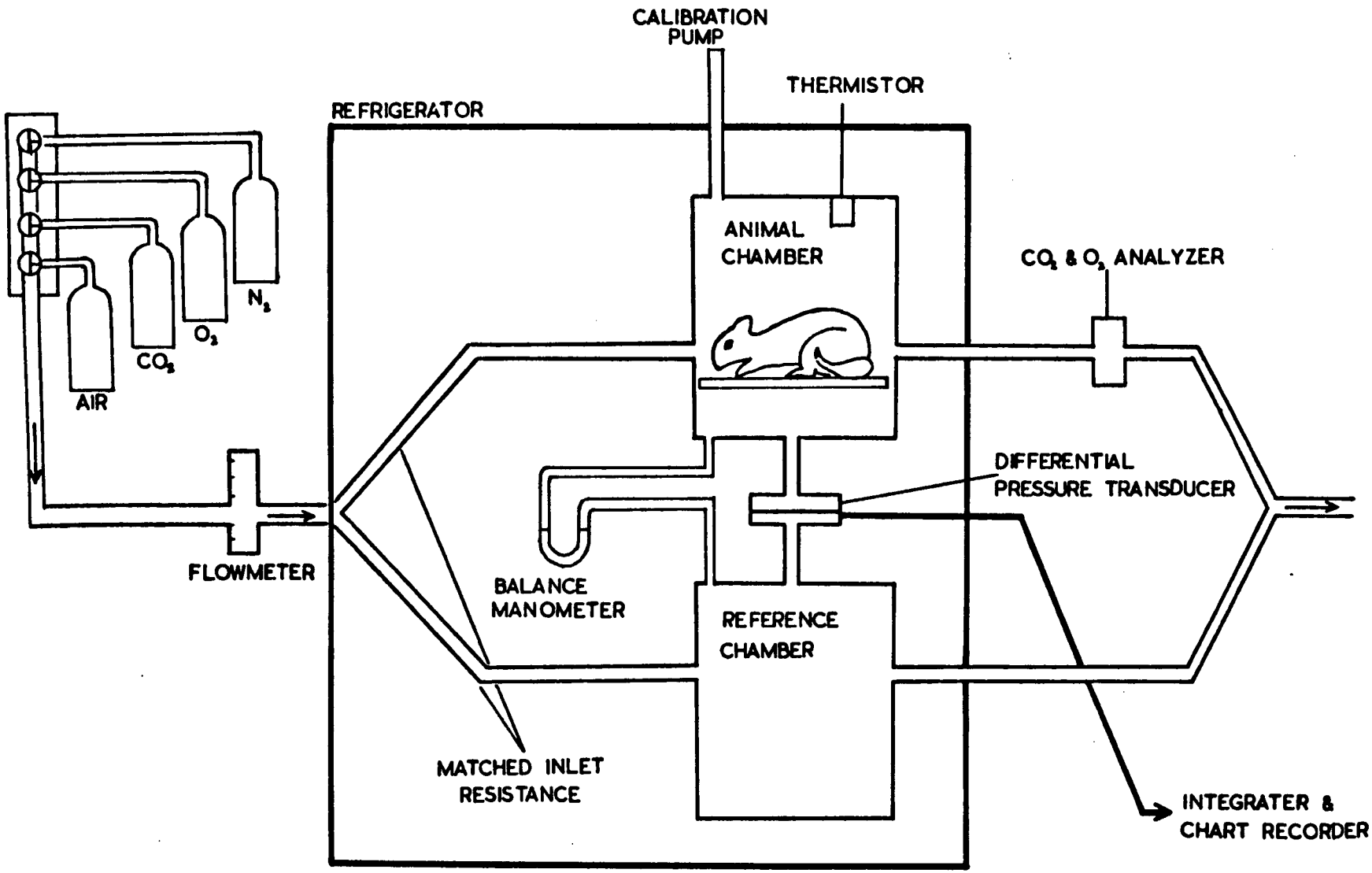
Measurement of Ventilation

Table 1 presents a summary of respiratory variables measured and notations made during the present study. In the awake ground squirrel, ventilation was measured using whole body plethysmograph as described by Epstein and Epstein (1980) and modified by Jacky (1978, 1980). A modified flow-through plethysmograph allowed constant flushing of the chamber to reduce O_2 depletion and CO_2 accumulation (Figure 3). The flow-through method also allowed for rapid changes in inspired gas mixtures without disturbing the ground squirrel. Two identical chambers were used; an animal chamber and a reference chamber (Figure 3). Both chambers (23 cm x 14 cm x 13 cm) had an inflow and outflow port (.9 cm in diameter) for the delivery of air and various gas mixtures. The top of each chamber was fitted with ports to allow each chamber to be connected to both a differential pressure transducer to measure respiration and a manometer to maintain equal pressures in the two chambers. The animal chamber had an additional port for a thermistor to measure chamber temperature (T_C). In addition, the animal chamber had a removable lid which was fitted with

TABLE 1. List of notation and units of respiratory variables measured and calculated in the golden-mantled ground squirrel (*S. lateralis*).

NOTATION	RESPIRATORY VARIABLE	UNITS
$\dot{V}$	Minute Ventilation	ml/100g/min
V_T	Tidal Volume	ml/100g
f	Respiratory frequency	breaths/min
T_I	Inspiratory time	sec
T_E	Expiratory time	sec
T_{TOT}	Total breath duration	sec
T_{E}'	Length of end inspiratory pause	sec
T_{NVP}	Length of nonventilatory period (interburst)	sec
T_{VP}	Length of breathing episode	sec
$\dot{V}_{O2}$	Oxygen consumption	ml O2/100g/hr
$\dot{V}_{CO2}$	CO_2 production	ml CO2/100g/hr
T_I/T_{TOT}	Duty cycle	-
$\dot{V}/\dot{V}_{O2}$	Air convection requirement	ml air/ml O2

Figure 3. Schematic diagram of whole body plethysmograph arrangement used to measure ventilation in awake S. lateralis. See text for explanation.


CALIBRATION PUMP
THERMISTOR
REFRIGERATOR
ANIMAL CHAMBER
CO_2 & O_2 ANALYZER
N_2
O_2
CO_2
AIR
DIFFERENTIAL PRESSURE TRANSDUCER
FLOWMETER
BALANCE MANOMETER
REFERENCE CHAMBER
MATCHED INLET RESISTANCE
INTEGRATER & CHART RECORDER

four latches and lined with neoprene to ensure an airtight seal. The entire plethysmograph system was placed in a dark refrigerator in order to minimize pressure disturbances from the room and disturbances to the animal.

Using the plethysmograph method (Drorbaugh and Fenn, 1955) pressure changes in the animal chamber relative to the reference chamber are created by the warming and humidifying of inspired air during normal respiration. This pressure change is considered to be proportional to tidal volume (V_T) and can be measured, as in our system, by a differential pressure transducer (Validyne model DP103-18, Northridge, California).

Calibrations of the system were performed at the end of each experiment with the animal present in the animal chamber. Known volumes of air were pumped into the chamber at a frequency similar to that of the animal's breathing. The calibration volume was chosen to produce a pressure delfection at least 10 times as great as the pressure deflection produced by the animal breathing (Jacky, 1978). Accordingly, for calibrations, the system gain was reduced by a factor of 10.

Calibration measurements were made while the air flow rate through the system was varied to ensure that the rate of air flow through the system had no effect on the

pressure deflections. In addition, measurements were made with and without the animal in the chamber to determine whether the presence of an animal in the chamber had any effect on the calibration pressure deflections.

Expired volume is calculated using the formula;

$$VE = \frac{P_m \times V_{cal} \times T_A(P_B - P_{CH2O})}{P_{cal}\,[T_A(P_B - P_{CH2O}) - T_c(P_B - P_{AH2O})]}$$

Drorbaugh and Fenn, 1955.

where P_m is the measured pressure deflection, P_{cal} is the calibrated pressure deflection, V_{cal} is 1/10 the calibrating volume, T_c is the temperature of the animal chamber (^oK), P_{CH2O} is the water vapour pressure at T_c, T_A is the body temperature of the animal and P_{AH2O} is the water vapour pressure at T_A (Jacky, 1978).

Epstein and Epstein (1978) concluded that this formula can lead to an underestimation of V_T if the assumption that expired gas returns to ambient temperature and humidity before a subsequent inspiration is not met. Epstein and Epstein (1978) therefore proposed that expired gas should be considered to be at nasal conditions in order to obtain accurate V_T measurements. Jacky (1980) derived a correction forumla to retrospectively correct tidal volume

estimates produced by the Drorbaugh and Fenn formula;

$$V_E/V_{COR} = 1 - (T_I/T_{TOT})(1 - G_A/G_N)$$

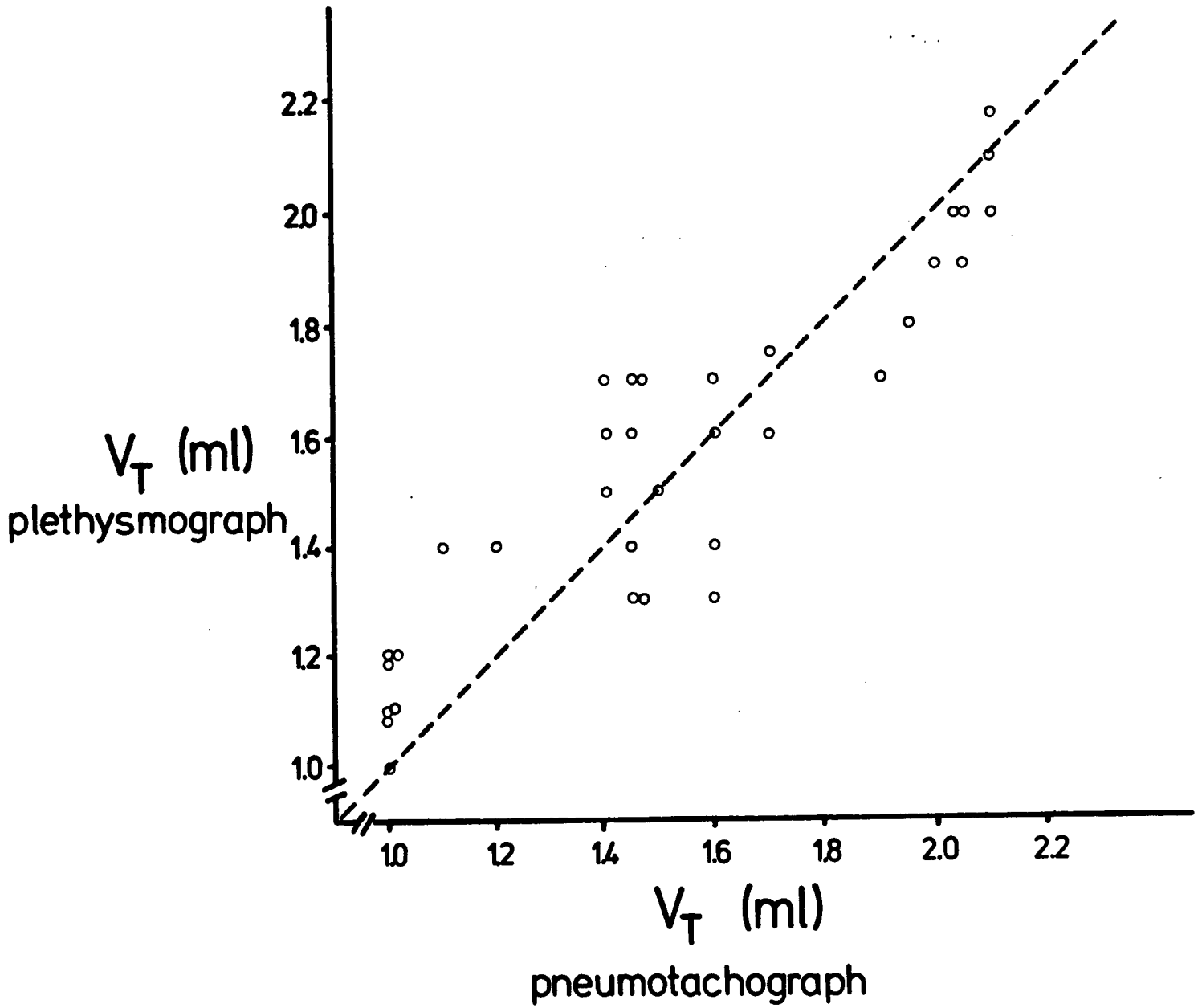
where G_A represents the pressure and temperature changes from alveolar to chamber conditions (as in the Drorbaugh and Fenn formula), G_N represents the pressure and temperature changes from alveolar to nasal conditions, T_I is inspiratory time and T_{TOT} is total breath duration. These two forumlas were used in the calculation of V_T.

Simultaneous measurements of V_T in anesthetized squirrels using both the plethysmograph and pneumotachograph mask unit indicated that the two measurements were in close agreement (Figure 4).

Experimental Protocol

All experiments were performed from 0700 to 1800 hours in a six week period in July and August 1985. At the start of each experiment a ground squirrel was placed in the animal chamber and left undisturbed on the control gas (either air or 17% O_2 and 4% CO_2 for control and CHH animals respectively) for a minimum of 1 hour to achieve resting conditions. Total gas flow through the chamber was approximately 1 litre per minute. This gas flow minimized the metabolic build-up of CO_2 and depletion of O_2 in the animal chamber. Respiratory frequency was recorded at

Figure 4. The relationship between tidal volume values calculated using the whole body plethysmograph and measured using a pneumotachograph in two anesthetized ground squirrels exposed to varying levels of CO_2. The dashed line represents the line of equality. See text for description of methods.


2.2
2.0
1.8
1.6
1.4
1.2
1.0
V_T (ml)
plethysmograph
1.0
1.2
1.4
1.6
1.8
2.0
2.2
V_T (ml)
pneumotachograph

moderate chart speed (2.5 mm/sec) over a 2 minute period. At the end of the initial control gas exposure a breathing trace, consisting of a minimum of 15 breaths at high chart speed (10mm/sec), was recorded to allow measurement of respiratory pressure deflection and respiratory timing variables. In addition, T_C was recorded for each gas exposure. The ground squirrel was then exposed to various gas mixtures (see below) in a random order and alternating with the control gas. The chambers were fully flushed with gas mixtures in about 5 minutes and the ground squirrel was maintained on the gas for an additional 15 minutes in order to achieve a steady state ventilatory response. Breathing traces were then recorded as previously described. Traces were only recorded if the animal was awake and nonactive.

Test gas mixtures were as follows;

hypoxia: 17, 12 and 8% fractional inspired O_2 (F_{IO2})

hypercapnia: 2, 4 and 6% fractional inspired CO_2 (F_{ICO2})

hypoxia/hypercapnia: 4% F_{ICO2} in 17, 12 and 8% F_{IO2}

hypoxia/Hypercapnia: 50% F_{IO2} in 0, 2 4 and 6% F_{ICO2}.

Gas mixtures were produced using flow meters to mix 100% N_2, CO_2 and/or O_2 with room air. Gas concentrations were continuously monitored with Beckman O_2 and CO_2 gas analysers. Inflow and outflow gases of the animal chamber

were monitored to ensure complete chamber flushing and adequate chamber flow. Barometric pressure (P_B) was recorded at the beginning and end of each experiment. If P_B varied over the course of the experiment an averaged value was used in V_T calculations. Nasal temperature (T_N) was measured on 3 occassions over the summer by inserting a calibrated thermistor bead approximately .5cm into the nostril. T_N measurements of 32°C in the awake ground squirrel are in agreement with measurements previously reported in our lab (McArthur, 1986) and reported by others (Schmid, 1976; Jacky, 1980; Fleming et al., 1983). All ground squirrels were weighed at the end of the experiment. On average experiments lasted 6 to 8 hours.

Data Analysis

The high speed respiratory traces for each gas exposure were analysed for inspired pressure deflection. Tidal volume (V_T) was calculated using the formula of Drorbaugh and Fenn (1955) as modified by Jacky (1980). The measurements of inspiratory time (T_I) and total breath duration (T_{TOT}) used in V_T calculations were obtained from work done by McArthur (1986) on S. lateralis. Values of T_I and T_{TOT} obtained in this study were checked against those of McArthur to ensure they were not significantly different. Frequency was calculated by counting breaths in at least six

ten second segments of steady state ventilation. Each segment was multiplied by 6 to give breaths per minute. Mean values for f and V_T were calculated for each individual squirrel and these mean values were used to calculate overall mean values for each experimental group. Minute ventilation ($\dot{V}$) was calculated as the product of V_T and f and expressed in ml/min/100g for each squirrel and group means were calculated. For the calculation of air convection requirement $\dot{V}/\dot{V}_{O2}$ in Figure 31 a value of 1.54 ml O_2/ml/min/100g was used for $\dot{V}_{O2}$ values; this was estimated from date on awake S. lateralis (Heller, 1978) and S. richardsonii (Wang, 1978).

Changes in ventilation within experimental groups and between experimental groups in response to gas mixtures were analysed using a single class analysis of variance (ANOVA) or a point to point 1-way ANOVA. Trends were considered to be significantly different at $P \leq .05$ level unless otherwise stated.

HIBERNATION PROTOCOL

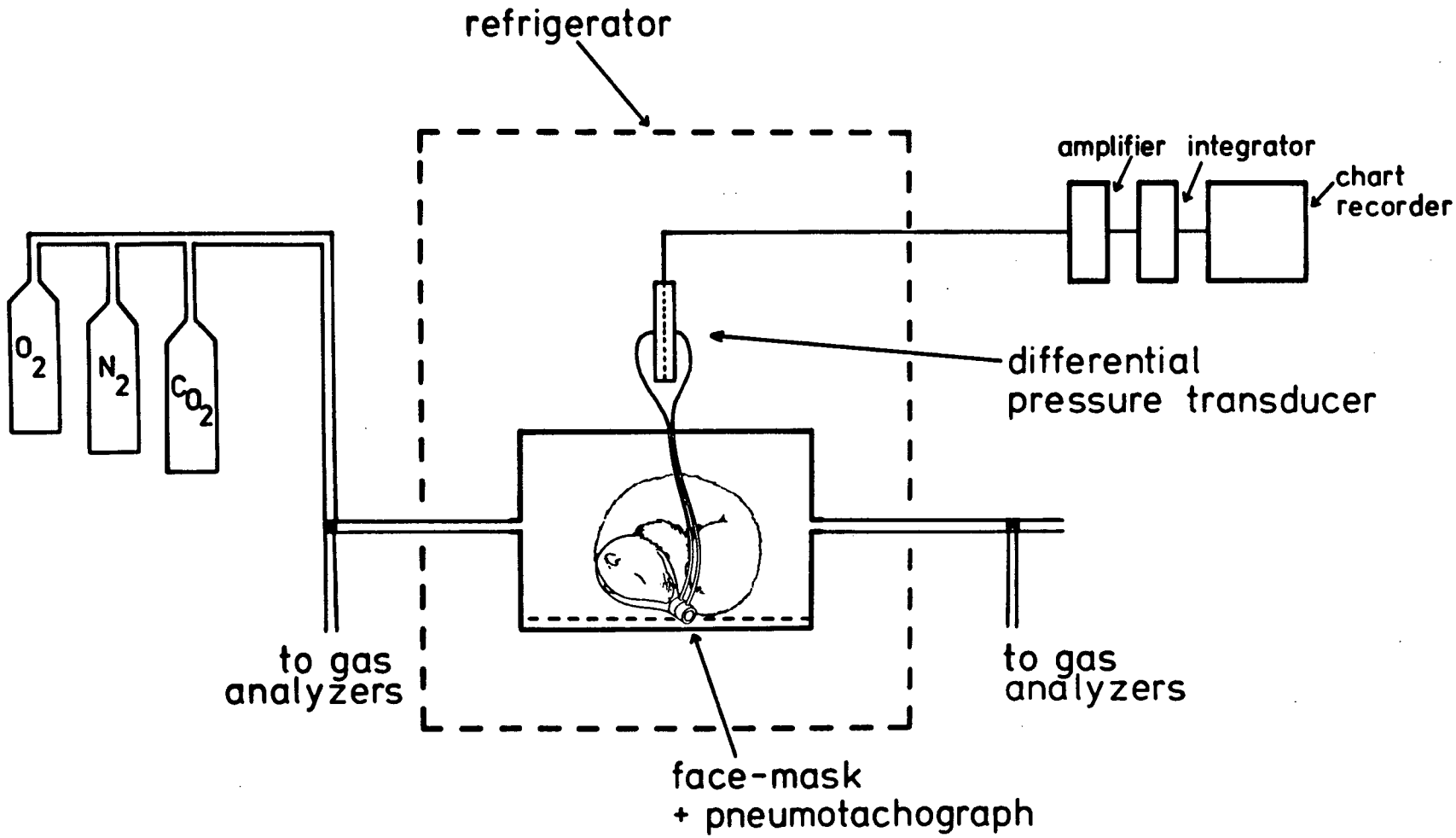
Animals were induced into hibernation in early November in two consecutive years. Over a 10 day period photoperiod was decreased from 12L:12D to 2L:22D and temperature was decreased from 20°C to 5°C. Most ground

squirrels entered hibernation before the induction period was complete and all squirrels were hibernating within one month of induction. Experiments were not started until 1 month after initial induction into hibernation. Handling of the ground squirrels was minimized except during experimental periods. Hibernation was terminated in May: photoperiod and temperature were gradually returned to summer levels.

Experimental Protocol

Ventilation during hibernation was measured using the pneumotachograph-mask unit described earlier (Figure 5). Only ventilatory responses of air control and air CBX squirrels were studied during hibernation. Hibernating animals were placed in a flow-through animal chamber (previously described) situated in a 500 cubic inch refrigerator. Temperature was controlled by an external rheostat. Gas exposure experiments were performed at two ambient temperatures; $6.0 \pm 0.1^{o}C$ and $2.4 \pm 0.1^{o}C$. The movement of the squirrels from the environment chamber into the laboratory refrigerator invariably caused arousal from hibernation. After arousal the squirrel was left for a minimum of 24 hours to allow re-entry into hibernation. After this time the pneumotachograph-mask unit (as previously described) was secured to the snout and T_B leads connected when possible. This procedure sometimes initiated

Figure 5. Schematic diagram showing the experimental arrangement used to record ventilation during hibernation in S. lateralis. See text for description.


refrigerator
amplifier
integrator
chart recorder
O_2
N_2
CO_2
differential pressure transducer
to gas analyzers
to gas analyzers
face-mask + pneumotachograph

arousal, but usually after some signs of disturbance (i.e. movement and increased ventilation) the ground squirrel re-entered deep hibernation. Once hibernating the ground squirrel was not disturbed unless necessary and in some cases animals wearing a mask would remain hibernating for up to 7 days.

When steady state ventilation was achieved at 6.0 $\pm$ 0.1^{o}C, a control respiratory trace was recorded from the animal while breathing air for 2 to 3 hours in the burst breathing state. Chart recorder speed during this period was fast enough for the analysis of ventilatory pattern (.25 mm/sec). Chart recorder speed was increased (2.0 mm/sec) and the integrated signal recorded to obtain breathing records for analysis of V_T and respiratory timing components. In burst breathing animals a minimum of one burst, approximately 20 breaths, was recorded at high speed. Airflow through the chamber was maintained at approximately 1 litre/minute for all gases. After air control traces were obtained various gas mixtures (see below) were introduced in a random order. Ground squirrels were exposed to air after every third exposure to a test gas. During burst breathing, when nonventilatory periods could last for 30 to 40 minutes, gas exposures lasted from 1.5 hours to 2 hours until a steady state response was achieved. Long air exposures were required after stressful gases (i.e. 8% F_{ICO2}) and the animal was often left overnight to recover. At the end of

each gas exposure, ventilatory traces were obtained as previously described. T_c and T_b (if available) were recorded for each gas mixture.

Test gases for hibernating animals were as follows;

hypoxia: 10, 5 and 3% F_{IO2}

hypercapnia: 2, 4, 6 and 8% F_{ICO2}

hypoxia/hypercapnia: 5% F_{IO2} in 21, 6 and 8% F_{ICO2}

hyperoxia/hypercapnia: 50% F_{IO2} in O, 2, 4, 6 and 8% F_{ICO2}

Gases were created and delivered by mixing 100% N_2, CO_2 and/or O_2 with air using calibrated flow meters. Gas composition was continuously monitored using Beckman O_2 and CO_2 gas analysers. Both inflow and outflow gas concentrations were monitored to ensure proper flushing of the animal chamber. Total experiment time was approximately 30 hours, but animals were often maintained overnight on air and the experiment continued the next day. If the animal aroused during the experiment, it was left in the animal chamber until it re-entered hibernation and the experiment was then continued. At the end of each experiment the weight of the ground squirrel was recorded and calibrations for tidal volume measurements were performed. Calibrations involved pumping known volumes of air across the pneumotachograph with a syringe and recording the integrated airflow signal.

The transition from a burst breathing state to single breath breathing state was examined simply by allowing animals to enter deep hibernation at an ambient temperature of 6.9 ± 0.1°C. Ambient temperature was then reduced by about 4°C to 1.8 ± 0.1°C. Ventilation was monitored as body temperature decreased passively by a similar magnitude, about 4°C. These experiments were carried out separately from gas response experiments.

Hibernating ground squirrels were fitted with a pneumotachograph and T_B leads and allowed to reach a steady state breathing pattern. Records of the burst breathing pattern and frequency were long, usually 2 to 3 hours. Chart recorder speed was increased at the end of this period to record V_T and respiratory timing components (as previously described). Chamber temperature was then decreased and the new steady state temperature was reached within 10 minutes. A short time later the body temperature of the squirrel began to decline. The changes in breathing pattern were monitored continuously at low chart recorder speed and every 15 minutes chart recorder speed was increased briefly to measure V_T and respiratory timing components. T_B was recorded, when possible, every 15 minutes. At the end of each experiment the weight of the ground squirrel was recorded, and calibrations of the pneumotachograph were performed as previously described.

Measurements of oxygen consumption ($\dot{V}_{O2}$) and C_{O2} production ($\dot{V}_{CO2}$) were obtained from hibernating squirrels at ambient temperatures of approximately 6°C and 1°C. Hibernating animals were placed in an airtight container fitted with two syringes. After a two hour period, if arousal had not been initiated, gas samples were extracted from the container. Changes in O_2 and CO_2 concentrations were measured with Beckman O_2 and CO_2 gas analysers. $\dot{V}_{O2}$ and $\dot{V}_{OC2}$ were calculated by subtracting the final O_2 and CO_2 concentrations from the initial concentrations and multiplying the difference by the total volume to give ml O_2 consumed and ml CO_2 produced over a known period.

Single breath breathing experiments were performed at ambient temperatures of 2.4 ± 0.1°C. Squirrels were maintained in the refrigerator, as previously described, until steady state respiration was achieved with the pneumotachograph - mask unit in place. Protocol for this set of experiments was identical to that used for burst breathing, except that gas exposures tended to be shorter (40 to 60 minutes in length) because nonventilatory periods were less than a minute during single breath breathing. At the end of the experiment the animal's weight was recorded and calibrations for V_T performed as previously described.

In order to observe the effects of anesthetic on the burst breathing pattern during hibernation, four S.

lateralis, hibernating at about 6°C, were exposed to vaporous halothane. Squirrels were maintained in the experimental set-up, wearing the penumotachograph-mask unit until steady state burst breathing was obtained. Only measurements of ventilatory pattern were recorded. Vaporized halothane was delivered to the animal chamber by passing the inflow air through a vaporizer calibrated to deliver anesthetic in volumes percent (vol. %). Each burst breathing squirrel was exposed to gradually increasing levels of halothane, until a change in the burst breathing pattern was observed, at about 3 vol. %. The squirrel was maintained on this level of halothane for approximately 30 minutes, to ensure steady state respiration. Halothane was then removed from the inflow gas and the animal was maintained on air until a burst breathing state had been achieved again.

Data Analysis

Breathing traces from both burst breathing and single breath breathing animals were analysed for f, V_T, T_I, T_{TOT} and T_E. Burst breathing traces were further analysed for burst frequency (B/min), breaths per burst (b/B), burst duration (T_{VP}), intraburst end inspiratory pause ($T_{\acute{E}}$) and length of the nonventilatory period (T_{NVP}). For the analysis of ventilatory pattern and overall f a

minimum of 40 minutes of recording for burst breathing animals and 20 minutes of recording for single breath breathing animals was used. For the analysis of other respiratory variables a minimum of 10-15 breaths (or 1 burst) was used. Minute ventilation ($\dot{V}_E$) was calculated from the product of f and V_T. Overall means for experimental groups were calculated as previously described.

Single breath data collected during the winters of 1984 and 1985 were compared using a one-way ANOVA and were not significantly different. The ventilatory responses to various gas mixtures were therefore combined for these groups. Statistical analysis, both within and between groups was performed as previously described.

RESULTS

AWAKE ANIMALS

Resting Ventilation

The resting ventilatory pattern of air breathing S. lateralis is shown in Figure 6. Table 2 summarizes respiratory variables for all four experimental groups of squirrels while breathing air. At rest, at an ambient temperature (T_C) of 22 to 25°C all squirrels show a similar continuous breathing pattern.

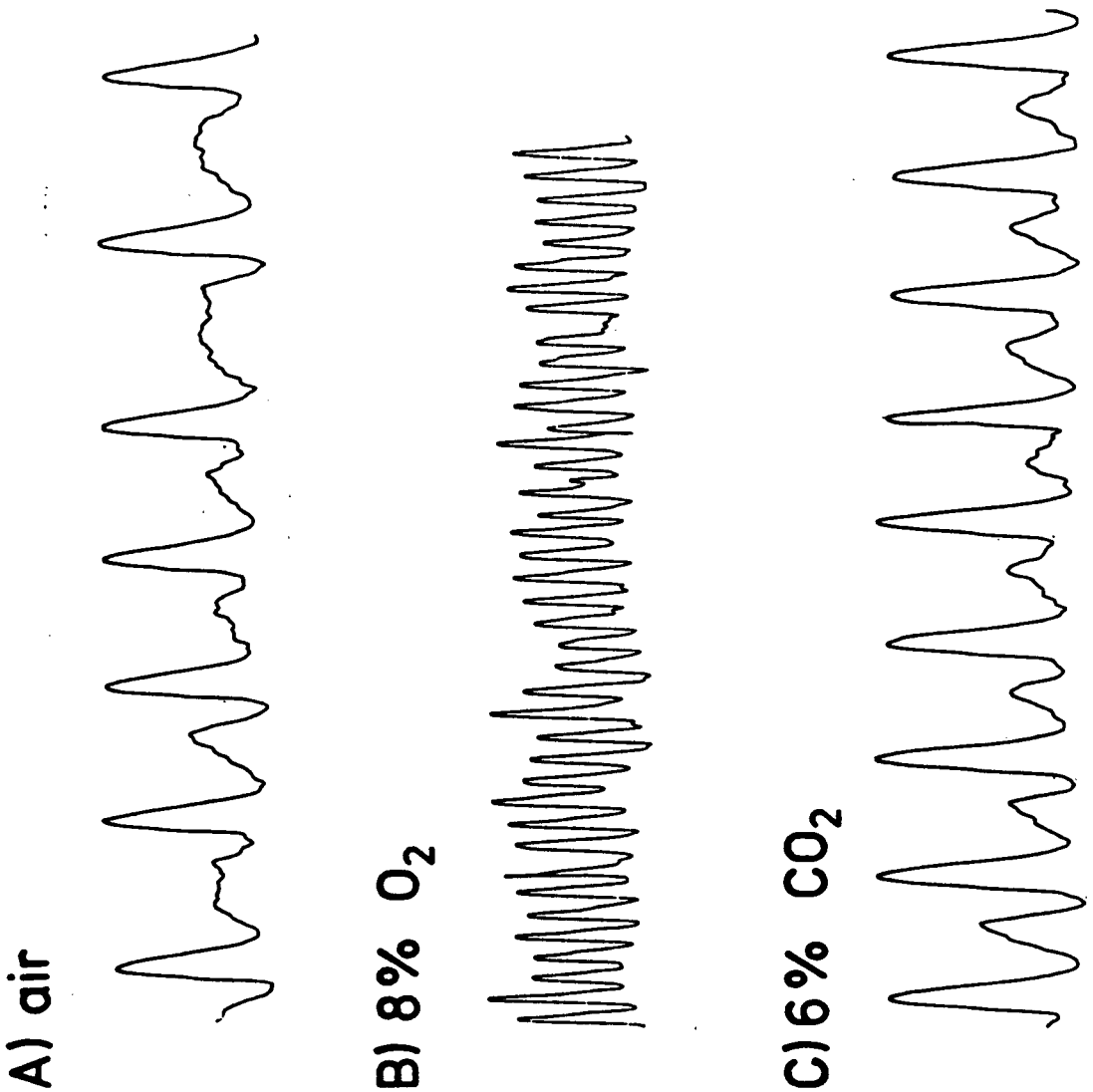
In both air breathing and chronic hypoxic and hypercapnic exposed (CHH) squirrels carotid body denervation (CBX) results in a decrease in resting $\dot{V}$ compared to intact animals (Table 1). In air breathing squirrels CBX reduces f, such that $\dot{V}$ is approximately 35% lower than in intact animals. CHH CBX squirrels show a slight decrease in V_T with little change in f such that overall $\dot{V}$ is only slightly decreased compared to CHH control squirrels.

Minute ventilatory responses to hypoxia and hypercapni are similar during acute and chronic exposure. The pattern of ventilatory response is slightly different in the two groups. Animals respond to acute exposure to hypoxia and hypercapnia with larger increases in f and

TABLE 2. Resting ventilatory variables during air exposure in awake golden-mantled ground squirrels (S. lateralis). All values are mean ± standard error. See Table 1 for explanation of symbols.

	AIR		CHRONIC	
	INTACT	CBX	INTACT	CBX
n	8	7	6	5
Mass (grams)	162 ± 6	174 ± 3	168 ± 8	161 ± 12
T_C (^oC)	25.7 ± 0.5	25.9 ± 0.7	25.4 ± 0.9	22.9 ± 0.6
f (breaths/min)	42 ± 3	28 ± 1	37 ± 4	43 ± 3
V_T (ml/100g)	0.8 ± 0.05	0.7 ± 0.04	1.1 ± 0.1	0.9 ± 0.1
$\dot{V}$ (ml/100g/min)	33 ± 2	21 ± 2	45 ± 4	37 ± 4

Figure 6. Representative breathing traces recorded by the whole body plethysmograph in S. lateralis at room temperature exposed to air (A), 8% inspired O_2 (B) and 6% inspired CO_2 (C).


A) air
B) 8% O_2
C) 6% CO_2
2 sec

smaller increases in V_t than are observed in CHH squirrels.

Animals chronically exposed to hypoxia and hypercapnia show an elevated resting $\dot{V}$ while exposed to air in both intact and CBX groups compared to air breathing control and CBX squirrels respectively. In CHH controls an increase of 35% in $\dot{V}$ over air breathing controls, is achieved through increases in V_T. In CHH CBX squirrels increases of 76% in $\dot{V}$ comapred to air breathing CBX squirrels are mediated by increases in V_T and f. (Table 1).

Response to Hypoxia

The effects of decreasing F_{IO2} on ventilation in air breathing and CHH S. lateralis is shown in Figure 7 and Figure 8. All four groups show a strong ventilatory response to hypoxia.

Air breathing intact S. lateralis respond to severe hypoxia (8% O_2) with a 140% increases in $\dot{V}$ over normoxic levels. The hypoxic response is mediated solely through increases in f (Table 3). Severe hypoxia (8% O_2) also causes a significant decrease in V_T. Ventilatory response threshhold for hypoxia falls between 17% and 21% O_2 in intact squirrels.

Figure 7. Effect of decreasing F_{IO2} on minute ventilation, tidal volume and frequency in awake air breathing S. lateralis (●) and awake air breathing CBX S. lateralis (O). All values are mean ± standard error for 7 to 8 animals.

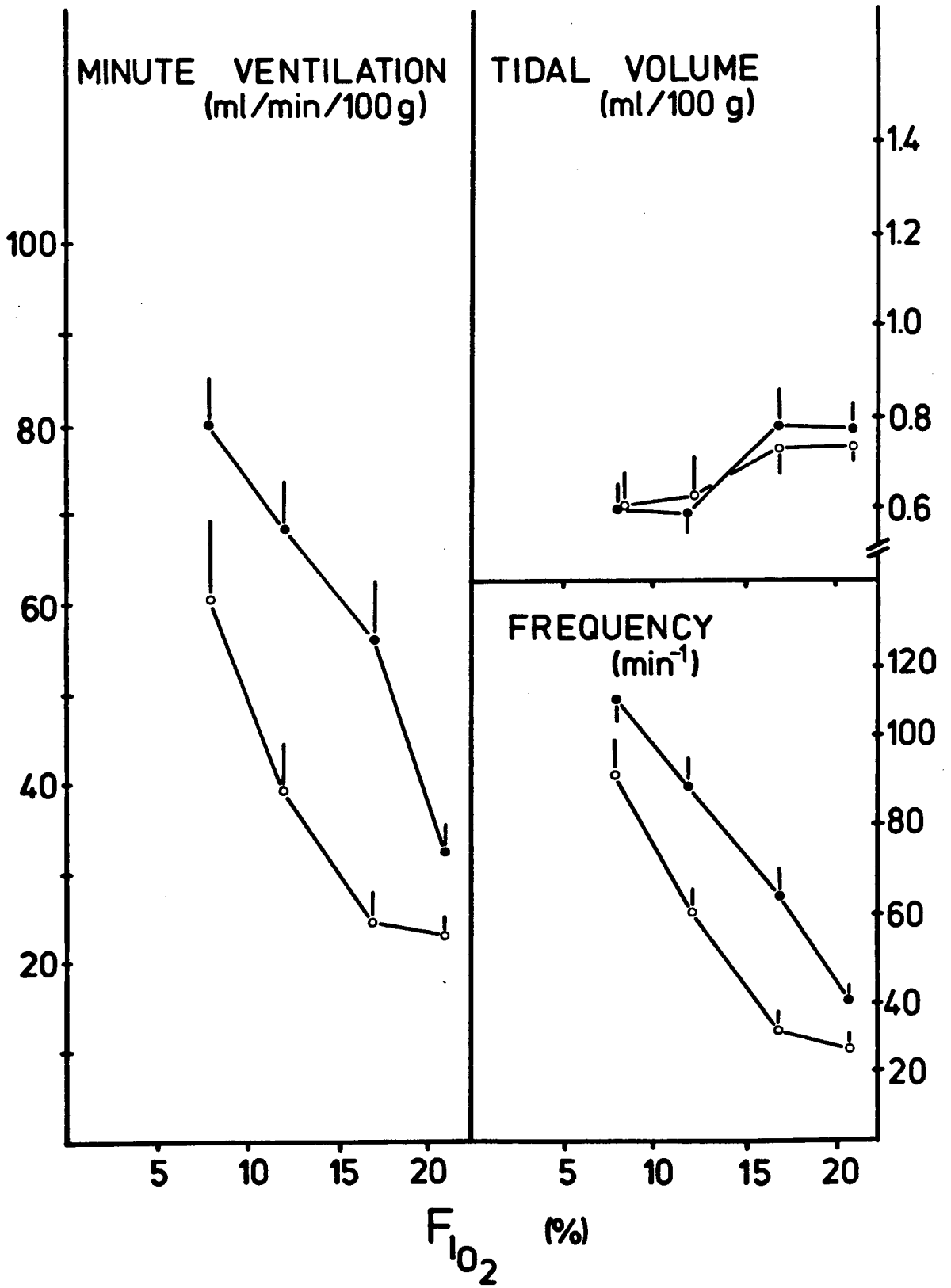

MINUTE VENTILATION
(ml/min/100g)
TIDAL VOLUME
(ml/100 g)
FREQUENCY
(min⁻¹)
100
80
60
40
20
1.4
1.2
1.0
0.8
0.6
120
100
80
60
40
20
5
10
15
20
5
10
15
20
FIO2 (%)

Figure 8. Effect of decreasing F_{IO2} on minute ventilation, tidal volume and frequency in awake CHH *S. lateralis* (●) and awake CHH CBX *S. lateralis* (○). All values are mean ± standard error for 6 animals.

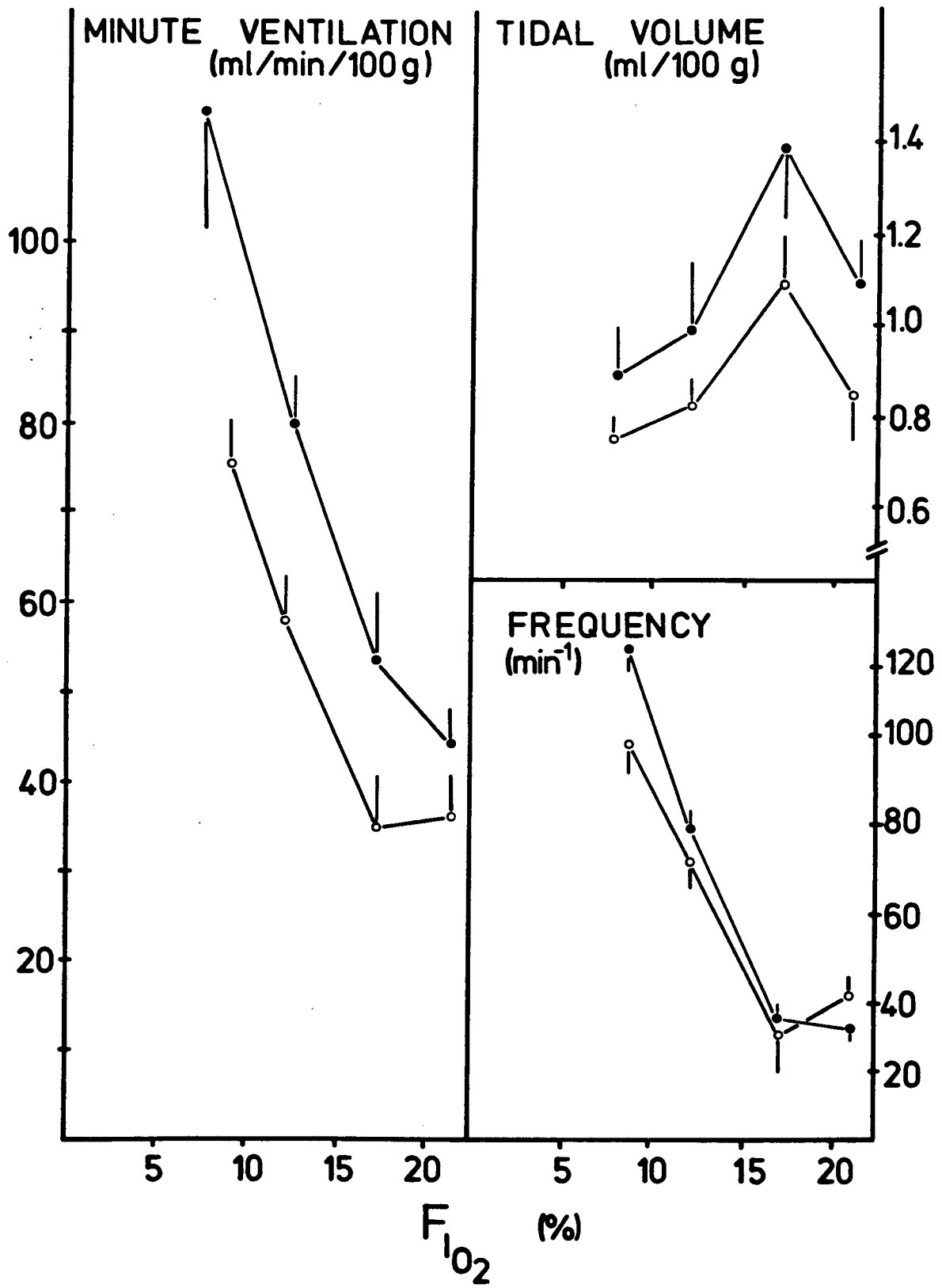

MINUTE VENTILATION
(ml/min/100 g)
TIDAL VOLUME
(ml/100 g)
FREQUENCY
(min⁻¹)
100
80
60
40
20
1.4
1.2
1.0
0.8
0.6
120
100
80
60
40
20
5
10
15
20
5
10
15
20
FIO2 (%)

TABLE 3. Effects of alteration in inspired gas composition on the frequency (f, breaths/minute), tidal volume (V_T, ml/100g) and minute ventilation ($\dot{V}$, ml/100g/min) of awake golden-mantled ground squirrels (S. lateralis). All values are mean ± standard error.

		AIR		CHRONIC	
GAS		INTACT	CBX	INTACT	CBX
n		8	7	6	6
AIR	f	42 ± 3	28 ± 1	37 ± 4	43 ± 3
	V_T	0.8 ± 0.05	0.7 ± 0.04	1.1 ± 0.1	0.9 ± 0.1
	$\dot{V}$	33 ± 2	21 ± 2	45 ± 4	37 ± 4
17% O_2	f	71 ± 9	33 ± 5	38 ± 2	32 ± 7
0% CO_2	V_T	0.8 ± 0.07	0.7 ± 0.07	1.4 ± 0.2	1.1 ± 0.1
	$\dot{V}$	56 ± 6	24 ± 3	53 ± 7	35 ± 9
12% O_2	f	105 ± 9	67 ± 8	80 ± 5	70 ± 7
0% CO_2	V_T	0.6 ± 0.04	0.6 ± 0.09	1.0 ± 0.1	0.8 ± 0.05
	$\dot{V}$	68 ± 5	39 ± 5	79 ± 4	58 ± 5
8% O_2	f	131 ± 5	109 ± 9	130 ± 9	99 ± 9
0% CO_2	V_T	0.6 ± 0.04	0.6 ± 0.07	0.9 ± 0.1	0.8 ± 0.03
	$\dot{V}$	80 ± 6	62 ± 7	119 ± 14	75 ± 5
21% O_2	f	40 ± 7	33 ± 4	37 ± 4	46 ± 8
2% CO_2	V_T	0.8 ± 0.07	0.9 ± 0.06	1.1 ± 0.09	1.0 ± 0.05
	$\dot{V}$	32 ± 4	28 ± 3	39 ± 5	45 ± 5

TABLE 3 cont...

21% O_2	f	43 ± 5	37 ± 6	46 ± 4	39 ± 6
4% CO_2	V_T	1.0 ± 0.07	0.9 ± 0.07	1.4 ± 0.1	1.2 ± 0.1
	$\dot{V}$	40 ± 5	33 ± 4	60 ± 6	51 ± 12
21% O_2	f	47 ± 6	49 ± 9	47 ± 4	52 ± 5
6% CO_2	V_T	1.1 ± 0.06	1.3 ± 0.08	1.3 ± 0.07	1.3 ± 0.1
	$\dot{V}$	49 ± 5	62 ± 10	61 ± 4	67 ± 11
50% O_2	f	27 ± 5	41 ± 6	36 ± 3	31 ± 7
0% CO_2	V_T	0.8 ± 0.09	1.1 ± 0.09	1.6 ± 0.09	1.1 ± 0.1
	$\dot{V}$	21 ± 3	50 ± 7	51 ± 12	33 ± 6
50% O_2	f	43 ± 5	29 ± 2	41 ± 3	45 ± 16
2% CO_2	V_T	0.8 ± 0.1	0.9 ± 0.07	1.4 ± 0.03	1.1 ± 0.1
	$\dot{V}$	31 ± 6	28 ± 3	50 ± 3	58 ± 11
50% O_2	f	36 ± 5	37 ± 3	45 ± 3	33 ± 6
4% CO_2	V_T	0.9 ± 0.11	1.2 ± 0.12	1.3 ± 0.09	1.3 ± 0.12
	$\dot{V}$	34 ± 4	43 ± 6	60 ± 5	41 ± 8
50% O_2	f	53 ± 7	46 ± 9	43 ± 11	41 ± 6
6% CO_2	V_T	1.1 ± 0.08	1.3 ± 0.11	1.6 ± 0.10	1.4 ± 0.16
	$\dot{V}$	50 ± 4	61 ± 13	69 ± 10	62 ± 12
17% O_2	f	65 ± 7	47 ± 5	47 ± 5	42 ± 6
4% CO_2	V_T	1.0 ± 0.09	0.9 ± 0.13	1.4 ± 0.05	1.2 ± 0.06
	$\dot{V}$	59 ± 3	38 ± 3	64 ± 3	52 ± 8
12% O_2	f	62 ± 4	48 ± 9	50 ± 5	57 ± 9
4% CO_2	V_T	0.9 ± 0.07	1.0 ± 0.11	1.4 ± 0.09	1.1 ± 0.08
	$\dot{V}$	60 ± 8	52 ± 8	70 ± 10	60 ± 14
8% O_2	f	105 ± 8	86 ± 9	98 ± 11	72 ± 9
4% CO_2	V_T	0.9 ± 0.07	0.8 ± 0.07	1.2 ± 0.12	1.0 ± 0.11
	$\dot{V}$	95 ± 4	64 ± 8	110 ± 10	68 ± 8

Carotid body denervation, in both air breathing and CHH squirrels, results in a downward shift of the entire ventilatory response curve with little change in the overall ventilatory sensitivity compared to intact controls in each respective group (Figures 7 and 8). The downshifted response curve is a result of lower respiratory frequencies at all levels of O_2 in air breathing CBX squirrels and lower tidal volumes at all levels of O_2 in CHH CBX squirrels. As in intact squirrels, all CBX animals responds to hypoxia through increases in f. In addition, CBX causes a slight left shift in the hypoxic response threshold down to between 12% and 17% O_2 in both air breathing and CHH groups.

Chronic exposure to hypoxia and hypercapnia leads to an upward shift in the hypoxic response curve in both intact and CBX squirrels compared to respective air breathing groups (Figures 7 and 8) resulting from a maintained increase in V_T at all levels of inspired O_2. The overall magnitude of the ventilatory response to 8% O_2 is slightly elevated in CHH control animals compared to air breathing control squirrels. In contrast, CHH exposure does not alter the sensitivity of CBX squirrels to hypoxia. Both groups of CHH squirrels respond to hypoxia solely by increases in f and both groups show slight decreases in V_T during severe hypoxia. CHH exposure does not alter hypoxic ventilatory threshold.

Response to Hypercapnia

Figure 9 and Figure 10 show the ventilatory responses of air breathing and CHH S. lateralis to increases in F_{ICO2}. All four groups exhibit an increase in $\dot{V}$ in response to hypercapnia.

Intact air breathing S. lateralis responds to 6% CO_2 with a comparatively moderate 58% increase in $\dot{V}$. The increase in $\dot{V}$ is acheived primarily through significant increases in V_T with only slight increases in f. The threshhold for the ventilatory response falls between 2% and 4% CO_2.

At low levels of CO_2, CBX squirrels show a downshifted ventilatory response curve compared to intact animals, but as levels of hypercapnia increase to 6% CO_2 there is no significant difference between absolute $\dot{V}$ values. The increased sensitivity to hypercapnia in CBX squirrels is due to an elevated frequency response than is seen in intact squirrels. both air breathing CBX and CHH CBX squirrels increase $\dot{V}$ through more or less equal increases in V_T and f. Hypercapnic response thresholds are slightly left shifted by carotid body denervations, such that $\dot{V}$ increases in response to hypercapnia at levels below 2% CO_2.

Figure 9. Effect of increasing F_{ICO2} on minute ventilation, tidal volume and frequency in awake air breathing S. lateralis (●) and awake air breathing CBX S. lateralis (O). All values are mean ± standard error for 7 to 8 animals.

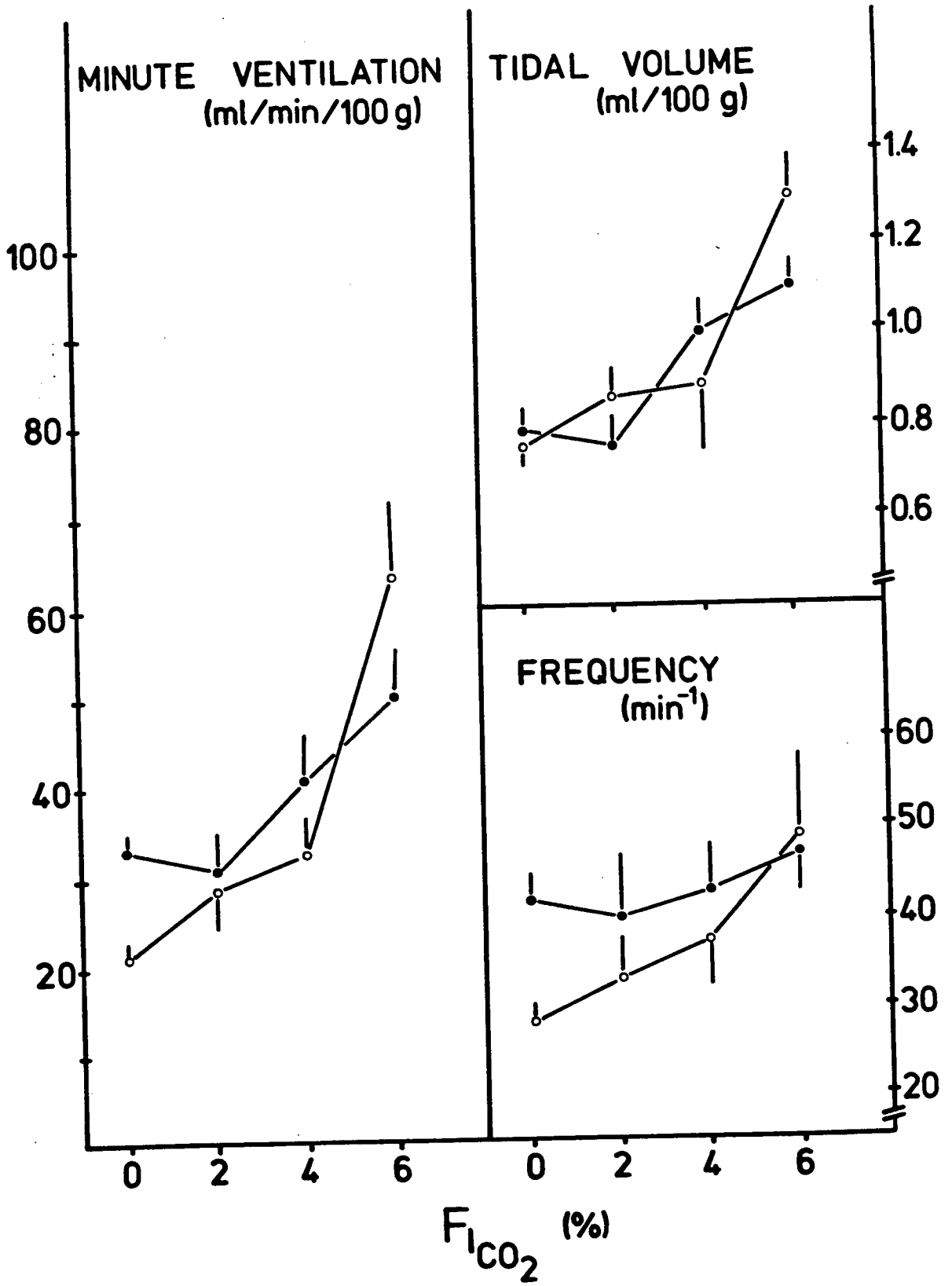
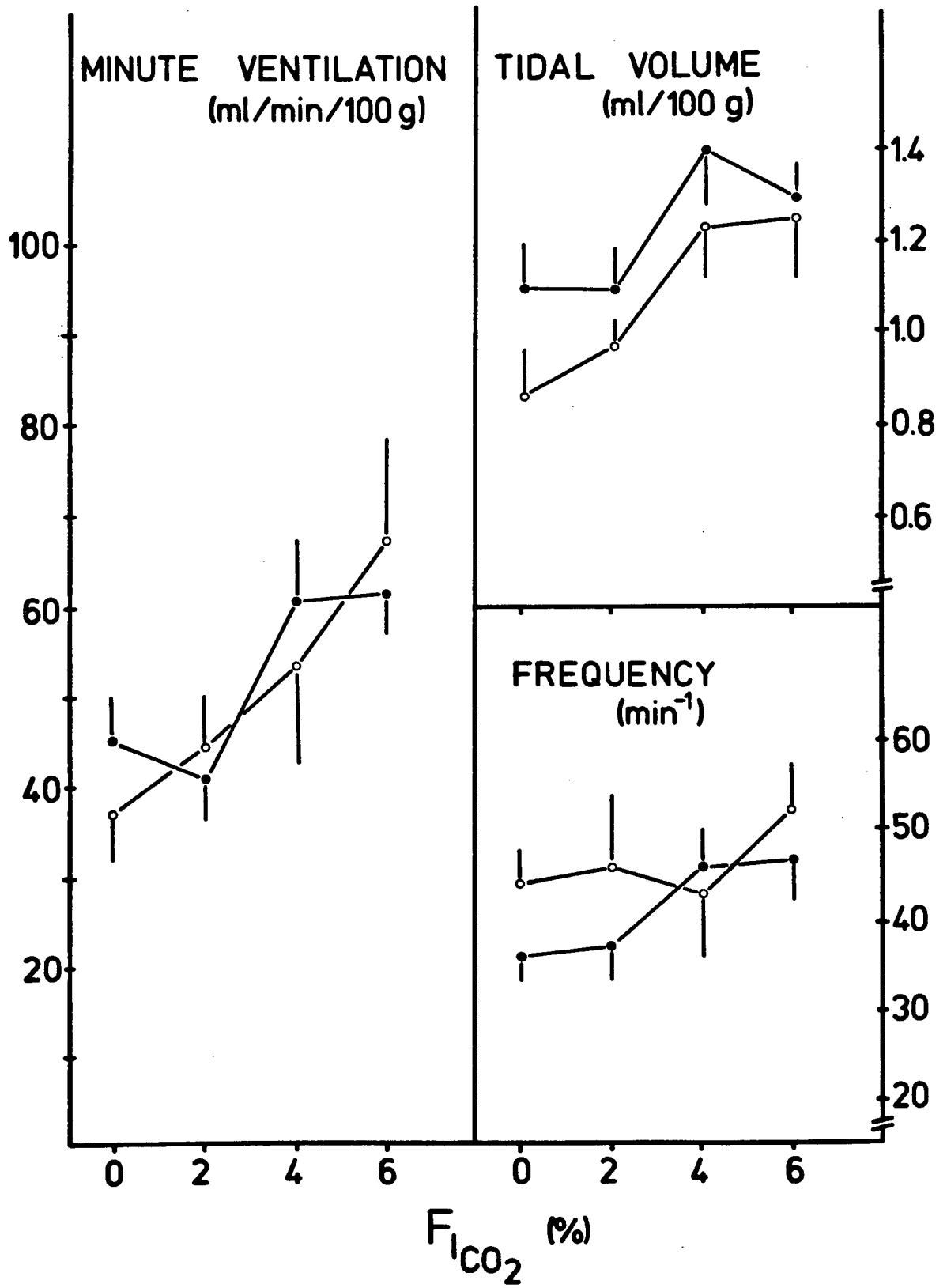

MINUTE VENTILATION
(ml/min/100 g)
TIDAL VOLUME
(ml/100 g)
FREQUENCY
(min⁻¹)
100
80
60
40
20
1.4
1.2
1.0
0.8
0.6
60
50
40
30
20
0
2
4
6
0
2
4
6
FICO2 (%)

Figure 10. Effect of increasing F_{ICO2} on minute ventilation, tidal volume and frequency in awake CHH *S. lateralis* (●) and awake CHH CBX *S. lateralis* (○). All values are mean ± standard error for 6 animals.


MINUTE VENTILATION
(ml/min/100 g)
TIDAL VOLUME
(ml/100 g)
FREQUENCY
(min⁻¹)
100
80
60
40
20
1.4
1.2
1.0
0.8
0.6
60
50
40
30
20
0
2
4
6
0
2
4
6
FI CO2 (%)

Chronic exposure to hypoxia and hypercapnia has little effect on overall sensitivity to hypercapnia. CHH exposure does result in an overall elevated $\dot{V}$ response curve due to a maintained increase in V_T at all levels of CO_2. Neither the hypercapnic response threshhold nor the pattern of ventilatory response was altered by CHH exposure.

Responses to hyperoxia, hyperoxic hypercapnia and hypoxic hypercapnia

The effects of hyperoxia, hyperoxic hypercapnia and hypoxic hypercapnia on ventilatory responses are given in Table 3. Figure 11 and Figure 12 shows the $\dot{V}$ responses of all four experimental groups to combined gas mixtures.

In air breathing control squirrels hyperoxia causes a 36% decrease in $\dot{V}$, which results solely from a significant decrease in f. In contrast, hyperoxia causes a 53% increase in $\dot{V}$ in air breathing CBX animals. Adjustments in $\dot{V}$ are produced primarily by increases in V_T in CBX squirrels. In CHH control animals hyperoxia causes a slight increase in $\dot{V}$, produced by an increase in V_T whereas in CHH CBX squirrels hyperoxia results in no overall change in $\dot{V}$. Generally the ventilatory responses to hyperoxia, particularly in CHH animals, are variable (Table 3).

Overall ventilatory responses to hypercapnia are not significantly altered by the addition of 50% O_2 in any group of S. lateralis (Figure 11 and Figure 12). Overall $\dot{V}$ responses to hypercapnia remained low relative to hypoxic responses. In air breathing control and CHH control squirrels, although a hyperoxic background has no significant effect on the overall ventilatory response, ventilatory threshhold for CO_2 response is shifted slightly to the right in both groups. As in normoxia CHH control and CHH CBX squirrels maintain an elevated hypercapnic response curve relative to air breathing groups mediated by an increased V_T at all levels of hypercapnia. In all four groups increases in $\dot{V}$ are still caused primarily by increases in V_T.

During acute exposure to 17% O_2 and 4% CO_2 (CHH conditions) air breathing squirrels and CHH squirrels exhibit similar $\dot{V}$ responses. The pattern of ventilatory response is slightly different in the two groups, with CHH squirrels having a significantly higher V_T and significantly lower f compared to air breathing control squirrels. Intact CBX squirrels respond to acute exposure of 17% O_2 and 4% O_2 with smaller increases in $\dot{V}$ and V_T comapred to CHH CBX squirrels.

In general, a constant hypercapnic background (4% CO_2) has little effect on the overall ventilatory response curve to hypoxia (Figure 11). Overall sensitivity to

Figure 11. Effect of decreasing F_{IO2} with a hypercapnic background (A, 4% CO_2) and increasing F_{ICO2} with a hyperoxic background (B, 50% O_2) on minute ventilation in awake air breathing CBX *S. lateralis* (●) and awake air breathing CBX *S. lateralis* (○). All values are mean ± standard error for 7 to 8 animals.

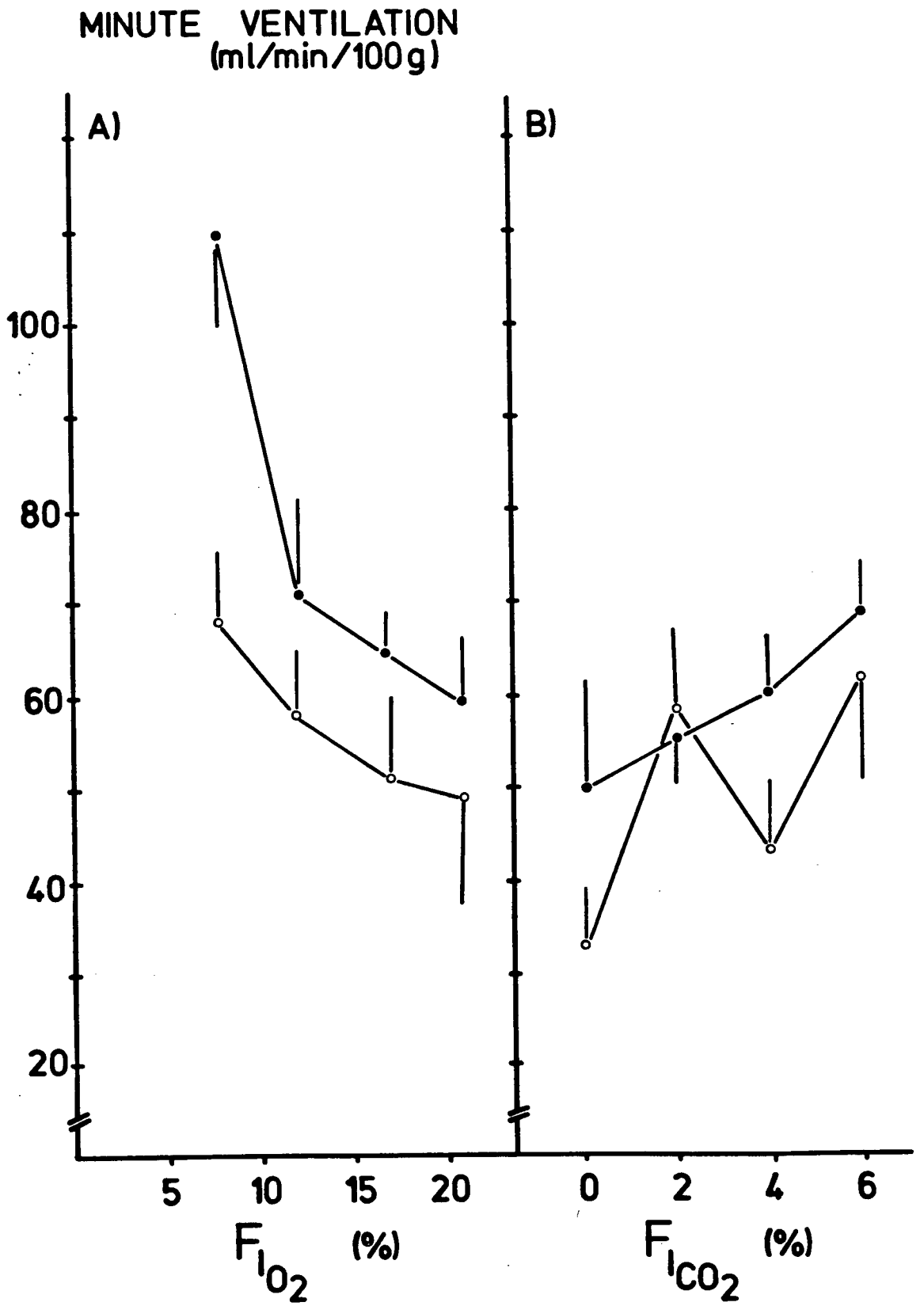
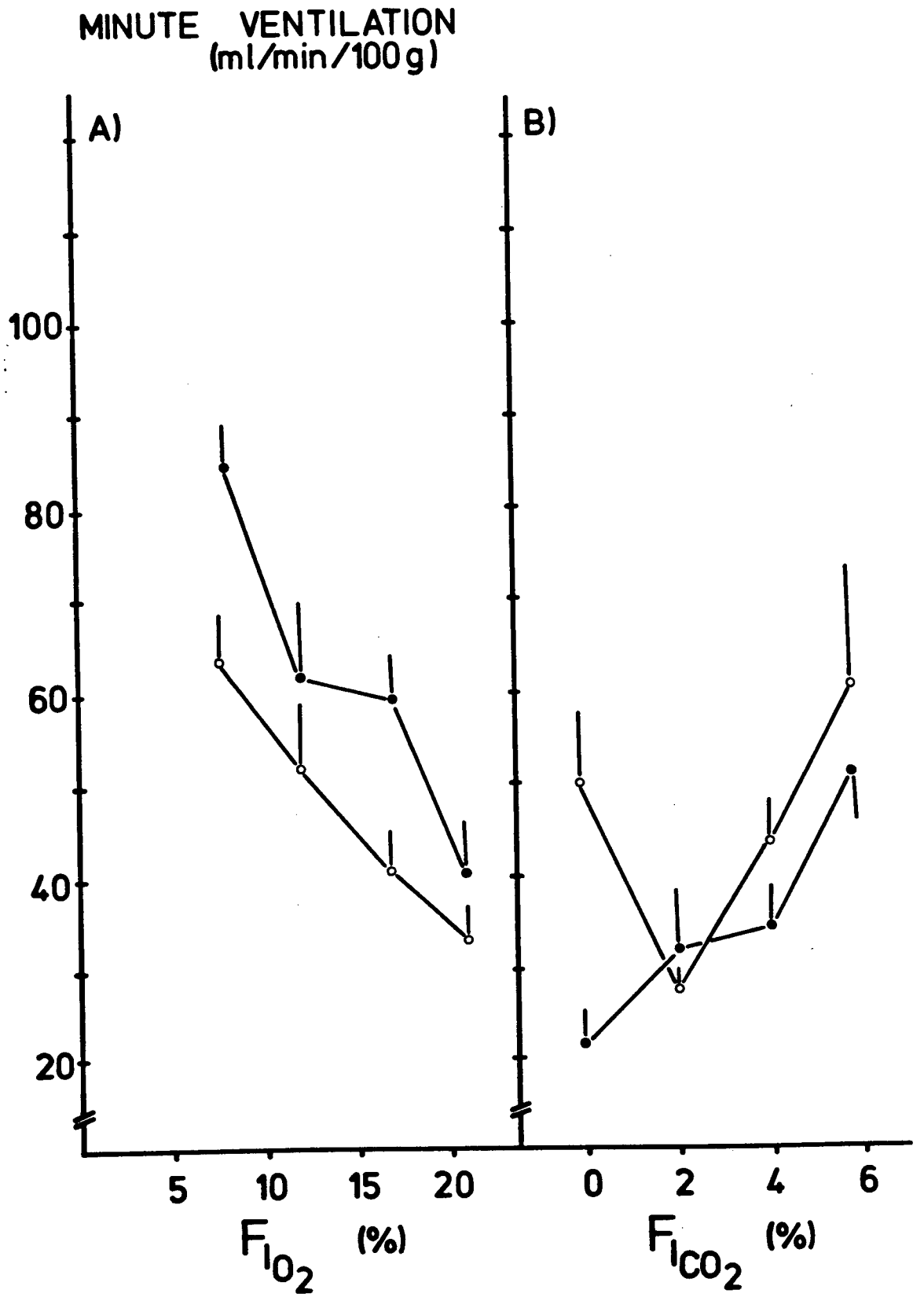

MINUTE VENTILATION
(ml/min/100g)
A)
B)
100
80
60
40
20
5
10
15
20
0
2
4
6
F_{IO_2} (%)
F_{ICO_2} (%)

Figure 12. Effect of decreasing F_{IO2} with a hypercapnic background (A, 4% CO_2) and increasing F_{ICO2} with a hyperoxic background (B, 50% O_2) on minute ventilation in awake CHH S. lateralis (●) and awake CHH CBX S. lateralis (○). All values are mean ± standard error for 6 animals.


MINUTE VENTILATION
(ml/min/100g)
A)
B)
100
80
60
40
20
5
10
15
20
0
2
4
6
F_{IO_2} (%)
F_{ICO_2} (%)

hypoxia is slightly reduced in all groups, except air breathing control squirrels. At moderate levels of hypoxia (21%, 17% and 12%) the hypercapnic background causes an increase in $\dot{V}$ due to an elevated V_T. V_T remains elevated in all groups at all levels of hypercapnic hypoxia relative to normocapnic hypoxia. A hypercapnic background also reduced the overall frequency response to hypoxia by 10% to 17% in all experimental groups. Thus, although absolute values of $\dot{V}$ were not reduced at 8% O_2, hypercapnia results in an elevated V_T at all levels of O_2 and a blunted f response.

Air breathing CBX squirrels show a blunted hypoxic response during hypercapnia compared to air breathing control squirrels (Figure 11). Air breathing CBX squirrels increase $\dot{V}$ 60% during hypercapnic hypoxia (8% O_2, 4% CO_2) while in control squirrels increase $\dot{V}$ 150%. The decrease in sensitivity in CBX squirrels is due to a reduced V_T and f response. CHH CBX squirrels exhibited a blunted hypoxic response compared to intact CHH controls, similar to that seen during normocapnia.

HIBERNATING ANIMALS

Resting Ventilation

At an ambient temperature (T_C) of 6°C and a body temperature of 7°C golden-mantled ground squirrels exhibit a

burst breathing pattern. Figure 13 illustrates a typical burst breathing pattern under resting conditions. Both control and CBX S. lateralis show similar bursting patterns (Figure 13). Corresponding resting ventilatory variables are presented in Table 4.

The burst breathing pattern consists of a series of rapid breaths followed by a long breath hold period. Individual bursts are variable not only in the number of breaths they contain, but also in V_T, f and timing components within the burst. Several bursts were analysed, breath by breath, to determine if they resembled a Cheyne-Stokes bursting pattern. Figure 14 illustrates a burst which has features typical of a CSR pattern. T_I and T_E do not change over the course of the burst, but there is a waxing and waning of both frequency (measured by $T_{\acute{E}}$) and V_T. Figure 15 represents a much more uniform burst, in which there is little change in T_I, T_E, $T_{\acute{E}}$ and V_T of individual breaths through the burst.

Variability of the burst breathing pattern is high both between different squirrels, and in an individual squirrel during a single hibernation bout. The number of breaths/burst (b/B) ranges from 2 or 3 to over 100 breaths/burst ($\bar{x}$ = 19 b/B in controls), while T_{NVP} ranges from less than 1 minute to over 40 minutes ($\bar{x}$ = 509 sec in controls). In spite of the large variability in pattern,

TABLE 4. Resting ventilatory variables in hibernating golden-mantled ground squirrels (S. lateralis). All values are mean ± standard error. See Table 1 for explanation of symbols.

	BURST BREATHING		SINGLE BREATH BREATHING	
	CONTROL	CBX	CONTROL	CBX
n	7	6	9	10
Mass (grams)	189 ± 7	179 ± 9	155 ± 7	162 ± 5
T_C (^oC)	6.3 ± 0.1	5.7 ± 0.1	2.5 ± 0.2	2.4 ± 0.2
T_b (^oC)	7.4 ± 0.1 ($n=3$)	7.2 ± 0.2 ($n=4$)	-	4.9 ± 0.2 ($n=4$)
Breaths/burst	19 ± 2	18 ± 4	-	-
T_{VP} (sec)	84 ± 9	79 ± 21	-	-
T_{NVP} (sec)	690 ± 118	503 ± 136	-	-
T_E' (sec)	-	-	23 ± 3	30 ± 4
f (breaths/min)	1.7 ± 0.2	2.2 ± 0.2	2.6 ± 0.2	2.1 ± 0.2
V_T (ml/100g)	0.67 ± 0.08	0.46 ± 0.02	0.63 ± 0.04	0.71 ± 0.05
$\dot{V}$ (ml/min/100g)	1.1 ± 0.08	1.0 ± 0.08	1.6 ± 0.1	1.4 ± 0.2
$\dot{V}_{O2}$ (ml/hr/100g)	2.0 ± 0.1	2.0 ± 0.1	combined = 2.3 ± 0.1	
$\dot{V}_{CO2}$ (ml/hr/100g)	1.8 ± 0.1	1.7 ± 0.1	combined = 2.1 ± 0.1	
T_{TOT} (sec)	2.5 ± 0.08	2.3 ± 0.09	5.5 ± 0.01	5.5 ± 0.2
T_I (sec)	1.0 ± 0.03	1.0 ± 0.04	2.2 ± 0.1	2.3 ± 0.1
T_E (sec)	1.5 ± 0.03	1.3 ± 0.07	3.3 ± 0.1	3.2 ± 0.1

Figure 13. Representative records of resting burst breathing patterns in hibernating control and CBX S. lateralis at a T_c of about 6°C.

BURST BREATHING DURING HIBERNATION

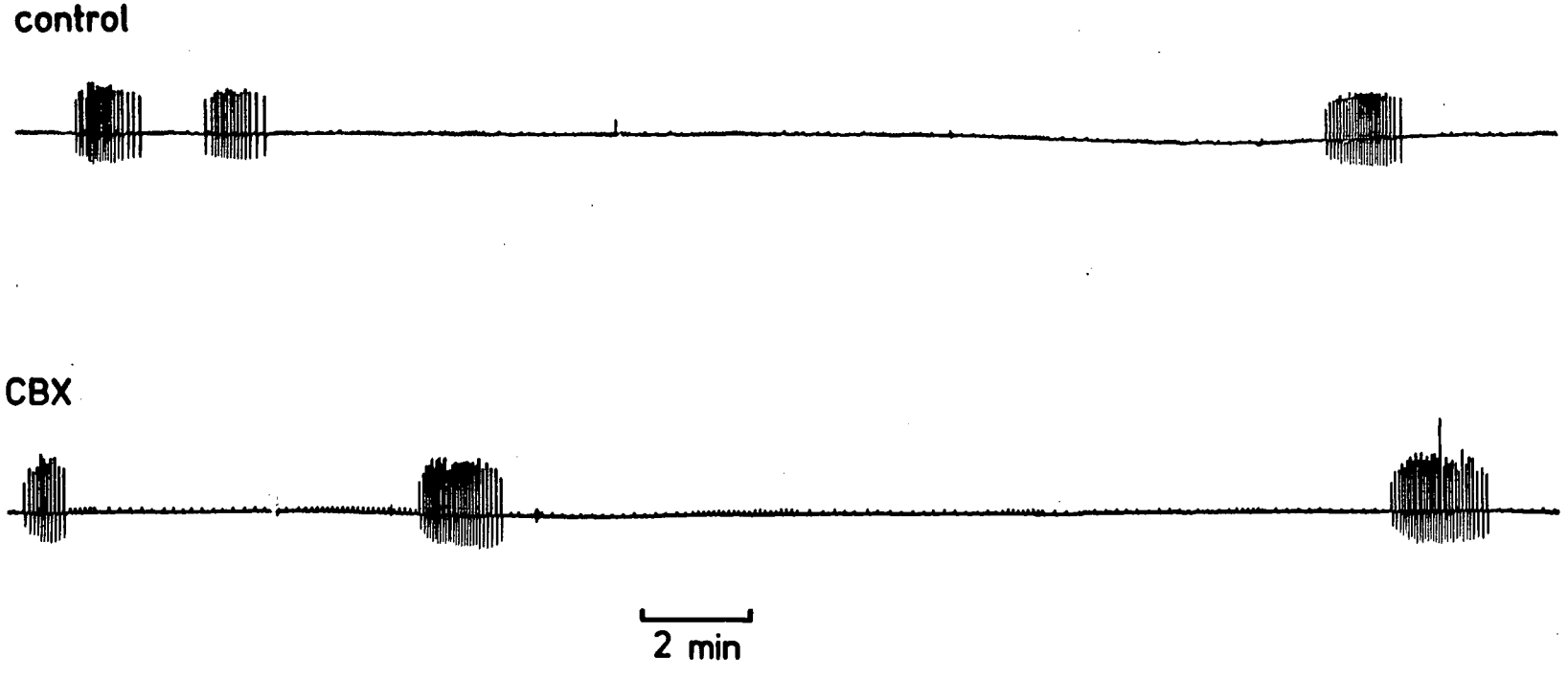


Figure 14. A single burst of breathing showing a Cheyne-Stokes pattern from S. lateralis hibernating at a T_C of about 6°C. The bottom panel represents a breath by breath analysis of inspiratory time (T_I), expiratory time (T_E), end inspiratory time (T_E') and tidal volume (V_T). Note the changes in respiratory frequency and depth through the burst.

BURST BREATHING PATTERNS

Cheyne-Stokes

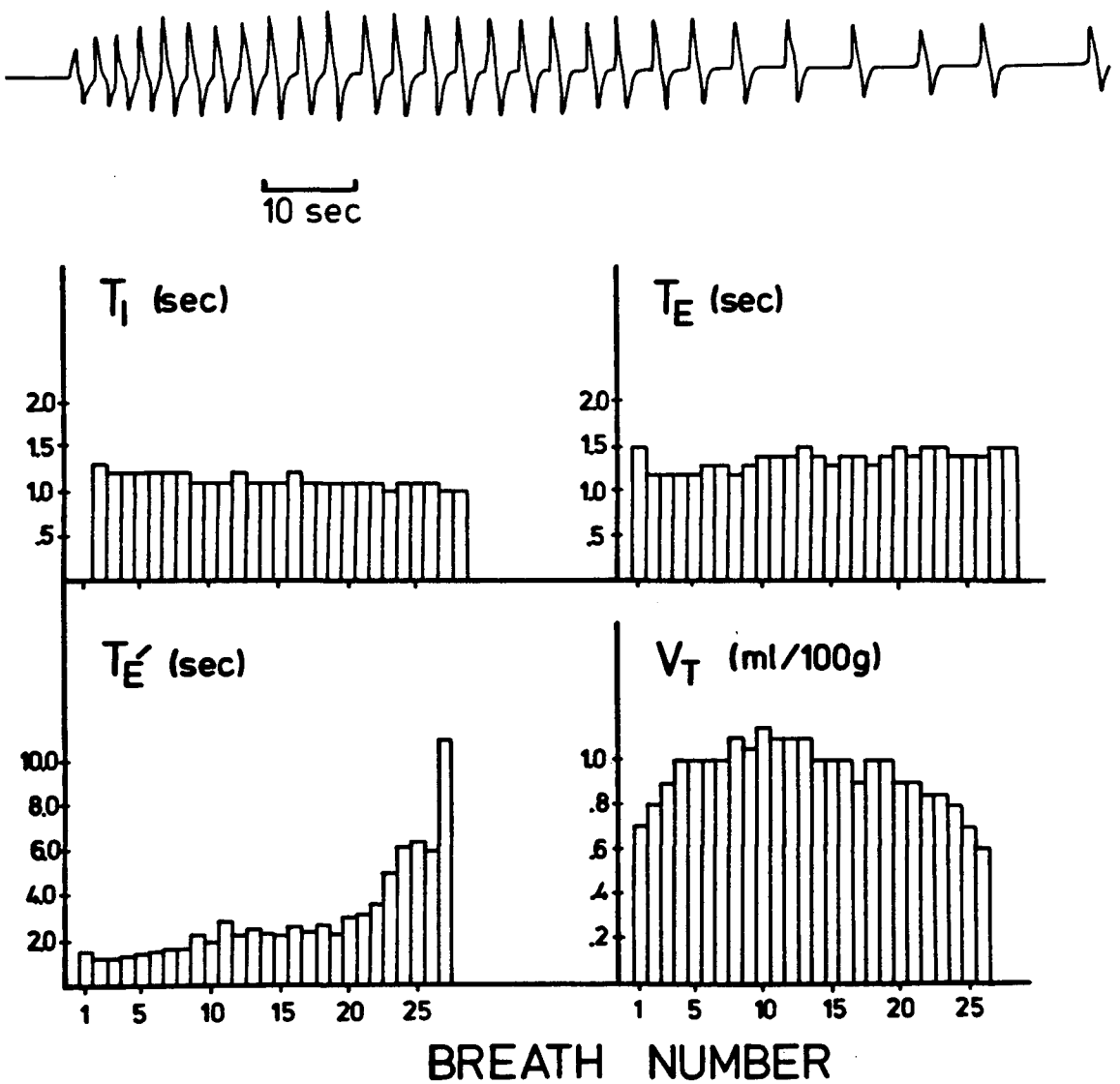
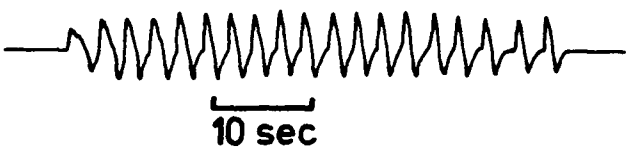
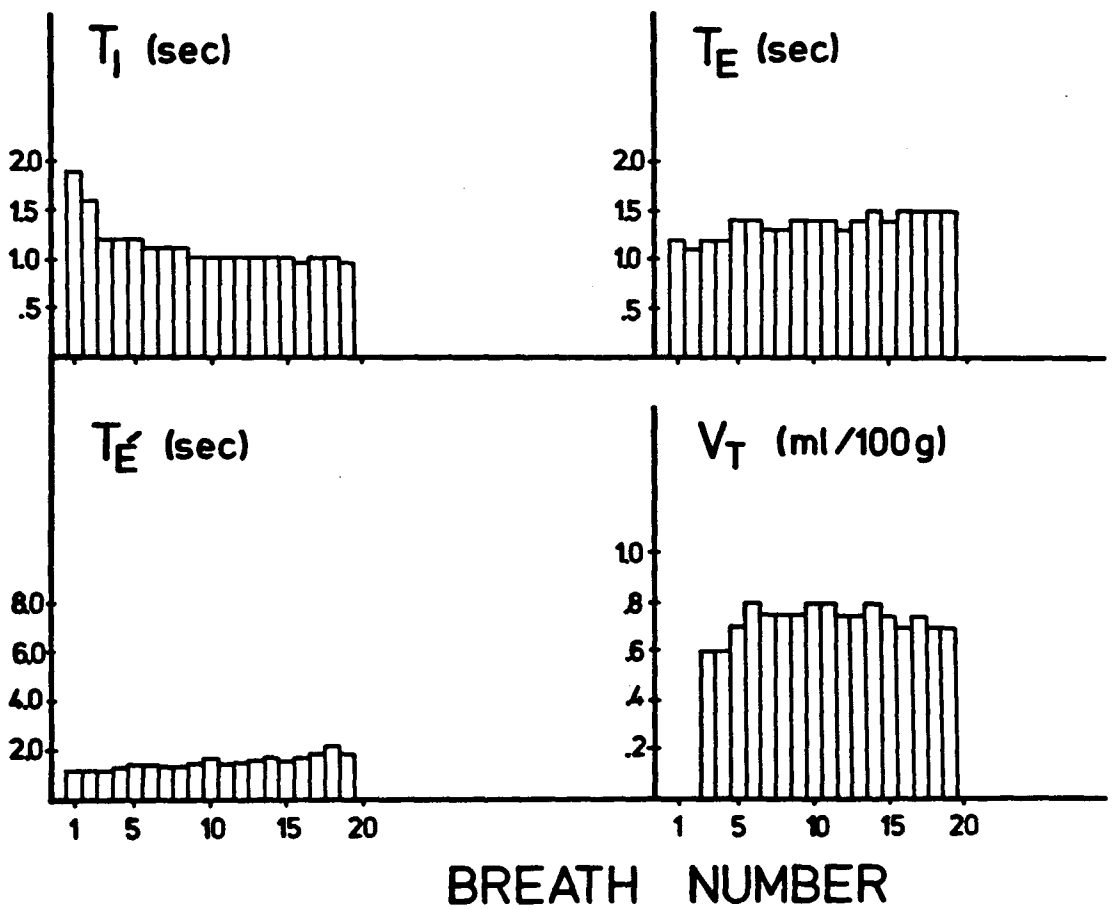


Figure 15. A single burst of breaths from S. lateralis hibernating at a T_C of about 6°C. The bottom panel represents a breath-by-breath analysis of inspiratory time (T_I), expiratory time (T_E), end inspiratory pause (T_E') and tidal volume (V_T). Note there is little change in respiratory frequency and depth through the burst.

BURST BREATHING PATTERNS

Regular





overall resting frequency remains fairly constant (Table 4). Ventilatory frequency is slightly lower, while V_T is significantly higher in control squirrels compared to CBX squirrels. This results in a slightly higher resting $\dot{V}$ in control squirrels (Table 4).

During burst breathing T_{TOT} is similar in both control and CBX squirrels. The ratio of T_I to T_E is .7 for both groups. The duty cycle (the ratio of T_I to T_{TOT}) is also similar in both groups being, on average, 0.4 (Table 5). T_{TOT}, T_I and T_E do not change significantly in response to any gas mixture. Changes in frequency are achieved solely by changes in the end inspiratory pause (T_E' or T_{NVP}).

Oxygen consumption ($\dot{V}_{O2}$) was not measured simultaneously with ventilation and it has been assumed that $\dot{V}_{O2}$ values obtained are accurate for animals in deep hibernation at a T_c of 6 to 7°C. $\dot{V}_{O2}$ values for control and CBX squirrels are not statistically different, the mean for both intact and CBX squirrels is 2.0 ml/hour/100g.

During hibernation single breath breathing occurs in S. lateralis at T_c of 2.5°C and T_b of about 4.9°C. Under normoxic and normocapnic conditions the respiratory pattern consists of single breaths followed by an end inspiratory pause (T_E') ranging from 10 to 60 seconds in length (Figure 16). Ventilatory f for control and CBX squirrels is

similar, as is V_T (Table 4). Thus, overall V is not different between control and CBX ground squirrels. In addition, $\dot{V}_{O2}$ values are not significantly different, being, on average, 2.2 ml/hour/100g.

Respiratory timing variables for control and CBX S. lateralis are not significantly different (Table 4). The timing variable did not change significantly in response to any gas mixture. In approximately 15% to 20% of experiments involving single breath breathing squirrels double inspirations were observed at one time or another (Figure 33). The double inspirations seldom continued over the course of the entire experiment and there was no apparent reason for their occurrence.

Transition Data

Since breathing patterns of control and CBX squirrels under normoxic conditions are not significantly different, transition data represents combined values collected from control and CBX squirrels. Table 5 presents the changes which occur in several respiratory variables during the transition from burst breathing (0% transition) to single breath breathing (100% transition). The entire transition requires between 3 to 4 hours to complete for most animals. As chamber temperature dropped from 6.9°C to 1.8°C body temperature underwent a similar magnitude drop

TABLE 5. Effects of decreasing body temperature (T_b) on respiratory variables in golden-mantled ground squirrels (*S. lateralis*). All values are mean ± standard error. See Table 1 or text for explanation of symbols.

	0% TRANSITION (burst breathing)	33% TRANSITION	66% TRANSITION	100% TRANSITION (single breath)
n	12	12	11	11
T_C (°C)	6.9 ± 0.1	-	-	1.8 ± 0.1
T_b (°C, n=4)	8.6 ± 0.2	6.2 ± 0.4	4.8 ± 0.5	4.4 ± 0.5
Transition Time (hours)	0	1.1 ± 0.4	2.2 ± 0.4	3.4 ± 0.2
Breaths/burst	20.6 ± 1.9	3.1 ± 0.2	1.4 ± 0.06	1.0
T_{VP} (sec)	89 ± 7	12.4 ± 2.0	9.3 ± 3.6	5.5 ± 0.4
Bursts/min	0.09 ± 0.01	0.5 ± 0.03	1.0 ± 0.06	2.0 ± 0.2
f (breaths/min)	1.7 ± 0.1	1.6 ± 0.09	1.6 ± 0.1	2.0 ± 0.1
V_T (ml/100g)	0.80 ± 0.03	0.85 ± 0.04	0.78 ± 0.05	0.74 ± 0.05
$\dot{V}$ (ml/100g/min)	1.4 ± 0.1	1.4 ± 0.1	1.3 ± 0.1	1.5 ± 0.1
T_{TOT} (sec)	2.4 ± 0.2	4.0 ± 0.1	4.7 ± 0.1	5.5 ± 0.1
T_I (sec)	1.0 ± 0.03	1.6 ± 0.06	1.9 ± 0.04	2.2 ± 0.04
T_E (sec)	1.5 ± 0.05	2.4 ± 0.06	2.8 ± 0.08	3.3 ± 0.08
T_I/T_E	0.67	0.67	0.68	0.67
Duty Cycle (T_I/T_{TOT})	0.42	0.40	0.40	0.40
Time actively breathing	7%	11%	13%	19%
V_T/T_I	0.8	0.5	0.4	0.3

Figure 16. Representative record of the breathing pattern transition from burst breathing at a T_c of 7°C to single breath breathing at a T_c of 2°C in S. lateralis during hibernation.

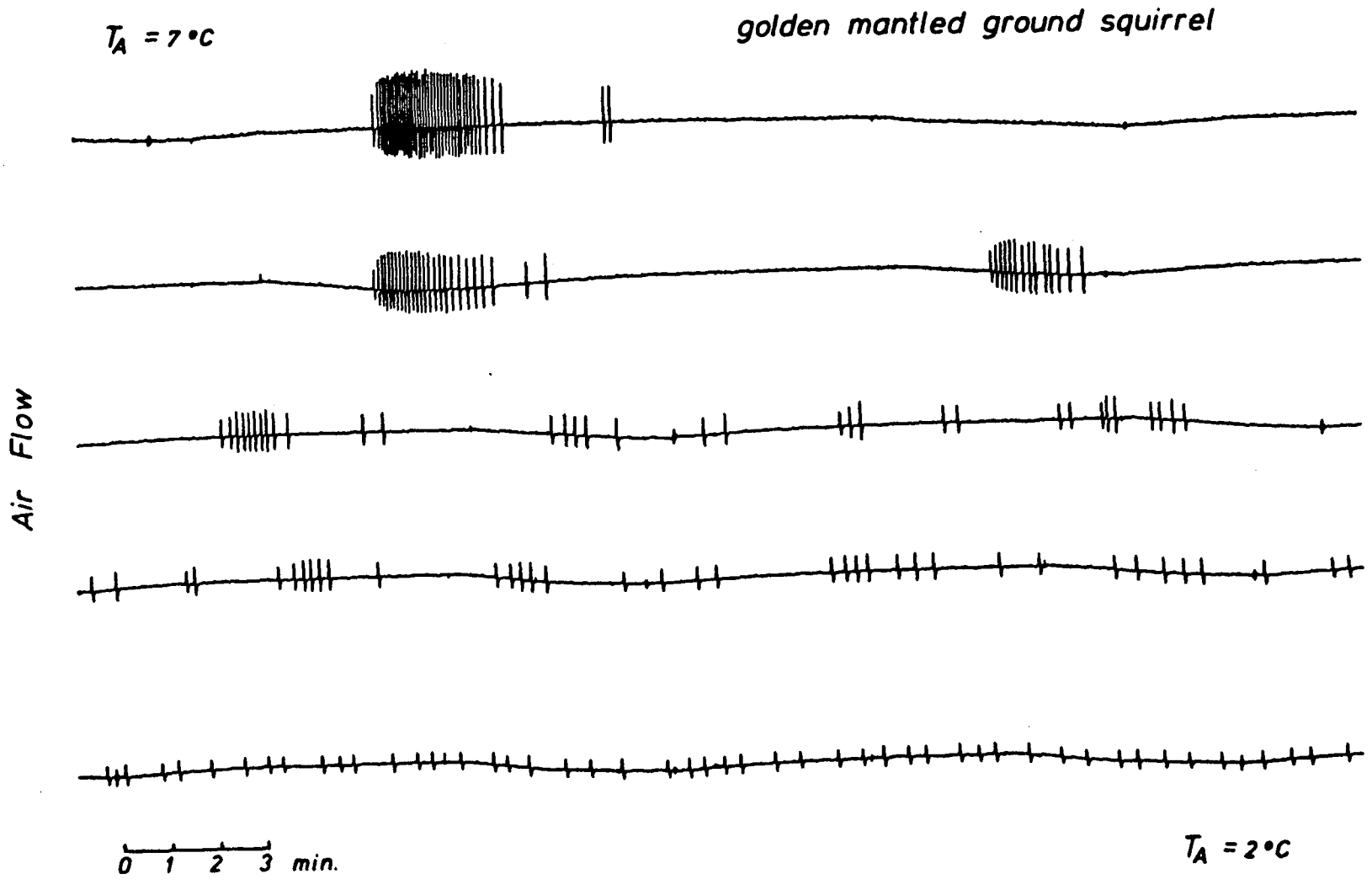

T_A = 7 °C
golden mantled ground squirrel
Air Flow
0 1 2 3 min.
T_A = 2 °C

Figure 17. Bar plot showing the effects of decreasing ambient and body temperature on respiratory variables; breaths per burst, bursts per minutes, overall frequency, tidal volume, minute ventilation, total breath duration (T_{TOT}) inspiratory time (T_I), expiratory time (T_E) and the ratio of T_I/T_E. 0% transition represents burst breathing at a T_b of 8.6 ± 0.2°C and 100% transition represents single breath breathing at a T_b of 4.4 ± 0.5°C. For 0% and 33% transition n=12 and for 66% and 100% transition n=11. The vertical line on each bar represents one standard error of the mean.

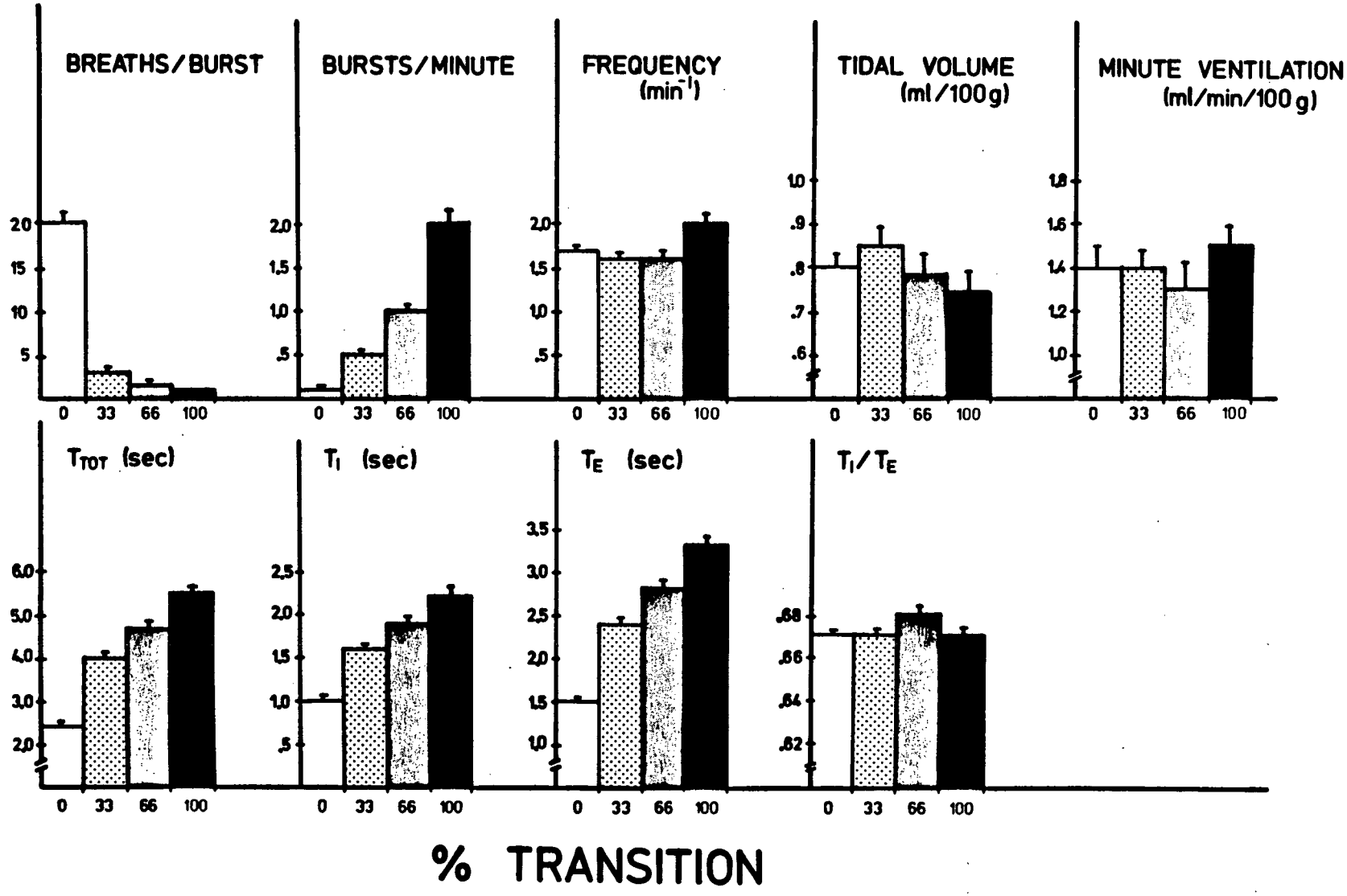
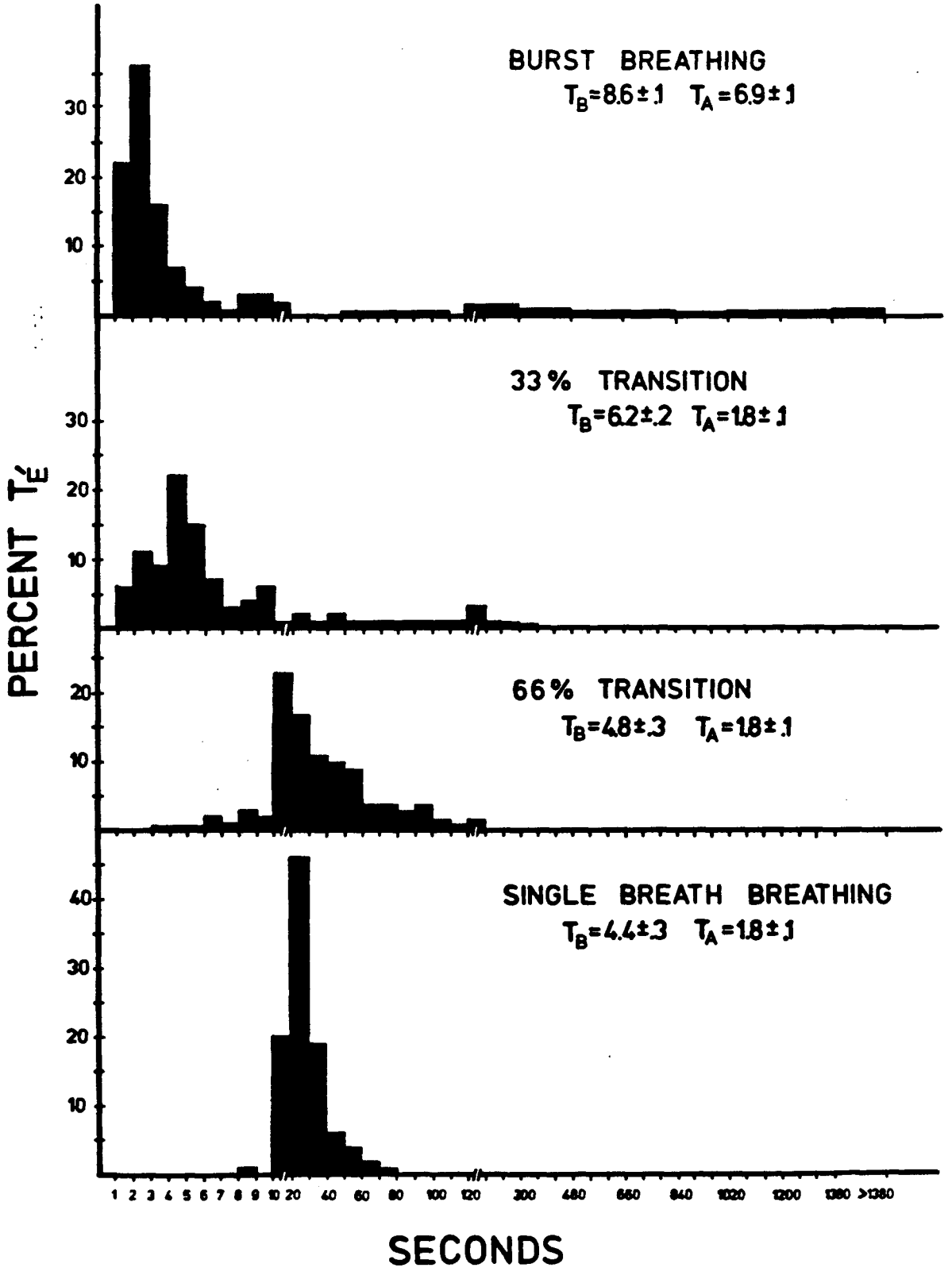

BREATHS/BURST
BURSTS/MINUTE
FREQUENCY (min⁻¹)
TIDAL VOLUME (ml/100g)
MINUTE VENTILATION (ml/min/100g)
T_{TOT} (sec)
T_I (sec)
T_E (sec)
T_I/T_E
0 33 66 100
% TRANSITION

Figure 18. Bar plot of the effects of decreasing ambient and body temperature on the end inspiratory pause (T_E') in S. lateralis during the transition from burst breathing to single breath breathing. The graph represents total values for 11 to 12 squirrels.

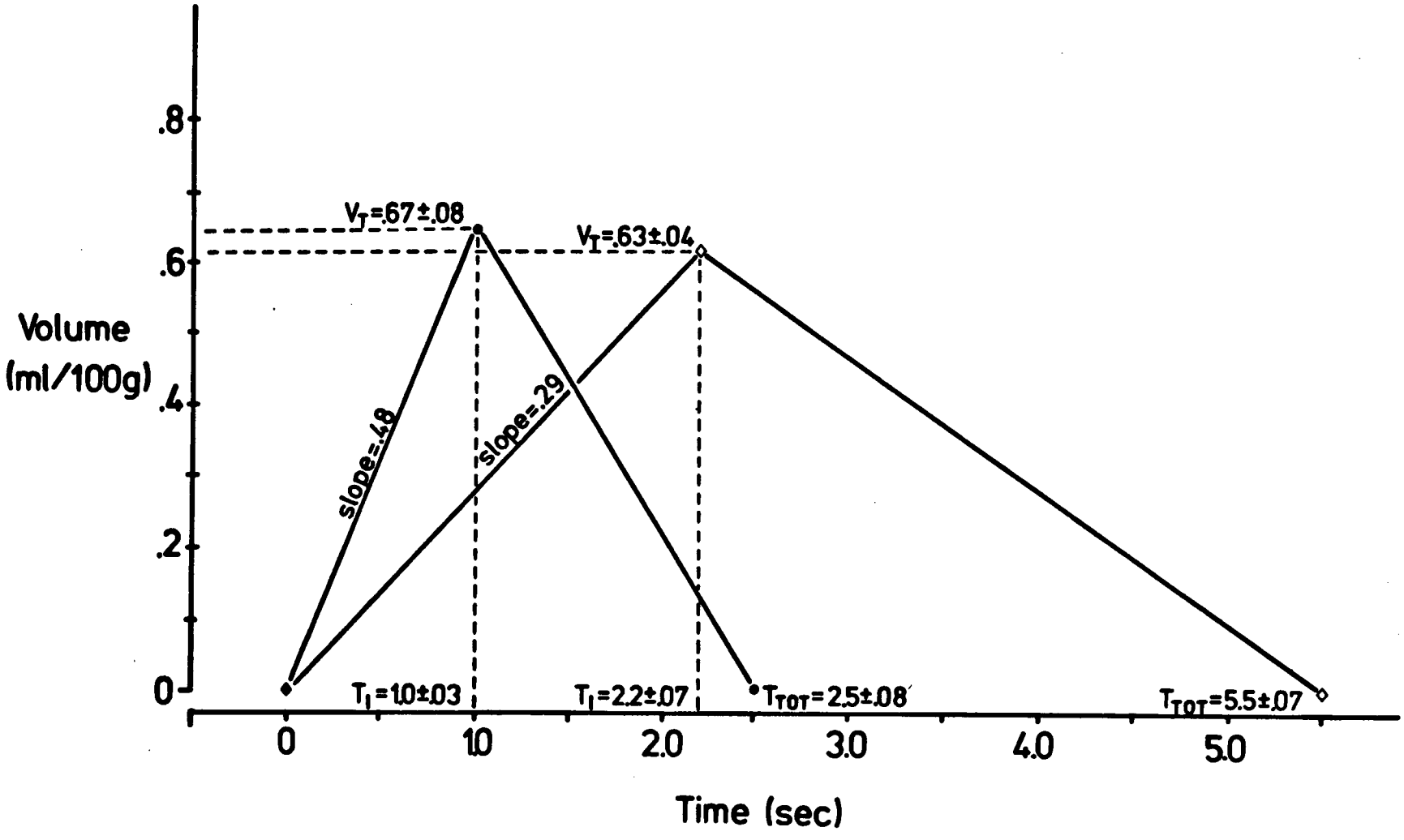

BURST BREATHING
T_B=8.6±.1 T_A=6.9±.1
33% TRANSITION
T_B=6.2±.2 T_A=1.8±.1
66% TRANSITION
T_B=4.8±.3 T_A=1.8±.1
SINGLE BREATH BREATHING
T_B=4.4±.3 T_A=1.8±.1
PERCENT T_E'
SECONDS
1 2 3 4 5 6 7 8 9 10 20 40 60 80 100 120 300 480 660 840 1020 1200 1380 >1380

from 8.6°C to 4.4°C. Although T_b is maintained slightly above T_c it does decrease passively as T_c is lowered.

Figure 16 and Figure 17 illustrate the changes in respiratory pattern which occur during the transition from burst breathing to single breath breathing. As T_b falls, the number of breaths/burst decreases until steady state single breath breathing is reached (Figure 16). During this time T_{NVP} decreases so that the bursts become closer together. These changes in T_{NVP} (or T_E') during the transition are further illustrated in Figure 18. During burst breathing intraburst T_E' is very short and interburst T_E' (T_{NVP}) is very long. As the transition proceeds the intraburst T_E' lengthens as the breaths within a burst gradually get farther apart. Interburst T_E' shortens as the bursts get closer together. This trend continues until steady state single breath breathing is achieved and "intraburst" and "interburst" T_E' are indistinguishable (Figure 18).

Despite large changes in respiratory pattern, f remains relatively constant throughout the transition (Figure 17). Conversely, V_T decreases slightly during the breathing pattern transition. Overall $\dot{V}$ does not change throughout the transition.

Figure 19. Relationship between tidal volume (V_T) and inspiratory time and total breath duration (T_I and T_{TOT} respectively) in the burst breathing (●) and single breath breathing (◇) S. lateralis during hibernation. Note the slope represents the relationship of V_T/T_I. All respiratory values represents mean standard error for 11 to 12 animals.


.8
.6
.4
.2
0
Volume
(ml/100g)
V_T=.67±.08
V_T=.63±.04
slope=.48
slope=.29
T_I=1.0±.03
T_I=2.2±.07
T_{TOT}=2.5±.08
T_{TOT}=5.5±.07
0
1.0
2.0
3.0
4.0
5.0
Time (sec)

T_{TOT} increases steadily through the transition from 2.4 seconds during burst breathing to 5.5 seconds during single breath breathing (Figure 19). Proportional increases in T_I and T_E occur such that T_I/T_E and T_I/T_{TOT} (the duty cycle) do not change through the transition. The increase in T_{TOT} with no increase in ventilatory f results in an increase in the total time spent actively breathing in single breath breathing squirrels. Single breath breathing animals spend approximately 18% to 24% of time actively breathing whereas burst breathing squirrels only spend 7% of time actively breathing (Table 5).

Given that V_T does not change but T_I increases the ratio of V_T/T_I decrease by a factor of 2 in single breath breathing animals relative to burst breathing animals (Table 5). Since V_T/T_I is a measure of ventilatory drive to breath, the decrease in this ratio indicates that the ventilatory drive has decreased in single breath breathing squirrels.

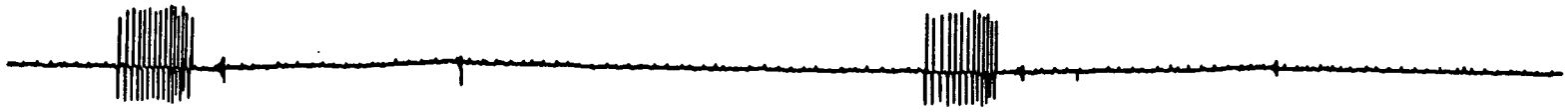
Response to hypoxia

Hypoxia has little effect on respiration in burst breathing squirrels until severe levels are reached (3% F_{IO2}). Figure 20 illustrates typical pattern changes which occur in response to severe hypoxia. Both control and CBX

Figure 20. Representative breathing traces recorded by pneumotachography in S. lateralis during hibernation at a T_c of about 6°C exposed to air (A), 3% inspired O_2 (B) and 8% inspired CO_2 (C).

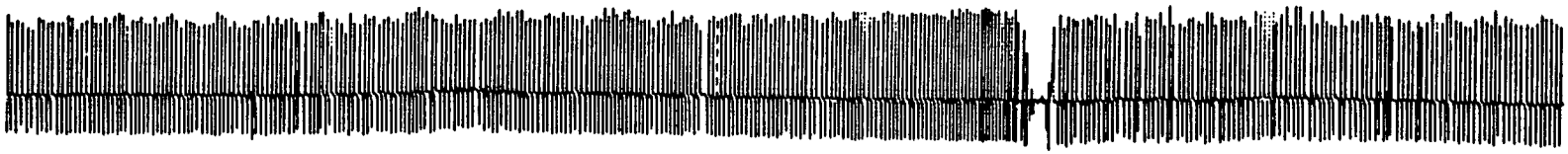
GOLDEN-MANTLED GROUND SQUIRREL
BURST BREATHING

A) air



B) 3% O_2

C) 8% CO_2

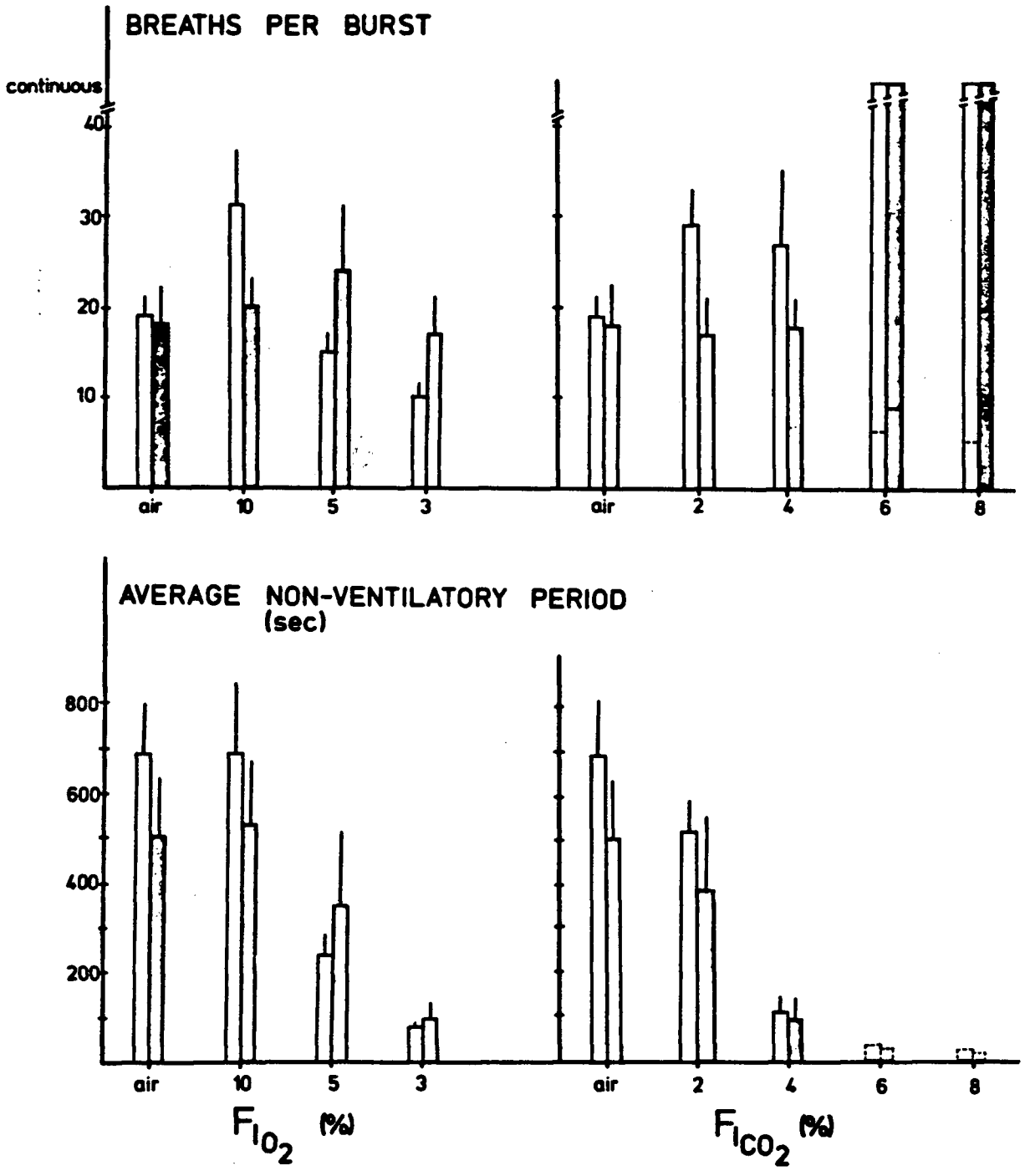


1 min

Figure 21. Bar graph showing the effects of changing F_{IO2} and F_{ICO2} on breaths per burst and average non-ventilatory period in intact and CBX S. lateralis during hibernation at a T_C of about 6°C. All values are mean ± standard error for 5 to 7 animals. Dashed lines represent intact (n=2) and CBX (n=1) animals who continued to show a bursting pattern at 6% CO_2 and 8% CO_2. See text for explanation.

□ = intact S. lateralis

▒ = CBX S. lateralis


BREATHS PER BURST
continuous
40
30
20
10
air
10
5
3
air
2
4
6
8
AVERAGE NON-VENTILATORY PERIOD (sec)
800
600
400
200
air
10
5
3
air
2
4
6
8
F_{IO_2} (%)
F_{ICO_2} (%)

squirrels retain a bursting pattern even at 3% O_2 (Figure 21). In intact squirrels as F_{IO2} decreases the number of breaths/burst (b/B) decreases as does T_{NVP} (Figure 21). These changes result in an overall increase in f (Figure 22, Table 6). CBX squirrels show a similar decrease in T_{NVP} in response to hypoxia, but show little change in the number of breaths/burst (Figure 13).

The overall $\dot{V}$ response to hypoxia in control and CBX burst breathing squirrels is plotted in Figure 22. The magnitude of the ventilatory response is similar in both control and CBX animals. In control squirrels hypoxic ventilatory threshhold occurs between 21% and 10% O_2, below which the response curve rises steeply. CBX squirrels exhibit a significant left shift in the hypoxic response threshold with little or no increase in $\dot{V}$ until F_{IO2} falls below 5% O_2. In both groups ventilatory responses to hypoxia are mediated exclusively by increases in f. The tidal volume of CBX squirrels remains significantly lower than controls over the range of hypoxic gases, resulting in a downshifted response curve.

During single breath breathing neither control nor CBX ground squirrels exhibit a significant ventilatory response to hypoxia, even at severe levels (3% O_2). Figure 23 illustrates the ventilatory responses to hypoxia during single breath breathing. Although at moderate levels of

TABLE 6. Effects of alterations of inspired gas composition on the frequency (f, breaths/minute), tidal volume (V_T, ml/100g), and minute ventilation ($\dot{V}$, ml/100g/min) in hibernating golden-mantled ground squirrels (S. lateralis). All values are mean ± standard error. Numbers in parentheses represent sample size.

		BURST BREATHING		SINGLE BREATH BREATHING	
		CONTROL	CBX	CONTROL	CBX
AIR	f	1.7 ± 0.2	2.2 ± 0.2	2.6 ± 0.2	2.1 ± 0.2
	V_T	0.67 ± 0.08	0.46 ± 0.02	0.63 ± 0.04	0.71 ± 0.05
	$\dot{V}$	1.1 ± 0.1	1.0 ± 0.1	1.6 ± 0.1	1.4 ± 0.2
		(7)	(7)	(10)	(10)
10% O_2	f	2.5 ± 0.4	2.3 ± 0.2	2.7 ± 0.4	1.8 ± 0.3
0% CO_2	V_T	0.66 ± 0.07	0.50 ± 0.02	0.73 ± 0.04	0.77 ± 0.06
	$\dot{V}$	1.6 ± 0.3	1.1 ± 0.1	1.9 ± 0.3	1.2 ± 0.2
		(5)	(6)	(8)	(9)
5% O_2	f	3.2 ± 0.4	2.5 ± 0.2	3.4 ± 0.5	2.0 ± 0.3
0% CO_2	V_T	0.66 ± 0.06	0.41 ± 0.02	0.60 ± 0.04	0.63 ± 0.06
	$\dot{V}$	2.1 ± 0.2	1.0 ± 0.1	1.8 ± 0.1	1.0 ± 0.1
		(6)	(6)	(8)	(9)
3% O_2	f	4.5 ± 0.4	5.4 ± 0.7	2.2 ± 0.4	2.2 ± 0.3
0% CO_2	V_T	0.64 ± 0.08	0.46 ± 0.05	0.64 ± 0.04	0.56 ± 0.04
	$\dot{V}$	2.4 ± 0.4	2.4 ± 0.3	1.5 ± 0.3	1.1 ± 0.2
		(6)	(5)	(7)	(7)
21% O_2	f	2.9 ± 0.4	3.2 ± 0.5	3.4 ± 0.4	2.6 ± 0.3
2% CO_2	V_T	0.71 ± 0.10	0.52 ± 0.06	0.66 ± 0.04	0.74 ± 0.05
	$\dot{V}$	1.9 ± 0.2	1.7 ± 0.3	2.2 ± 0.3	1.8 ± 0.2
		(6)	(6)	(8)	(10)

TABLE 6 cont...

21% O_2	f	6.4 ± 0.6	7.1 ± 1.3	4.8 ± 0.4	3.8 ± 0.2
4% CO_2	V_T	0.75 ± 0.09	0.52 ± 0.04	0.77 ± 0.06	0.89 ± 0.05
	$\dot{V}$	4.6 ± 0.8	3.6 ± 0.5	3.6 ± 0.3	3.1 ± 0.3
		(6)	(6)	(8)	(10)
21% O_2	f	10.4 ± 1.5	9.9 ± 1.5	5.0 ± 0.5	4.3 ± 0.4
6% CO_2	V_T	0.77 ± 0.08	0.60 ± 0.02	0.96 ± 0.03	0.88 ± 0.07
	$\dot{V}$	7.6 ± 1.4	5.9 ± 1.0	4.8 ± 0.6	4.4 ± 0.6
		(6)	(6)	(8)	(10)
21% O_2	f	10.4 ± 1.4	9.8 ± 1.4	5.2 ± 0.4	5.6 ± 0.4
8% CO_2	V_T	0.90 ± 0.08	0.81 ± 0.06	1.00 ± 0.11	1.10 ± 0.13
	$\dot{V}$	9.3 ± 1.4	8.0 ± 1.3	4.9 ± 0.4	6.2 ± 0.9
		(6)	(7)	(5)	(5)
50% O_2	f	1.9 ± 0.2	2.3 ± 0.2	2.9 ± 0.2	2.5 ± 0.3
0% CO_2	V_T	0.66 ± 0.04	0.52 ± 0.06	0.63 ± 0.05	0.71 ± 0.05
	$\dot{V}$	1.3 ± 0.1	1.2 ± 0.1	1.7 ± 0.2	1.7 ± 0.3
		(6)	(6)	(8)	(10)
50% O_2	f	2.8 ± 0.3	3.7 ± 0.8	3.2 ± 0.2	2.6 ± 0.3
2% CO_2	V_T	0.64 ± 0.04	0.52 ± 0.01	0.73 ± 0.05	0.75 ± 0.05
	$\dot{V}$	1.9 ± 0.2	1.9 ± 0.4	2.3 ± 0.2	1.8 ± 0.2
		(6)	(6)	(8)	(10)
50% O_2	f	5.5 ± 0.5	6.9 ± 1.0	4.7 ± 0.3	3.6 ± 0.2
4% CO_2	V_T	0.73 ± 0.05	0.54 ± 0.04	0.83 ± 0.07	0.87 ± 0.06
	$\dot{V}$	3.9 ± 0.3	3.7 ± 0.5	4.0 ± 0.4	3.1 ± 0.3
		(5)	(6)	(7)	(10)
50% O_2	f	11.3 ± 1.1	10.5 ± 1.7	5.0 ± 0.3	4.9 ± 0.4
6% CO_2	V_T	0.88 ± 0.08	0.64 ± 0.05	0.91 ± 0.08	1.0 ± 0.08
	$\dot{V}$	9.7 ± 1.2	6.6 ± 1.3	4.4 ± 0.5	5.3 ± 0.6
		(6)	(6)	(8)	(8)

TABLE 6 cont...

50% O_2	f	10.4 ± 1.9	10.8 ± 1.8	6.1 ± 0.8	5.0 ± 0.2
8% CO_2	V_T	0.87 ± 0.1	0.77 ± 0.07	1.1 ± 0.13	1.3 ± 0.19
	$\dot{V}$	8.9 ± 1.0	8.2 ± 1.5	6.0 ± 0.6	6.1 ± 0.8
		(6)	(6)	(4)	(5)
5% O_2	f	5.0 ± 0.6	5.5 ± 1.0	4.6 ± 0.3	3.5 ± 0.2
4% CO_2	V_T	0.76 ± 0.08	0.57 ± 0.04	0.80 ± 0.03	0.84 ± 0.06
	$\dot{V}$	3.8 ± 0.5	3.0 ± 0.4	3.6 ± 0.3	2.9 ± 0.3
		(6)	(6)	(8)	(10)
5% O_2	f	11.3 ± 1.1	10.4 ± 1.7	4.4 ± 0.2	4.9 ± 0.3
6% CO_2	V_T	0.76 ± 0.04	0.59 ± 0.03	0.94 ± 0.05	0.97 ± 0.12
	$\dot{V}$	8.2 ± 0.7	6.2 ± 1.3	4.2 ± 0.3	5.1 ± 0.8
		(6)	(5)	(5)	(5)
5% O_2	f	9.3 ± 1.2	10.8 ± 1.7	5.0 ± 0.2	5.3 ± 0.3
8% CO_2	V_T	1.0 ± 0.1	0.70 ± 0.05	0.96 ± 0.07	1.0 ± 0.14
	$\dot{V}$	8.4 ± 1.1	7.3 ± 0.9	4.7 ± 0.2	5.6 ± 0.8
		(6)	(6)	(5)	(5)

Figure 22. Effect of decreasing F_{IO2} on minute ventilation, tidal volume and frequency in burst breathing intact S. lateralis (●) and burst breathing CBX S. lateralis (△) during hibernation at T_c of about 6°C. All values are mean ± standard error for 5 to 7 animals.

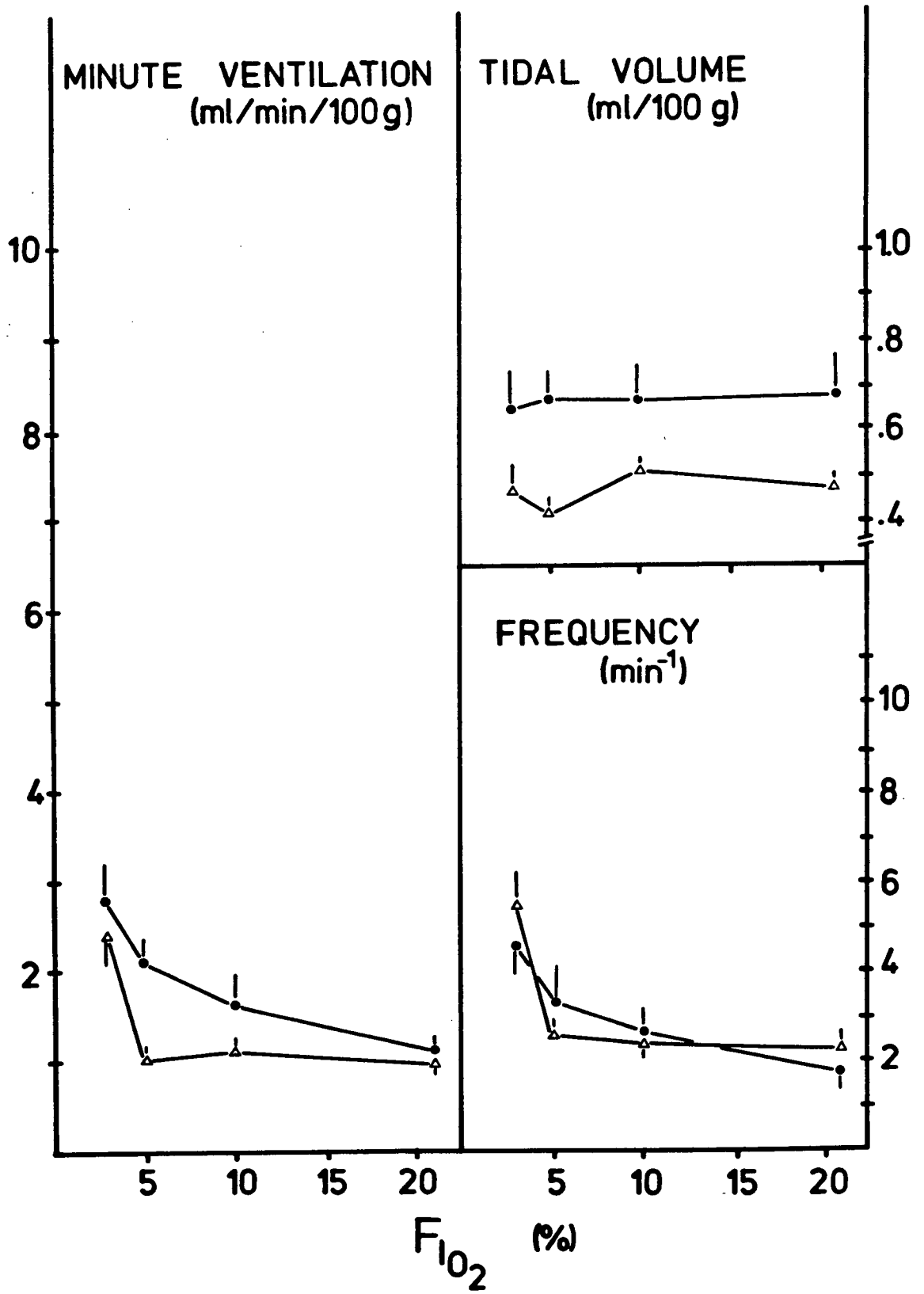
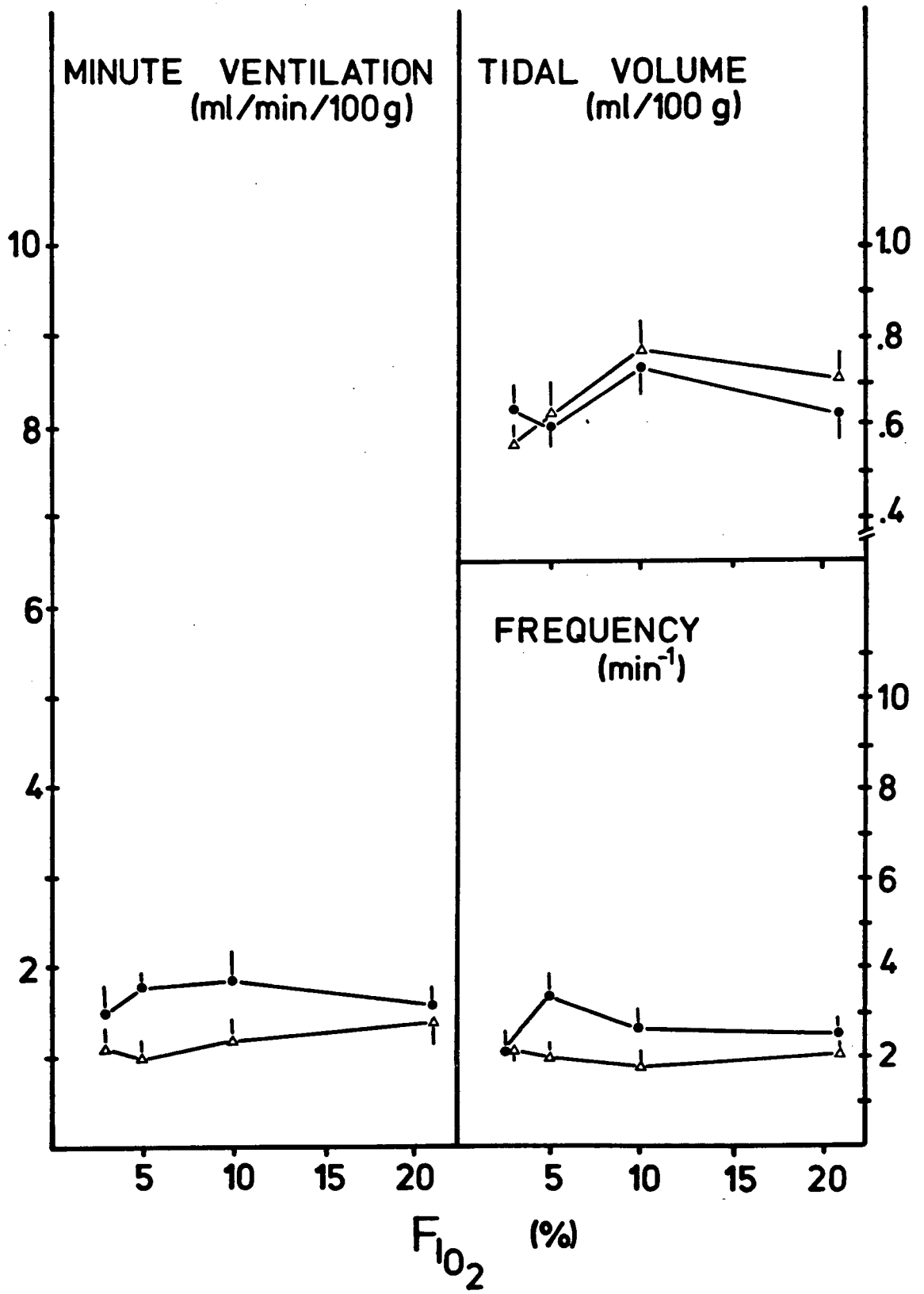

MINUTE VENTILATION
(ml/min/100g)
TIDAL VOLUME
(ml/100 g)
FREQUENCY
(min⁻¹)
10
8
6
4
2
1.0
.8
.6
.4
10
8
6
4
2
5
10
15
20
5
10
15
20
FIO2 (%)

Figure 23. Effect of decreasing F_{IO2} on minute ventilation, tidal volume, and frequency in single breath breathing intact S. lateralis (●) and single breath breathing CBX S. lateralis (Δ) during hibernation a T_C of about 2°C. All values are mean ± standard error for 7 to 10 animals.


MINUTE VENTILATION
(ml/min/100g)
TIDAL VOLUME
(ml/100 g)
FREQUENCY
(min-1)
10
8
6
4
2
1.0
.8
.6
.4
10
8
6
4
2
5
10
15
20
5
10
15
20
FIO2 (%)

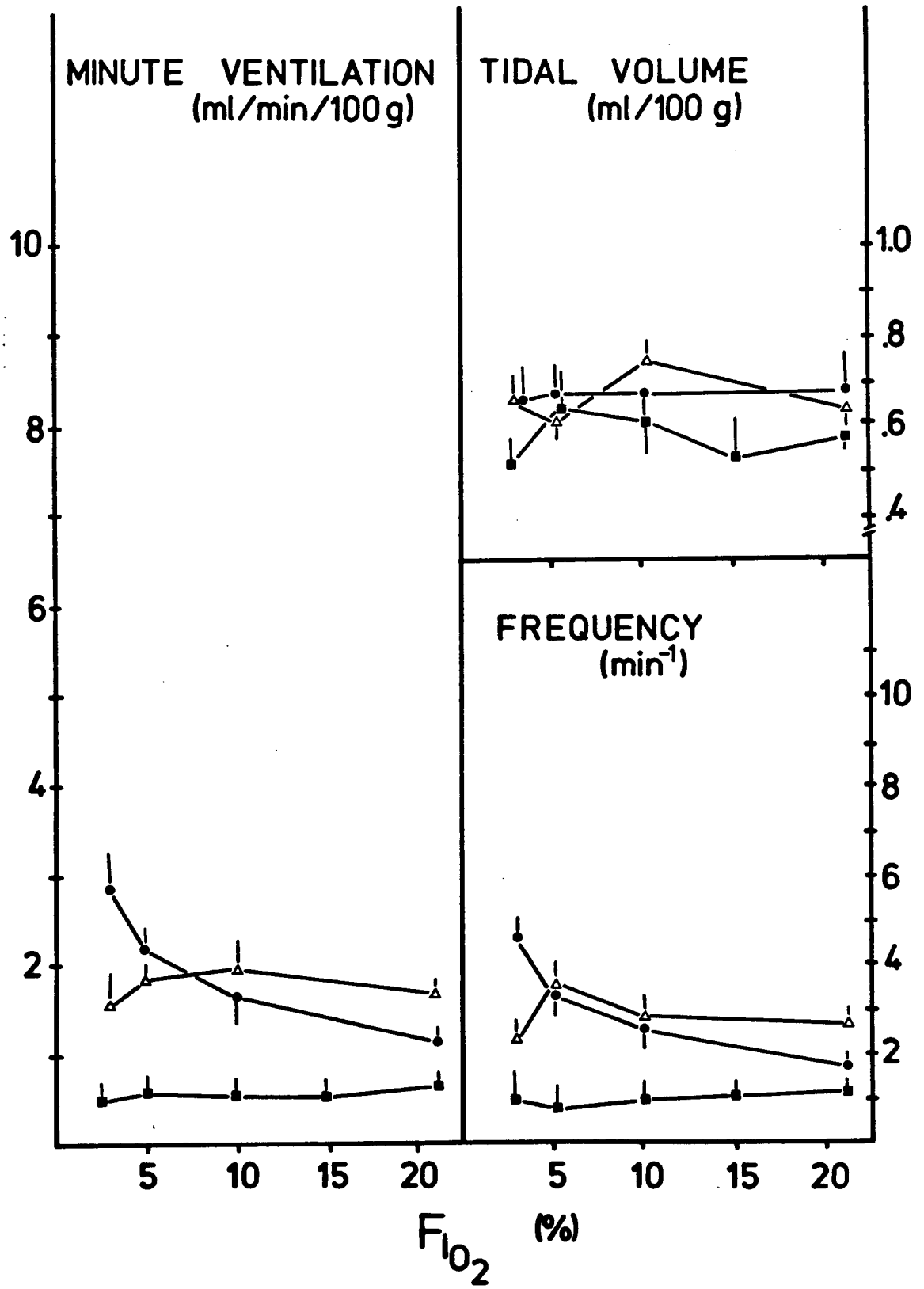
hypoxia (10% and 5% O_2) control squirrels exhibit a slight increase in $\dot{V}$, at severe levels (3% O_2) $\dot{V}$ is not significantly different from normoxic levels. CBX squirrels do not respond to hypoxia at moderate levels, but show a 21% decrease in $\dot{V}$ at 3% O_2 mediated by a decrease in V_T (Figure 23).

Figure 24 compares the ventilatory responses of burst breathing and single breath breathing control S. lateralis to hypoxia. Ventilatory responses of Spermophilus columbianus, a species of ground squirrel which only exhibits a single breath pattern, have been included for comparison (McArthur, 1986). Burst breathing S. lateralis exhibit a moderate ventilatory response to severe hypoxia, while single breath breathing S. lateralis show no significant ventilatory response at any level of hypoxia tested. Similar to the hypoxic ventilatory response of single breath S. lateralis, S. columbianus does not show a ventilatory response to hypoxia, even at severe levels.

Response to Hypercapnia

Burst breathing control and CBX S. lateralis show a strong ventilatory response to hypercapnia. In most squirrels respiration becomes continuous at high levels of F_{ICO2} (Figure 20). Figure 21 illustrates the changes in

Figure 24. Effect of decreasing F_{IO2} on minute ventilation, tidal volume, and frequency in burst breathing S. lateralis (●) hibernating at a T_c of about 7°C, single breath breathing S. lateralis (Δ) hibernating at a T_c of about 2°C, and single breath breathing Spermophilus columbianus (■) hibernating at a T_c of about 6°C (McArthur, 1986). All values are mean ± standard error for 5 to 10 animals.

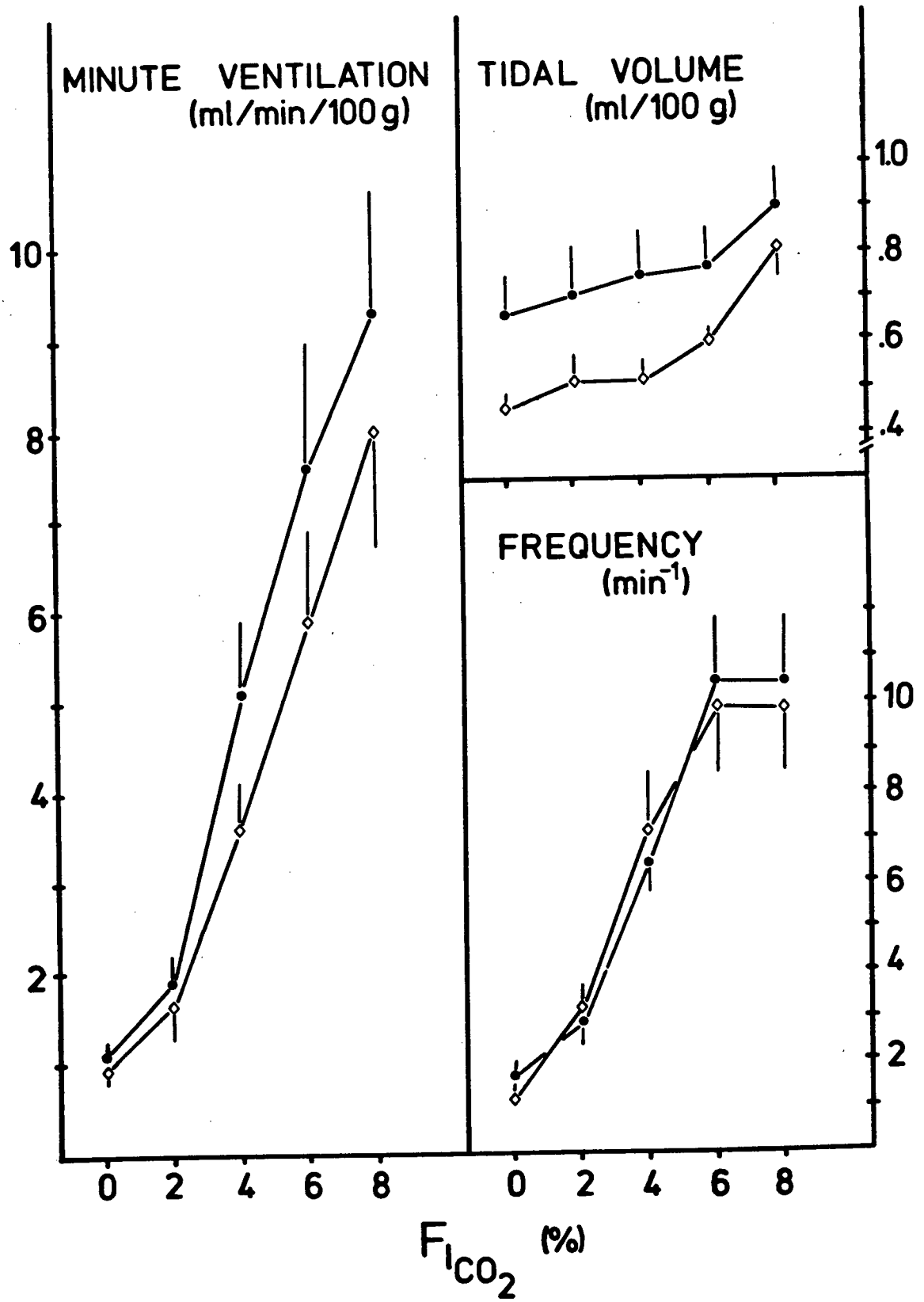

MINUTE VENTILATION
(ml/min/100 g)
TIDAL VOLUME
(ml/100 g)
FREQUENCY
(min⁻¹)
10
8
6
4
2
1.0
.8
.6
.4
10
8
6
4
2
5
10
15
20
5
10
15
20
FIO2 (%)

respiratory pattern with increasing F_{ICO2}. Below 4% CO_2 the number of breaths/burst remains relatively constant while T_{NVP} becomes shorter (Figure 21). In both groups, above 4% CO_2 breathing becomes continuous. Several animals (n=2 for controls and n=1 for CBX) maintain a burst breathing pattern up to 8% CO_2. In these animals, at 6% and 8% CO_2, the number of breaths/burst is small ($\bar{x}$ = 6 b/B) and T_{NVP} is short ($\bar{x}$ = 29 sec) (Figure 21).

Minute ventilation at 8% C_{O2} increased, on average, 745% and 700% over resting levels in burst breathing control and CBX squirrels respectively (Table 5). Both groups show little change in ventilation at 2% CO_2 and a more or less linear increase in $\dot{V}$ above 2% CO_2 (Figure 25). Increases in both V_T and f contribute to the overall respiratory response. Below 6% CO_2 f increases substantially, whereas V_T increases only slightly. Increases in f are achieved by decreases in T_{NVP}. Above 6% CO_2 f no longer increases, but large increases in V_T contribute to the rise in $\dot{V}$. This trend is similar in both control and CBX squirrels.

CBX squirrels maintain a lower V_T than the control squirrels throughout the hypercapnic exposure (Figure 25). Thus at most levels of hypercapnia the CBX squirrels have a slightly downshifted response curve.

Figure 25. Effect of increasing F_{ICO2} on minute ventilation, tidal volume, and frequency in burst breathing intact S. lateralis (●) and burst breathing CBX S. lateralis (◇) hibernating at a T_c of about 7°C. All values are mean ± standard error for 5 to 7 animals.


MINUTE VENTILATION
(ml/min/100 g)
TIDAL VOLUME
(ml/100 g)
FREQUENCY
(min⁻¹)
10
8
6
4
2
1.0
.8
.6
.4
10
8
6
4
2
0
2
4
6
8
0
2
4
6
8
FICO2 (%)

In single breath breathing S. lateralis both control and CBX squirrels exhibit similar responses to hypercapnia (Figure 26). In both groups $\dot{V}$ increases only slightly between 0% and 2% CO_2 and above 2% CO_2 $\dot{V}$ increases in a linear fashion. Increases in both f and V_T contribute to the changes in $\dot{V}$. Tidal volume increases steadily over the range of hypercapnic gases (Figure 26) while f does not increase above 4% CO_2.

Although single breath breathing S. lateralis shows a strong hypercapnic response, it is blunted compared to burst breathing S. lateralis (Figure 27). At 8% CO_2 both burst breathing and single breath breathing squirrels exhibit continuous respiration. Burst breathing squirrels increase $\dot{V}$ more than 700% above normocapnic conditions at 8% CO_2, whereas single breath breathing squirrels increase $\dot{V}$ only 200% to 300% over normocapnic conditions at 8% CO_2. Both burst breathing and single breath breathing S. lateralis show similar strong increase in V_T, but the single breath breathing squirrels show a blunted frequency response compared to burst breathing squirrels at 8% CO_2 (Figure 27).

S. columbianus has a slightly higher ventilatory sensitivity to hypercapnia than single breath breathing S. lateralis, but a blunted $\dot{V}$. response relative to burst breathing S. lateralis (Figure 26, McArthur, 1986). This relativey blunted hypercapnic sensitivity is due to a low frequency response in S. columbianus.

Figure 26. Effect of increasing F_{ICO2} on minute ventilation, tidal volume, and frequency in single breath breathing intact S. lateralis (●) and single breath breathing CBX S. lateralis (◇) hibernating at a T_c of about 2°C. All values are mean ± standard error for 5 to 10 squirrels.

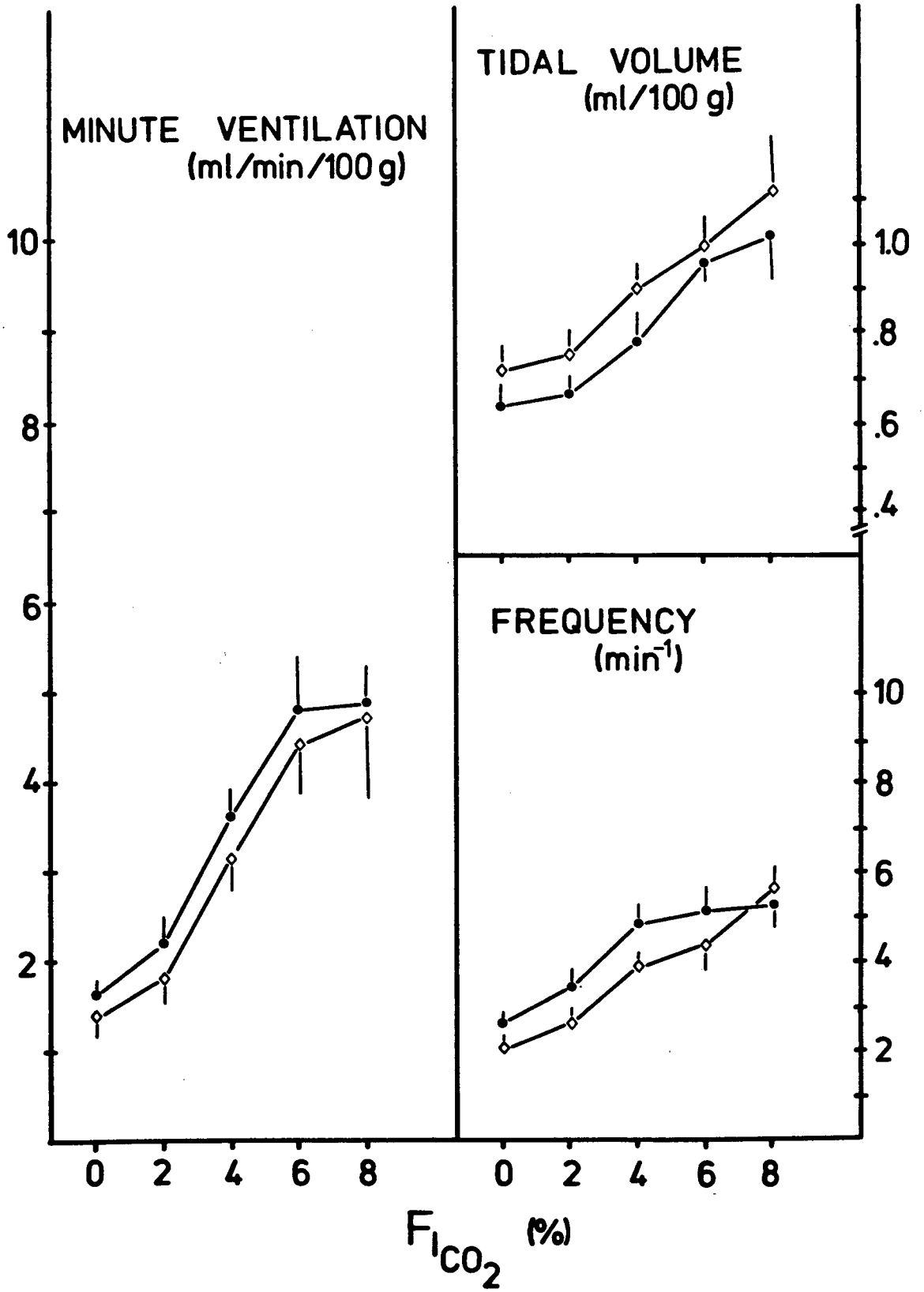
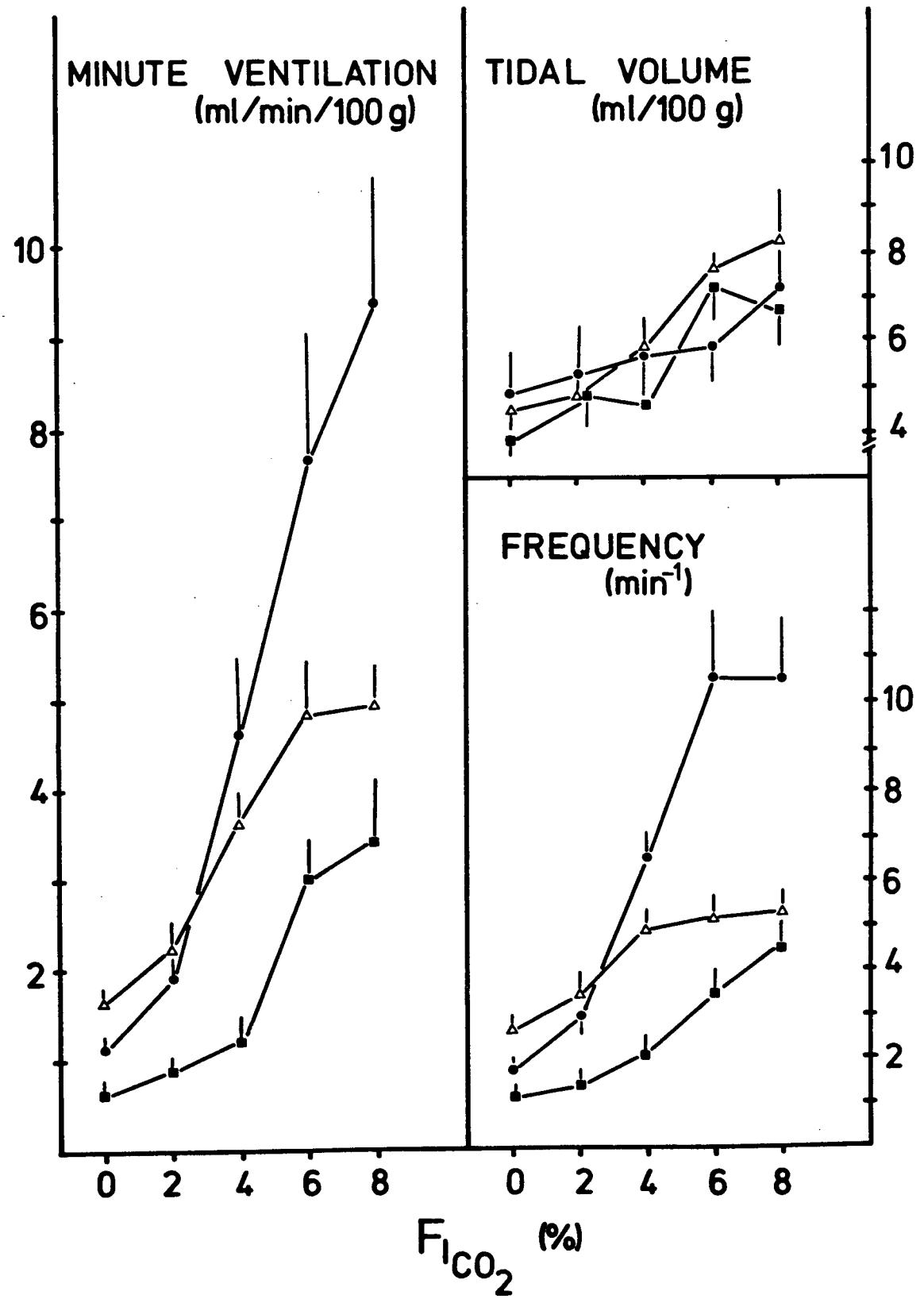

MINUTE VENTILATION
(ml/min/100 g)
TIDAL VOLUME
(ml/100 g)
FREQUENCY
(min⁻¹)
10
8
6
4
2
1.0
.8
.6
.4
10
8
6
4
2
0
2
4
6
8
0
2
4
6
8
FICO2 (%)

Figure 27. Effect of increasing F_{ICO2} on minute ventilation, tidal volume, and frequency in burst breathing S. lateralis (●) hibernating at a T_c of about 6°C, single breath breathing S. lateralis (△) hibernating at a T_c of about 2°C and single breath breathing S. columbianus (■) hibernating a T_c of about 6°C (McArthur, 1986). All values are means ± standard error for 5 to 10 animals.


MINUTE VENTILATION
(ml/min/100 g)
TIDAL VOLUME
(ml/100 g)
FREQUENCY
(min⁻¹)
10
8
6
4
2
0
2
4
6
8
FICO2 (%)

Responses to hyperoxia, hyperoxic hypercapnia and hypoxic hypercapnia

Hyperoxia alone has little effect on respiration in burst breathing control squirrels (Table 6). In burst breathing CBX squirrels hyperoxia causes a slight increase in V corresponding to a slight increase in V_T.

In single breath breathing control and CBX squirrels hyperoxia does not cause any significant changes in ventilation (Table 6). The hyperoxic response in single breath CBX animals is mediated by a small increase in f.

Figure 28 illustrates $\dot{V}$ responses to increasing hypercapnia with a normoxic, hyperoxic or hypoxic background in burst breathing control squirrels. The various O_2 backgrounds have little effect on the hypoxic response curve in burst breathing control or CBX S. lateralis (Table 6). Ventilatory threshold and the overall hypercapnic $\dot{V}$. response are unaltered by the various oxygen backgrounds.

In single breath breathing animals hyperoxia and hypoxia also have no effect on the hypercapnic response threshold or on the overall $\dot{V}$ response to hypercapnia in either control (Figure 28) or CBX squirrels (Table 6). The contributions of f and V_T to the increases in ventilation

Figure 28. Effect of increasing F_{ICO2} in combination with 21% O_2 (O), 50% O_2 (▲) and 5% O_2 (■) on minute ventilation in burst breathing S. lateralis hibernating at a T_a of about 7°C (A) and single breath breathing S. lateralis hibernating at a T_a of about 2°C (B).

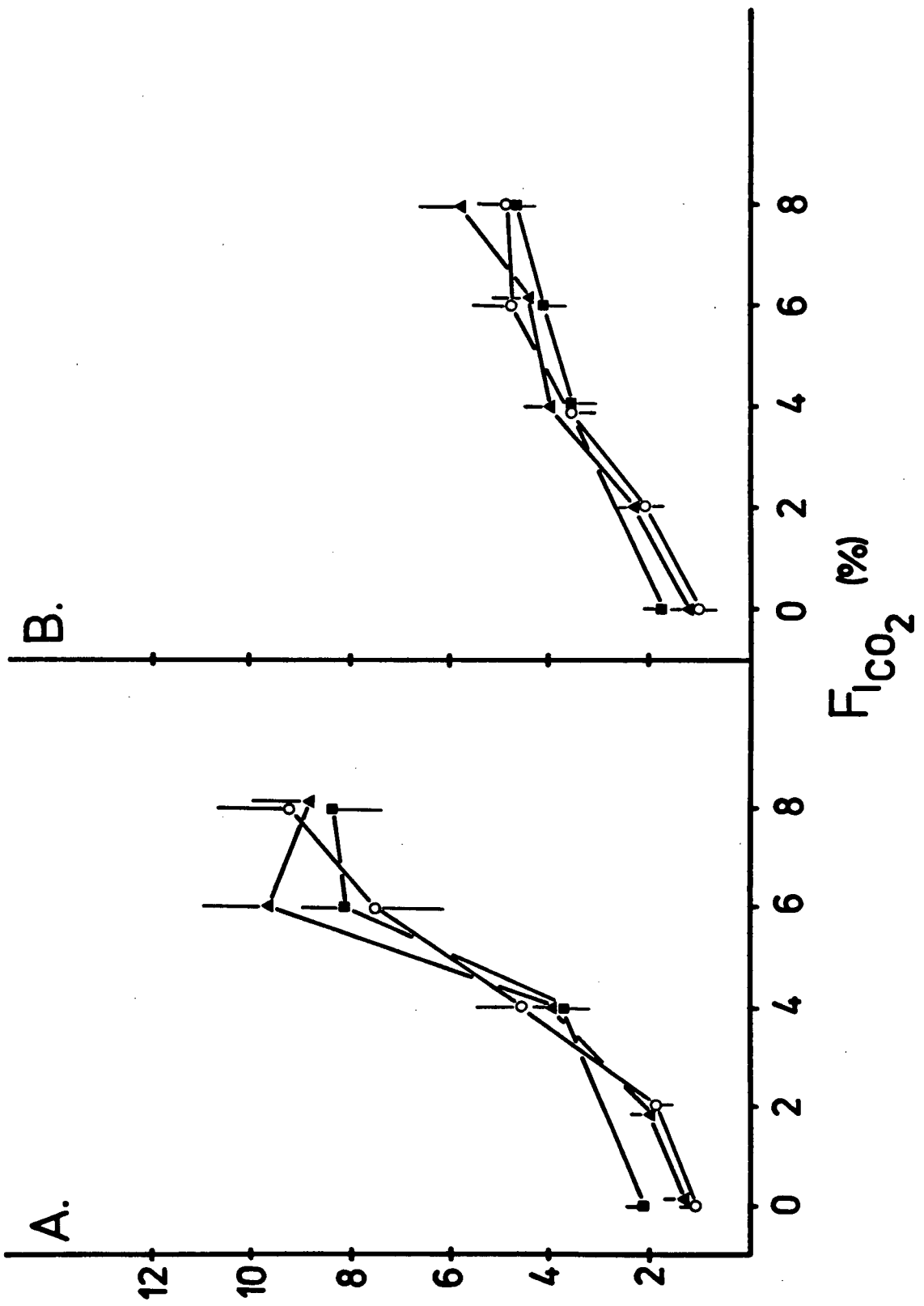
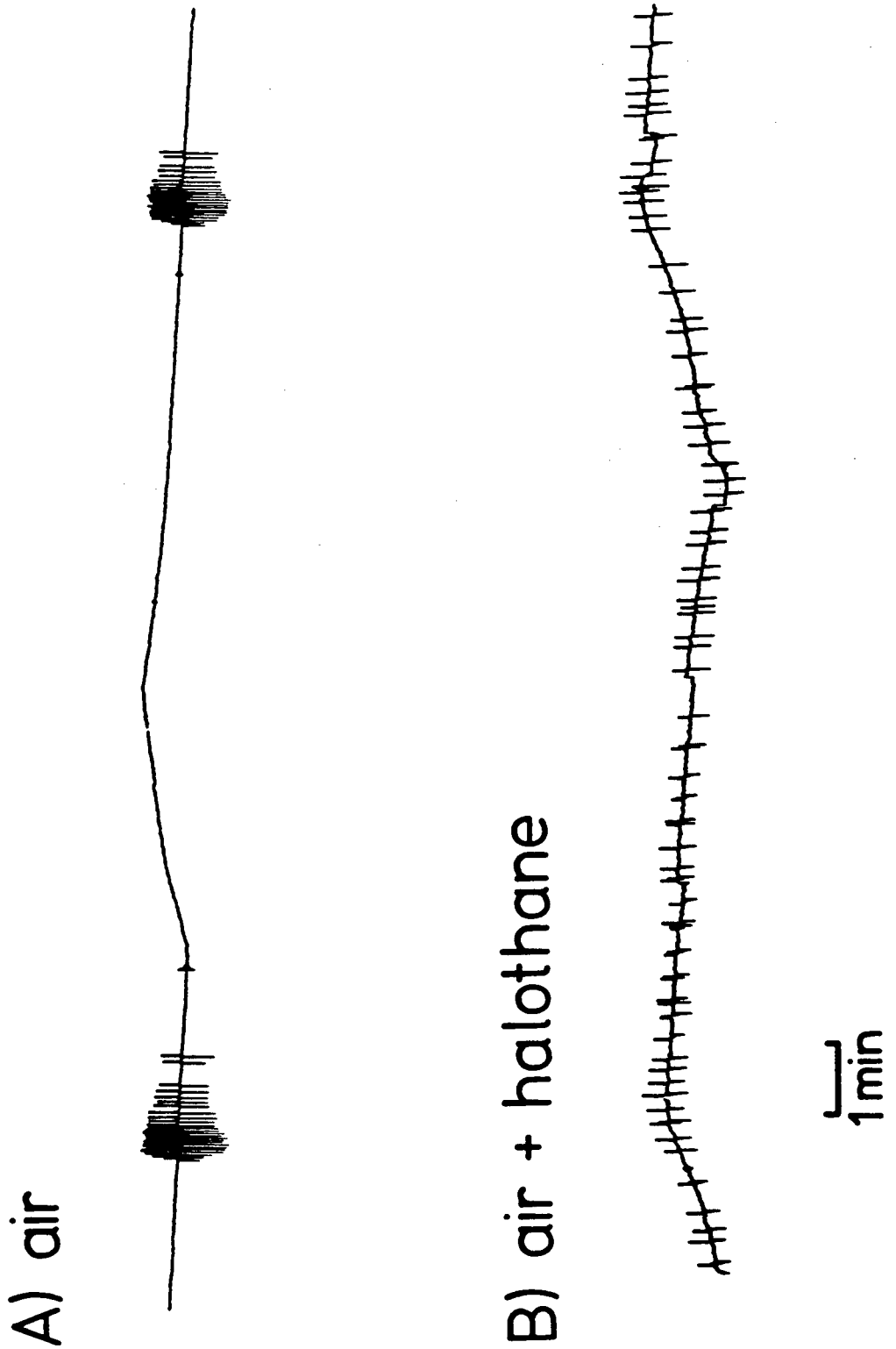

A.
B.
12
10
8
6
4
2
0
2
4
6
8
0
2
4
6
8
F_{ICO_2} (%)

Figure 29. Representative records of breathing patterns in hibernating S. lateralis at T_c of about 7°C during exposure to air (A) and exposure to air plus 3 vol % halothane (B).


A) air
B) air + halothane
1min

are not significantly different from those seen during normoxia.

Effect of Anesthetics

Four burst breathing golden mantled ground squirrels hibernating at 6.5°C were exposed to varying levels of vaporized halothan anesthetic. At levels of about 2.5 to 3 vol % halothane the normal burst breathing pattern is converted to a single breath pattern (Figure 29). Further increases in halothane concentrations above 3.5 vol % cause a loss of respiratory activity. Removal of the anesthetic resulted in a gradual return to a bursting pattern.

DISCUSSION

AWAKE ANIMALS

At room temperature awake golden-mantled ground squirrels breathe continuously. Acute exposure to hypoxia produces a strong ventilatory response, while acute exposure to hypercapnia produces a comparatively blunted ventilatory response. Carotid body denervation results in a reduction in resting ventilation and a slight reduction in ventilatory sensitivity to hypercapnia. Chronic exposure to hypoxic and hypercapnic conditions (CHH) results in a maintained increase in minute ventilation through changes in both tidal volume and frequency but respiratory sensitivity to hypoxia and hypercapnia is unaltered.

Methodology

The whole body plethysmograph has been used in many respiratory studies involving small mammals (Birchard et al., 1984; Blake and Banchero, 1985; Darden, 1972; Holloway and Heath, 1984; Lai et al., 1981; Walker et al., 1985). This method allows for measurement of both respiratory pattern and tidal volume in unanesthetized, unrestrained animals. Both anesthetics and devices used to measure respiration such as face masks and neck seals, can produce undesirable alterations in normal ventilation (Gautier, 1976; Gilbert et al., 1972; Fleming et al., 1983). In

addition, the experimental set-up can be placed in a closed area so that changes in gas mixtures and measurements of T_C and T_b can be performed without tactile or visual contact with the animal.

The accuracy of the calculated tidal volume using the plethysmograph method depends on the reliability of measurements of independent variables such as P_B, T_C and particularly T_b and T_N (Malan, 1973). Direct measurements of body and nasal temperature were not taken during the course of the experiments. T_b and T_N of active squirrels were assumed to be relatively constant at 37°C and 32°C respectively regardless of changes in ambient temperature, breathing frequency or gas exposure.

Exposure to severe levels of hypoxia and hypercapnia have been reported to cause decreases in body temperature (Faleschini and Whitten, 1975; Jennings, 1979, Lia et al., 1975). For example, Faleschini and Whitten (1975) reported a 3°C drop in body temperature in S. lateralis during a 30 minute hypoxic exposure. In addition it is possible that during severe hypoxia or hypercapnia when respiratory frequency is extremely high, inspired and expired air are not fully saturated or desaturated across the nasal passages. These alterations may have led to an underestimation of V_T.

Changes in respiratory timing variables can also increase the error factor in tidal volume measurements with this technique by up to 30% (Jacky, 1980). Although comprehensive measurements of T_I, T_E and T_{TOT} were not taken in this study, values were available from the study by McArthur (1986) in S. lateralis during exposure to similar hypoxic and hypercapnic gases.

Despite these potential sources of error in the plethysmograph method used in the present study, simultaneous measurements of V_T made using both the plethysmograph and pneumotachograph methods were similar (Figure 4).

Resting Ventilation

Intact control animals

Values obtained for resting minute ventilation in S. lateralis are about 50% smaller than $\dot{V}$ values predicted by Stahl (1967) using scaling equations for all mammals. Tidal volume measurements in the present study are about 0.8 ml/100g. This value falls within the range predicted by Stahl (1967) for rats (0.4 to 1.0 ml/100g). Frequency values, however, fall slightly below the range of 45 to 100 breaths/minute predicted by Stahl (1967) and well below the reported values of 97 to 115 breaths/minute for 250 gram rats (Stahl, 1967).

Low resting values for minute ventilation are commonly observed in fossorial and semi-fossorial species (Arieli and Ar, 1979; Darden, 1972, Holloway and Heath, 1984; Schlencher, 1985; Walker et al., 1985). In most studies the reduction in $\dot{V}$ is attributed primarily to decreases in resting ventilatory frequency. The effect of a fossorial environment on V_T is more variable. Walker et al. (1985) and Arieli and Ar (1979) found V_T values in semi-fossorial and fossorial animals which are higher than V_T values in comparable sized rats. In contrast, Schlenker (1985) reported V_T values in the Djungarian hamster which are smaller than V_T values of similar sized mice. These results suggest there may be no effect of burrow-dwelling on V_T. The combination of a relatively small breathing frequency and large V_T commonly observed in many fossorial mammals may result in a more effective alveolar ventilation than is observed in other mammals (Tenney and Bogg, 1986).

Previous measurements of V_T in S. lateralis performed in our laboratory using similar plethysmograph techinques yielded V_T values about 60% smaller than those obtained in the present study (McArthur, 1986). This discrepancy in V_T measurements may reflect differences in the specific plethysmograph used, differences in the measurement of pressure deflections or differences in the calibration of the pressure deflections caused by respiration. Resting frequency is about 40% higher than

those found by McArthur (1986) suggesting that resting frequency may not have been achieved in the previous study. These changes in breathing pattern result in resting $\dot{V}$ values which are 50% smaller than those reported in the present study. These discrepencies in f, V_T and $\dot{V}$ values may be due to differences in methodology or in the physiological state of the squirrels during the study. Panting, which allows furred animals to dissipate heat, results in a rapid, shallow breathing pattern similar to those reported by McArthur (1986). This may be one factor contributing to differences in breathing pattern and overall $\dot{V}$ in the two studies.

CBX Animals

Carotid body denervations result in a relative hypoventilation under resting conditions. A 40% reduction in minute ventilation is achieved through decreases in respiratory frequency with no significant changes in V_T. Qualitatively similar decreases in $\dot{V}$ after CBX have been observed in a large variety of animals (Bisgard et al., 1980; Bouverot and Bureau, 1975; Bouverot et al., 1973; Fordyce and Tenney, 1984; Forster et al., 1981; Miller and Tenney, 1975), although a few studies have reported no change in $\dot{V}$ after CBX (Watt et al., 1942) or only acute decreases in $\dot{V}$ which return to preoperative levels over time (Bisgard et al., 1980; Smith and Mills, 1980). The hypoventilation which often results from CBX is accompanied by an increase in P_{aCO2} up to levels which are known to

normally act as a ventilatory stimulus (Bouverot and Bureau, 1975; Bouverot et al., 1973; Miller and Tenney, 1975). The increase in P_{aCO2} and its effects on ventilation does not compensate for the loss of carotid body chemoreceptors and their influence on resting ventilation (Miller and Tenney, 1975).

Thus, it appears that in S. lateralis carotid body chemoreceptors are important in the maintenance of normal levels of ventilation. Under normoxic and normocapnic conditions carotid body chemoreceptors provide a tonic excitatory influence on central respiratory mechanisms, particularly those influencing breathing frequency.

CHH Animals

The overall ventilatory response during both chronic and acute exposure to hypoxia and hypercapnia is similar, but the ventilatory pattern is slightly altered during CHH exposure. CHH exposure results in an elevated $\dot{V}$ under normoxic and normocapnic conditions, due to a maintained increase in V_T.

Although there are no studies which have examined the ventilatory acclimation to chronic hypoxia and hypercapnia together in other species of mammal, there are many studies which have looked at ventilatory responses to chronic hypoxia and hypercapnia separately.

Mammals, other than humans, exposed to long term hypoxia generally exhibit a sustained increase in $\dot{V}$ which approximates the level of ventilation achieved during acute hypoxic exposure (Fordyce and Tenney, 1984; Bouverot et al., 1973; Forster et al., 1981; Mortola et al., 1986). Most of these studies report that the elevated $\dot{V}$ is produced by sustained increases in both V_T and f (Fordyce and Tenney, 1984; Bouverot et al., 1973, Mortola et al., 1986). Increases in V_t associated with long term hypoxic exposure lead to more efficient pulmonary ventilation relative to the acute hypoxic response. Dempsey and Forster (1982) suggest that the mechanism behind these long term changes during hypoxia could result from changes in the excitability of medullary respiratory neurons or changes in the suprapontine or brain stem mechanisms. In general, changes to ventilation during chronic hypoxia appear to involve complex adjustments to respiratory mechanisms (Dempsey and Forster, 1982).

Relatively few studies have looked at ventilatory acclimation to chronic hypercapnia. Most evidence indicates that during chronic hypercapnia ventilation increases and remains elevated throughout the chronic exposure (Dempsey and Forster, 1982). The increases in ventilation during chronic CO_2 exposure equal or exceed the increases in ventilation during acute CO_2 exposure (Dempsey and Forster; 1982). Similar pattern changes to those observed in the

present study in response to chronic hypercapnia have been reported both in man (Schaefer et al., 1966) and laboratory animals (Dodd and Milsom, 1987; Lai et al., 1981). In these studies chronic hypercapnia caused sustained increases in alveolar minute ventilation ($\dot{V}_A$), while dead space (V_D) initially increased and subsequently returned to normal levels over time. In S. lateralis, assuming that there is no change in dead space during exposure to either chronic or acute hypoxia and hypercapnia, $\dot{V}_A$ increases during initial (acute) exposure and then is further elevated after chronic exposure to hypoxia and hypercapnia primiarily by further increases in V_T (Figure 30). $\dot{V}_A$ has been calculated assuming that under resting conditions the ratio of V_D to V_T is approximately .33 (Stahl, 1966; Tenney and Boggs, 1986) and that this ratio is unaltered by gas exposure. Increases in $\dot{V}_A$ during CHH exposure are similar in both intact and CBX squirrels.

Increases in $\dot{V}_A$ serve to reduce the alveolar P_{O2} and P_{CO2} to arterial P_{O2} and P_{CO2} differences (Tenney and Boggs, 1986). These changes increase O_2 loading and CO_2 unloading in the lungs and thus minimize changes from normal levels of arterial P_{O2} and P_{CO2}. During chronic hypercapnia both plasma and cerebral spinal fluid H^+ concentration return to near normal levels, making it unlikely that CO_2 mediated changes in H^+ concentration are responsible for the hypernea (Dempsey and Forster, 1982). Most evidence to date

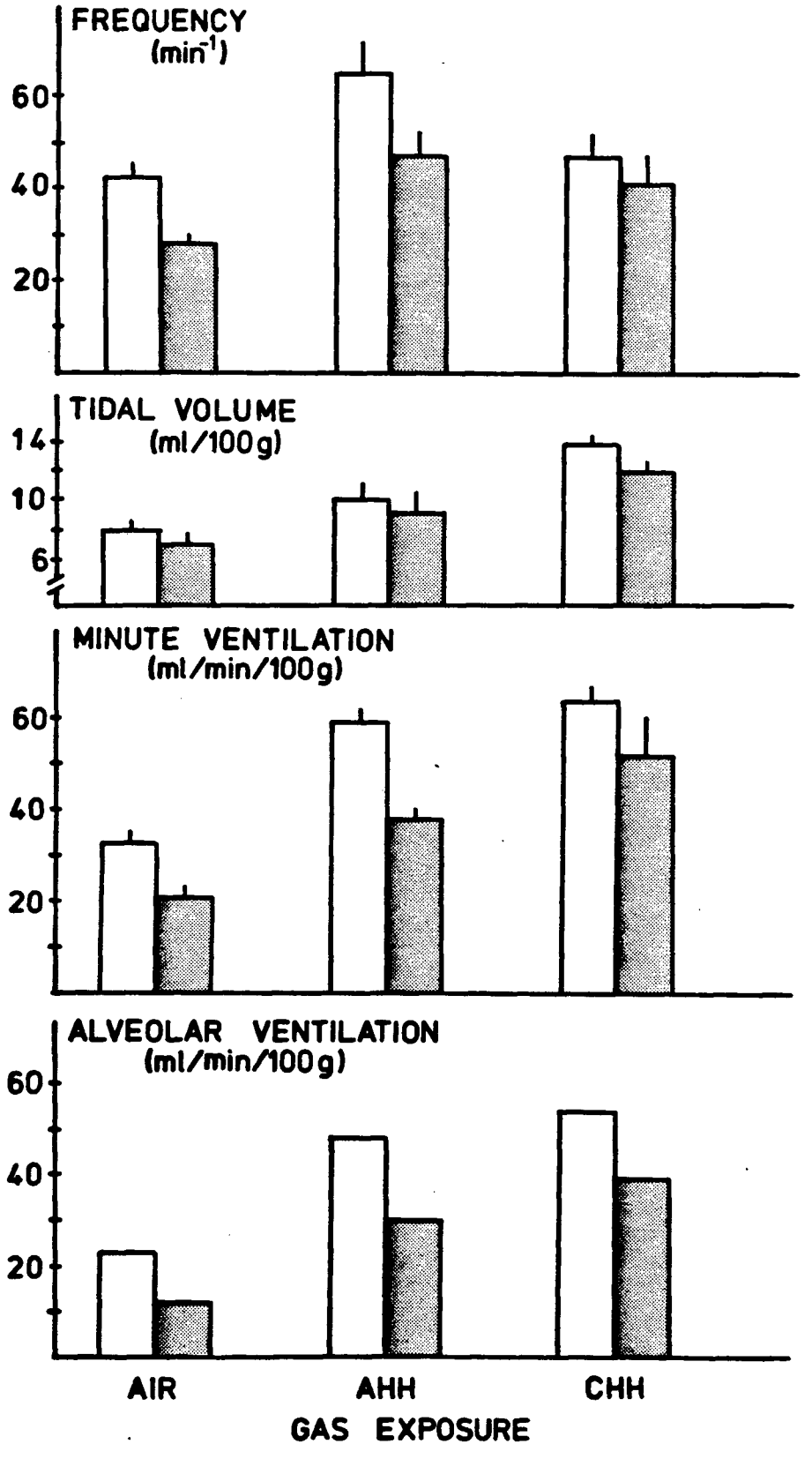
Figure 30. Bar plot showing changes in frequecy, tidal volume, minute ventilation and alveolar minute ventilation during exposure to air, acute hypoxia and hypercapnia (AHH, 17% O_2 and 4% CO_2) and chronic hypoxia and hypercapnia (CHH, 17% O_2 and 4% CO_2). Alveolar ventilation was calculated from the equation:

$$\dot{V}_A = (V_T - V_D) \times f$$

where V_D was assumed to be 33% of normal resting V_T (Tenney and Boggs, 1986) and it was assumed that V_D did not change with exosure to different gas mixtures.

□ = intact S. lateralis

▒ = CBX S. lateralis


FREQUENCY
(min⁻¹)
60
40
20
TIDAL VOLUME
(ml/100g)
14
10
6
MINUTE VENTILATION
(ml/min/100g)
60
40
20
ALVEOLAR VENTILATION
(ml/min/100g)
60
40
20
AIR
AHH
CHH
GAS EXPOSURE

suggests that the mechanisms underlying respiratory pattern changes during chronic CO_2 exposure could involve a disequilibrium between cerebral spinal fluid and interstitial spinal fluid at the site of intercranial chemoreceptors, a change in the contribution of pulmonary CO_2 sensing mechanisms or a change in chemoreceptor activity which directly or indirectly alters medullary respiratory neuron functioning (Dempsey and Forster, 1982). In general, acclimation to both chronic hypoxia and hypercapnia involves complex changes in regulation of breathing pattern and ventilation.

Response to Hypoxia

Intact Control Animals

Intact S. lateralis show a strong ventilatory reponse to decreases in inspired O_2. Increases in $\dot{V}$ during hypoxia are achieved solely by increases in f (Figure 7). Tidal volume during severe hypoxia actually decreases. Two studies on ventilatory responses in semi-fossorial golden hamsters also report hypoxic hyperventilation (Holloway and Heath, 1984; Walker et al., 1985). Increases exclusively in frequency in response to hypoxia have also been reported in several non-fossorial species such as the rat (Cragg and Drysdale, 1983) and the cat (Holloway and Heath, 1984). In contrast, increases in $\dot{V}$ mediated by increases in both V_T and f have been observed in the fossorial mole rat (Arieli and Ar, 1979) the Djungarian hamster (Schlencher, 1985), the

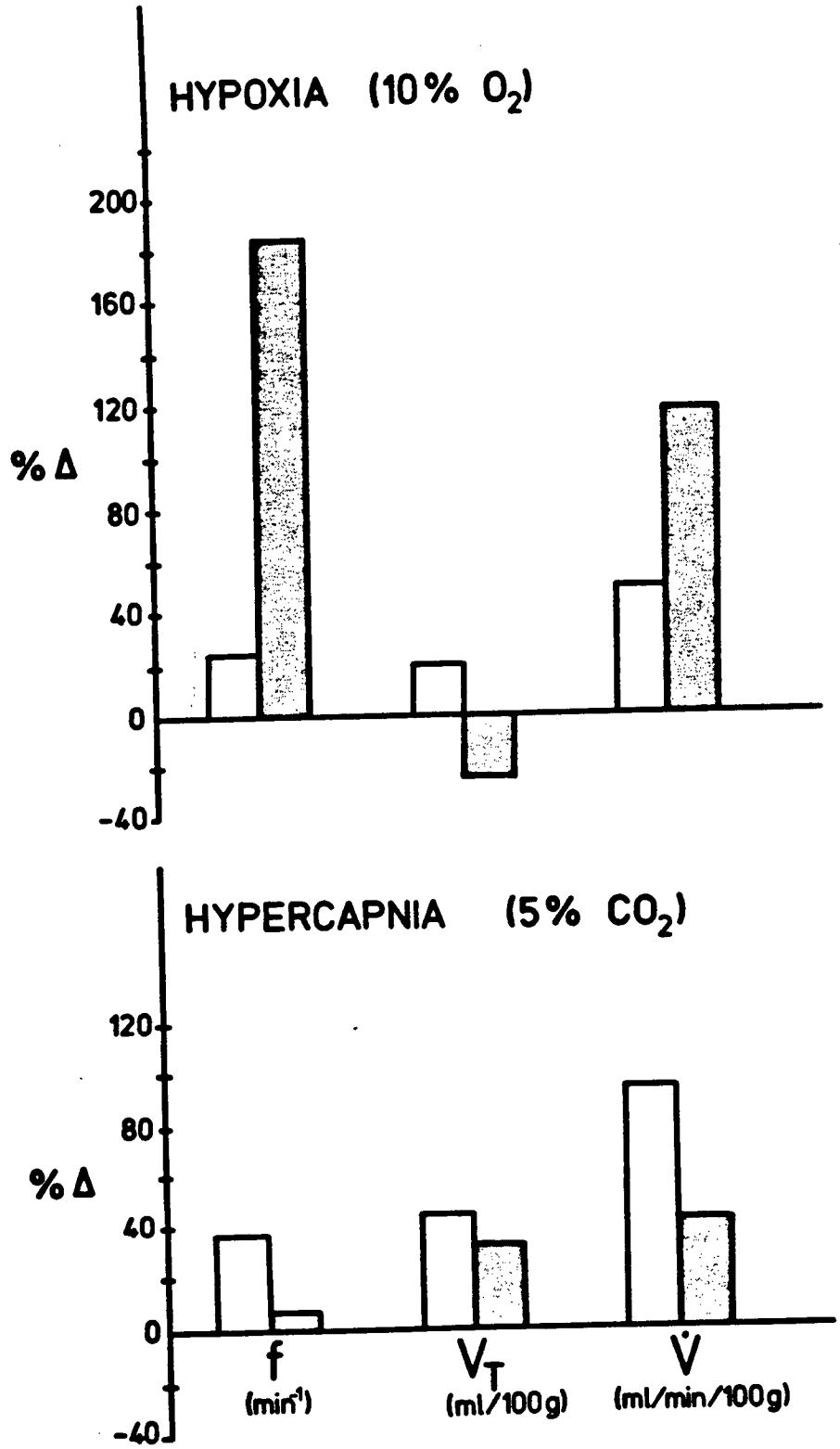
golden-mantled ground squirrel (McArthur, 1986) and the rat (Holloway and Heath, 1984; Maskrey et al., 1981; Walker et al., 1985). Thus, the relative contribution of V_T and f to the hypoxic ventilatory response appears to be study specific, possibly resulting from different methods used to measure respiration in the various studies. In general, large increases in frequency in response to hypoxia are common to most mammals.

The overall ventilatory response of S. lateralis to hypoxia at 8% F_{IO2} is a 140% increase in $\dot{V}$. Increases of similar magnitude in $\dot{V}$ in response to hypoxia have been observed in both semi-fossorial species (Holloway and Heath, 1984; McArthur, 1986; Schlenker, 1985; Walker et al., 1985) and non-fossorial species such as mice and rats (Holloway and Heath, 1984; Schlenker, 1985; Walker et al., 1985). The hypoxic sensitivity of S. lateralis and other semi-fossorial mammals is equivalent to, or greater than, hypoxia sensitivity in non-fossorial species. This conclusion has been confirmed in studies directly comparing hypoxic ventilatory responses from similar sized semi-fossorial and non-fossorial species (Holloway and Heath, 1979; Schlenker, 1984; Walker et al., 1985). Figure 31 illustrates the hypoxic ventilatory response of a semi-fossorial species, the golden-mantled ground squirrel (this study) to that of the white rat (Pappenheimer, 1977).

Figure 31. Bar plot showing the percent change from normoxic and normocapnic conditions in frequency (f), tidal volume (V_T) and minute ventilation ($\dot{V}$) in response to hypoxia (10% O_2) and hypercapnia (5% CO_2) in awake golden-mantled ground squirrels compared to the white rat. Rat ventilation data taken from Pappenheimer (1977).

▒ = golden-mantled ground squirrels

□ = white rat


HYPOXIA (10% O_2)
200
160
120
% Δ
80
40
0
-40
HYPERCAPNIA (5% CO_2)
120
80
% Δ
40
0
-40
f
(min^{-1})
V_T
(ml/100g)
$\dot{V}$
(ml/min/100g)

CBX Animals

Carotid body denervations do not greatly affect the overall magnitude of the hypoxic ventilatory response of either air breathing or CHH animals. Although CBX squirrels exhibit a lower $\dot{V}$ at all levels of F_{IO2} due to a lower respiratory frequency, intact and CBX groups exhibit similar increases in $\dot{V}$. CBX in both air breathing and CHH animals, however, does result in a leftshift in the ventilatory response threshold,

Many previous studies have examined the role of carotid body chemoreceptors during acute ventilatory response to hypoxia. It is classically assumed that CBX produces a long term decrease in hypoxic sensitivity. Decreases in ventilation in response to severe hypoxia after CBX are the result of low P_{aO2} levels causing central depression of ventilation (Fordyce and Tenney, 1984). Loss or reduction of ventilatory stimulation during hypoxia in carotid body denervated animals has been observed in goats (Tenney and Brooks, 1966), rats (Chioccho et al., 1984; Sapru and Krieger, 1977), dogs (Bouverot et al., 1973) and cats (South and Mills, 1980; Fordyce and Tenney, 1982). In view of these results, ventilatory responses to hypoxia in intact animals have been assumed to reflect a balance between facilitory carotid body chemoreceptor input and the direct central depression caused by low central P_{O2} (Gautier, 1976).

Moyer and Beecher (1942) observed that in CBX cats hypoxia produced large increases in f and small decreases in V_T. Similar results have since been obtained in cats (Miller and Tenney, 1975; Sorenson and Mines; 1970) and in dogs (Davenport et al., 1947; Watt et al., 1943). In general most of these studies report that hypoxia does depress ventilation intially, but then stimulates ventilation after a long period of latency. The present study supports the idea that after carotid body denervation hypoxia can act centrally to stimulate respiratory frequency and at severe levels may inhibit V_T. It is also possible that the reduced V_T is purely a function of high frequency respiration.

CHH Animals

In S. lateralis chronic exposure to hypoxia and hypercapnia results in an upward shift of the entire hypoxic response curve. There is little change in overall hypoxic sensitivity in CHH CBX animals, and there is a slight increase in sensitivity in the intact CHH animals. Studies of chronic hypoxia suggest that there is little alteration in ventilatory sensitivity to hypoxia (Dempsey and Forster, 1982), but chronic hypoxia does displace the entire ventilatory response curve upward (Lahiri et al., 1971; Lahiri et al., 1983; Mortola et al., 1986). Few studies have looked at the effects of chronic hypercapnia on hypoxic sensitivity. Falchuk et al. (1966) found that 48 hours of hypercapnic exposure did not effect the hypoxic response at

comparable P_{ACO2} levels. In general the central adjustments which result in a high V_T in CHH animals are affected by acute hypoxia in a similar fashion as air breathing squirrels exposed to acute hypoxia. Additionally, CHH CBX squirrels do not exhibit an elevated hypoxic sensitivity, suggesting that peripheral chemoreceptors may be involved in the increased hypoxic sensitivity in CHH control squirrels.

The combination of 4% CO_2 with increasing levels of hypoxia causes a slight elevation in the absolute $\dot{V}$ at each level of F_{IO2} in all groups of animals. The slightly increased $\dot{V}$ results from the effect of hypercapnia on V_T. Hypercapnia also caused a slight decrease in the overall frequency response at 8% O_2. As in this study, Maskrey et al. (1981) found that a 4% hypercapnic background did not significantly alter the overall response to 10% O_2 in rats. In addition, hypercapnia had a slight negative interactive effect on frequency which persisted after carotid sinus denervation (Maskrey et al., 1981). It appears that hypoxia and hypercapnia interact centrally to decrease the frequency response normally associated with hypoxia alone. In contrast, a large amount of literature suggests that positive hypoxic and hypercapnic interactions occur primarily at the carotid body chemoreceptors and result in increases in the slope of the hypoxic resonse curve (Dempsey and Forster, 1982; Falchuk et al., 1966; Fitzgerald and Lahiri, 1986). If carotid body chemoreceptors are the site

of hypoxic and hypercapnic interactions, given the small contribution of carotid body chemoreceptors to hypoxic and hypercapnic responses in S. lateralis, it is not surprising that interactions appear to be small and to be centrally produced.

Response to Hyperoxia

Hyperoxia alone causes a decrease in $\dot{V}$ in intact S. lateralis. The decrease in $\dot{V}$ is mediated solely through a reduction in frequency. A similar decrease in ventialtion has been observed in dogs and rabbits (Bouverot et al., 1973; Watt et al., 1943). Inspiration of 50% O_2 results in an increase in P_{aO2}, and providing that a low P_{aO2} has a stimulatory role on ventilation during normoxia, hyperoxia should decrease the chemoreceptor hypoxic drive. The hypoxic drive during normoxia in S. lateralis appears to be mediated entirely by carotid body chemoreceptors, since CBX and hyperoxia both produce similar adjustments in $\dot{V}$ (Miller and Tenney, 1975).

Although during normoxia CBX lowers $\dot{V}$, during hyperoxia CBX animals exhibit an elevated $\dot{V}$. These increases in $\dot{V}$ are mediated exclusively through increases in V_T. Miller and Tenney (1975) found similar results in CBX cats and suggested that O_2 acts to stimulate central regions of the brain concerned with respiratory tidal volume control. Thus in the absence of carotid bodies these

regions are relatively depressed during normoxia. Miller and Tenney (1975) also suggested that the region sensitive to hyperoxia may be within the brain stem structure and that the discharge activity may be limited in part by the availability of O_2.

Response to hypercapnia

Intact Control Animals

In S. lateralis the relationship between $\dot{V}$ and F_{ICO2} is more or less linear. $\dot{V}$ rises primarily through increases in V_T and small but significant increases in f. Similar response patterns to hypercapnia have been found in a number of animals including the laboratory rat (Maskrey et al., 1981), the golden hamster (Holloway and Heath, 1984; Walker et al., 1985) and the burrowing owl (Boggs and Kilgore, 1983). Other studies report more or less equal increases in V_T and f (Arieli and Ar, 1977; Darden, 1977; Holloway and Heath, 1984; Schlenker, 1985). Thus, the relative contribution of V_T and f to the hypercapnic ventilatory response is variable and appears to be species specific.

The overall magnitude of the hypercapnic ventilatory response is lower in S. lateralis than most non-fossorial mammals. Blunted hypercapnic sensitivity appears to be a common adaptation to fossorial or semi-

fossorial existance (Boggs et al., 1984) and has been documented in a variety of species including the burrowing owl (Boggs and Kilgore, 1982), the pocket gopher (Darden, 1972), the mole rat (Arieli and Ar, 1979), the marmot (Leitner and Malan, 1973), the golden-mantled and columbian ground squirrels (McArthur, 1986), and the golden hamster (Holloway and Heath; 1984; Walker et al., 1985). A blunted hypercapnic response has not been observed in the semi-fossorial Djungerian hamster (Schlenker, 1985). Figure 30 illustrates typical hypercapnic $\dot{V}$ repsonses in a semi-fossorial species, the golden-mantled ground squirrel (the present study) and a non-fossorial species, the white rat (Pappenheimer, 1977).

The overall reduction in sensitivity at all levels of CO_2 could result from decreases in chemoreceptor sensitivity (Darden, 1972; Holloway and Heath, 1984), low V_D to V_T ratios or increases in the buffering capacity of the blood. As mentioned previously, semi-fossorial species, including S. lateralis, tend to breathe with a small frequency and large tidal volume (Arieli and Ar, 1979; Darden, 1972) or a reduced V_D (Darden, 1972). These ventilatory adjustments result in a higher $\dot{V}_A$ relative to non-fossorial mammals. Changes in inspired CO_2 may result in smaller changes in arterial P_{CO2}, and this may contribute to the reduced hypercapnic response curve (Tenney and Boggs, 1986). Changes in buffering capacity may occur through

increases in blood buffers such as bicarbonate or through renal adjustments (Boggs et al., 1984; Chapman and Bennett, 1975; Falchuk et al., 1966).

Hyperoxia has little effect on the overall hypercapnic response curve. In both intact and CBX animals hyperoxia alone significantly alters $\dot{V}$ from normoxic levels. The addition of 2% CO_2 to hyperoxia returns $\dot{V}$ to levels which are not significantly different from those seen in normoxia at 2% CO_2. Thus, in intact squirrels hyperoxia alone may depress peripheral chemoreceptor input, but this depression is abolished with increases in CO_2. Similarly in CBX animals the increases in V_T resulting from hyperoxia are abolished with the addition of low levels of CO_2. In general, it appears that the peripheral and central mechanisms involved in the hyperoxic response are sensitive to and overridden by increases in CO_2.

CBX Animals

Carotid body denervations do not significantly alter the pattern of ventilatory response to hypercapnia. A slight increase in overall hypercapnic sensitivity indicates that peripheral chemoreceptors may have a slight inhibitory effect on central hypercapnic sensitivity. Maskrey et al. (1981) found that CBX in rats caused a slight decrease in hypercapnic sensitivity through decreases in both V_T and f responses. Berchenbosch et al., (1979) found that carotid

body chemoreceptors contributed about 40% to hypercapnic ventilatory responses during hyperoxia. Other studies have reported reduced hypercapnic sensitivity after CBX although the magnitude of the reduction is extremely variable (Fitzgerald and Lahiri, 1986). The role of carotid body chemoreceptors in hypercapnic responses remains uncertain (Dempsey and Forster, 1982), but results from the present study suggest that carotid body chemoreceptors do not play an important role in ventilatory responses to CO_2 in the golden-mantled ground squirrel. It is possible that the relativey small role of peripheral chemoreceptors in hypercapnic responses in S. lateralis contributes to the overall blunted hypercapnic sensitivity.

CHH Animals

Chronic exposure to hypoxia and hypercapnia has little effect on the overall response to hypercapnia compared to air breathing S. lateralis. Intact CHH animals exhibit an elevated V_T at all levels of hypercapnia relative to air breathing control squirrels.

Chronic hypoxia alone does not appear to alter carotid chemoreceptor responses to hypercapnia (Lahiri et al., 1983). Chronic hypercapnia results in a shift in the hypercapnic response threshold to higher levels of P_{CO2}, but has little effect on ventilatory sensitivity in humans (Falchuk et al., 1966; Kellogg, 1960). Results of the

present study indicating that acclimation to CHH conditions has little effect on hypercapnic sensitivity is supported by previous studies on acclimation to both chronic hypoxia and chronic hypercapnia (Dempsey and Forster, 1982, Jenning and Chen, 1975; Schaefer et al., 1963).

Most studies on fossorial and semi-fossorial mammals involve maintaining animals outside of burrow conditions for long periods of time before measuring O_2 and CO_2 sensitivities. These studies generally assume that no "deacclimatization" occurs over this period with respect to ventilatory control (Boggs et al., 1982). Deacclimatization has been documented in humans and other mammals upon return to air after long term hypoxic exposure (Lahiri et al. 1976) and long term hypercapnic exposure (Jennings and Chen, 1976). For example, Lahiri et al. (1982) found that even after being raised at high altitude (hypoxic conditions), deacclimatization occurred in humans moved to sea level (normoxic conditions). If the reduced CO_2 sensitivity typical of burrow-dwelling animals is a result of long term exposure to hypoxia and hypercapnia, it is possible that deacclimatization could occur.

Birchard et al. (1984) exposed perinatal rats to chronic hypercapnic conditions to determine if modification to CO_2 ventilatory sensitivity could be caused by prolonged exposure during development. No changes in the buffer base

or in the responses to hypercapnia could be detected between rats raised under hypercapnic conditions or those raised under normocapnic conditions. Birchard et al. (1986) concluded that alterations in the hypercapnic response curve may be genetic in origin rather than developmental. Farber et al. (1972), working on developing opposums, concluded that chronic hypercapnia during development did not affect adult respiratory responses to CO_2. In addition, laboratory raised hamsters still retain a reduced hypercapnic sensitivity, implying the differences between fossorial and non-fossorial species represents a genetically determined characteristic (Ariel and Ar, 1979; Birchard et al., 1984; Boggs et al., 1984). Results from the present study support the idea that CO_2 sensitivities are genetically determined.

HIBERNATING ANIMALS

The major finding of this portion of the study is that during hibernation the respiratory pattern of S. lateralis is temperature sensitive. Two patterns of steady state respiration are observed; at relatively high ambient and body temperatures a burst breathing pattern is observed, and at lower ambient and body temperatures a single breath breathing pattern is observed. Large changes in respiratory pattern do not greatly alter overall levels of resting ventilation or ventilatory responses to hypoxia or

hypercapnia. Generally, hibernating S. lateralis exhibit a greatly reduced ventilatory sensitivity to hypoxia and a comparatively high ventilatory sensitivity to hypercapnia. Carotid body chemoreceptors are not important in determining resting ventilation or overall ventilatory responses to hypoxia or hypercapnia.

Methodology

Quantitative measurements of respiration during hibernation have been hampered by the sensitivity of hibernating animals to handling (Steffen and Riedesel, 1984). Tidal volume has seldom been measured since the methods employed to measure V_T often cause arousal (Lyman, 1982). In addition, periodic or burst breathing patterns are known to be sensitive to disturbance (Kristofferson and Sovio, 1964; Pajunen, 1970; Pembrey and Pitts, 1899). The pneumotachograph-mask unit used in the present study to measure breathing pattern and tidal volume did not appear to disturb the hibernating animal. Squirrels could be maintained in hibernation for up to 7 days while wearing the mask. In addition, respiratory pattern and respiratory values observed during hibernation at ambient temperatures of about $6^{o}C$ are comparable to previously reported values for S. lateralis (Hammel et al., 1968; McArthur, 1986; Steffen and Reidesel, 1982).

Several studies have observed a high variability in breathing at constant ambient temperatures, particularly in burst breathing hibernators. Lyman (1951) working with hibernating hamsters and golden-mantled ground squirrels found that frequency could vary as much as 25% from the mean. Malan et al. (1973) found high variability in f even when there was no measurable change in oxygen consumption or ambient temperature. Thus it appears that frequency can fluctuate without changes in the depth of hibernation.

Pajunen (1984) noted that the pattern of periodic breathing during hibernation changed over the hibernation season. In addition, irritability to external stimuli increased during a bout of hibernation (Lyman, 1982). It is possible that the amount of time spent in "deep" hibernation, both seasonally and during an individual hibernation bout, may effect respiratory pattern and respiratory sensitivity to hypoxia and hypercapnia.

During the present study an attempt was made to reduce possible variability in respiratory responses resulting from seasonal variability and progressive irritability. Although experiments examining the ventilatory responses to various gases were carried out over a 4 to 5 month period, no responses were tested until animals had been in deep hibernation for at least 1 month. Throughout the hibernating season squirrels undergo periodic

arousals approximately every 7 to 8 days. In order to reduce the possible effects of progressive irritability through a hibernation bout animals used in experiments had always entered hibernation only 1 to 2 days prior to the study. Responses to gas stimuli appeared to be relatively constant both within an experimental groups and within an individual animal.

Respiratory Pattern

The occurrence of two very different intermittent breathing patterns in hibernating animals has been observed for many years (Pembrey and Pitts, 1899). The two patterns, burst and single breath breathing, have generally been assumed to be species specific (Malan, 1982). Observations that changes in the burst breathing pattern are the first sign that an animal has been disturbed (Pajunen, 1970) have been used to explain the occurrence of the two different breathing patterns in one hibernating species.

Several authors have noted the importance of ambient and body temperature on respiratory pattern (Hammel et al., 1968); Kristofferson and Sovio, 1966; Pajunen, 1984). The "optimum" hibernation temperature has been determined for most hibernators at between 4°C to 7°C (Kayser, 1961, in Lyman, 1982). Consequently, most studies maintain hibernating animals in this temperature range.

Increases in ambient temperature above this range (T_a=10^{o}C) result in decreases in the breath-hold length (Kristofferson and Sovio, 1969; Pajunen, 1984). Decreases in ambient temperature down to 0^{o}C cause respiration in the dormouse to breathe in a single breath breathing pattern for long periods of time, up to 17 hours, interspersed with short breath hold periods (Pajunen, 1984). During the first one-half to two-thirds of these breathing periods frequency was very low, less than 1 breath/minute (Pajunen, 1984). In addition, preliminary experiments suggested that at slightly lower ambient temperatures (-2^{o}C) the burst breathing pattern becomes a continuous single breath pattern (Pajunen, 1984). Kristofferson and Sovio (1964) working with hedgehogs found that in "deeply hypothermic" animals (T_a = -5^{o}C) respiration also took on a single breath pattern. Hammel et al. (1968) reported that above hypothalamic temperatures of 5.5^{o}C S. lateralis exhibit a burst breathing pattern. At slightly lower hypothalamic temperatures (3.5^{o}C) breathing became increasingly regular and lost its periodic pattern.

Hammel et al. (1968) suggested that the change in respiratory pattern observed in S. lateralis as body temperature declines could be indicative of a transitional state leading to arousal should ambient temperature continue to decrease. Results from the present study do not suggest that the single breath breathing is a transitional state preceeding arousal. S. lateralis can be maintained for

several days at lower body temperatures with steady state ventilation. In addition, measurements of overall minute ventilation are not significantly different between the two states. The observation that the burst breathing pattern is extremely temperature sensitive indicates the importance of measurement of ambient and body temperatures during respiratory studies.

The level or depth of hibernation does not significantly change during the transition from burst breathing to single breath breathing. Overall minute ventilation, V_T, and f remain constant while oxygen consumption increases slightly. At an ambient temperature of 2°C S. lateralis maintain only a small temperature gradient between T_b and T_a, therefore no additional energy for maintenance of T_b should be required. Thus, the reduction in T_b should result in a further decrease in tissue metabolism and, if anything, a reduction in $\dot{V}_{O2}$.

Large increases in respiratory timing components occur during single breath breathing. No overall changes occur in the T_I/T_E ratio or in the duty cycle during the transition from burst breathing to single breath breathing. This suggests that there is no loss of the integrity of the respiratory system. The increase in breath duration could result from the direct effects of reduced body temperature on central mechanisms or indirect effects on pulmonary

mechanics or both. The decrease in V_T/T_I in the single breath breathing state implies that the ventilatory drive to breathe is reduced. A two-fold increase in the time spent actively breathing is seen during single breath breathing. This implies that the work or energy required for respiration has also increased. The suggestion that respiratory work increases during single breath breathing is supported by the small but significant increase in $\dot{V}_{O2}$.

The transition between patterns occurs in the presence or absence of carotid body chemoreceptors implicating the CNS as the site mediating the pattern changes. Little is known about CNS structures involved in the production of burst breathing. Some work has been done in an attempt to elucidate the central structures involved in the production of burst breathing patterns in crocodiles (Naifeh et al., 1971a, 1971b). Naifeh et al. (1971b) found that brain stem lesions in the rostral medulla above the nucleus laminarus resulted in the conversion of a periodic bursting pattern into a single breath breathing pattern. Thus, the bursting pattern in crocodiles appears to be controlled by higher CNS centers. Naifeh et al. (1971a) also used anesthetics to look at the central production of the bursting pattern in crocodiles. Both chloroform and pentobarbital, at low levels, produced a regular, single breath pattern. The regularity of the single breath pattern and the length of time the pattern could be sustained lead

to the suggestion that the single breath pattern may be the basic pattern of crocodilian respiration when uninfluenced by other CNS centres. Similar effects of anaesthetics on the burst breathing pattern of Chrysemys picta (the western painted turtle) have been observed (Milsom and Webb, unpublished observations). The burst breathing pattern of S. lateralis seen during hibernation is very similar to that seen in Chrysemys picta (Figure 32) and preliminary observations suggest that anesthetics also have profound effects on the burst breathing pattern seen in S. lateralis. Exposure to anesthetics transforms the burst breathing pattern observed at an ambient temperature of 6°C into a regular breathing pattern consisting of single or double breaths followed by a ventilatory pause (Figure 33). The pattern is similar to the single breath pattern of S. lateralis hibernating at 2°C. During anesthetic exposure individual breaths were often characterized by double inspirations similar to those observed in about 20% of single breath breathing animals at reduced body temperatures. Figure 33 illustrates the double inspirations in both single breath breathing groups. Removal of the anesthetic leads to a gradual return to a burst breathing pattern in S. lateralis.

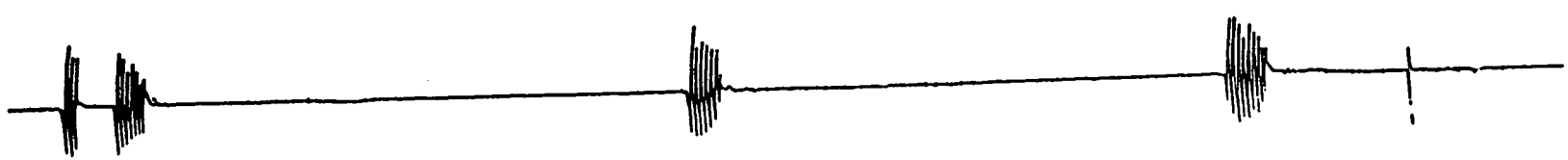
Thus, anesthetics (in S. lateralis, turtles and crocodiles), reduced body temperature (in S. lateralis), and brain stem lesions (in crocodiles) all will convert burst

Figure 32. Representative record of the burst breathing pattern observed in S. lateralis (the golden-mantled ground squirrel) during hibernation at a T_b of about 7°C and C. picta (the western painted turtle) under resting conditions at a T_b of about 22°C.

A) Spermophilus lateralis

1 min

B) Chrysemys picta

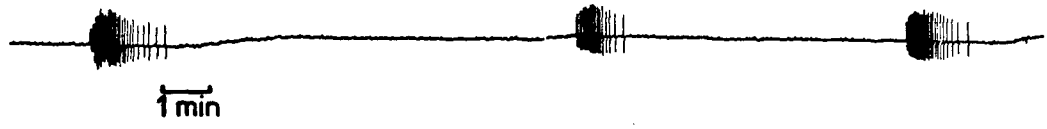


1 min

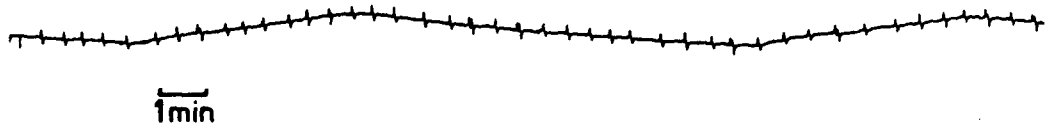
Figure 33. Representative records of the effects of decreasing ambient temperature from 7°C to 2°C on breathing pattern in S. lateralis during hibernation (A) and the effects of anesthetic exposure (halothane) on the burst breathing pattern of S. lateralis (B) during hibernation at a T_a of 7°C. Note that both decreased ambient temperature and anesthetic exposure can result in double inspirations during single breath breathing.

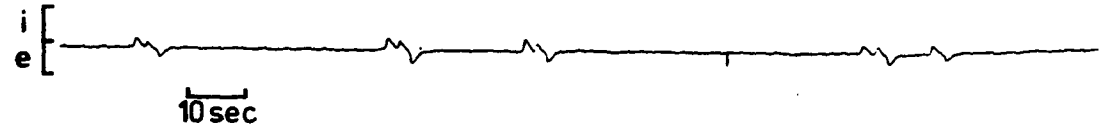
A) EFFECTS OF AMBIENT TEMPERATURE

a) air T_a=7°C



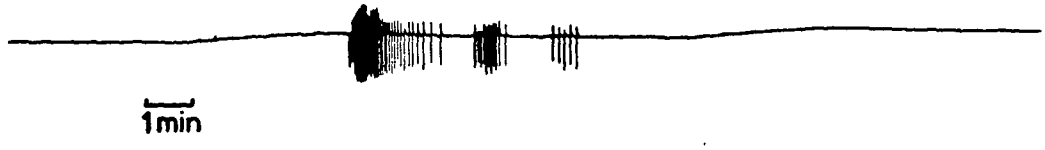
b) air T_a=2°C



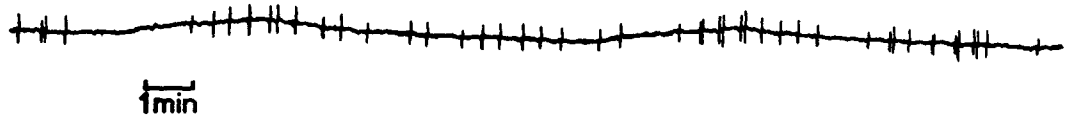


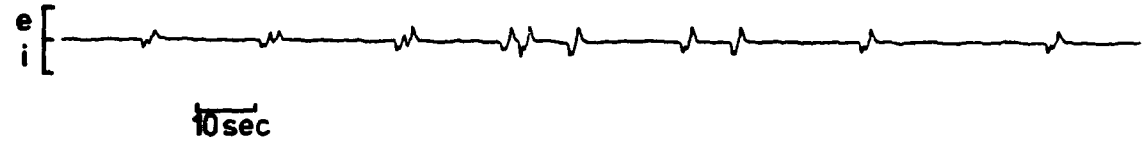
B) EFFECTS OF HALOTHANE

a) air T_a=7°C



b) air + halothan T_a=7°C





i= inspiration
e= expiration

breathing to a single breath breathing pattern. It is conceivable that all of these treatments act to block the influence of higher brain centres on the basic respiratory pattern produced in the brain stem region (Feldman, 1986). In S. lateralis at T_b of about 6-7°C, spontaneous electrical activity can still be recorded from the CNS (Strumwasser, 1959). Indeed, the maintained electrical activity in the CNS at very low T_b may be related to specific modifications of the brain tissue during hibernation (Aloia, 1979). In spite of possible cellular modifications which ensure neural firing, a body temperature much below 4 to 5°C must be nearing the lower limits of neural function. It is possible that the small drop in T_b from about 8°C down to about 4°C causes inhibition of neural transmission. Some peripheral neurons are known to be more sensitive to changes in temperature than others (Ruch, 1973). Similar differences in temperature sensitivity may be found in the CNS neurons, and the areas involved with the production of a burst breathing pattern may no longer function at reduced body temperatures. This suggestion is supported by the observation that both anesthetics and cold have reversible effects on breathing pattern.

The burst breathing pattern observed during hibernation is often referred to as Cheyne-Stokes Respiration (CSR). True CSR in mammals is generally associated with pathological conditions (Lyman, 1982). The

CSR pattern by definition consists of a gradual increase in frequency and depth of breathing, followed by a gradual decrease in frequency and depth. During hibernation, any one burst may contain elements common to CSR, but just as often respiration starts and stops with little change in the depth or rate of respiration (Figure 15; Lyman, 1982). Thus breathing pattern observed during hibernation is not a true CSR pattern, and the term burst breathing is a simpler and more accurate term for this type of respiration.

The production and control of periodic patterns in euthermic mammals has been the topic of many clinical and experimental studies. Several theories on the production of periodic breathing have been proposed (Cherniak and Longobardo, 1973; Longobardo et al., 1960), but only one model attempts to account for the occurrence of all types of periodic breathing (Khoo et al., 1982). This model suggests that in the euthermic awake state in mammals the respiratory system is relatively stable. Decreases in the stability of the respiratory system result in the production of periodic patterns of respiration. Decreased stability in the respiratory system can be acheived by abnormally long circulatory delays, changes in peripheral and central chemoreceptor drive and decreases in total body or lung CO_2 and O_2 stores (Khoo et al., 1982; Cherniak and Longobardo, 1973). Although changes in all of these factors probably contribute to an increased respiratory instability during

hibernation, to what degree, if any, they promote a periodic pattern is uncertain. In view of this limited information it is difficult to apply any models involving production of periodic breathing in euthermic mammals to the periodic breathing seen during hibernation.

The effects of reductions of body temperature and metabolism on respiratory patterns in either hibernating or non-hibernating mammals has not been well studied. Cherniak et al. (1979) induced periodic breathing in anaesthetized cats by cooling the ventral medullary surface. In addition, it has been suggested that periodic breathing in reptiles may be associated with reduced metabolic rates and O_2 requirements and that burst breathing patterns may be a strategy to reduce the cost of breathing (Milsom, 1984). It is possible that changes in body temperature and metabolic rate could contribute to the mechanisms concerned with the production of burst breathing patterns during hibernation.

Resting Ventilation

The respiratory values measured for ventilation during normoxia in S. lateralis are similar to those reported in studies by Hammel et al. (1968) and Steffen and Reidesel (1984). Non-ventilatory periods in the present study lasted, on average, 11 to 12 minutes compared to 8 minutes reported by Steffen and Riedesel (1984) and about 10

minutes reported by Hammel et al. (1968). Nonventilatory periods in each of these studies are about two to three times longer than those reported by McArthur (1986) for S. lateralis. Overall ventilatory frequency in burst breathing and single breath breathing S. lateralis is within the range of .5 to 3.0 breaths/minute reported for most hibernating animals (Kristofferson and Sovio, 1966; Landau and Dawe, 1958, Lyman, 1941; Malan et al., 1973; Pajunen, 1984; Steffen and Riedesel, 1984). Tidal volume measurements in S. lateralis are slightly lower than those measured by Steffen and Reidesel (1984), 1.2 ml/100g, but slightly higher than those measured by McArthur (1986), 0.5 ml/100g. Variations in respiratory variables may be related to differences in ambient temperatures used in different studies. Oxygen consumption measurements of about 2.0 ml 0_2/100g/hour for both single breath and burst breathing squirrels were also in the range of 2.1 to 3.5 ml/100g/hour previously reported for S. lateralis (Hammel et al., 1968; McArthur, 1986; Steffen and Riedesel, 1984).

Carotid body denervations do not result in significant changes in normoxic minute ventilation in burst breathing or single breath breathing S. lateralis. In burst breathing squirrels CBX caused a reduction in V_T in air and during all gas exposures. This suggests that carotid body chemoreceptors may be important in determining base line tidal volume during burst breathing. Overall, carotid body

chemoreceptors do not play an important role in determining breathing pattern or levels of ventilation in air. This conclusion is further supported by the observation that hyperoxia, in intact animals, has no effect on respiration. If carotid body chemoreceptors are active during normoxia, hyperoxia would decrease the existing ventilatory drive by decreasing peripheral chemoreceptor input and therefore reduce $\dot{V}$.

Response to hypoxia

Ventilatory responses to decreases in inspired O_2 are low in all groups of S. lateralis. Burst breathing squirrels show a moderate increase in $\dot{V}$ at 5% O_2 and a 100% increase in $\dot{V}$ at 3% O_2. The increase in $\dot{V}$ is achieved solely by decreases in T_{NVP} leading to increases in f. The observed hypoxic hyperventilation is similar to observations in other studies which report moderate ventilatory stimulation by hypoxia. Tahti et al. (1975) observed ventilatory stimulation below 16% O_2 in the hedgehog, with breathing becoming continuous at 1.7% to 3% O_2. McArthur (1986) also observed a doubling in $\dot{V}$ at 3% O_2 in burst breathing S. lateralis. In contrast, during single breath breathing, S. lateralis do not show an increase in $\dot{V}$ at any level of F_{IO2} used in this experiment. Similarly McArthur (1986) found no increase in $\dot{V}$ in the single breath breathing S. columbianus at 3% F_{IO2}.

Carotid body denervations do not significantly alter the overall ventilatory response to hypoxia in either burst breathing or single breath breathing squirrels. A significant left-shift in the response threshold at 5% O_2 in burst breathing S. lateralis indicates that carotid body chemoreceptors may be involved in the hypoxic frequency response at relatively moderate levels of hypoxia. The frequency response associated with severe levels of hypoxia in burst breathing squirrels appears to be centrally produced.

The low hypoxic sensitivity in hibernators is not surprising in view of the effects of temperature on metabolic rate and the hemoglobin-O_2 (HbO_2) dissociation curve. As an animal enters hibernation, metabolic rate drops to about 1% to 2% that of a euthermic animal (Malan, 1982; Wang, 1978). The decline in metabolic rate greatly reduces the tissue demand for O_2. As T_b drops there is a large left-shift in the HbO_2 dissociation curve. Several studies indicate that the partial pressure at which the hemoglobin is 50% saturated with O_2 at a body temperature of $5^{\circ}C$ is less than 10 Torr (Clausen and Ersland, 1968; Musacchia and Volker, 1971). This partial pressure corresponds to inspired O_2 levels of about 1%. Thus, arterial blood remains saturated with O_2 down to very low partial pressures.

Levels of inspired O_2 required to cause desaturation of the blood are unlikely to be found under natural conditions. In addition, even during long breath hold periods, levels of P_{aO2} are unlikely to fall to levels required to cause desaturation of the blood. Steffen and Riedesel (1982) report that end-tidal P_{O2} at the end of a breath-hold period was about 90-95 Torr. Similar arterial partial pressures of between 88 and 120 Torr have been reported at end breath-hold (Musacchia and Volkert, 1971; Tahti, 1978 in Steffen and Riedesel 1984). These levels of P_{O2} are not likely to result in less than 100% saturation and it is, therefore, unlikely that P_{O2} acts as an important chemical stimulus to breathe during hibernation.

The differences in the hypoxic sensitivity between burst breathing and single breath breathing S. lateralis may result from slightly reduced body temperatures in the single breath breathing squirrels. It is conceivable that at a T_b of 7^{o} to $8^{o}C$ blood is not fully saturated at 3% to 5% F_{IO2}, thus causing a slight ventilatory response. A decrease in T_b to about $4^{o}C$ may further left-shift the HbO_2 dissociation curve such that blood remains fully saturated even at 3% F_{IO2}.

Response to Hypercapnia

Both burst breathing and single breath breathing S.

lateralis exhibit a relatively high sensitivity to hypercapnia. The pattern of hypercapnic ventilatory response is similar in both groups, consisting of a large frequency response and a slight V_T response. Increases in frequency are achieved by changes in T_{NVP} with no adjustment to the duration of individual breaths.

The overall ventilatory response to hypercapnia appears to be qualitatively similar to responses reported for hedgehogs (Biorch et al., 1956; Tahti, 1975), the golden hamster (Lyman, 1951), the marmot (Endres and Taylor, 1930) and the 13-lined, columbian and golden-mantled ground squirrels (Lyman, 1951; McArthur, 1986). Most studies report that levels of 1% to 3% CO_2 stimulate respiration through decreases in T_{NVP} and that respiration is continuous above 5% CO_2 (Biork et al., 1956; Lyman, 1951; McArthur, 1986; Tahti, 1975). The magnitude of the frequency response varies greatly, from a 200% increase in frequency reported for golden hamsters at 5% CO_2 (Lyman, 1951) to a 550% increase in frequency reported in the present study at 6% CO_2. Tidal volume responses have not been measured in most respiratory studies, but V_T has been shown to increase between 35% and 60% during hypercapnia by McArthur (1986) and in the present study.

During single breath breathing S. lateralis show a lower overall sensitivity to hypercapnia than during burst

breathing. The decreased sensitivity is a result of a smaller overall increase in the breathing frequency. At 6% CO_2 both groups breathe continuously and further increases in CO_2 do not result in further increases in f. It appears that in each group maximum f is determined by the duration of T_{TOT}. During single breath breathing the breath duration is about two times that during burst breathing (5.5 seconds and 2.5 seconds respectively), and therefore the maximum breathing frequency is about one half that during burst breathing (5.2 breaths/minute and 10.4 breaths/minute). A similar trend can be seen in the columbian ground squirrel which also breathes with a single breath pattern; total breath duration is 6.5 seconds and maximum breathing frequency under similar hypercapnic conditions is about 4.5 breaths/minute (McArthur, 1986).

CBX does not significantly alter overall ventilatory responses in either burst breathing or single breath breathing S. lateralis. Although burst breathing CBX squirrels had slightly reduced tidal volumes at all levels of CO_2, hypercapnia still acted centrally to produce increases in V_T. Thus, the major effect of hypercapnia was a central stimulation of ventilatory frequency and tidal volume.

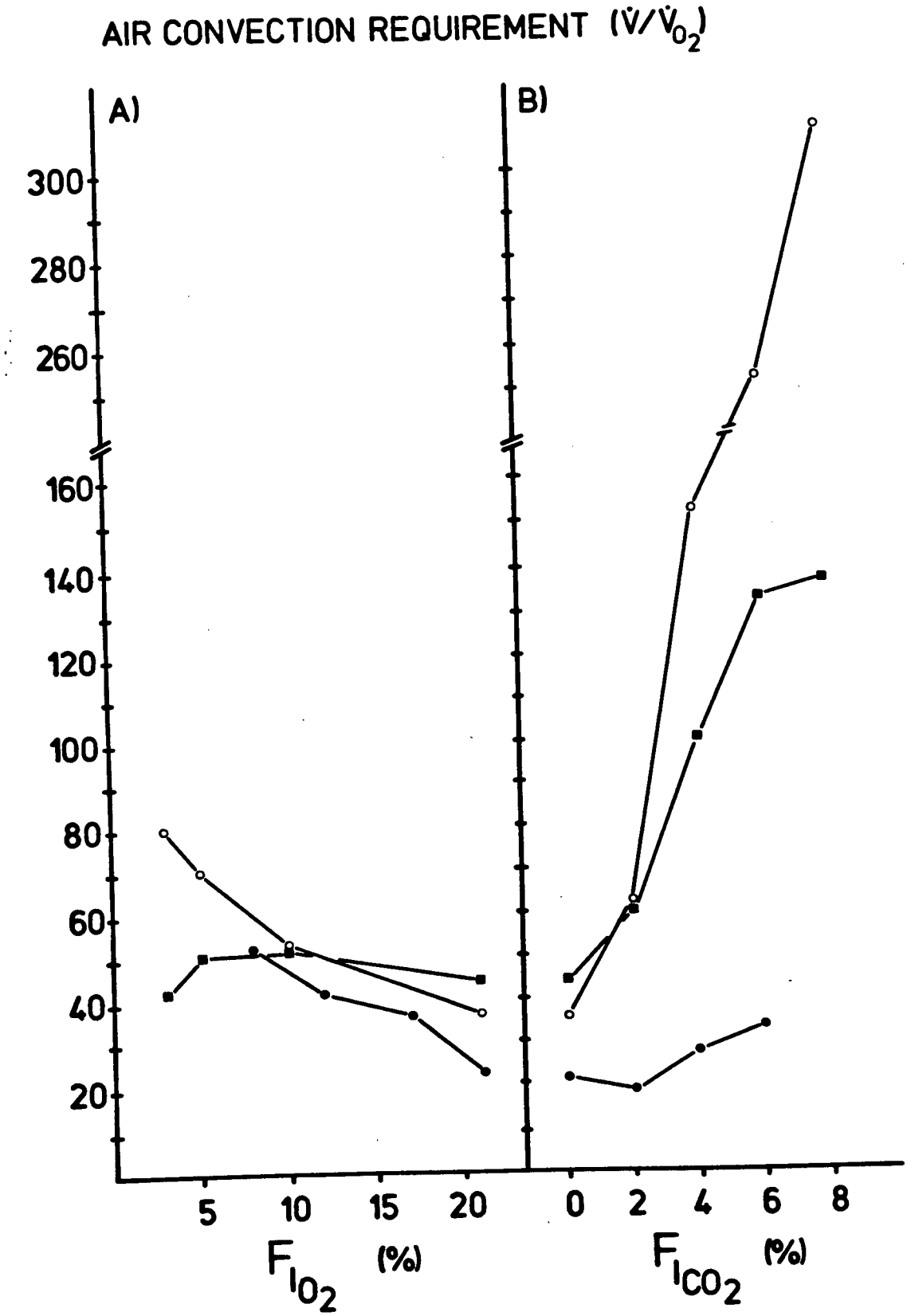
Although there is a great deal of variability in the actual magnitude of the hypercapnic response in

hibernating animals, it is evident that hypercapnic sensitivity is high relative to hypoxic sensitivity. Observations of a high CO_2 sensitivity imply that the length of the breath hold period may be related to levels of either arterial P_{CO2} or pH (McArthur, 1986; Steffen and Riedesel, 1984; Tahti, 1982). It has been suggested that ventilation in burst breathing animals is initiated by a rise in P_{aCO2} or fall in pH beyond a critical or threshold level of P_{aCO2} or pH. Increases in the inspired CO_2 fraction alter P_{aCO2} or pH such that the threshold for respiration is reached more quickly over the breath hold period and T_{NVP} becomes shorter (McArthur, 1986). Therefore, during hibernation PCO_2 or pH act as the primary chemical stimulus in controlling breathing (McArthur, 1986; Tahti, 1982) and specifically breath hold duration.

GENERAL DISCUSSION

The chemical control of breathing in awake S. lateralis appears to be fundamentally different from the chemical control of breathing during hibernation. Figure 34 illustrates the difference in respiratory sensitivities to hypoxia and hypercapnia in the two states. Air convection requirement takes into account the differences in metabolic rate between hibernation and euthermia, and therefore can be used to compare ventilatory responses in the two states. Air convection requirements represents the number of mls. of

Figure 34. Comparison of the effects of decreasing F_{IO2} (A) and increasing F_{ICO2} (B) on air convection requirement ($\dot{V}/\dot{V}_{O2}$) in awake S. lateralis (●), and during burst breathing in S. lateralis (○) hibernating at T_c of about 7°C and during single breath breathing in S. lateralis (■) hibernating at a T_c of about 2°C.


AIR CONVECTION REQUIREMENT ($\dot{V}/\dot{V}_{O_2}$)
A)
B)
300
280
260
160
140
120
100
80
60
40
20
5
10
15
20
0
2
4
6
8
F_{IO_2} (%)
F_{ICO_2} (%)

air which are moved per ml. of O_2 which is consumed.

Awake euthermic S. lateralis show a strong hypoxic ventilatory response and a comparatively blunted hypercapnic ventilatory response (Figure 34). As with most hibernating species, S. lateralis are also semi-fossorial and normally live in chronic hypoxic and hypercapnic conditions (Boggs et al., 1984). Many of the ventilatory adaptations observed in fossorial species appear to minimize respiratory work while ensuring adequate delivery of O_2 to the tissues (Boggs et al., 1984). Resting ventilation is low compared to predicted values based on body weight (Boggs et al., 1984). The relatively high hypoxic sensitivity ensures that under hypoxic conditions there is adequate pulmonary ventilation. Reduced sensitivity to CO_2, on the other hand, may be a means to reduce the work of breathing since in an environment of high CO_2 increases in ventilation would not serve to reduce CO_2 in the blood much further (Boggs et al., 1982). The relatively low frequency to tidal volume relationship may enhance alveolar ventilation and makes O_2 loading and CO_2 unloading in the lungs more effective. This adjustment may also serve to decrease ventilatory sensitivity to increases in inspired CO_2. In addition, reduced sensitivity to CO_2 may be due to an increase in the levels of buffer base in the body (Tenney, 1954), or it may be an adaptation at the cellular level in the central chemoreceptors (Darden, 1972; Tenney and Boggs, 1986).

Chronic exposure to hypoxia and hypercapnia does not alter the CO_2 sensitivity of golden-mantled ground squirrels. This suggests that the reduced responsiveness to hypercapnia involves long term adaptations or genetic adaptations through modifications to the buffering capacities of the blood and renal system, the efficiency of alveolar ventilation under resting conditions, or the chemoreceptor characteristics. Carotid body chemoreceptors in several mammals contribute up to 50% to the acute hypercapnic response. The observation that in S. lateralis peripheral chemoreceptors do not contribute to the hypercapnic response suggests a possible mechanism for the decrease in overall hypercapnic sensitivity.

Chronic exposure to hypoxia and hypercapnia does result in modification of the frequency and tidal volume components of respiration which may further increase O_2 loading and CO_2 unloading in the lungs under borrow gas conditions as described above.

Although peripheral chemoreceptors do play an excitatory role during resting ventilation and during moderate levels of hypoxia, they are not necessary for the production of a hypoxic hyperventilation. Hypoxic ventilatory responses, mediated solely by increases in frequency, can be produced centrally in S. lateralis. Only a limited number of studies have reported central excitation

in response to hypoxia. Tenney and Miller (1975) suggest that the site responsible for hypoxic sensitivity may not be in the brain stem region but rather in a higher brain centre such as the diencephalon. It is unknown if low peripheral chemosensitivity and high central hypoxic sensitivity are common to other fossorial species. It is also unclear what the adaptive significance of adjustments in the relative roles of peripheral and central chemoreceptor sensitivities compared to non-fossorial mammals may be.

As S. lateralis enter hibernation at 5°C continuous breathing is converted into a periodic pattern. The reduced ventilation during hibernation does not imply a loss of ventilatory control as suggested by Hammel et al. (1968) and Lyman (1972). Figure 34 illustrates that ventilatory sensitivity to hypercapnia is at least 3 times that of the awake animal.

The hypoxic sensitivity during hibernation at a T_b of 8°C is similar to that of the awake animal once reductions in metabolic rate are accounted for (Figure 34). This implies that decreased tissue demand for O_2 may be the major factor in the high hypoxic tolerance typical of hibernating species. The decrease in hypoxic sensitivity in ground squirrels hibernating at 2°C could result from a further left shift in the HbO_2 dissociation curve. Alternatively, if hypoxic chemosensitive areas are located

in higher brain centres, such as the diencephalon, it is possible that nerve transmission in or from these areas is temperature sensitive. Therefore, as in the production of a burst breathing pattern, decreased body temperature may block input from these areas. It is interesting to note that in euthermic animals the centrally produced hypoxic hyperventilation reported by several authors can be abolished by anesthetics (Moyer and Beecher, 1942; Miller and Tenney, 1975). It is unclear whether the site of central hypoxic sensitivity in the hibernating animal is similar to that seen in the euthermic animal.

The relative hypercapnic response is much greater during hibernation than in euthermia (Figure 34). The levels of CO_2 reported for burrows during the summer period, about 3% to 4% CO_2, would not stimulate respiration significantly in the euthermic animal. Levels of 3% to 4% CO_2 during hibernation would dramatically increase respiration. In the winter, when metabolic rate is extremely low, however, several studies report that burrow conditions are no longer hypoxic and hypercapnic except during periods of arousal and euthermia (Kuhen, 1986; Williams and Rausch, 1973). Thus, the relatively high CO_2 sensitivity during hibernation would not result in elevated ventilation.

Current data suggests that CO_2 is important in ventilatory control and that flucuations in P_{CO2} or pH may determine the length of the breath hold period in burst breathing animals (McArthur, 1986; Steffen and Riesedel 1984; Tahti, 1982). It is difficult to apply this mechanism of ventilatory control to the single breath breathing. During burst breathing the breath hold period is, on average, 10 minutes long. During this time even at low metabolic rates arterial P_{CO2} could change enough to initiate respiration. During single breath breathing in S. lateralis breath hold periods are only about 40 seconds long. This breath hold period is not long enough to allow substantial changes in P_{CO2}. Undoubtedly either the threshold which intiates respiration or the stimulus which initiates respiration has changed at the lower temperatures. It is evident that long term overall minute ventilation is maintained constant despite a considerable change in ventilatory pattern. What the mechanism for such precise ventilatory control is remains unknown, although it is evidently centered in the CNS.

Whether there is an adaptive significance to the two observed respiratory patterns during hibernation is also unknown. Burrow or hibernaculum temperatures have been reported to reach levels far below those used in most laboratories (Maclean, 1981; Williams and Rausch, 1973). Hibernating S. lateralis could therefore be exposed to

temperatures which would produce single breath breathing.

It is evident that the respiratory patterns observed during hibernation are produced in part, by central mechanisms. The burst breathing pattern may involve influences of higher CNS areas on the basic respiratory pattern produced by the brain stem, and the transition between the two patterns involves the direct effects of temperature on neural transmission in the CNS.

Studies involving selective CNS cooling during hibernation are required to clarify the role of the CNS in pattern generation during hibernation. The contribution of pulmonary information and the effects of decreases in body temperature on pulmonary mechanics and the energetic cost of breathing in both burst breathing and single breath breathing may help to assess the adaptive importance of burst breathing during hibernation.

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