INTERACTIVE EFFECTS OF HYPOXIA AND COCAINE TREATMENT ON VENTILATORY CHEMOREFLEXES AND LOCOMOTOR SENSITISATION

by

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Abstract

This study investigated two hypotheses. First, that chronic cocaine treatment would mimic the changes in breathing that are associated with ventilatory acclimatisation to chronic hypoxia (VAH). Second, that pre-treatment with a hypoxic stressor would bring about cross-sensitisation to cocaine. To address the first hypothesis, rats were exposed to either chronically hypoxic or chronically normoxic conditions and treated with either cocaine or saline for a 14 day period. Following this period, acute breathing trials were performed to measure resting ventilation and ventilatory chemoreflexes. The results demonstrated that chronic cocaine treatment did not induce the changes in breathing associated with VAH. To address the second hypothesis rats were exposed to a hypoxic stressor for 10 days (either intermittent hypoxia or chronic hypoxia) after which cocaine sensitisation was measured via locomotor sensitisation trials. The results demonstrated that cross-sensitisation between a hypoxic stress and cocaine was observed for intermittent but not chronic hypoxia.
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List of Abbreviations

ACTH: adrenocorticotropic hormone
AMPA: alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA: analysis of variance
CC: cocaine control group
CCH: cocaine chronic hypoxia group
CH: chronic hypoxia group
CNS: central nervous system
CRF: corticotropin-releasing factor
CSF: cerebral spinal fluid
D_{2}-R: dopamine 2 receptor
DA: dopamine
DRG: dorsal respiratory group
f\text{fr}: breathing frequency
GABA: gamma-aminobutyric acid
HCVR: hypercapnic ventilatory response
HPA: hypothalamic-pituitary-adrenal
HVR: hypoxic ventilatory response
i.c.v.: intracerebroventricular
i.p.: interperitoneal
IH: intermittent hypoxia group
LTF: long term facilitation
MK-801: dizocilpine maleate

mPFC: medial prefrontal cortex

mRNA: messenger ribonucleic acid

NAcc: nucleus accumbens

NMDA: N-methyl-D-aspartate

No CH: no chronic hypoxia control group

No IH: no intermittent hypoxia control group

NTS: nucleus of the solitary tract

PCPA: parachlorophenylalanine

PaO₂: partial pressure of oxygen in arterial blood

PiCO₂: partial pressure of inspired carbon dioxide

PiO₂: partial pressure of inspired oxygen

PRG: pontine respiratory group

PSR: pulmonary stretch receptors

PVN: paraventricular nucleus

s.c.: subcutaneous

SC: saline control group

SCH: saline chronic hypoxia group

SEM: standard error of the mean

TE: expiratory time

TI: inspiratory time

TTOT: total breath time

VAH: ventilatory acclimatisation to hypoxia
VI: minute ventilation
VRG: ventral respiratory group
VT: tidal volume
VT/Ti: ventilatory drive
VTA: ventral tegmental area
WGA-HRP: wheat germ agglutinin - horseradish peroxidase
Chapter 1

Introduction
1. Overview of the Thesis

Cocaine is a psychostimulant drug that serves as a potent reuptake inhibitor of dopamine, serotonin and noradrenaline. By blocking monoamine reuptake, the drug increases the concentration of transmitters in the synaptic cleft available for receptor binding (Moore et al., 1977). The powerful reinforcing effects of cocaine have been specifically attributed to its effects on the inhibition of dopamine reuptake within the brain’s reward pathway, the so-called mesocorticolimbic dopamine system (this pathway will be described below) (Kalivas and Stewart, 1991; Vanderschuren and Kalivas, 2000; Steketee, 2003). The effects of the drug on various other cognitive and basic biological functions, including induction of anxiety (Goeders, 1992), alterations in appetite (Orford, 2001), sleep (Morgan and Malison, 2007), and cardiovascular function (Billman, 1990), have been attributed to its facilitation of activity within numerous other neurotransmitter systems (e.g., serotonin, noradrenaline, glutamate, gamma-aminobutyric acid, acetylcholine, and a variety of neuropeptides).

Cocaine can exert profound effects on virtually all biological functions via its widespread actions within the central nervous system (CNS), not the least being control of breathing. Indeed, cocaine acts acutely to induce rapid, shallow breathing, or tachypnea, via excitation of the respiratory centres of the brainstem (Harper, 1991). Moreover, these effects on respiration are known to persist, and may even escalate, with chronic exposure to the drug.

Given its pervasive effects within the CNS, and ability to mobilise stress-related neurotransmitter systems, it is not surprising that repeated exposure to cocaine is associated with long-lasting changes in the response of the CNS to various forms of stress.
(physical, environmental, pharmacological) and, conversely, that repeated exposure to stress influences subsequent responses to cocaine (MacLennan and Maier, 1983). As such, studies of the short- and long-term interactive effects of cocaine and stress on CNS function have been the focus of considerable attention.

Given the acute effects of cocaine on respiratory function and its known effects on responses to various forms of stress, it is of interest to consider how exposure to hypoxia (i.e., low levels of O₂) might interact with cocaine to produce changes in respiratory or behavioral responses to the drug. Indeed, hypoxia produces profound alterations in breathing (Bisgard and Neubauer, 1995; Powell et al., 1998) and, by producing such internal disruptions in homeostasis, serves as a potent form of stress to the organism.

The primary objective of the present work was to explore the effects of different schedules of exposure to chronic hypoxia and cocaine on subsequent ventilatory chemoreflexes. Given this primary focus, this chapter will begin with a comprehensive review of the structures, transmitters, cell types, receptors and mechanisms involved in the control of breathing. Subsequently, the rationale for the central experiment of the thesis, that being to examine the interactive effects of chronic cocaine and hypoxia on the regulation of breathing (Experiment 1), will be developed. Finally, the rationale for a secondary objective of the thesis, that being to study the long-lasting effects of different schedules of exposure to chronic hypoxia on a behavioral response to cocaine, will be developed.

2. Voluntary and Involuntary Control of Breathing
The main muscles (i.e., the diaphragm, intercostal muscles and muscles of the upper airway) involved in breathing are under both voluntary and involuntary control. Voluntary control occurs mostly within the motor and pre-motor cortices (Mitchell and Berger, 1975). Voluntary control of breathing is necessary for functions such as speech, breath holding and swallowing food (Butler, 2007). Clinical cases where either autonomic control is lost, such as Ondine’s curse (Severinghaus and Mitchell, 1962), or voluntary control, such as the “locked-in syndrome” (Plum and Posner, 1972), illustrate that autonomic and voluntary control over breathing are in fact controlled via independent pathways. Voluntary breathing is proposed to initiate within the motor cortex and output from this region of the brain effects the respiratory muscles via a direct corticospinal pathway. Evidence of this direct pathway has been observed in the cat brain where direct connections between motor cortical pyramidal cells and thoracic motoneurons exist (Rikard-Bell et al., 1985). Evidence for a direct pathway in humans has been based on the comparison of conduction time. Specifically, the conduction time from the motor cortex to respiratory motoneurons has been shown to be similar to the conduction time, from the cortex, to motor neurons for proximal muscles that are known to be controlled via a direct corticospinal pathway (Gandevia and Rothwell, 1987).

Conversely, the involuntary control of breathing is a much more complex system, encompassing a large network of CNS structures including the pre-Bötzinger Complex, the ventral respiratory group (VRG), the dorsal respiratory group (DRG), the pontine respiratory group (PRG), and the nucleus of the solitary tract (nucleus tractus solitarius; NTS) (Mitchell and Berger, 1975; Butler, 2007).
3. The Central Control of Breathing

3A. The pre-Bötzinger Complex

The site of respiratory rhythm generation is postulated to be immediately rostral to the VRG in the ventrolateral medullary region known as the pre-Bötzinger complex (Smith et al., 1991). Within the pre-Bötzinger complex there are a group of neurons which are considered to form the “pacemaker kernel” which display the spontaneous burst activity that is believed to be the root of the respiratory rhythm (Rekling and Feldman, 1998). Evidence for the pre-Bötzinger Complex’s role as the respiratory rhythm generator comes from in vitro studies that analysed excitatory neurotransmitter activity in rat medullary slices. Specifically, Funk et al. (1993) illustrated that the reciprocal inhibitory connections which produce a rhythmic output are dependent on endogenously released excitatory neurotransmitters acting at non-NMDA glutamatergic receptors within the pre-Bötzinger complex. In vivo studies on rats have shown that the breathing rhythm is generated by neurokinin-1 receptor-expressing neurons in the pre-Bötzinger complex (Gray et al., 2001). In that study, the bilateral destruction of these neurons (by treatment with the neurokinin, Substance P, conjugated to a toxin, saporin), resulted in an ataxic breathing pattern although it did not lead to complete apnoea (Gray et al., 2001).

More recently, a group-pacemaker hypothesis has been proposed which suggests that the respiratory rhythm is produced via an inspiratory oscillator, located in the pre-Bötzinger complex, and an expiratory oscillator, located just rostral in the area of the retrotrapezoid nucleus (Feldman and Del Negro, 2006).

3B. The Ventral and Dorsal Respiratory Groups (VRG and DRG)
The VRG is a group of nuclei (*nucleus ambiguous; nucleus retroambiguous;* Bötzinger Complex) which extend along the ventral rostro-caudal axis, forming a distributed network which interconnects many of the neurons involved in respiration (Richter and Spyer, 2001). Different regions (neurons) in the VRG are active during both inspiration and expiration, specifically playing an important role in the initiation of inspiration (Richter and Spyer, 2001). In contrast, a similar network-like structure, the dorsal respiratory group located within the dorsomedial medulla is composed of inspiratory neurons and has not been implicated in expiration (Oku et al., 1992).

3C. The Pontine Respiratory Group (PRG)

The pontine respiratory group (PRG) is composed of nuclei found within the dorsolateral pons (the parabrachial nucleus and the Kölliker-Fuse nucleus) and has been shown to play an important role in the termination of inspiration (Feldman, 1986). The PRG has many afferent projections to the VRG, specifically the Bötzinger complex and rostral VRG. These connections have been demonstrated in rats with retrograde tracing using an injection of WGA-HRP (wheat germ agglutinin - horseradish peroxidase) (Kalia, 1977; Zheng et al., 1998). Anterograde tracing from the PRG with WGA-HRP has uncovered projections to respiratory-related sub-nuclei of the NTS (Herbert et al., 1990; Krukoff et al., 1993). Finally, efferent projections from the Kölliker-Fuse nucleus to inspiratory neurons in the C5 ventral horn have also been shown to exist in cats (Song and Aoki, 2001). Although normally inactive during quiet breathing, the PRG neurons, when activated, act by exciting inspiratory off-switch neurons that terminate inspiration via inhibition of the DRG inspiratory neurons (Cohen, 1979). Breathing frequency can be
modulated by the PRG via its control over inspiratory termination. However, the PRG has been shown to play a redundant role in inspiratory termination for it is not ordinarily active during normal breathing; it acts as a fail-safe mechanism becoming critical only after the breakdown of vagal volume feedback (described below) (Song and Poon, 2004).

3D. The Nucleus of the Solitary Tract (NTS)

The nucleus of the solitary tract (nucleus tractus solitarius; NTS) is a collection of sub-nuclei (i.e., central subnucleus, commissural subnucleus, parvicellular subnucleus etc.) situated within the dorsomedial medulla at the level of the obex spanning both the medulla and spinal cord (Paxinos and Watson, 1998). Overall, the NTS is sub-divided into the caudal and rostral NTS, named for their obvious relative orientations as well as the different afferent fibres that project to each region. It is one of the main sites within the brainstem that regulates the hypoxic ventilatory response (HVR; i.e., the increase in breathing during acute exposure to hypoxia) (Dempsey and Forrester, 1982; Powell, et al., 1998). The NTS is a highly connected region of the brainstem; of chief importance to respiration are the afferent projections from the carotid body chemoreceptors (Finlay and Katz, 1992) and the pulmonary stretch receptors (Bonham et al., 1993). As described below, the carotid body is responsible for measuring arterial blood O2 levels. The NTS receives afferent input from the carotid body chemoafferent projections via the carotid sinus nerve which meets the glossopharyngeal nerve and continues to the NTS (Housley and Sinclair, 1988). These afferent fibres, which release glutamate as their primary neurotransmitter, have their first synapse within the caudal NTS and, to a lesser extent, within the ventrolateral medulla. It is the input from these afferent fibres, and the
integration of this input within the NTS, that plays an important role in the HVR. The NTS’s efferent connections enable it to exert control over ventilation via bulbospinal projections which innervate the phrenic motor neurons (Dobbins and Feldman, 1994) that, in turn, drive the major respiratory muscle in mammals, the diaphragm. The NTS also contains projections to other brainstem respiratory centres, such as the propriobulbar (premotor) neurons that project to the VRG, which allows it to exert influence over breathing pattern formation (Carroll et al., 1996).

4. Pulmonary Stretch Receptors

The pulmonary stretch receptors (PSR) are mechanoreceptors located in the walls of the lungs which provide integral sensory information about the degree of lung inflation to the respiratory centres of the brain, such as the NTS (Bonham et al., 1993). As the lungs inflate, PSR’s increase their rate of firing and send increased levels of afferent input to the brain. This signal prevents over-inflation of the lungs by initiating the Hering-Breuer inspiratory termination reflex (Breuer, 1868a, b; Bianchi and Barillot, 1975). PSR’s initiate the Hering-Breuer reflex via projections through the vagus nerves to both the apneustic centre of the pons and the NTS where they cause the inhibition of inspiratory neurons thus inhibiting inspiration and allowing expiration to occur (Bianchi and Barillot, 1975).

5. Chemoreceptors

The respiratory centres of the brain require constant input that informs them of the levels of CO₂ and O₂ in the blood as well as the levels of CO₂ (and pH) in the cerebral
spinal fluid (CSF). This task is performed by peripheral (arterial) (Heymans et al., 1930; Gonzalez et al., 1995) and central (brain) chemoreceptors (Nattie, 1999). Some chemoreceptors sense the partial pressure of either O₂ or CO₂ but others (i.e., the central chemoreceptors) indirectly measure CO₂ by monitoring pH (Gonzalez et al., 1995). Carbon dioxide levels within the blood or CSF can be inferred from pH because an equilibrium exists within the blood and CSF according to the equation, CO₂ + H₂O ⇌ H⁺ + HCO₃⁻. The hydration of CO₂ to a proton and bicarbonate ion (and the reverse reaction depending on levels of the various constituents within the equation) is catalysed by the enzyme carbonic anhydrase which is located in the red blood cells as well as in central chemoreceptor cells and on the capillary walls within the brain. Therefore, an increase in CO₂ levels usually produces a drop in pH (although this will depend on buffering within any individual compartment).

5A. Peripheral Chemoreceptors

There are two separate sets of peripheral (arterial) chemoreceptors, one located within the carotid bodies and the second inside the aortic arch (Gonzalez et al., 1995). Of these two sets, the carotid body chemoreceptors are more sensitive to low oxygen levels in the arterial blood and show a greater response (i.e., action potential firing) under hypoxic conditions. These chemoreceptors are also stimulated by high levels of CO₂ in the blood. The carotid bodies are composed of two different cell types; Type I glomus cells and Type II sustentacular cells. Only the glomus cells possess the ability to sense the partial pressure of O₂ and they are the primary site of O₂ chemoreception in the mammalian body (Gonzalez et al., 1995; Lahiri et al., 2006), while the sustentacular cells
play a supportive role similar to glial cells in the CNS (Eyzaguirre and Zapata, 1984). Carotid body chemoafferents project via the carotid sinus nerve which meets the glossopharyngeal nerve and continues to the NTS (Housley and Sinclair, 1988). Two main mechanisms have been suggested which allow the glomus cells of the carotid body to sense the level of $O_2$ in the arterial blood. These are the mitochondrial mechanism and the heme protein mechanism.

The mitochondrial mechanism is based on the fact that during the metabolic process of oxidative phosphorylation, which occurs within the mitochondria, oxygen is required as the final electron acceptor. The “Pasteur effect” states that when oxygen is unavailable, cells must switch from oxidative phosphorylation to solely glycolysis in order to produce energy. This results in several changes within the cell. The hypotheses that have been proposed regarding how mitochondrial $O_2$ sensing occurs suggest that, during hypoxia, a change in cytosolic redox state, the production of reactive $O_2$ species or the change in energy state initiate a signal transduction pathway which signals a change in $[O_2]$ (Ward, 2008). Ultimately, the signal transduction pathway would lead to an elevation of cytosolic calcium levels which, in turn, trigger neurotransmitter release.

The heme protein mechanism postulates that the $O_2$ sensor is based on a heme protein that exists in two readily interconvertable states. When the $[O_2]$ is low, $O_2$ dissociates from the heme protein producing an increase in the deoxy conformation which then activates effectors either directly or through a signaling cascade (Ward, 2008). The most attractive hypothesis suggests that the dissociation of $O_2$ from a plasma membrane-bound heme protein causes the closure of $K^+$ channels within the plasma membrane. This leads to an increase in intracellular $K^+$, plasma membrane depolarisation,
the opening of voltage-dependent Ca\(^{2+}\) channels and, ultimately, the release of neurotransmitters.

5B. Central Chemoreceptors

The central pH/CO\(_2\)-sensitive chemoreceptors are located within the CNS primarily within the ventrolateral medulla although many regions of the brainstem are now suspected of having respiratory-related chemosensitivity (Kawai et al., 1996). These chemoreceptors are located on the ventral surface of the medulla where they are immersed in the CSF and they continually sample the CSF in order to detect changes in pH and [CO\(_2\)] (Lipscomb and Boyarsky, 1972). More recently, other putative central respiratory-related chemosensitive sites have been discovered including the retrotrapezoid nucleus (Li et al., 1999), the NTS and surrounding area (Coates et al., 1993) and the locus coeruleus and surrounding area (Coates et al., 1993).

Unlike the peripheral chemoreceptors in the carotid body or aortic arch, the central chemoreceptors are unable to sense the O\(_2\) level of the CSF. There are, however, possible central O\(_2\) chemoreceptors within the pre-Bötzing complex that may help to regulate metabolic rate (Solomon et al., 2000).

Carbonic anhydrase, which is present in the chemoreceptor cells and on the walls of brain capillaries, allows for CO\(_2\), H\(^+\) and HCO\(_3^-\), to quickly reach equilibrium according to the CO\(_2\) hydration-dehydration equation listed above (Nattie, 1995). Central chemoreceptors exert their greatest influence on breathing when an animal is exposed to high levels of inspired CO\(_2\), when CO\(_2\) production is increased via changes in metabolic rate or by a reduction in gas exchange (i.e., if lung function is compromised by disease or
if breathing is reduced/absent during sleep). Central chemoreceptors are thought to act via the following putative mechanism. Inspired and metabolic waste CO₂ diffuses from blood to CSF and then into chemoreceptor cells. It reaches an equilibrium with H⁺ and HCO₃⁻ as described above. A membrane Na⁺/H⁺ exchanger than removes the H⁺ from the cell while bringing in Na⁺. The Na⁺ is then removed via a Na⁺/Ca²⁺ exchanger which brings Ca²⁺ into the cell. The buildup of intracellular calcium then triggers neurotransmitter release (Lahiri et al., 2003). This release of neurotransmitters then triggers an appropriate increase in breathing termed the hypercapnic ventilatory response (HCVR) (Dempsey and Forrester, 1982). Also a decrease in blood [CO₂] can lead to a decrease in breathing via the same pathway.

6. Acute Exposure to Hypoxia or Hypercapnia

When mammals encounter a hypoxic (i.e., low O₂) environment (e.g., at high altitudes) they breathe in air with a decreased partial pressure of inspired oxygen (PIO₂). Depending upon the severity of the hypoxia, this may also lead to arterial hypoxaemia (a decrease in the concentration of O₂ in the blood or a decrease in the saturation of haemoglobin with oxygen). The PaO₂ of mammalian arterial blood at sea level, under normoxic conditions, is approximately 100 mmHg. When PaO₂ falls below this level, there is an increase in breathing (frequency and/or tidal volume) (Bisgard and Neubauer, 1995; Powell et al., 1998). Breathing frequency is defined as the number of breaths taken per unit time (usually minutes) while tidal volume is the volume of an individual breath (usually expressed in ml of inspired gas per kg body weight). This increase in breathing is
called the acute hypoxic ventilatory response (HVR) and can occur within 1-2 inspirations of hypoxic gas (Powell et al., 1998).

Acute exposure to a hypercapnic (i.e., high CO₂) gas mixture also elicits an increase in ventilation termed the hypercapnic ventilatory response (Rigatto, 1977; Dempsey and Forster, 1982; Klein et al., 2002). In air-breathing mammals, hypercapnia is generally a more potent respiratory stimulus than hypoxia. The increase in partial pressure of inspired carbon dioxide (PICO₂) leads to an increased arterial PCO₂ which produces an increase in [CO₂] in the CSF. This stimulates the central chemoreceptors via the mechanism described above which, in turn, leads to the increase in breathing (Loeschcke, 1982; Millhorn and Eldridge, 1986).

7. Ventilatory Acclimatisation to Hypoxia (VAH)

7A. Changes in Breathing Associated with VAH

Chronic exposure to hypoxic conditions leads to a slowly developing, but long lasting, response termed ventilatory acclimatisation to hypoxia (VAH) (Bisgard and Neubauer, 1995; Powell et al., 1998). VAH is the time-dependant increase in ventilation that is fully developed after approximately nine days of hypoxia in both rats and humans (Dwinell and Powell, 1999). The changes associated with VAH persist for a number of weeks following a return to normoxic conditions and represent changes to respiratory control systems (see below). Three characteristic changes occur with the manifestation of VAH. First, there is an increase in overall levels of ventilation during normoxia (i.e., under resting conditions). This phenomenon is sometimes referred to as an offset in breathing and leads to a decreased arterial partial pressure of CO₂ because the
hyperventilation (i.e., the increase in breathing) hastens the removal of CO$_2$. Second, there is an enhanced hypoxic ventilatory response (HVR). In other words, the magnitude of the acute HVR is greater when animals (or humans) are exposed to subsequent bouts of acute hypoxia following the development of VAH. This is sometimes referred to as an increase in the gain of the HVR. However, this increase in gain usually only occurs under isocapnic conditions when arterial PCO$_2$ levels are experimentally maintained at normal levels rather than being allowed to fall due to hyperventilation. Under poikilocapnic conditions (when the increased breathing leads to a reduction in arterial PCO$_2$), the increase in the magnitude of the HVR is rarely observed. Third, there is a decrease in the CO$_2$ threshold for breathing (Duffin and Mahamed, 2003). In other words, there is a reduction in the level of CO$_2$ in the blood that is needed to turn breathing on or off (i.e., the threshold is the arterial PCO$_2$ level below which breathing stops and above which breathing occurs).

7B. Mechanisms of Ventilatory Acclimatisation to Hypoxia

Two main mechanisms have been shown to underlie the changes in breathing that occur with VAH. The first is an increase in the sensitivity of the carotid body O$_2$ chemoreceptors (i.e., glomus cells) to low levels of O$_2$ in the arterial blood. This means that for any given level of arterial PO$_2$, the level of afferent input from the carotid body, to the respiratory centres in the CNS (i.e., the NTS), is greater in animals that have undergone VAH compared to control animals kept under normoxic conditions (Bisgard, 2000). There are two main possible mechanisms that have been proposed to explain the increase in the sensitivity of the carotid body to hypoxia. First, there is an increase in
endothelin activity within the carotid body. When exogenously applied, endothelin has been shown to increase the afferent output from the carotid body (McQueen et al., 1995). Chronic hypoxia has been shown to produce a significant increase in endothelin (He et al., 1996) and an upregulation of endothelin receptor mRNA (Chen et al., 2002) within O2 sensing glomus cell of the carotid body. Therefore, endothelin could be responsible for the increased sensitivity of the carotid body to hypoxic stimulation following VAH. The other mechanism that has been proposed to explain the increase in the sensitivity of the carotid body to hypoxia is an increase in ion conductance within the glomus cells. Glomus cells exposed to chronic hypoxia have been shown to be more easily depolarized, and this has been postulated to be due to increased Na+ conductance (Hempleman et al., 1995). This increase in ion conductance, with chronic hypoxia, may lead to a greater sensitivity to hypoxia in these O2 sensing cells after VAH.

The second mechanism (the first being the change in carotid body chemosensitivity) that has been proposed to lead to VAH is an increase in the responsiveness of the CNS to the afferent input that it receives from the carotid body chemoreceptors (Dempsey and Forester, 1982; Dwinnel and Powell, 1999). Although this mechanism was initially proposed by Dempsey and Forester (1982), it was only definitively demonstrated by relatively recent experiments by Dwinnel and Powell (1999). These authors (Dwinnel and Powell, 1999) recorded phrenic nerve activity from an anesthetised, artificially-ventilated rat preparation. Given that the phrenic nerve drives the diaphragm, motor output from this nerve serves as an index of breathing. In those experiments they also severed the carotid body from the carotid sinus nerve. Electrical stimulation of the carotid sinus nerve served as mock afferent input from the carotid
body. For any given level of electrical carotid sinus nerve stimulation, phrenic nerve output (frequency and amplitude) was greater in those rats that had been exposed to chronic hypoxia compared to control normoxic rats. Given that the carotid body was not providing input the CNS, these experiments demonstrated that the afferent input from the carotid sinus nerve was being integrated in a different manner, by the CNS, following VAH (Dwinell and Powell, 1999).

It has been proposed (Reid and Powell, 2005; Chung et al., 2006), that the increased sensitivity of the CNS to afferent input from the carotid body (or carotid sinus nerve under electrical stimulation), during VAH, is due to changes in neurotransmitter function/action within the NTS (Dwinnel et al., 2000; Reid and Powell, 2005; Chung et al., 2006) since the NTS is the primary site of synapse of the carotid body afferents (Housley and Sinclair, 1988; Finlay and Katz, 1992). Potential changes in neurotransmitter systems during VAH will be discussed below.

8. Neurotransmitters and Breathing

A number of neurotransmitters within the NTS have been shown to be important in the control of breathing, however, research into VAH has focused mainly on the glutamatergic (Mizusawa et al., 1994; Reid and Powell, unpublished) and GABAergic systems (Tolstykh et al., 2004; Chung et al., 2006; Reid et al., unpublished) and, to a lesser extent, the catecholamines, mainly dopamine (Huey et al., 2003).

8A. Glutamate
Glutamate receptors have been localised in the medullary respiratory centres (Monaghan and Cotman, 1985) and administration of exogenous glutamate to this region produces an increase in tidal volume (Gatti et al., 1985). If degradation of glutamate is pharmacologically blocked, minute ventilation (i.e., overall breathing) increases, suggesting that endogenous glutamate plays a role in increasing breathing. When peripheral chemosensitive fibers from the carotid body are stimulated, they cause the release of glutamate into brainstem respiratory centres (i.e., the NTS and ventrolateral medulla) leading to an increase in breathing (Housley and Sinclair, 1988). Mizusawa et al. (1994) also observed an increase in breathing in response to the in vivo release of glutamate within the caudal NTS (Mizusawa et al., 1994).

A growing body of literature suggests an important role for N-methyl-D-aspartate (NMDA)-type and non-NMDA-type (i.e., kainate and AMPA) glutamatergic receptors in the regulation of the acute hypoxic ventilatory response (HVR). For example, intraperitoneal injections of the NMDA receptor channel blocker MK-801 have been shown to reduce the HVR in awake rats, implying a role for glutamate in mediating the increase in breathing during the acute HVR (Ohtake et al., 1998). Microinjections of MK-801 directly into the caudal NTS attenuated the increase in tidal volume, but not breathing frequency, during exposure to acute hypoxia in awake rats (Mizusawa et al., 1994). Microinjection of kynurenic acid (an excitatory neurotransmitter receptor antagonist) produced an even greater decrease in tidal volume, compared to the MK-801-induced decrease, during exposure of rats to acute hypoxia (Mizusawa et al., 1994). In anesthetised rats, antagonism of both NMDA and non-NMDA glutamate receptors,
within the NTS, eliminated the ventilatory response to carotid body stimulation (Vardhan et al., 1993).

The glutamatergic system has also been implicated in ventilatory acclimatisation to hypoxia (VAH), illustrated by experiments in which chronic hypoxia augmented the effects of MK-801 on the HVR, implying that glutamate’s effect on breathing was greater following chronic hypoxia (Reid and Powell, 2005). Furthermore, the HVR has been shown to be abolished by microinjections of MK-801 directly into the NTS of chronically hypoxic, but not control, rats (Reid, unpublished). These results demonstrate that, after the formation of VAH, NMDA receptors might play a more important role in regulating the acute HVR than they did prior to exposure to chronic hypoxia.

8B. Gamma-Aminobutyric Acid (GABA)

Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian CNS. Endogenous GABA can activate three independent sets of receptors termed GABA_A, GABA_B, and GABA_C which, due to their separate mechanisms of action, can mediate different responses (Chebib and Johnston, 1999). Application of exogenous GABA to the ventral surface of the medulla produces a decrease in breathing, an effect that is reversed by the application of bicuculline (a GABA_A receptor antagonist) (Yamada et al., 1982). When aminooxyacetic acid (a blocker of GABA degradation) is administered directly into the cerebral ventricles it produces a decrease in breathing (Hedner et al., 1984). Further studies have elucidated the neuroanatomical basis of GABA’s effect on breathing, by demonstrating that GABA receptive neurons near the
ventral surface of the medulla have projections to medullary respiratory motoneurons and, when activated, decrease tidal volume (Kazemi and Hoop, 1991).

The role of GABA in the NTS, in mediating the HVR, is evident from experiments involving microinjections of muscimol, a GABA$_A$ receptor agonist (Chitravanshi et al., 1994). The micro-injections of muscimol into the NTS abolished the increase in phrenic nerve discharge (the phrenic nerve drives the diaphragm) that normally occurs in response to acute hypoxia. Furthermore, this effect was reversed by the application of bicuculline, a GABA$_A$ receptor antagonist, into the NTS.

The direct administration, into the NTS, of both GABA$_A$ and GABA$_B$ agonists, muscimol and baclofen, respectively, resulted in a reduction in the HVR in rats acclimatised to chronic hypoxia (Tabata et al., 2001). On the other hand, the application of GABA$_A$ and GABA$_B$ antagonists elicited the opposite response, i.e., an increase in the HVR, when administered under the same conditions (Tabata et al., 2001). A possible role for GABA, in the NTS, in VAH was described by Chung et al. (2006). These authors observed that exposure to chronic hypoxia changed GABA’s role in regulating resting breathing but not breathing during exposure to acute hypoxia. They observed that a microinjection, into the NTS, of the GABA$_A$ receptor antagonist, bicuculline, alone or a combination of bicuculline and CGP-35348 (a GABA$_B$ receptor antagonist) prevented the increase in resting breathing (i.e., breathing under normoxic conditions) that occurred following chronic hypoxia. On the other hand, neither bicuculline nor bicuculline/CGP-35348 affected breathing during acute exposure to hypoxia (following the long-term exposure to chronic hypoxia). Furthermore, the injection of these antagonists into the NTS only produced an effect on breathing in those animals previously exposed to chronic
hypoxia and not those that remained in a normoxic (control) environment (Chung et al., 2006).

On the other hand, a role for the GABA<sub>B</sub> receptor in VAH has been suggested by another study in which direct infusion of GABA and the GABA<sub>B</sub> receptor agonist baclofen, into the NTS continually for 9 days under normoxic conditions, resulted in an increase in both normoxic ventilation and the HVR (Reid et al., unpublished). These changes in breathing mirror those which occur after the natural formation of VAH suggesting that changes in GABA<sub>B</sub>-mediated neurotransmission are sufficient to induce VAH-like changes in breathing in the absence of chronic hypoxia.

8C. Serotonin

Serotonin has been shown to act as a ventilatory stimulant within the CNS, producing a long lasting increase in respiration during normoxia (Millhorn et al., 1980). However, blockade of serotonin production via parachlorophenylalanine (PCPA), an inhibitor of tryptophan hydroxylase (an enzyme involved in serotonin synthesis), has also been shown to produce an increase in ventilation (Olson, 1987). Serotonin receptor antagonism has been shown to produce an increase in the HVR in goats (Herman, 1998). Finally, in rats exposed to chronic hypoxia, treatment with PCPA was unable to block the formation of ventilatory acclimatisation to hypoxia (Olson, 1987). Taken together, the above results indicate that the serotonergic system may not play a role in the formation of VAH.

Intermittent exposure to hypoxia has also been shown to produce lasting changes in breathing including an increase in baseline ventilation (Ling et al., 2001) and an
increased HVR (Peng et al., 2003). One of the proposed mechanisms underlying these effects is the long term facilitation (LTF) of phrenic nerve output which has been shown to occur with intermittent hypoxia (Bach and Mitchell, 1996). Furthermore, this LTF has been shown to be dependent on the serotonergic system as it does not occur if methysergide (a serotonin receptor antagonist) is administered prior to hypoxic exposure (Bach and Mitchell, 1996). Further evidence that the serotonergic system is responsible for the LTF, associated with exposure to intermittent hypoxia, is that intermittent serotonin administration alone is also sufficient to produce phrenic nerve LTF (Lovett-Barr et al., 2006).

8D. Noradrenaline

Noradrenaline has a complex effect on breathing acting at the carotid bodies as a stimulant (Eldridge and Gill-Kunar, 1980) and within the CNS as a depressant (Champagnat et al., 1978). Noradrenaline has been implicated in having a possible role in the development of VAH as noradrenaline turnover is significantly increased in the caudal NTS after 14 days of chronic hypoxia (Schmitt et al., 1994).

8E. Dopamine

8E-1. Acute and Chronic Hypoxia’s Effects on Dopamine Levels

Acute hypoxia stimulates dopamine release from the oxygen-sensitive glomus cells of the carotid bodies (Gonzalez et al., 1995) and from the chemoafferent fibers that project from the carotid bodies into the NTS (Goiny et al., 1991). Chronic hypoxia increases extracellular dopamine levels in the carotid body (Gonzalez et al., 1995) and the
CNS (Olson et al., 1983). Chronic hypoxia has also been shown to increase the amount of tyrosine hydroxalase, the enzyme responsible for the rate-limiting step in dopamine synthesis, in both the carotid bodies (Czyzyk-Krzeska et al., 1992) and the caudal NTS (Schmitt et al., 1994).

8E-2. The Effect of Carotid Body Dopamine on Resting Breathing

Dopamine infusion into the carotid bodies decreases breathing, most likely due to dopamine acting on inhibitory D₂ autoreceptors on the glomus cells, which, in turn, decreases the output from glomus cell which leads to a decrease in breathing (Conforti et al., 1999). Carotid body D₂-R blockade renders the inhibitory D₂ autoreceptors inoperable leading to an increase in glomus cell chemoafferent output which significantly increases breathing. Based on this, it is assumed that basal levels of dopamine release within the carotid bodies produce D₂-R inhibition of carotid body output which, in turn, has an inhibitory effect on breathing (Bee and Pallot, 1995).

8E-3. Dopamine’s Ventilatory Role in the CNS

Dopamine in the CNS stimulates breathing, demonstrated by the observations that CNS D₂-R blockade with domperidone depresses normoxic ventilation and that CNS administration of apomorphine (dopamine agonist) stimulates ventilation (Hedner et al., 1982). D₂-receptors in the CNS tonically stimulate ventilation at low levels of chemoafferent input and are thought to, at least in part, facilitate the translation of increases in chemoafferent input into increases in ventilation. This is illustrated by the
observation that increases in carotid sinus nerve activity do not produce corresponding increases in ventilation if CNS D<sub>2</sub>-receptors are blocked (Dwinell et al., 2000).

8E-4. Dopamine’s Role in the HVR and VAH

A possible mechanism underlying VAH could involve decreased dopaminergic activity at D<sub>2</sub>-receptors within the carotid body, where they have an inhibitory effect on breathing. In other words, chronic hypoxia may cause a decrease in D<sub>2</sub>-receptor numbers or their efficacy; this will lead to a decrease in the inhibitory dopaminergic activity within the carotid body and, ultimately, lead to increased afferent activity which produces an increase in ventilation.

This suggestion is supported by the observation that 1 – 2 days of chronic hypoxia decreased carotid body dopaminergic inhibition of breathing in rats (Bee and Pallot, 1995). In humans however, 8 h of chronic hypoxia did not decrease dopaminergic inhibition (Pedersen et al., 1999). Therefore changes in D<sub>2</sub>-receptor gene expression may require a time course of many days of hypoxia in order to significantly decrease D<sub>2</sub>-receptor mRNA in the rat carotid body (Huey and Powell, 2000). Decreased D<sub>2</sub>-receptor mRNA means that there are less D<sub>2</sub>-receptors on the glomus cells. Therefore, when dopamine is released from the glomus cells there are less inhibitory D<sub>2</sub>-autoreceptors for it to act on resulting in a decreased level of inhibition, which leads to less of an effect on glomus cell output and ultimately breathing.

Dwinnel and Powell (2000) demonstrated that two days of chronic hypoxia decreased the CNS D<sub>2</sub>-receptor facilitation of the HVR, illustrated by the fact that CNS D<sub>2</sub>-receptor blockade did not alter the relationship between carotid sinus nerve
stimulation and phrenic nerve output after 2 days of chronic hypoxia. However the facilitation was restored after a further 6 days of chronic hypoxia. Therefore, CNS facilitation of the HVR does not contribute to VAH in the early stages of exposure to chronic hypoxia but may be involved in establishing VAH as the time-course of chronic hypoxia is increased (Dwinell et al., 2000).

Huey et al. (2000) presented data illustrating that mice genetically lacking D2-receptors do not exhibit an increase in resting ventilation or an augmentation of the HVR, characteristic signs of VAH, after 8 days of chronic hypoxia. Further studies illustrated that this effect was not due to a developmental problem but due to the fact that the D2-receptors were missing during the chronic exposure to hypoxia (Huey et al., 2003). This indicates that the D2-receptors are necessary for the proper formation of VAH in mice. However the D2-receptor deficient mice still exhibited an intact HVR and therefore D2-receptors are not necessary for the HVR (just for it’s augmentation following VAH).

8E-5. Dopamine Summary

Dopamine is release in the carotid body by glomus cells and acts via negative feedback by activating the D2 autoreceptors on the glomus cells thereby decreasing their chemoafferent output and, ultimately, breathing. In the CNS, dopamine acts as a stimulant to breathing and facilitates, via D2-receptors, the translation of increases in chemoafferent activity from the carotid body to increases in breathing. Chronic hypoxia increases dopamine within both the CNS and carotid body. A possible dopaminergic mechanism underlying VAH would be a decrease in the D2 autoreceptor activity within the carotid body and an increase in the D2-R facilitation of the HVR within the CNS.
9. Rationale for the Experiments

9A Experiment 1: An examination of the interactive effects of concurrent administration of hypoxia and cocaine on ventilatory chemoreflexes

The primary aim of this thesis was to assess the interactive effects of cocaine treatment and chronic hypoxia on ventilatory chemoreflexes and breathing. Cocaine’s effect on ventilation is complicated by the opposing effects of dopamine and noradrenaline within the CNS and the carotid body. Dopamine acts to decrease breathing within the carotid body, but increases breathing when acting in the CNS. Noradrenaline, on the other hand, has the opposite effects, acting to increase breathing when released from the carotid body (Eldridge and Gill-Kunar, 1980) but decreasing breathing via its actions in the CNS (Champagnat et al., 1978). The acute effect of cocaine on breathing is an extreme tachypnea (i.e., rapid breathing) with sporadic sustained inspiratory bouts (Harper, 1991). These stimulatory effects on breathing are caused by cocaine acting to increase dopamine and serotonin excitation of the respiratory centres in the brainstem and, more specifically, stimulate the inspiratory/expiratory phase-switching mechanisms within the PRG (Richard et al., 1991).

In contrast to the normoxic ventilatory pattern of cocaine-exposed rats, respiratory challenges such as acute hypoxia or hypercapnia, following cocaine administration, elicited a slow and deep pattern of breathing which resulted in a greater level of minute ventilation compared to that prior to cocaine exposure (Trippenbach and Kelly, 1994). Chronic cocaine administration was able to attenuate the HVR but only after at least 3 weeks of exposure, most likely due to a down-regulation of the CNS dopamine system.
which is thought to facilitate the HVR (Trippenbach and Kelly, 1994). In previous studies, the effects of cocaine on breathing have been attributed primarily to its effects on the dopaminergic system. For example, administration of the dopamine receptor antagonist, haloperidol, prior to cocaine exposure, reduced the normal stimulatory effect of cocaine on breathing (Trippenbach and Kelly, 1994).

As described in earlier sections, during exposure to chronic hypoxia, changes in neurotransmitter function within the brainstem are involved in the development of the changes in breathing associated with VAH. Furthermore, dopamine (Huey et al., 2003) and noradrenaline (Schmitt et al., 1994) have been implicated in the formation of VAH. Since cocaine acts to prevent the uptake of these neurotransmitters from the synaptic cleft (Moore et al., 1977), it is possible that exposure to chronic hypoxia (i.e., the development of VAH) may alter the effects of cocaine on resting ventilation and the acute hypoxic and hypercapnic ventilatory responses.

In a previous experiment, we studied the effects of concurrent administration of chronic cocaine and hypoxia on ventilatory chemoreflexes and breathing (Knight, Erb and Reid, unpublished). In that experiment, rats were given daily interperitoneal (i.p.) injections of cocaine (30mg/kg) for 14 days in either a normoxic (21% O₂) or a hypoxic (hypobaric hypoxia; equivalent to 10% O₂ at sea level) environment. Acute breathing trials, to measure resting ventilation and the acute hypoxic and hypercapnic ventilatory responses, were conducted after the 14-days of cocaine exposure. VAH-like changes in breathing were not observed in the group that was administered cocaine and remained in a normoxic environment. However, as seen in Figure 1A below, animals treated with cocaine, relative to those treated with saline, tended to exhibit an increase in ventilation
during both resting ventilation (21% and 30% \(O_2\)) and exposure to acute hypoxia (8% \(O_2\)). For comparison, Figure 1B illustrates breathing levels in chronically hypoxic rats treated with saline and cocaine.

Whereas there was a tendency for cocaine-treated animals maintained in a normoxic environment to show an increase in ventilation in response to acute hypoxia, these same animals exhibited a significant decrease in breathing frequency in response to acute hypercapnia (see Figure 2A). This effect of cocaine on the acute hypercapnic response, however, was abolished in animals that were maintained for the 14-day treatment period in a hypoxic environment (see Figure 2B).

Taken together, the findings from this study suggest that the effects of chronic cocaine treatment on respiratory responses to acute hypoxia or hypercapnia are, if anything, suppressed by maintenance in a hypoxic environment. Thus, the findings support the hypothesis that exposure to chronic hypoxia produces changes in neurotransmitter function that, in turn, may be involved in the formation of ventilatory acclimatisation to hypoxia (VAH).
Figure 1. Minute ventilation (ml/min/kg) during exposure to acute hypoxia in (A) control (chronically normoxic) and (B) chronically hypoxic rats treated with either saline (open circles) or cocaine (closed circles) i.p for 14 days. The data are shown as the mean ± 1 standard error of the mean (SEM). Letters (a, b and c) indicate significant differences, within any group, between different levels of inspired O2. A plus sign (+) indicates that, at any given level of O2, the value in the chronic hypoxia group is different from the corresponding value in the control group. An ampersand (&) indicates that, at any given level of O2, the value in the cocaine control (normoxia) group is different from the corresponding value in the saline chronic hypoxia group. Abbreviations: SC (saline control group), CC (cocaine control group), SCH (saline chronically hypoxic group) and CCH (cocaine chronically hypoxic group).
Figure 2. Breathing frequency (breaths/min) during exposure to acute hypercapnia in (A) control (chronically normoxic) and (B) chronically hypoxic rats treated with either saline (open circles) or cocaine (closed circles) i.p. for 14 days. The data are shown as the mean ± 1 standard error of the mean (SEM). Letters (a, b and c) indicate significant differences, within any group, between different levels of inspired O₂. A plus sign (+) indicates that, at any given level of O₂, the value in the chronic hypoxia group is different from the corresponding value in the control group. A number sign (#) indicates that, at any given level of O₂, the value in the cocaine group is different from the corresponding value in the saline group. Abbreviations: SC (saline control group), CC (cocaine control group), SCH (saline chronically hypoxic group) and CCH (cocaine chronically hypoxic group).
In the study just described, the effects of the chronic cocaine treatment on acute respiratory responses to hypoxia and hypercapnia were either non-significant (i.e., acute hypoxia and normoxia) or relatively modest (i.e., acute hypercapnia). These modest effects may be attributed to the nature of the cocaine injection protocol that we employed. More specifically, in our previous study, animals were administered cocaine once daily in a single bolus, i.p. injection. It is possible that this treatment protocol, which would induce a short-lived elevation in the blood concentration of cocaine, was not sufficient to induce the necessary changes in monoamine function required to bring about VAH-like changes in breathing (i.e., an increase in resting ventilation or an augmentation of the acute hypoxic ventilatory response).

Based on the outcome of our previous study, in the present experiment we modified the cocaine treatment protocol in an effort to maximise observing interactive effects of chronic cocaine and hypoxia on subsequent respiratory responses to acute hypoxia and hypercapnia. In this experiment, animals were maintained for the 14-day procedure in either a hypoxic or normoxic environment. However, animals assigned to the cocaine treatment group were implanted subcutaneously with mini-osmotic pumps containing cocaine that permitted constant drug infusion and stable elevations of blood concentrations of cocaine (Joyner et al., 1993). Thus, unlike in the previous study, cocaine-treated animals received continuous exposure to cocaine throughout the treatment period.

Thus, Experiment 1 was designed to test the following 3 hypotheses:

1) That continuous exposure to cocaine would induce changes in breathing characteristic of those that are observed during VAH.
2) That the failure of the intraperitoneal injections of cocaine to accomplish this in the previous study (Knight, Erb and Reid, unpublished) was the result of the treatment protocol being insufficient to raise blood (brain) cocaine levels for a long enough period of time to enact the required changes in monoamine neurotransmission.

3) That the effects of cocaine on breathing in control animals will be altered by exposure to chronic hypoxia.

9B Experiment 2: An examination of the cross-sensitising effects of hypoxia and cocaine

A secondary aim of this thesis was to assess the interactive effects of cocaine and hypoxia on a well-characterised behavioral response to cocaine; namely, the induction of locomotor activity. Whereas in Experiment 1 we studied the effects of concurrent administration of hypoxia and cocaine on respiratory responses, in this experiment we were interested in studying the effects of exposure to hypoxia on a behavioral response to cocaine that occurred after a period of time following the return of the animal to normoxic conditions. Because repeated exposures to stressors, like hypoxia, and to cocaine are associated with long-lasting neuroadaptations within common brain circuitry, it was of interest to determine whether neuroadaptations brought about as a consequence of prior exposure to hypoxia would induce changes in a well-characterised behavioral response to cocaine; namely, locomotor activity. Locomotor activity is known to be related to changes within pathways mediating the motivational and rewarding effects of psychostimulant drugs, such as cocaine. The changes in brain function that occur as a consequence of repeated exposure to cocaine, or other drugs of abuse, have been the subject of intense study. One of the best-established animal models of drug-induced
neuronal plasticity is behavioral sensitisation, a phenomenon characterised by the augmentation of behavioral responses to a drug challenge, most typically measured as a change in locomotor activity, as a result of prior, repeated administration of that drug (Robinson and Becker, 1986; Robinson and Berridge, 1993). Behavioral, or locomotor, sensitisation has been shown to persist after long periods of drug abstinence (Paulson et al., 1991) and is reliably associated with neuronal adaptations in induced neurotransmitter release (Vanderschuren and Kalivas, 2000), induced activation of second messengers and transcription factors (McClung and Nestler, 2008), and changes in cellular morphology (Robinson and Kolb, 2004; Thomas et al., 2008). Thus, it has been proposed that the processes underlying the development and expression of sensitisation represent, at least in part, a neurobiological substrate of addiction. In this light, the high propensity for relapse that characterises drug addiction may reflect the expression of a sensitisation phenomenon (Robinson and Berridge, 1993; Kalivas et al., 1998).

Research on the neurobiological underpinnings of sensitisation have, to a large extent, focused on the long-lasting changes induced by drugs that occur within a network of dopaminergic and glutamatergic interconnections between the ventral tegmental area (VTA), nucleus accumbens (NAcc) and medial prefrontal cortex (mPFC). This network of neurons comprises the so-called mesocorticolimbic system and is known to mediate the rewarding effects of psychostimulant and opiate drugs (Kalivas and Stewart, 1991; Vanderschuren and Kalivas, 2000; Steketee, 2003).

The expression of drug-induced sensitisation can be elicited by a challenge dose of the drug of experience, or by challenge doses of other drugs that have similar pharmacological actions within the mesocorticolimbic system. Such “cross-sensitisation”
effects have been observed with psychostimulants and opiates, as well as with alcohol (e.g., Vezina and Stewart, 1990; Itzhak, 1997; Manley and Little, 1997; Vanderschuren et al., 1999). Similar cross-sensitisation effects have also been observed with exposure to physical or environmental stressors and drugs of abuse. For example, repeated exposure to footshock results in a potentiated locomotor response to an acute injection of cocaine, amphetamine or morphine (MacLennan and Maier, 1983; Herman et al., 1984; Leyton and Stewart, 1990). Similarly, food restriction or restraint stress can induce augmented behavioral responses to later challenges with amphetamine or morphine (Deroche et al., 1992; Deroche et al., 1993). These findings are consistent with findings of cross-sensitisation between the effects of repeated injections of the stress-related neuropeptide, corticotropin-releasing factor (CRF), or repeated restraint stress on amphetamine-induced locomotor activation. For example, repeated i.c.v. injections of CRF produce a sensitised response to an amphetamine challenge after an 8-day drug-free period (Cador et al., 1993). In the same manner that repeated exposure to stressors can produce sensitised responses to a cocaine challenge at test, repeated exposure to cocaine can produce sensitised responses to a subsequent stressor. For example, it was recently reported that animals given 7 daily injections of cocaine (15-30mg/kg, i.p., per day) exhibited a sensitised locomotor response to a central injection of CRF after drug-free periods of 10-28 days (Erb et al., 2003; Erb and Brown, 2006).

Hypoxia can be considered as a form of systemic stress which is perceived by the organism as an internal threat of homeostasis and, thereby, serves to mobilise stress-related circuitry of the CNS that is aimed at restoring homeostasis. This circuitry includes brainstem structures such as the NTS. Activation of the NTS in response to hypoxia
induces direct activation of CRF-containing neurons in the paraventricular nucleus (PVN) of the hypothalamus which, in turn, stimulates the endocrine stress response (i.e., activation of the hypothalamic-pituitary-adrenal [HPA] axis). Activation of the HPA-axis is initiated by CRH being released from neurosecretory nerve terminals at the median eminence where it enters the portal blood vessel system of the hypophyseal stalk. CRH reaches the anterior pituitary where it stimulates the secretion of adrenocorticotropic hormone (ACTH) from corticotrope cells. ACTH is released into the blood stream and travels through it until reaching the adrenal cortex of the adrenal gland, located atop the kidney, where it stimulates the biosynthesis of corticosteroids such as cortisol. To date, studies exploring the cross-sensitising effects of cocaine and stress have tended to focus on so-called “psychogenic”, rather than “systemic”, stressors. Psychogenic stressors, such as exposure to restraint, footshock, environmental novelty, or conditioned fear, are not perceived by the organism as a direct internal threat to homeostasis and require cognitive processing in order to be construed as stressful. Although psychogenic stressors, like systemic stressors, mobilise stress-related responses of the CNS, including activation of the HPA axis, they do so via recruitment of distinct neural pathways. Whereas systemic stressors act initially at the level of the brainstem, psychogenic stressors are processed via activation of the limbic forebrain and frontal cortex.

Although the preponderance of studies to date on the cross-sensitising effects of cocaine and stressors have focused on psychogenic stressors, there is good reason to explore whether exposure to systemic stressors, such as hypoxia, induce neuroadaptations that can result in long-term and enhanced sensitivity to cocaine. Ultimately, both psychogenic and systemic stressors converge on common stress-responsive pathways of
the CNS, most notably the PVN. In fact, stress-induced activation of the HPA axis has been implicated in some aspects of long-term behavioral sensitisation to psychostimulants, including cocaine (Stohr et al., 1999; Przegaliński, 2000). Przegaliński et al. (2000) observed that adrenalectomy prior to cocaine treatment blocked the manifestation of cocaine induced sensitisation, a result that was reversed with pre-treatment with corticosterone before each daily cocaine administration. Furthermore, Przegaliński et al. (2000) also observed cross-sensitisation between cocaine and corticosterone, with pretreatment of corticosterone producing an increased response to the cocaine challenge dose and vice versa. In addition, there is evidence that exposure to anoxic stress during the perinatal period can induce profound and long-lasting changes in the function of the mesocorticolimbic dopamine system in adulthood (Brake et al., 1997; Flores et al., 2002), the system which, as mentioned, mediates the reinforcing effects of psychostimulant drugs such as cocaine. Flores et al. (2002) showed that perinatal anoxia enhanced the effect of stress during adulthood on the VTA, therefore they proposed that perinatal anoxia’s alteration of dopaminergic function within the mesocorticolimbic system may mimic the naturally occurring development of sensitisation to stimulant drugs.

The sections above have illustrated that hypoxia can act as a potent systemic stressor and that stress can produce an activation of the mesocorticolimbic dopamine system, similar to the rewarding effects of cocaine. Furthermore, there is some evidence that alterations in environmental levels of O₂ can produce neuroadaptations within the mesocorticolimbic dopamine system, the system thought to mediate the cross-sensitising effects of cocaine and stress. Thus, Experiment 2 was designed to test the hypothesis that
exposure to hypoxia will produce a sensitised locomotor response to an acute cocaine challenge following a prolonged drug-free period (i.e., 21 days).
Chapter 2

Materials and Methods
1. Experimental Animals

   Male Sprague-Dawley rats (n = 72; 40 for the breathing trials and 32 for the behavioural trials) were obtained from Charles River, Quebec and transported to the University of Toronto, Scarborough. All rats arrived with their weight between 250g and 275g. They were individually housed within standard rat cages in the UTSC vivarium. Food (Labdiet 5001 Rodent chow) and water were provided ad libitum. The vivarium was maintained under standard conditions for temperature (20-21°C) and humidity (30-35%) with a 12h:12h light-dark cycle. All experiments were approved by the University of Toronto Local Animal Care Committee, the University of Toronto Animal Care Committee and were performed in accordance with the guidelines established by the Canadian Council for Animal Care.

2. Exposure to Hypoxia

   A custom-built hypobaric chamber (Submersible Systems Technology), maintained at a pressure of 0.5atm, was used to simulate a high altitude hypoxic environment. The 150cm x 66cm x 47cm chamber was composed of an aluminium frame with aluminium sides and bottom and a clear Plexiglas top and door. A vacuum pump withdrew air from the chamber resulting in a decreased pressure (0.5atm) within the chamber. A “release” valve, on the opposite side of the chamber from the vacuum pump, was adjusted so that it would open when the pressure in the chamber reached 0.5atm thus allowing the intake of fresh air. In this manner, once the pressure in the chamber reached 0.5atm, the vacuum pump would continue to remove air from the chamber while fresh air flowed in through the release valve. In addition to maintaining the pressure at 0.5atm, this
flow through system facilitated the removal of CO₂ and the replenishment of O₂. As a
failsafe mechanism, if the pressure within the chamber reached 0.484 atm, which would
only occur if the release valve failed to open, the vacuum pump would shut off. In this
case, a safety valve would disconnect from the inlet of the vacuum pump connection to
the chamber to allow fresh air to enter the chamber even when the pump was not active
and the release valve was closed. The atmospheric pressure of 0.5atm within the
hypobaric chamber was equivalent to breathing 10% O₂ at sea level. The hypobaric
chamber was housed within a room maintained under the same lighting, temperature and
humidity as the UTSC vivarium.

Animals assigned to chronic hypoxia conditions (Experiments 1 and 2A) were
housed in the hypobaric chambers for 24 hours per day. In this case, the hypobaric
chamber was opened once each day for any necessary maintenance (i.e., cage cleaning
and provision of food/water). Animals assigned to intermittent hypoxia conditions
(Experiment 2B), were placed in the chamber for 1 hour each day; otherwise, they were
housed in the vivarium.

3. Experiment 1: Measuring the Effects of Chronic Hypoxia and Cocaine Treatment
on Breathing

3A. Description of the Barometric Method of Plethysmography

The barometric method of plethysmography was used to measure the animals’
breathing during acute breathing trials (described below). Plethysmography is a method
which translates the changes in pressure that occur during each breath when an animal is
placed within an enclosed chamber (plethysmograph), into relevant respiratory measures
When an animal inspires, the air that is taken into the lungs is warmed and humidified. This warming/humidification causes the air in the lungs to expand (i.e., increase in volume). This, in turn, causes an increase in the overall volume of the rat which causes an increase in pressure within the plethysmograph. Breathing is therefore measured as the change in pressure associated with the change in volume that is caused when air is warmed and humidified during inspiration.

The plethysmograph used in this study was a modified cylindrical plexiglass Buxco Electronics Inc. plethysmograph (PLYUN1R/U; Buxco Electronics Inc., Sharon, CT, USA), 24cm in diameter by 12.5cm in height with an internal volume of 5.6L. Gas flow through the plethysmograph was maintained at 1.0L/min. The flow rate of three different gases (O₂, N₂, and CO₂) were independently regulated by digital mass flow controllers (N₂, Top Trak 822, Sierra Instruments Inc; O₂, GFC Mass Flow Controller GFC171, Aalborg; CO₂, GFC Mass Flow Controller GFC171S, Aalborg). Each mass flow controller was calibrated for the specific gas flowing through it. The flow was regulated by setting each mass flow controller to a specific flow rate. A single gas line came from a compressed gas cylinder, (N₂, O₂, and CO₂) to each respective flow controller before they converged into a single line and entered the plethysmograph via a port at the top. Outflowing gas left the plethysmograph through a second port on the top and then passed through a column containing anhydrous calcium sulfate (Drierite; W.A. Hammond Drierite Company LTD.) in order to remove all water vapour. The outflowing gas then flowed through oxygen (BIOPAC Systems, Inc. O₂100C-1) and carbon dioxide analysers (BIOPAC Systems, Inc. CO₂100C-1) in order to monitor the gas levels within the plethysmograph. In this manner, the gas levels flowing into the plethysmograph were
set using the mass flow controllers while the outflowing gas levels were monitored with
the gas analysers. The inflowing levels of O₂ and CO₂ within the plethysmograph were
set using the flow controllers based on the outflowing levels monitored with the gas
analysers. In theory, the outflowing O₂ level will be slightly less than the inflowing O₂
level and the outflowing CO₂ level will be slightly higher than the inflowing CO₂ level
due to the animal’s consumption of O₂ and production of CO₂.

Given that the measurement of breathing with plethysmography is based on the
pressure change due to the humidification and warming of inspired air within the lungs, it
was necessary to measure the humidity and temperature of the air within the
plethysmograph. This was accomplished with a hydrometer/temperature probe (VWR;
35519-048) placed inside the plethysmograph. Temperature and humidity were recorded
at the beginning of the final minute of each trial section.

A pressure transducer (Validyne DP103; Northridge, CA, USA), connected to the
plethysmograph, measured the changes in pressure associated with breathing. The
pressure transducer produced an analog signal which was sent to a Validyne carrier
demodulator (model no. CD12) which digitised, filtered (1Hz) and amplified the signal.
The digital signal was sent from the carrier demodulator to a BIOPAC MP150 data
acquisition system (MP150CE; BIOPAC Systems, Inc.). The sampling frequency of the
MP150 system was 200Hz.

The recorded changes in pressure (i.e., the amplitude of the pressure signal) were
converted into a physiologically relevant measure, tidal volume (volume of an individual
breath). This was accomplished by calibrating the pressure transducer at the end of each
experiment. Prior to calibration, a sealed 250ml flask was placed inside the
plethysmograph to displace approximately the same volume of air as a standard-sized experimental rat. The plethysmograph was then sealed and calibration was accomplished by injecting 1.0ml of air into the plethysmograph at rates between 1ml in 0.14s to 1ml in 0.5s. This range of injection times was designed to mimic normal inspiratory times under a variety of levels of respiratory drive (i.e., when the drive to breathe was high, inspiratory times were low and vice versa). A linear regression was then performed on the calibration data with calibration injection time (to mimic inspiratory time; sec) on the x-axis and the amplitude of the pressure change (caused by the calibration injection; mV) on the y-axis. The linear regression produced a line of best fit in the form of $y = mx + b$. This formula was then used in the conversion of breath amplitudes (i.e., pressure changes associated with each breath; mV) to actual volumes of inspired air (tidal volume; ml) using the equations of Drorbaugh and Fenn (1955).

In order to measure tidal volume (the volume of each breath) using plethysmography, a measure of body temperature must be obtained. This was accomplished by implanting a radio telemeter into the body cavity. The telemeter emits a radio signal proportional to body temperature. The radio signal from the telemeter was received by a PhysioTel Receiver (Data Sciences; RPC-1) placed underneath the plethysmograph, then sent to a Data Exchange Matrix (Data Sciences) where it was relayed to a computer that converted it into a temperature and recorded it via Dataquest A.R.T. Bronze Acquisition (Data Sciences) software.

3B. Animal Preparation Required to Measure Breathing Using Plethysmography

3B-1. Anaesthesia
Animals were anaesthetised within an induction chamber containing 3.5% gaseous isoflurane in oxygen. The isoflurane was delivered to the induction chamber from a Tech 3 vapouriser which, in turn, was supplied with oxygen from a compressed O₂ tank. Once anaesthetised, the animal was removed from the induction chamber and placed onto a surgery table where anesthesia was maintained with 2.5% isoflurane (in oxygen) delivered through a mask (nose cone) placed over the animals’ snout. Before, and throughout surgery, the level of anaesthesia was routinely assessed using the toe pinch pain test and by monitoring the animals breathing rate. The rat was initially placed on its back on a Deltaphase isothermal pad (37°C) used to maintain the animal’s body temperature. Body temperature was not monitored during surgery.

3B-2. Telemeter Implantation

The first surgical procedure was the implantation of a Data Science radio telemeter (TA10TA-F20) within the abdomen. This procedure was necessary because the barometric method of plethysmography used during the study to measure breathing required a measure of the animal’s body temperature in order to calculate tidal volume. The abdominally-implanted telemeters emit a radio frequency that is proportional to body temperature.

Using hair clippers, a 3cm square patch was shaved on the animal’s abdomen. The exposed skin was cleansed with a 7.5% iodine/detergent surgical scrub (Prepodyne Betadine) and swabbed with 95% alcohol. This was repeated three times. The area was then disinfected with a 10% solution of iodine (Prepodyne Betadine). An incision, approximately 2cm in length, through both the skin and muscle layers was made along
the ventral midline. The Data Science telemeter was placed into the abdominal cavity. In order to close the wound, 3-0 black braided surgical silk was used to suture both the muscle and skin layers independently. Lastly the closed incision was cleansed with alcohol swabs and treated with the 10% solution of Prepodyne Betadine.

3B-3. Implantation of the Mini-Osmotic Pump to Deliver Cocaine/Saline

The second surgical procedure was the implantation of a mini-osmotic pump into a subcutaneous pocket between the scapulae. These pumps were used to administer the cocaine and/or saline (see below).

Prior to surgery, an Alzet® (model 2002) mini-osmotic pump (3.0cm in length x 0.7cm in diameter, 1.1g, 0.5μl/h pump rate) was filled with either cocaine hydrochloride (30mg/ml; Medisca Pharmaceuticals, St. Laurant, QC) dissolved in saline (0.9% NaCl; Baxter Corporation) or saline (0.9% NaCl; Baxter Corporation). The mini-osmotic pumps are composed of two chambers separated by an impermeable membrane. The inner chamber contains the pharmacological agent to be administered (i.e., cocaine or saline) whereas the outer chamber contains a highly osmotic solution. The inner chamber has a port at the one end through which the solution within it exits the pump. The chamber’s outer wall is composed of a semi-permeable membrane that allows body fluid to diffuse through it. When these pumps are implanted into an animal, the body fluid surrounding the pump diffuses into the outer chamber, moving into an area of greater osmolarity. This causes the outer chamber to increase in volume. The increased volume of the outer chamber exerts an inward pressure on the inner chamber which causes the solution within
the inner chamber to leave the pump via the exit port and move into the surrounding tissue/body fluid, from which it can then diffuse into the blood.

Following implantation of the telemeter (see above), the animal was placed on it’s ventral surface (still on the isothermal pad) exposing it’s dorsal surface. Hair clippers were used to remove the fur from a 2.0cm x 1.0cm area on the dorsal surface between the scapulae. The shaved surface was cleansed with an iodine scrub, swabbed with 95% alcohol and disinfected with a 10% solution of Prepodyne Betadine. A 1cm long incision was made, with a scalpel, between the scapulae along the dorsal midline. The skin was then separated from the underlying muscle creating a subcutaneous pocket into which the mini-osmotic pump was placed. Finally, the incision was closed using 3-0 black braided surgical silk, cleansed with alcohol swabs and treated with a 10% Prepodyne Betadine solution.

Once the surgical procedures (telemeter and mini-osmotic pump implantation) were complete, the isofluorane was turned off and 100% O₂ was delivered through the nose cone until the animal recovered from anaesthesia. The animal was then placed into a clean cage on a sheet of paper towel and the cage was placed under a heat lamp for one hour. After this recovery period the animal was returned to the vivarium.

3C. Experimental Protocol

3C-1. Experimental Groups

Animals were randomly assigned into the following four experimental groups (cocaine and saline were administered subcutaneously via the mini-osmotic pumps as described above):
1. Saline Control (SC) Group (n = 10); administered 0.9% NaCl (12μl/day;) and housed under normoxic conditions.

2. Cocaine Control (CC) Group (n = 10); administered cocaine hydrochloride (30mg/kg per day) and housed under normoxic conditions.

3. Saline Chronic Hypoxia (SCH) Group (n = 10); administered 0.9% NaCl (12μl/day) and housed under chronically hypoxic conditions (in the hypobaric chamber).

4. Cocaine Chronic Hypoxia (CCH) Group (n = 10); administered cocaine hydrochloride (30mg/kg per day) and housed under chronically hypoxic conditions (in the hypobaric chamber).

Cocaine hydrochloride was acquired with Dr. Suzanne Erb’s license. It was handled and stored according to the guidelines and regulations issued by Health Canada.

3C-2. Exposure to Chronic Hypoxia (or Control Chronic Normoxia) and Cocaine/Saline-Delivery

One day after surgery (designated as day 1), the animals in the chronically hypoxic groups (SCH and CCH) were placed into the hypobaric chamber (described above in Section 2) while the control (normoxic; SC and CC) animals were housed within the same room as the hypobaric chamber, under normobaric normoxic conditions. The
previously implanted mini-osmotic pumps administered their contents (saline or cocaine) for 14 days. On the thirteenth day of the protocol, while the pumps were still administering their agents, each animal underwent acute breathing trials (described below), after which they were returned to the previously ascribed conditions; either chronic hypoxia (SCH and CCH) or normoxia (SC and CC). On the fifteenth day, one day after the pumps have theoretically stopped delivering their contents, each animal underwent a second round of acute breathing trials (described below).

3C-3. Acute Breathing Trials

Acute breathing trials were performed on the 13th and 15th experimental days (i.e., the 13th and 15th days following surgery). These days were chosen to allow sufficient time for the animals to undergo ventilatory acclimatisation to hypoxia which has been shown to be established within 9 days for rats (Powell et al., 1998), and for the cocaine treatment to exert whatever effects it may have on breathing (Trippenbach and Kelly, 1994). In addition, cocaine was still being administered to the animals during the day 13 breathing trials, whereas it was not during the day 15 breathing trials (as the pumps had theoretically stopped delivering their contents by that time). Thus, performing the trials on days 13 and 15 permitted assessment of the effects of cocaine on breathing both in the presence of chronic administration of the drug (day 13) and during a period of time following the acute withdrawal of the drug (day 15).

The acute breathing trials were designed to measure the animals’ resting ventilation, as well as their ventilatory response to acute bouts of hypoxia (low inspired O₂), hyperoxia (high inspired O₂) and hypercapnia (high inspired CO₂). Prior to placing
animals in the plethysmograph, each animal was weighed with a triple beam balance (OHAUS 700/800). The first 45 minute period of the breathing trial was a stabilisation period where the plethysmograph was maintained at an oxygen environment which matched that in which the animal was kept for the past 14 days, either 21% O₂ (SC and CC groups) or 10% O₂ (SCH and CCH groups; equivalent to being in the hypobaric chamber at an atmospheric pressure of 0.5 atm). Following this 45 minute period, the animals were exposed to 15 minute periods in which the O₂ or CO₂ level in the plethysmograph was altered to produce conditions of acute hypoxia, hyperoxia, hypercapnia (interspersed with periods of normoxic normocapnia). Table 1 below lists the individual gas levels during each subsequent 15 minute period. The acute exposure to hyperoxia (30% O₂) was utilised in order to completely silence the arterial chemoreceptors as there is no chemoreceptor discharge (carotid body input to the brain) at an inspired O₂ level of 30% (Ling et al., 1997).
<table>
<thead>
<tr>
<th>[O₂] (%)</th>
<th>[CO₂] (%)</th>
<th>[N₂] (%)</th>
<th>Length of Period (min)</th>
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<td></td>
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<td></td>
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<tr>
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</table>

**Table 1.** The gas (O₂, CO₂ and N₂) levels used to create conditions of acute hypoxia (low inspired O₂), hyperoxia (high inspired O₂) and hypercapnia (high inspired CO₂) during the acute breathing trials. Note, the normoxic (normal oxygen) condition occurs at 21% O₂ and the normocapnic (normal CO₂) condition occurs at 0% CO₂.
The change from one period of gas levels to another was performed efficiently in order to rapidly set the \([O_2]\) and \([CO_2]\) to the levels for the next period. The gas change consisted of purging the plethysmograph for a calculated length of time with pure \(N_2\), to bring the \(O_2\) levels down to 10% / 8%, with pure \(O_2\), to bring the \(O_2\) levels up to 21% or 30%, or with pure \(CO_2\), in order to increase the \(CO_2\) levels to 6% / 8%. Finally, during the gas purges, the flow controllers were set to the appropriate values in order to maintain the desired gas percentages throughout the subsequent period. All gas change procedures were determined during pilot experiments (without any animals in the plethysmograph) in order to perfect the rapid transition from one set of gases to the next.

4. Experiment 2: Examining the Long-Lasting Effects of Chronic versus Intermittent Hypoxia on Cocaine-Induced Locomotor Activity

4A. Experimental Protocol

These experiments were carried out in 3 phases: 1) Exposure to hypoxia or no hypoxia; 2) “Rest” period; 3) Test for locomotor response to an acute cocaine challenge.

In Experiment 2A, animals were assigned to conditions of chronic hypoxia (or no hypoxia), whereas in Experiment 2B, animals were assigned to conditions of intermittent hypoxia (or no hypoxia).

4A-1. Experimental Groups

For each experiment, animals were randomly assigned to one of two experimental groups, as follows:
Experiment 2A

1. No Chronic Hypoxia (No CH) Control Group (n = 8); Housed under normoxic conditions throughout the experiment.

2. Chronic Hypoxia (CH) Group (n = 8); Housed under chronically hypoxic conditions (in the hypobaric chamber).

Experiment 2B

1. No Intermittent Hypoxia (No IH) Control Group (n = 8); Housed under normoxic conditions throughout the experiment.

2. Intermittent Hypoxia (IH) Group (n = 8); Housed in hypoxic conditions (in the hypobaric chamber) for 1 hour each day.

4A-2. Phase 1: Pre Exposure to Chronic or Intermittent Hypoxia

   In Experiment 2A, CH animals were housed in the hypobaric chamber (described above in Section 2) for 10 days, whereas their controls (No CH) were housed within the same room for the 10-day period, but under normoxic conditions. For this experimental protocol, the chronic hypoxia treatment is also referred to as chronic continuous hypoxia. This was done to differentiate it in relation to previous literature where repeated exposure to a daily stressor has been termed “chronic” when in comparison with the current study that protocol would be similar to our intermittent exposure. In Experiment 2B, IH animals were transferred from the *vivarium* to the room housing the hypobaric chamber, and
placed in the chamber under hypoxic conditions, for 1 hour each day (13:00 – 14:00).
Following the 1-hour period, the animals were returned to the *vivarium*. This procedure
was repeated for 10 days. Control animals (No IH) were treated in exactly the same
manner as IH animals, except that they were placed just outside the hyperbaric chamber,
under normoxic conditions, for the 1-hour period.

4A-3. Phase 2: “Rest” Period

After the 10-day pre-exposure period, animals in both experiments were left
undisturbed in home cages in the *vivarium* for a 21-day period. This duration of “rest”
period is standard for studies examining the long-term and cross-sensitising effects of
stressors and psychostimulants.

4A-4. Phase 3: Sensitisation Test for Locomotor Response to an Acute Cocaine
Challenge

Phase 3 occurred in the 2 days immediately following the 21-day rest period.
Procedures were identical for animals in Experiments 2A and 2B. On the first day,
animals were habituated to the testing manipulations; on the second day, they were tested
for their locomotor response to an acute cocaine challenge. For the habituation session,
animals were transferred from the *vivarium* to the room housing the locomotor activity
boxes, and were placed in the boxes for a 60min period. They were then administered an
i.p. injection of saline (1ml/kg) and placed back into the chamber for an additional 60min.
Subsequently, they were returned to the *vivarium*. The next day, the test day, animals
were again transferred to the activity chambers, as they had been the previous day. On
this day, however, after the initial 60min period in the chamber, all animals were injected with cocaine (10mg/kg, i.p.) and placed back in the chambers for an additional 60min. During both the habituation and test sessions, total distance travelled (cm) both before and after the injection, was measured and recorded via an infrared video camera linked to EthoVision software (Version 3, Noldus, The Netherlands). Thus, in both Experiments 2A and 2B, locomotor responsivity to a cocaine challenge was compared in animals that either had or had not been previously exposed to conditions of hypoxia.

5. Data Analysis

5A. Experiment 1: Breathing Trials

The following variables were measured under each gas condition: 1) breathing frequency (fR; breaths/min); 2) inspiratory time (TI; sec); 3) total breath time (TTOT; sec) and 4) breath amplitude (mV). From these measurements the following values were calculated: 1) expiratory time (TE; sec) as the difference between TTOT and TI; 2) tidal volume (VT; ml/kg), using the equations developed by Drorbaugh and Fenn (1955); 3) minute ventilation (VI; ml/kg/min), as the product of breathing frequency; and 4) tidal volume and ventilatory drive as tidal volume divided by inspiratory time (VT/TI; ml/kg/s). The data were illustrated in graphic form with the mean values ± the standard error of the mean (SEM) plotted for each group as a function of either inspired O₂ or inspired CO₂ levels.

5B. Experiment 2: Behavioural Trials
For the locomotor activity data, the total mean distance travelled during the 60min post-injection periods, and the mean distance travelled during each 10min of the 60min sessions, was calculated. The data were illustrated in a graph form with the mean values ±SEM plotted for each treatment group.

6. Statistical Analysis

6A. Experiment 1: Breathing Trials

All statistical testing of the breathing data was performed using commercial software (SigmaStat 3.0; SPSS). The data were first analysed to ensure normality and homogeneity of variance within groups. Two sets of statistical tests were then performed on the ventilatory data. First, a one-way repeated measures analysis of variance (ANOVA) was performed to compare the effects of the different concentrations of O₂ and CO₂ on ventilation within each experimental group (i.e., within the SC, CC, SCH and CCH groups). The second set of tests was a two-way repeated measures analysis of variance which compared the effects of each of the experimental treatments. The two-way ANOVAs examined: 1) The effects of cocaine treatment in the control groups (SC/CC X inspired O₂ or CO₂) and chronically hypoxic groups (SCH/CCH X inspired O₂ or CO₂). 2) The effects of chronic hypoxia in the saline-treated animals (SC/SCH X inspired O₂ or CO₂) and the cocaine-treated animals (CC/CCH X inspired O₂ or CO₂). Finally, where appropriate, a Student-Newman-Keuls test was used for all post-hoc multiple comparisons. For all statistical tests, significance was taken to be 5% (p < 0.05).

6B. Experiment 2: Behavioural Trials
All statistical testing of the behavioural data was performed using commercial software (SigmaStat 3.0; SPSS). All the data were first analysed to ensure normality and homogeneity of variance within groups and any outliers (defined as > 3 standard deviations away from the mean) were removed. Separate independent samples t-tests were performed to compare total distance travelled in CH versus No CH (Exp 2A) and IH versus No IH (Exp 2B). To assess for changes in activity as a function of time, the 60min post-injection activity counts were separated into 10min time bins and these values were entered into repeated measures ANOVAS for the factors of Time (10min bins from Time 0-60) and Group (CH, No CH; IH, No IH). Subsequent one-way ANOVAs and Student-Newman-Keuls post-hoc tests were carried out as appropriate. For all statistical tests, significance was taken to be 5% (p < 0.05).
Chapter 3

Results
1. Ventilatory Responses on the Thirteenth Day of Cocaine Treatment

The day 13 breathing trials occurred after 13 days of each of the group’s respective treatments.

1A. Responses to Acute Hypoxic Conditions

1A-1. Breathing Frequency (fR)

Figures 3A and 3B illustrate that fR, in all groups, increased significantly during acute exposure to 8% O₂ when compared with that recorded during exposure to 21% and 30% O₂ (SC, p < 0.001; CC, p < 0.001; SCH, < 0.001; CCH, p < 0.001). fR in the SCH group was also significantly greater than that of the SC group at all O₂ levels (30%, p < 0.001; 21%, p = 0.001; 8%, p < 0.001). fR in the CCH group was significantly greater than that in the CC group at all levels of O₂ (30%, p < 0.001; 21%, p = 0.001; 8%, p < 0.001). Lastly, no significant differences in fR were found between the SC and CC groups (p = 0.533) or the SCH and CCH groups (p = 0.992) for any concentration of O₂.

1A-2. Tidal Volume (VT)

As seen in Figures 4A and 4B, there were no significant differences observed in VT within any of the treatment groups at any O₂ level, (SC, p = 0.755; CC, p = 0.589; SCH, = 0.901; CCH, p = 0.445). Also, no significant differences in VT were observed between the SC and SCH groups (30%, p = 0.373; 21%, p = 0.157; 8%, p = 0.231) or the CC and CCH groups (p = 0.087) at any level of O₂. Finally, no significant differences in VT were observed between the SC and CC groups (p = 0.873) or the SCH and CCH groups (p = 0.691) at any level of O₂.
1A-3. Minute Ventilation (VI)

As seen in Figures 5A and 5B, VI (the product of fR and VT) increased in all groups during acute exposure to 8% O₂ when compared to VI when breathing 21% and 30% O₂ (SC, p < 0.001; CC, p < 0.001; SCH, p = 0.005, CCH, p = 0.005). VI in the CCH group was significantly elevated over that in the CC group during exposure to all levels of O₂ (30%, p = 0.041; 21%, p = 0.031; 8%, p = 0.003). VI in the SCH group was significantly greater than that of the SC group at both 8% and 30% O₂ but not at 21% O₂ (30%, p = 0.011; 21%, p = 0.169; 8%, p = 0.014). Finally, no significant differences in VI were observed between SC and CC groups (p = 0.907) or the SCH and CCH groups (p = 0.572) at any level of O₂.

1A-4. Inspiratory Time (TI)

Figures 6A and 6B demonstrate that acute exposure to 8% O₂ resulted in a significant decrease in TI for all groups compared to that recorded when the animals were breathing 21% and 30% O₂ (SC, p < 0.001; CC, p < 0.001; SCH, p = 0.013, CCH, p = 0.002). TI in the SCH group was significantly less than that in the SC group for every level of inspired O₂ (30%, p < 0.001; 21%, p < 0.001; 8%, p < 0.001). Also the TI of the CCH group was significantly less than that of the CC group at all O₂ levels (30%, p < 0.001; 21%, p < 0.001; 8%, p < 0.001). However, no significant differences in TI were observed between the CS and the CC groups (p = 0.474) or the SCH and CCH groups (p = 0.649) at any level of O₂.
1A-5. Expiratory Time (TE)

Figures 7A and 7B illustrate that acute exposure to 8% O₂ caused a significant decrease in TE in the CC, SCH, and CCH treatment groups compared to the values recorded at 21% and 30% O₂ (CC, p < 0.001; SCH, p < 0.001, CCH, p < 0.001). TE in the SC group was significantly different at every level of O₂, decreasing at both 21% and 8% over the value observed at 30% O₂ (p < 0.001). The TE in the SCH group was significantly less than that of the SC group for every level of O₂ (30%, p < 0.001; 21%, p < 0.001; 8%, p = 0.003). Also the TE in the CCH group was significantly less than that of the CC group at all O₂ levels (30%, p < 0.001; 21%, p < 0.001; 8%, p = 0.003). However, no significant differences in TE were observed between the SC and CC groups (p = 0.600) or the SCH and CCH groups (p = 0.966) at any level of O₂.

1A-6. Ventilatory Drive (VT/TI)

Figures 8A and 8B illustrate that VT/TI increased in the SC, CC, and CCH groups during exposure to 8% O₂ when compared to the values recorded at 21% and 30% O₂ (SC, p = 0.002; CC, p = 0.006; CCH, p = 0.037). No significant changes occurred in VT/TI within the SCH group at any level of O₂ (p = 0.054). VT/TI in the SCH group was significantly greater than in the SC group at 8% and 30% O₂, but not 21% O₂ (30%, p = 0.006; 21%, p = 0.135; 8%, p = 0.029). VT/TI in the CCH group was significantly greater than in the CC group for all levels of O₂ (30%, p = 0.029; 21%, p = 0.034; 8%, p = 0.005). Finally, no significant differences in VT/TI were observed between SC and CC groups (p = 0.841) or between the SCH and CCH groups (p = 0.780) at any level of O₂.
**Figure 3.** Breathing frequency (breaths/min) during exposure to acute hypoxia in (A) control (chronically normoxic) and (B) chronically hypoxic rats treated with either saline (open circles) or cocaine (closed circles) for 13 days. The data are shown as the mean ± 1 standard error of the mean (SEM). Letters (a, b and c) indicate significant differences, within any group, between different levels of inspired O₂. A plus sign (+) indicates that, at any given level of O₂, the value in the chronic hypoxia group is different from the corresponding value in the control group. Abbreviations: SC (saline control group), CC (cocaine control group), SCH (saline chronically hypoxic group) and CCH (cocaine chronically hypoxic group).
Figure 4. Tidal volume (ml/kg) during exposure to acute hypoxia in (A) control (chronically normoxic) and (B) chronically hypoxic rats treated with either saline (open circles) or cocaine (closed circles) for 13 days. The data are shown as the mean ± 1 standard error of the mean (SEM). Letters (a, b and c) indicate significant differences, within any group, between different levels of inspired O₂. Abbreviations: SC (saline control group), CC (cocaine control group), SCH (saline chronically hypoxic group) and CCH (cocaine chronically hypoxic group).
Figure 5. Minute ventilation (ml/min/kg) during exposure to acute hypoxia in (A) control (chronically normoxic) and (B) chronically hypoxic rats treated with either saline (open circles) or cocaine (closed circles) for 13 days. The data are shown as the mean ± 1 standard error of the mean (SEM). Letters (a, b and c) indicate significant differences, within any group, between different levels of inspired O2. A plus sign (+) indicates that, at any given level of O2, the value in the chronic hypoxia group is different from the corresponding value in the control group. Abbreviations: SC (saline control group), CC (cocaine control group), SCH (saline chronically hypoxic group) and CCH (cocaine chronically hypoxic group).
Figure 6. Inspiratory time (s) during exposure to acute hypoxia in (A) control (chronically normoxic) and (B) chronically hypoxic rats treated with either saline (open circles) or cocaine (closed circles) for 13 days. The data are shown as the mean ± 1 standard error of the mean (SEM). Letters (a, b and c) indicate significant differences, within any group, between different levels of inspired O₂. A plus sign (+) indicates that, at any given level of O₂, the value in the chronic hypoxia group is different from the corresponding value in the control group. Abbreviations: SC (saline control group), CC (cocaine control group), SCH (saline chronically hypoxic group) and CCH (cocaine chronically hypoxic group).
Figure 7. Expiratory time (s) during exposure to acute hypoxia in (A) control (chronically normoxic) and (B) chronically hypoxic rats treated with either saline (open circles) or cocaine (closed circles) for 13 days. The data are shown as the mean ± 1 standard error of the mean (SEM). Letters (a, b and c) indicate significant differences, within any group, between different levels of inspired O₂. A plus sign (+) indicates that, at any given level of O₂, the value in the chronic hypoxia group is different from the corresponding value in the control group. Abbreviations: SC (saline control group), CC (cocaine control group), SCH (saline chronically hypoxic group) and CCH (cocaine chronically hypoxic group).
Figure 8. Ventilatory drive (ml/kg/s) during exposure to acute hypoxia in (A) control (chronically normoxic) and (B) chronically hypoxic rats treated with either saline (open circles) or cocaine (closed circles) for 13 days. The data are shown as the mean ± 1 standard error of the mean (SEM). Letters (a, b and c) indicate significant differences, within any group, between different levels of inspired O₂. A plus sign (+) indicates that, at any given level of O₂, the value in the chronic hypoxia group is different from the corresponding value in the control group. Abbreviations: SC (saline control group), CC (cocaine control group), SCH (saline chronically hypoxic group) and CCH (cocaine chronically hypoxic group).
1B. Responses to Acute Hypercapnic Conditions

1B-1. Breathing Frequency ($f_R$)

Figure 9A illustrates that $f_R$ in the SC and CC groups increased significantly during exposure to both 6% and 8% CO$_2$ when compared with the value recorded at 0% CO$_2$ (SC, $p < 0.001$; CC, $p < 0.001$). However in the SCH and CCH groups (Fig. 9B), $f_R$ was not significantly different during exposure to any of the different levels of inspired CO$_2$ (SCH, $p = 0.178$; CCH, $p = 0.324$). $f_R$ in the SCH group was significantly greater than that of the SC group for all CO$_2$ conditions (0%, $p = 0.003$; 6%, $p = 0.023$; 8%, $p = 0.049$). $f_R$ in the CCH group was only significantly elevated over that in the CC group at 0% CO$_2$ (0%, $p = 0.006$; 6%, $p = 0.138$; 8%, $p = 0.144$). Finally, no significant differences in $f_R$ were observed between the SC and CC groups ($p = 0.910$) or the SCH and CCH groups ($p = 0.546$) at any level of CO$_2$.

1B-2. Tidal Volume ($V_T$)

Figures 10A and 10B illustrate that $V_T$, in all groups, was significantly different at every level of CO$_2$, compared to the value at 0% CO$_2$ (SC, $p < 0.001$; CC, $p < 0.001$; SCH, $p < 0.001$; CCH, $p < 0.001$). No significant differences in $V_T$ were observed between the CS and SCH groups ($p = 0.722$) or the CC and CCH groups ($p = 0.138$) at any level of CO$_2$. Also, no significant differences in $V_T$ were observed between the SC and CC groups ($p = 0.860$) or the SCH and CCH groups ($p = 0.413$) at any level of CO$_2$.

1B-3. Minute Ventilation ($V_I$)
As illustrated in Figures 11A and 11B, $V_1$ in all groups was significantly different at every level of CO$_2$ (SC, $p < 0.001$; CC, $p < 0.001$; SCH, $p < 0.001$, CCH, $p < 0.001$). $V_1$ in the SCH group was significantly greater than that in the SC group at 6% and 8% CO$_2$ levels ($0\%$, $p = 0.313$; $6\%$, $p = 0.018$; $8\%$, $p < 0.001$). $V_1$ in the CCH group was significantly elevated over that in the CC group at both 6% and 8% CO$_2$ ($0\%$, $p = 0.051$; $6\%$, $p = 0.001$; $8\%$, $p < 0.001$). Lastly, no significant differences in $V_1$ were observed between the SC and CC groups ($p = 0.863$) or the SCH and CCH groups ($p = 0.798$) at any level of CO$_2$.

1B-4. Inspiratory Time (TI)

Figure 12A illustrates that acute exposure to 6% and 8% CO$_2$ caused a significant decrease in TI in both the SC and CC groups when compared to the value recorded at 0% CO$_2$ (SC, $p < 0.001$; CC, $p = 0.004$). There was no significant difference in TI, at any CO$_2$ level within the CCH and SCH groups (Fig. 12B; SCH, $p = 0.328$, CCH, $p = 0.445$). TI in the SCH group was significantly less than that in the SC group at every CO$_2$ level ($0\%$, $p < 0.001$; $6\%$, $p = 0.024$; $8\%$, $p = 0.045$). TI in the CCH group was significantly less than that in the CC group at 0% and 6% CO$_2$ level but not at 8% ($0\%$, $p = 0.002$; $6\%$, $p = 0.039$; $8\%$, $p = 0.220$). Finally, there were no significant differences in TI between the SC and CC groups ($p = 0.979$) or the SCH and CCH groups ($p = 0.416$) at any level of CO$_2$.

1B-5. Expiratory Time (TE)
Figures 13A and 13B illustrate that exposure to 6% and 8% CO\textsubscript{2} caused a significant decrease in TE in all groups when compared with the value recorded at 0% CO\textsubscript{2} (SC, p < 0.001; CC, p < 0.001; SCH, p = 0.012, CCH, p = 0.040). TE in the SCH group was significantly less than that of the SC group at 0% CO\textsubscript{2} (0%, p < 0.001; 6%, p = 0.084; 8%, p = 0.125). TE in the CCH group was significantly less than that in the CC group at 0% CO\textsubscript{2} (0%, p < 0.001; 6%, p = 0.364; 8%, p = 0.590). No significant differences in TE were observed between the SC and CC groups (p = 0.676) or the SCH and CCH groups (p = 0.524) at any level of CO\textsubscript{2}.

1B-6. Ventilatory Drive (VT/TI)

Figures 14A and 14B illustrate that VT/TI, in all groups, was significantly different at each level of CO\textsubscript{2} (SC, p < 0.001; CC, p < 0.001; SCH, p < 0.001; CCH, p < 0.001). In the SCH group, VT/TI was significantly greater than the values in the SC group at 6 and 8% CO\textsubscript{2} (0%, p = 0.292; 6%, p = 0.021; 8%, p = 0.001). In the CCH group, VT/TI was also significantly greater than in the CC group at both the 6% and 8% CO\textsubscript{2} (0%, p = 0.060; 6%, p < 0.001; 8%, p < 0.001). Finally, no significant differences in VT/TI were observed between SC and CC groups (p = 0.642) or the SCH and CCH (p = 0.871) groups at any level of CO\textsubscript{2}. 
Figure 9. Breathing frequency (breaths/min) during exposure to acute hypercapnia in (A) control (chronically normoxic) and (B) chronically hypoxic rats treated with either saline (open circles) or cocaine (closed circles) for 13 days. The data are shown as the mean ± 1 standard error of the mean (SEM). Letters (a, b and c) indicate significant differences, within any group, between different levels of inspired CO2. A plus sign (+) indicates that, at any given level of CO2, the value in the chronic hypoxia group is different from the corresponding value in the control group. Abbreviations: SC (saline control group), CC (cocaine control group), SCH (saline chronically hypoxic group) and CCH (cocaine chronically hypoxic group).
Figure 10. Tidal Volume (ml/kg) during exposure to acute hypercapnia in (A) control (chronically normoxic) and (B) chronically hypoxic rats treated with either saline (open circles) or cocaine (closed circles) for 13 days. The data are shown as the mean ± 1 standard error of the mean (SEM). Letters (a, b, and c) indicate significant differences, within any group, between different levels of inspired CO₂. Abbreviations: SC (saline control group), CC (cocaine control group), SCH (saline chronically hypoxic group) and CCH (cocaine chronically hypoxic group).
Figure 11. Minute ventilation (ml/min/kg) during exposure to acute hypercapnia in (A) control (chronically normoxic) and (B) chronically hypoxic rats treated with either saline (open circles) or cocaine (closed circles) for 13 days. The data are shown as the mean ± 1 standard error of the mean (SEM). Letters (a, b and c) indicate significant differences, within any group, between different levels of inspired CO2. A plus sign (+) indicates that, at any given level of CO2, the value in the chronic hypoxia group is different from the corresponding value in the control group. Abbreviations: SC (saline control group), CC (cocaine control group), SCH (saline chronically hypoxic group) and CCH (cocaine chronically hypoxic group).
Figure 12. Inspiratory time (s) during exposure to acute hypercapnia in (A) control (chronically normoxic) and (B) chronically hypoxic rats treated with either saline (open circles) or cocaine (closed circles) for 13 days. The data are shown as the mean ± 1 standard error of the mean (SEM). Letters (a, b and c) indicate significant differences, within any group, between different levels of inspired CO₂. A plus sign (+) indicates that, at any given level of CO₂, the value in the chronic hypoxia group is different from the corresponding value in the control group. Abbreviations: SC (saline control group), CC (cocaine control group), SCH (saline chronically hypoxic group) and CCH (cocaine chronically hypoxic group).
Figure 13. Expiratory time (s) during exposure to acute hypercapnia in (A) control (chronically normoxic) and (B) chronically hypoxic rats treated with either saline (open circles) or cocaine (closed circles) for 13 days. The data are shown as the mean ± 1 standard error of the mean (SEM). Letters (a, b and c) indicate significant differences, within any group, between different levels of inspired CO₂. A plus sign (+) indicates that, at any given level of CO₂, the value in the chronic hypoxia group is different from the corresponding value in the control group. Abbreviations: SC (saline control group), CC (cocaine control group), SCH (saline chronically hypoxic group) and CCH (cocaine chronically hypoxic group).
Figure 14. Ventilatory drive (ml/kg/s) during exposure to acute hypercapnia in (A) control (chronically normoxic) and (B) chronically hypoxic rats treated with either saline (open circles) or cocaine (closed circles) for 13 days. The data are shown as the mean ± 1 standard error of the mean (SEM). Letters (a, b and c) indicate significant differences, within any group, between different levels of inspired CO₂. A plus sign (+) indicates that, at any given level of CO₂, the value in the chronic hypoxia group is different from the corresponding value in the control group. Abbreviations: SC (saline control group), CC (cocaine control group), SCH (saline chronically hypoxic group) and CCH (cocaine chronically hypoxic group).
2. Day 15 Acute Breathing Trials

The day 15 breathing trials occurred after 14 days of each of the group’s respective treatments and 1 day after the mini-osmotic pumps had finished pumping.

2A. Responses to Acute Hypoxic Conditions

2A-1. Breathing Frequency (fR)

Figures 15A and 15B illustrate that fR, in all groups, increased significantly during acute exposure to 8% O₂ when compared with the values recorded at 21% and 30% O₂ (SC, p < 0.001; CC, p < 0.001; SCH, < 0.001; CCH, p < 0.001). fR in the SCH group was significantly elevated over that in the SC group at all levels of inspired O₂ (30%, p < 0.001; 21%, p < 0.001; 8%, p = 0.002). fR in the CCH group was significantly greater than that in the CC group at all O₂ levels (30%, p < 0.001; 21%, p = 0.001; 8%, p < 0.001). No significant differences in fR were found between the SC and CC groups (p = 0.289) or the SCH and CCH groups (p = 0.895) at any level of O₂.

2A-2. Tidal Volume (VT)

Figures 16A and 16B illustrate that there were no significant differences in VT within any of the groups at any level of O₂ (SC, p = 0.739; CC, p = 0.980; SCH, p = 0.882; CCH, p = 0.430). No significant differences in VT were observed between the SC and SCH groups (p = 0.834) or the CC and CCH groups (p = 0.895) at any level of O₂. No significant differences in VT were observed between the SC and CC groups (30%, p = 0.242; 21%, p = 0.134; 8%, p = 0.088) or the SCH and CCH groups (p = 0.118) at any level of O₂.
2A-3. Minute Ventilation (VI)

Figures 17A and 17B illustrate that VI increased in all groups at 8% O₂ when compared to 21% and 30% O₂ (SC, p < 0.001; CC, p < 0.001; SCH, p = 0.017; CCH, p < 0.001). VI in the SCH group was significantly greater than that in the SC at all levels of O₂, (30%, p = 0.031; 21%, p = 0.041; 8%, p = 0.027). VI in the CCH group was significantly greater than that in the CC group at both 8% and 21% O₂ levels (30%, p = 0.054; 21%, p = 0.004; 8%, p < 0.001). No significant differences in VI were observed between the SC and CC groups (p = 0.104) or the SCH and CCH groups (p = 0.081) at any level of O₂.

2A-4. Inspiratory Time (TI)

Figures 18A and 18B demonstrate that acute exposure to 8% O₂ caused a significant decrease in TI in the CC, CCH, and SCH when compared to the values recorded at 21% and 30% O₂ (CC, p < 0.001; SCH, p = 0.007; CCH, p = 0.001). In the SC group, TI was significantly reduced at 8% O₂ compared to 30% O₂ (p = 0.035). TI in the SCH group was significantly less than that in the SC group at both 8% and 30% O₂ (30%, p = 0.004; 21%, p = 0.260; 8%, p = 0.003). TI in the CCH group was significantly less than that in the CC group at each level of inspired O₂, (30%, p < 0.001; 21%, p < 0.001; 8%, p = 0.001). No significant differences in TI were observed between SC and CC groups (p = 0.151) or the SCH and CCH groups (p = 0.957) at any level of O₂.

2A-5. Expiratory Time (TE)
Figures 19A and 19B illustrate that exposure to 8% O\textsubscript{2} caused a significant decrease in TE for all groups when compared to the values recorded at 21% and 30% O\textsubscript{2} (SC, p < 0.001; CC, p < 0.001; SCH, p < 0.001; CCH, p < 0.001). The TE in the SCH group was significantly less than that in the SC group at every level of inspired O\textsubscript{2} (30%, p < 0.001; 21%, p < 0.001; 8%, p = 0.028). TE in the CCH group was significantly less, at all levels of O\textsubscript{2}, than that in the CC group (30%, p < 0.001; 21%, p < 0.001; 8%, p < 0.001). No significant differences in TE were observed between the SC and CC groups (p = 0.178) or the SCH and CCH groups (p = 0.646) at any level of O\textsubscript{2}.

2A-6. Ventilatory Drive (VT/TI)

As illustrated in Figures 20A and 20B, VT/TI increased in the SC, CC and SCH groups during exposure to 8% O\textsubscript{2} when compared with the values recorded at 21% and 30% O\textsubscript{2} (SC, p = 0.010; CC, p = 0.013; SCH, p = 0.039). An increase in VT/TI was also observed in the CCH group during exposure to 8% O\textsubscript{2} but only when compared to the value at 30% O\textsubscript{2} (CCH, p = 0.011). There were no significant differences between the SCH and SC groups (30%, p = 0.100; 21%, p = 0.095; 8%, p < 0.051). VT/TI was significantly greater in the CCH group compared to the corresponding values in the CC group at 8% and 21% O\textsubscript{2} (30%, p = 0.072; 21%, p = 0.005; 8%, p = 0.001). No significant differences in VT/TI were observed between the SC and CC groups (p = 0.180) or the SCH and CCH groups (p = 0.066) at any level of O\textsubscript{2}. 
**Figure 15.** Breathing frequency (breaths/min) during exposure to acute hypoxia in (A) control (chronically normoxic) and (B) chronically hypoxic rats treated with either saline (open circles) or cocaine (closed circles) 15 days after the initiation of cocaine (or saline). The data are shown as the mean ± 1 standard error of the mean (SEM). Letters (a, b, and c) indicate significant differences, within any group, between different levels of inspired O₂. A plus sign (+) indicates that, at any given level of O₂, the value in the chronic hypoxia group is different from the corresponding value in the control group. Abbreviations: SC (saline control group), CC (cocaine control group), SCH (saline chronically hypoxic group) and CCH (cocaine chronically hypoxic group).
Figure 16. Tidal volume (ml/kg) during exposure to acute hypoxia in (A) control (chronically normoxic) and (B) chronically hypoxic rats treated with either saline (open circles) or cocaine (closed circles) 15 days after the initiation of cocaine (or saline). The data are shown as the mean ± 1 standard error of the mean (SEM). Letters (a, b and c) indicate significant differences, within any group, between different levels of inspired O₂. Abbreviations: SC (saline control group), CC (cocaine control group), SCH (saline chronically hypoxic group) and CCH (cocaine chronically hypoxic group).
Figure 17. Minute ventilation (ml/min/kg) during exposure to acute hypoxia in (A) control (chronically normoxic) and (B) chronically hypoxic rats treated with either saline (open circles) or cocaine (closed circles) 15 days after the initiation of cocaine (or saline). The data are shown as the mean ± 1 standard error of the mean (SEM). Letters (a, b and c) indicate significant differences, within any group, between different levels of inspired O₂. A plus sign (+) indicates that, at any given level of O₂, the value in the chronic hypoxia group is different from the corresponding value in the control group. Abbreviations: SC (saline control group), CC (cocaine control group), SCH (saline chronically hypoxic group) and CCH (cocaine chronically hypoxic group).
Figure 18. Inspiratory time (s) during exposure to acute hypoxia in (A) control (chronically normoxic) and (B) chronically hypoxic rats treated with either saline (open circles) or cocaine (closed circles) 15 days after the initiation of cocaine (or saline). The data are shown as the mean ± 1 standard error of the mean (SEM). Letters (a, b and c) indicate significant differences, within any group, between different levels of inspired O₂. A plus sign (+) indicates that, at any given level of O₂, the value in the chronic hypoxia group is different from the corresponding value in the control group. Abbreviations: SC (saline control group), CC (cocaine control group), SCH (saline chronically hypoxic group) and CCH (cocaine chronically hypoxic group).
Figure 19. Expiratory time (s) during exposure to acute hypoxia in (A) control (chronically normoxic) and (B) chronically hypoxic rats treated with either saline (open circles) or cocaine (closed circles) 15 days after the initiation of cocaine (or saline). The data are shown as the mean ± 1 standard error of the mean (SEM). Letters (a, b and c) indicate significant differences, within any group, between different levels of inspired O₂. A plus sign (+) indicates that, at any given level of O₂, the value in the chronic hypoxia group is different from the corresponding value in the control group. Abbreviations: SC (saline control group), CC (cocaine control group), SCH (saline chronically hypoxic group) and CCH (cocaine chronically hypoxic group).
Figure 20. Ventilatory drive (ml/kg/s) during exposure to acute hypoxia in (A) control (chronically normoxic) and (B) chronically hypoxic rats treated with either saline (open circles) or cocaine (closed circles) 15 days after the initiation of cocaine (or saline). The data are shown as the mean ± 1 standard error of the mean (SEM). Letters (a, b and c) indicate significant differences, within any group, between different levels of inspired O₂. A plus sign (+) indicates that, at any given level of O₂, the value in the chronic hypoxia group is different from the corresponding value in the control group. Abbreviations: SC (saline control group), CC (cocaine control group), SCH (saline chronically hypoxic group) and CCH (cocaine chronically hypoxic group).
2B. Responses to Acute Hypercapnic Conditions

2B-1. Breathing Frequency (fR)

Figures 21A and 21B illustrate that fR in the SC, CC and CCH groups increased significantly during exposure to both 6% and 8% CO₂ compared to the value at 0% CO₂ (SC, p < 0.001; CC, p < 0.001; CCH, p = 0.022). In the SCH group, fR was only greater during 6% CO₂ when compared to 0% (p = 0.038). fR in the SCH group was also significantly greater than that in the SC group at all CO₂ levels except 8% (0%, p = 0.002; 6%, p = 0.003; 8%, p = 0.080). fR in the CCH group was significantly greater than that in the CC group for all levels of inspired CO₂ (0%, p < 0.001; 6%, p = 0.020; 8%, p = 0.005). No significant differences in breathing frequency were found between the SC and CC (p = 0.295) groups or the SCH and CCH groups (p = 0.425) at any concentration of CO₂.

2B-2. Tidal Volume (VT)

Figures 22A and 22B illustrate that VT in the CC and CCH groups was significantly different at every level of CO₂, increasing at both 6% and 8% over VT at 0% CO₂ (CC, p < 0.001; CCH, p < 0.001). In contrast, in the SC and SCH groups, VT was greater at both 6% and 8% CO₂ when compared to 0% (SC, p = 0.002; SCH, p = 0.010) but the 6% and 8% values were not different from one another. No significant differences in VT were observed between the SC and SCH groups (p = 0.302) or the CC and CCH groups (0.222) at any level of CO₂. No significant differences in VT were observed between the SC and CC groups (p = 0.073) or the SCH and CCH groups (p = 0.382) at any level of CO₂.
2B-3. *Minute Ventilation (V₁)*

As illustrated in Figures 23A and 23B, V₁ in the SC, CC and CCH groups was significantly different at every level of CO₂, increasing at both 6% and 8% over the value observed at 0% CO₂ (SC, p < 0.001; CC, p < 0.001; CCH, p < 0.001). The SCH group had V₁ values that were the same at 6% and 8% CO₂ but both were greater than the value recorded at 0% (p = 0.001). V₁ in the SCH group was also significantly greater than that in the SC group at 6% and 8% CO₂ (0%, p = 0.282; 6%, p = 0.037; 8%, p = 0.027). For all levels of inspired CO₂, V₁ in the CCH group was significantly elevated over that in the CC group (0%, p = 0.018; 6%, p = 0.021; 8%, p < 0.001). No significant differences in V₁ were observed between SC and CC groups (p = 0.267) or the SCH and CCH groups (p = 0.533) at any level of CO₂.

2B-4. *Inspiratory Time (T₁)*

Figure 24A illustrates that exposure to 8% CO₂ caused a significant decrease in T₁ in the SC group when compared to both the values recorded at 6% and 0% CO₂ (p < 0.001). In the CC group (Fig. 24A), T₁ was significantly decreased during exposure to 6% and 8% CO₂ when compared to the value recorded at both 0% CO₂ (p = 0.039). There were no significant differences between any of the CO₂ levels in the SCH and CCH groups (SCH, p = 0.189; CCH, p = 0.410). T₁ in the SCH group was significantly less than that in the SC group at both 0% and 6% CO₂ (0%, p < 0.001; 6%, p < 0.001; 8%, p = 0.903). For every CO₂ level, T₁ in the CCH group was significantly less than the corresponding values in the CC group (0%, p < 0.001; 6%, p = 0.013; 8%, p = 0.007). No
significant differences in TI were observed between the SCH and CCH groups at any level of CO$_2$ ($p = 0.883$). During exposure to 8% CO$_2$, TI in the CC group was significantly greater than that in the CS group (0%, $p = 0.301$; 6%, $p = 0.103$; 8%, $p = 0.007$).

2B-5. Expiratory Time (TE)

Figures 25A and 25B illustrate that exposure to 6% and 8% CO$_2$ caused a significant decrease in TE in all groups when compared to the value at 0% CO$_2$ (SC, $p = 0.002$; CC, $p < 0.001$; SCH, $p = 0.020$; CCH, $p = 0.002$). TE in the SCH group was significantly less than that in the SC group at 0% and 6% inspired CO$_2$ (0%, $p = 0.002$; 6%, $p = 0.030$; 8%, $p = 0.271$). TE in the CCH group was significantly less than that in the CC group at 0% CO$_2$ but not at 6 or 8% (0%, $p < 0.001$; 6%, $p = 0.139$; 8%, $p = 0.063$). No significant differences in TE were observed between the SC and CC groups ($p = 0.462$) or the SCH and CCH groups ($p = 0.453$) at any level of CO$_2$.

2B-6. Ventilatory Drive (VT/TI)

As illustrated in Figures 26A and 26B, VT/TI in the CC, CS and CCH groups was significantly different at every level of inspired CO$_2$ (SC, $p < 0.001$; CC, $p < 0.001$; CCH, $p < 0.001$). An increase in VT/TI was observed in the SCH group during both the 6% and 8% CO$_2$ conditions when compared to the 0% CO$_2$ condition (SCH, $p = 0.002$) but the 6% and 8% values were not different from one another. The SCH group had a significantly greater VT/TI than then SC group at 6% and 8% CO$_2$ (0%, $p = 0.376$; 6%, $p = 0.031$; 8%, $p = 0.022$). In the CHH group, VT/TI was significantly greater than the CC
group at all levels of CO\(_2\) (0\%, p = 0.014; 6\%, p = 0.009; 8\%, p < 0.001). Finally, no
significant differences in VT/TI were observed between the SC and CC groups (p = 0.362) or the SCH and CCH groups (p = 0.430) at any level of CO\(_2\).
**Figure 21.** Breathing frequency (breaths/min) during exposure to acute hypercapnia in (A) control (chronically normoxic) and (B) chronically hypoxic rats treated with either saline (open circles) or cocaine (closed circles) 15 days after the initiation of cocaine (or saline). The data are shown as the mean ± 1 standard error of the mean (SEM). Letters (a, b and c) indicate significant differences, within any group, between different levels of inspired CO₂. A plus sign (+) indicates that, at any given level of CO₂, the value in the chronic hypoxia group is different from the corresponding value in the control group. Abbreviations: SC (saline control group), CC (cocaine control group), SCH (saline chronically hypoxic group) and CCH (cocaine chronically hypoxic group).
Figure 22. Tidal volume (ml/kg) during exposure to acute hypercapnia in (A) control (chronically normoxic) and (B) chronically hypoxic rats treated with either saline (open circles) or cocaine (closed circles) 15 days after the initiation of cocaine (or saline). The data are shown as the mean ± 1 standard error of the mean (SEM). Letters (a, b and c) indicate significant differences, within any group, between different levels of inspired CO₂. Abbreviations: SC (saline control group), CC (cocaine control group), SCH (saline chronically hypoxic group) and CCH (cocaine chronically hypoxic group).
Figure 23. Minute ventilation (ml/min/kg) during exposure to acute hypercapnia in (A) control (chrono-chronically normoxic) and (B) chronically hypoxic rats treated with either saline (open circles) or cocaine (closed circles) 15 days after the initiation of cocaine (or saline). The data are shown as the mean ± 1 standard error of the mean (SEM). Letters (a, b and c) indicate significant differences, within any group, between different levels of inspired CO2. A plus sign (+) indicates that, at any given level of CO2, the value in the chronic hypoxia group is different from the corresponding value in the control group.

Abbreviations: SC (saline control group), CC (cocaine control group), SCH (saline chronically hypoxic group) and CCH (cocaine chronically hypoxic group).
**Figure 24.** Inspiratory time (s) during exposure to acute hypercapnia in (A) control (chronically normoxic) and (B) chronically hypoxic rats treated with either saline (open circles) or cocaine (closed circles) 15 days after the initiation of cocaine (or saline). The data are shown as the mean ± 1 standard error of the mean (SEM). Letters (a, b and c) indicate significant differences, within any group, between different levels of inspired CO₂. A plus sign (+) indicates that, at any given level of CO₂, the value in the chronic hypoxia group is different from the corresponding value in the control group. A number sign (#) indicates that, at any given level of CO₂, the value in the cocaine group is different from the corresponding value in the saline group. Abbreviations: SC (saline control group), CC (cocaine control group), SCH (saline chronically hypoxic group) and CCH (cocaine chronically hypoxic group).
Figure 25. Expiratory time (s) during exposure to acute hypercapnia in (A) control (chronically normoxic) and (B) chronically hypoxic rats treated with either saline (open circles) or cocaine (closed circles) 15 days after the initiation of cocaine (or saline). The data are shown as the mean ± 1 standard error of the mean (SEM). Letters (a, b and c) indicate significant differences, within any group, between different levels of inspired CO2. A plus sign (+) indicates that, at any given level of CO2, the value in the chronic hypoxia group is different from the corresponding value in the control group. Abbreviations: SC (saline control group), CC (cocaine control group), SCH (saline chronically hypoxic group) and CCH (cocaine chronically hypoxic group).
Figure 26. Ventilatory drive (ml/kg/s) during exposure to acute hypercapnia in (A) control (chronically normoxic) and (B) chronically hypoxic rats treated with either saline (open circles) or cocaine (closed circles) 15 days after the initiation of cocaine (or saline). The data are shown as the mean ± 1 standard error of the mean (SEM). Letters (a, b and c) indicate significant differences, within any group, between different levels of inspired CO\(_2\). A plus sign (+) indicates that, at any given level of CO\(_2\), the value in the chronic hypoxia group is different from the corresponding value in the control group. Abbreviations: SC (saline control group), CC (cocaine control group), SCH (saline chronically hypoxic group) and CCH (cocaine chronically hypoxic group).
3. Experiment 2: Cocaine Sensitisation

3A. Chronic Hypoxia Pre-Exposure

Figure 27 shows the mean (±SEM) distance travelled (cm) during the habituation session (A and B) and test for cross-sensitisation to cocaine (C and D), following exposure to chronic continuous hypoxia. Graphs A and C show the distance travelled during each 10min of the 60min habituation and test sessions, respectively. Graphs B and D show the total distance travelled during the respective 60min sessions. An ANOVA carried out on distance travelled during the habituation session revealed an effect of Time (p < 0.001), but no effect of Group or Time by Group. Thus, CH and No CH animals showed comparable baseline levels of activity during the habituation session. An ANOVA carried out on distance travelled during the test session also revealed only a significant effect of Time (p < 0.001). Thus, CH and No CH animals did not differ in their locomotor response to an acute cocaine challenge.

3B. Intermittent Hypoxia Pre-Exposure

Figure 28 shows the mean (±SEM) distance travelled (cm) during the habituation session (A and B) and test for cross-sensitisation to cocaine (C and D), following exposure to intermittent hypoxia. Graphs A and C show the distance travelled during each 10min of the 60min habituation and test sessions, respectively. Graphs B and D show the total distance travelled during the respective 60min sessions. An ANOVA carried out on distance travelled during the habituation session revealed an effect of Time (p < 0.001), but no effect of Group or Time by Group. Thus, IH and No IH animals showed comparable baseline levels of activity during the habituation session. An
ANOVA carried out on distance travelled during the test session also revealed significant effects of Time (p < .001) and Group (p < 0.03). In this case, IH animals showed an enhanced locomotor response to an acute cocaine challenge, relative to No IH animals.
Figure 27. The mean (±SEM) distance travelled (cm) during the habituation session (A and B) and test for cross-sensitisation to cocaine (C and D), following exposure to 10 days of chronic continuous hypoxia. Abbreviations: CH (Chronic hypoxia group), No CH (No chronic hypoxia control group).
Figure 28. The mean (±SEM) distance travelled (cm) during the habituation session (A and B) and test for cross-sensitisation to cocaine (C and D), following exposure to 10 days of intermittent hypoxia. Symbols: An asterisk (*) indicates a difference between the IH and No IH group within that test. Abbreviations: IH (Intermittent hypoxia group), No IH (No Intermittent hypoxia control group)
Chapter 4

Discussion
1. Summary

The results of this thesis indicate:

1) That minute ventilation (i.e., overall breathing) increases during exposure to acute hypoxia and hypercapnia.

2) The changes in minute ventilation are due to increases in breathing frequency rather than tidal volume.

3) The increase in breathing frequency (during acute hypoxia and hypercapnia) in the control animals was due to decreases in inspiratory time (Ti) and expiratory time (Te).

4) In the chronically hypoxic animals, the increase in breathing frequency under normoxic, hyperoxic and hypoxic conditions was due to a decrease in Ti and Te.

5) In the chronically hypoxic animals, the increase in breathing frequency during acute hypercapnia was due only to decreases in Ti with no change in Te.

6) Exposure to chronic hypoxia caused increases in breathing. These changes were one of “offset” rather than “gain”.

7) Cocaine treatment did not induce VAH-like changes in breathing in control rats.

8) Cocaine treatment increased Ti during acute hypercapnia in the control rats on day 15. This was abolished by exposure to chronic hypoxia.

9) Chronic hypoxia treatment did not produce a significant difference in the locomotor response to an acute cocaine challenge.

10) Intermittent hypoxia treatment produced an increase in the locomotor response to an acute cocaine challenge.

2. Experiment 1: Effects of Chronic Hypoxia and Cocaine Treatment on Breathing
2A. Effects of Acute Hypoxia

Both normoxic control groups, saline control (SC) and cocaine control (CC), exhibited a significant increase in minute ventilation (Figs. 5A and 17A) during exposure to acute hypoxia (8% O₂) on both day 13 and day 15. These observations are consistent with the well-established literature on the hypoxic ventilatory response (HVR) in mammals (i.e., during acute hypoxia there is an increase in breathing) (Aaron and Powell, 1993; Mizusawa et al., 1994; Ohtake et al., 1998). These increases in minute ventilation were not a consequence of a change in tidal volume, as tidal volume did not significantly increase during acute exposure to 8% O₂ in either control group (Figs. 4A and 16A). Instead, the increases in breathing were due to corresponding increases in breathing frequency (Figs. 3A and 15A). This change was also observed to underlie the poikilocapnic HVR in previous studies (Aaron and Powell, 1993; Ohtake et al., 1998) although some studies have also found an increase in tidal volume along with breathing frequency (Mizusawa et al., 1994).

The increases in breathing frequency during acute hypoxia were due to a decrease in both inspiratory time (Figs. 6A and 18A) and expiratory time (Figs. 7A and 19A) which is consistent with the observations of Ohtake et al. (1998) and Wu et al. (2005). During resting ventilation, inspiration is an active process (driven by contraction of the diaphragm and external intercostal muscles) whereas expiration is generally a passive process driven by the relaxation of the diaphragm and thoracic cavity. Under conditions of elevated respiratory drive (i.e., during acute hypoxia and hypercapnia) expiration becomes an active process driven by contraction of the internal intercostal muscles. The
decrease in expiratory time during acute hypoxia suggests that expiration has indeed gone from a passive to active process.

The chronically hypoxic groups, saline chronic hypoxic (SCH) and cocaine chronic hypoxic (CCH), showed a similar responses to acute hypoxia as did the control groups; a significant increase in minute ventilation (Figs. 5B and 17B) on both day 13 and day 15. Once again, these increases were due to increases in breathing frequency (Figs. 3B and 15B) with no change in tidal volume (Figs. 4B and 16B). These results indicate that exposure to chronic hypoxia has not altered the regulation of breathing pattern (i.e., breathing frequency versus tidal volume).

In order to examine the acute HVR, rats were exposed to 8% O₂. This O₂ level was chosen because it is slightly less than the level of hypoxia that the chronically hypoxic groups were exposed to within the hypobaric chamber (i.e., 0.5 atm in the hypobaric chamber is the equivalent of breathing 10% O₂ under normobaric conditions). As such, acute exposure to 8% O₂ represents a subsequent (to the chronic hypoxia), yet more severe, bout of hypoxia. Additionally 8% O₂ is the lowest O₂ level that is reasonably tolerated by the non-acclimatised control animals. During acute breathing trials the animals were also exposed to an acute hypoxic level of 10% O₂ however these data were not analysed nor reported because the 10% period was only conducted to remain consistent with previous protocols (Reid and Powell, 2005; Chung et al., 2006).

No differences in minute ventilation were observed when comparing values during exposure to 30% O₂ and 21% O₂ for either of the control groups. The acute exposure to hyperoxia (30% O₂), in addition to exposure to 21% O₂ (indicative of the O₂ level of room air at sea level), was performed in order to completely silence the arterial
chemoreceptors in the carotid body, as previous studies have demonstrated that there is no chemoreceptor discharge (carotid body input to the brain) at an inspired O$_2$ level of 30% (Ling et al., 1997). Given this, one might expect that the maximum possible HVR would be observed when comparing 30% O$_2$ to 8% O$_2$ as opposed to 21% O$_2$ to 8% O$_2$. A slight decrease, albeit non-significant, in minute ventilation was observed for the control groups during exposure to 30% O$_2$ compared to 21% (Figs. 5A and 17A). This suggests that there is a minimal level (but not zero) of chemoreceptor input, from the carotid body to the brain, during exposure to 21% O$_2$ resulting in a slightly increased level of minute ventilation when compared to that during exposure to 30% O$_2$ when, I assumed, that no chemoreceptor input was present. However, the data also suggest that the chemoreceptor input during exposure to 21% O$_2$ was not enough to cause a statistically different level of breathing between 30% and 21% O$_2$.

The changes observed in breathing for the control groups during the acute hypoxia trials were similar for both the day 13 and day 15 tests (see section below on day 13 versus day 15).

2B. Effects of Acute Hypercapnia

The plotted data for 0% CO$_2$ in the acute hypercapnic breathing trials is the same as the plotted data for 21% O$_2$ in the acute hypoxic breathing trials. Both conditions represent a situation in which the animals were breathing a gas mixture of 21% O$_2$, 0% CO$_2$ and 79% N$_2$ (i.e., room air at sea level).

Both normoxic control groups, SC and CC, displayed a significant increase in breathing (minute ventilation) under both acute hypercapnic conditions, 6% and 8% CO$_2$,
on both day 13 and day 15 (Figs. 11A and 23A) which is characteristic of a normal hypercapnic ventilatory response (HCVR) (Mizusawa et al., 1994; Ohtake et al., 1998; Kinkead et al., 2001). Unlike the increase in breathing during acute hypoxia, the acute HCVR was due to a combination of increases in both tidal volume (Figs. 10A and 22A) and breathing frequency (Figs. 9A and 21A). Previous studies observed similar increases in both tidal volume and breathing frequency during acute hypercapnia (Mizusawa et al., 1994; Ohtake et al., 1998; Kinkead et al., 2001). The increase in breathing frequency was due to a decrease in both inspiratory (Figs. 12A and 24A) and expiratory time (Figs. 13A and 25A). Again, these changes in TI and TE were also observed to underlie the increase in breathing frequency during acute hypercapnia in previous studies (Ohtake et al., 1998; Wu et al., 2005).

The chronically hypoxic groups, SCH and CCH, exhibited results similar to the control groups with exposure acute hypercapnia; both demonstrated a significant increase in minute ventilation (Figs. 11B and 23B) on day 13 and day 15. Once again these increases were due to increases in both breathing frequency (Figs. 9B and 21B) and tidal volume (Figs. 10B and 22B).

During the acute hypercapnic breathing trials the animals were exposed to both 6% and 8% CO₂ levels. These levels were chosen for consistency with previous studies (Eskander and Reid, unpublished; Reid et al; unpublished). Furthermore, exposure to 4% CO₂ has a moderate (sub-maximal) effect on minute ventilation while levels of CO₂ greater than 8% do not generally cause any further increase in breathing.

2C. Effects of Chronic Hypoxia
In general, exposure to chronic hypoxia caused an increase in overall breathing (minute ventilation) during normoxic/hyperoxic normocapnia (i.e., 21% or 30% O₂; 0% CO₂), acute hypoxia (8%) and acute hypercapnia (6 and 8% CO₂) in both the saline-treated and cocaine-treated groups on both days 13 and 15 of the experimental breathing trials. Although several individual data points in the chronically hypoxic groups did not achieve statistical significance, in terms of their elevation above the control values, the overall data set clearly indicates that exposure to chronic hypoxia has increased overall levels of breathing. Therefore, the data indicate that the rats in the chronically hypoxic groups have undergone ventilatory acclimatisation to hypoxia.

However, the change in breathing caused by exposure to chronic hypoxia was one of “offset” rather than “gain”. In other words, breathing has increased under all conditions but exposure to chronic hypoxia did not increase the magnitude (i.e., the slope) of either the acute hypoxic ventilatory response (i.e., the slope from 21% O₂ to 8% O₂) or the acute hypercapnic ventilatory response (i.e., the slope from 0% CO₂ to 6 or 8% CO₂). However, this was not entirely unexpected. Although changes in “offset” (i.e., increases in breathing under resting conditions that continue to manifest during acute hypoxia and hypercapnia) and “gain” (an increase in the slope of acute ventilatory responses) are hallmarks of ventilatory acclimatisation to hypoxia, the increase in “gain” is often only observed under isocapnic (constant levels of arterial pCO₂) rather than poikilocapnic (fluctuation levels of arterial pCO₂) conditions.

The acute hypoxia experiments in this thesis were conducted under poikilocapnic conditions. In other words, the animals were made acutely hypoxic without any attempt to artificially clamp arterial CO₂ levels at the level indicative of acute normoxic
conditions. As such, when the animals increased their breathing as a result of the low inspired O\textsubscript{2} level, they also increased their rate of CO\textsubscript{2} excretion. Although I did not measure arterial blood gas levels in this thesis, it is reasonable to assume that arterial CO\textsubscript{2} levels fell during acute hypoxia. The reduction in the CO\textsubscript{2}-drive to breathe likely prevented the increase in the slope of the acute hypoxic ventilatory response. An increase in the slope of the acute hypercapnic ventilatory response has not been described as being a hallmark of VAH although Eskander and Reid (unpublished) have demonstrated such a response. It is unclear why such a response was not observed in this study although subtle experimental differences between the studies may account for this. Aaron and Powell (1993) found similar results when rats were tested under poikilocapnic conditions; chronic hypoxia produced an increase in breathing during resting ventilation and acute hypoxia with no change in the gain of the HVR. Conversely, Barer et al. (1976), Wach et al. (1989) and Hsieh et al. (2004) reported that chronic hypoxia produced an attenuation in the poikilocapnic HVR in anaesthetised rats. However, the previous studies need to be considered carefully as the mechanisms underlying breathing have been shown to be different under anesthesia when compared to conscious animals (Goodman and Black, 1987). Similar conscious animal versus anaesthetised animal differences can also occur in cardiovascular regulation. For example, Machado and Bonagamba (1998) observed that glutamate injections into the NTS caused an increase in blood pressure in conscious rats but a decrease in anaesthetised rats indicating that anaesthesia can indeed alter NTS-regulated responses.

As mentioned in the introduction, two main mechanisms may have underlain the changes in breathing produced by chronic hypoxic in this study. First, there may have
been an increase in the sensitivity of the carotid body O₂ chemoreceptors to low levels of O₂ in the arterial blood (Dempsey and Forester, 1982). Second, there may have been an increase in the responsiveness of the CNS to the afferent input that it receives from the carotid body chemoreceptors (Dempsey and Forester, 1982; Dwinnel and Powell, 1999). These two mechanisms are not mutually exclusive and both may have occurred in the current study.

Under all conditions (resting breathing, acute hypoxia and acute hypercapnia), in both the saline-treated and cocaine-treated groups on days 13 and 15, the chronic hypoxia-induced increase in minute ventilation was due to an increase in breathing frequency rather than an increase in tidal volume. As such, minute ventilation was modulated under acute conditions (acute hypoxia and hypercapnia) and chronic conditions (chronic hypoxia) by changes in breathing frequency rather than tidal volume. This might suggest that tidal volume in these rats was already at a maximal level under normoxic normocapnic conditions prior to exposure to chronic hypoxia. In other words, there may have been no, or limited, scope for tidal volume to increase in any situation. This is unlikely to be the case, however, because Wu et al. (2005) have shown that tidal volume can increase during acute hypoxia/hypercapnia and chronic hypoxia to levels of 14.2ml/kg. In this study, tidal volume ranged between 5.7ml/kg and 11.8ml/kg suggesting that a mechanical limitation did not prevent an increase in tidal volume. Aaron and Powell (1993) observed that the chronic hypoxia-induced increase in ventilation was produced by an increase in tidal volume and not breathing frequency. On the other hand, Olson and Dempsey (1978) and Reeves et al. (2003) observed an increase in both tidal volume and breathing frequency after treatment with chronic hypoxia. However, each of
these experiments had methodologies which differed in one way or another from the current thesis. Chung et al. (2006) used a very similar method of chronic hypoxia treatment and ventilatory measurement, as that used in the current thesis, and found similar changes in breathing pattern underlying the increase in minute ventilation during VAH; an increase in breathing frequency and no change in tidal volume.

Under resting conditions (i.e., 21% or 30% O₂ and 0% CO₂) and during exposure to acute hypoxia (8% O₂), the chronic hypoxia-induced increases in breathing frequency were due to decreases in both inspiratory time and expiratory time. However, during exposure to acute hypercapnia, the chronic hypoxia-induced increase in breathing frequency was due, with one exception, exclusively to a decrease in inspiratory time with no change in expiratory time. As mentioned above, the increases in breathing frequency during acute hypoxia and hypercapnia (in control rats) were due to decreases in both T₁ and Tₑ. In combination with the results from the chronically hypoxic animals, this means that exposure to chronic hypoxia has not altered the regulation of breathing frequency under acute normoxic, hyperoxic or hypoxic conditions but has altered the regulation of breathing frequency under acute hypercapnic conditions. It is possible that exposure to chronic hypoxia has altered the central mechanisms responsible for the switch from inspiration to expiration under acute hypercapnic conditions but not under normoxic, hyperoxic or hypoxic conditions.

2D. The Lack of an Effect of Cocaine on Breathing

I initially hypothesised that chronic cocaine-treatment in control (i.e., chronically normoxic) animals would induce changes in breathing characteristic of those that are
observed during VAH. In other words, I hypothesised that cocaine-treatment would increase breathing either through changes in “offset”, “gain” or both. The rationale behind this hypothesis is that one of the mechanisms of VAH is an increase in neurotransmission (i.e., glutamate, Reid and Powell, 2005; GABA, Chung et al., 2006) in the CNS. Given that cocaine prevents the reuptake of monoamines (dopamine, noradrenaline and serotonin), it was possible that cocaine-treatment would cause an increase in neurotransmission mediated by these monoamines thereby causing an increase in breathing. I had tested this hypothesis in a previous study (Knight, Erb and Reid, unpublished) using daily intraperitoneal injections of cocaine. That protocol did not cause VAH-like changes in breathing. However, I also hypothesised that the failure of the daily intraperitoneal injections to induce VAH-like changes in breathing was the result of the treatment protocol being insufficient to raise blood (brain) cocaine levels for a long enough period of time to enact the required changes in monoamine neurotransmission. In my thesis, the hypothesis was that the constant infusion of cocaine via the subcutaneously-implanted mini-osmotic pumps would raise blood (brain) cocaine to a high enough level in order to induce VAH-like changes in breathing.

Cocaine treatment did not have a significant effect on any breathing variable during the acute hypoxic trials. Neither did cocaine exposure have any effect on any breathing variable during the acute hypercapnic exposure with the exception of inspiratory time during the day 15 trial, which was significantly greater at 8% CO₂ in the cocaine control group when compared with the saline control (Fig. 24A). Furthermore, the cocaine-treated chronically hypoxic group did not show a similar increase in inspiratory time at 8% CO₂ when compared with the saline-treated chronically hypoxic
group. Taken together, these data indicate that the chronic exposure to cocaine led to an increase in expiratory time during acute hypercapnia and that this effect was abolished by chronic hypoxia and VAH.

In general then, the results of this thesis do not support the hypothesis that the chronic administration of cocaine in the absence of chronic hypoxic would lead to VAH-like changes in breathing. Furthermore, the results to not support the hypothesis that the previous study’s (Knight, Erb and Reid, unpublished) failure to induce VAH-like changes in breathing with chronic intraperitoneal injections of cocaine was due to the treatment protocol being unable to sufficiently raise blood (brain) cocaine levels. Although I did not measure blood cocaine levels in this study, it is unlikely that the failure of the cocaine treatment to alter breathing was a result of an insufficient blood/brain cocaine concentration because previous studies have used similar concentrations and observed significant changes in breathing (Trippenbach and Kelly, 1994). Trippenbach and Kelly (1994) observed similar results in normoxic control animals, where chronic cocaine treatment produced no change in ventilation. They did, however, observe an attenuation in the HVR following cocaine treatment but that was only after three weeks of administration (compared to the 14 days in the current study).

The lack of VAH-like changes in breathing in the cocaine control group (compared to the saline control group) was possibly due to cocaine acting (in opposite ways on breathing) at both the CNS and carotid bodies. Cocaine blocks the reuptake of dopamine and an increase in dopamine within the carotid bodies has been shown to cause a decrease in the HVR, an effect that opposes the increase in the HVR brought on by VAH (Bascom et al., 1991). Additionally, noradrenaline has also been shown to have
opposite effects on breathing; increasing breathing when released within the carotid body (Eldridge and Gill-Kumar, 1980) but decreasing breathing via its actions in the CNS (Champagnat et al., 1978). Therefore it is possible that the cocaine treatment produced competing effects within the CNS and carotid bodies and these opposing effects resulted in only a slight non-significant increase in ventilation in cocaine-treated animals compared to the saline controls. Lastly, even thought it has been shown that the D2-receptors are necessary for the formation of VAH (Huey et al., 2003), it may be that an increase in dopamine alone is not sufficient in the absence of chronic hypoxia to produce VAH.

Alternate routes of systemic administration would be unlikely to change the results observed in the current study, as intravenous injection or inhalation would ultimately still distribute the cocaine to both the CNS and carotid bodies. A possible route of administration, that would limit the effects of cocaine to the CNS, would be direct infusion into respiratory centres of the brain at doses low enough to prevent significant diffusion of cocaine out of the specific target areas. Although this approach was considered, it was rejected due to three possible complications. First, cocaine acts as a local anesthetic by blocking sodium channels (Andresen et al., 1994). Therefore administering it directly to the respiratory centres of the brain may lead to a cessation in breathing and death. Second, cocaine is administered in it’s salt form, cocaine hydrochloride, which is acidic and therefore when administered to the respiratory centres of the brain may stimulate breathing due to the localised acidification. These acidic effects on breathing would be very difficult to separate from any possible effects on respiration due to changes in monoamine neurotransmission. Third, cocaine treatment
(especially into the brain) can lead to increases in locomotor activity. The measurement of breathing via plethysmography requires that the animal sit relatively still within the plethysmograph. Any animal movement results in a distorted pressure signal making it very difficult, if not impossible, to accurately measure breathing.

The results of the cocaine treatment do, however, partly support the hypothesis that the role of monoamines in breathing is changed by the formation of VAH. Specifically, during the acute hypercapnic trials on day 15, there was an increase in inspiratory time in the control cocaine treatment group that did not appear in the chronically hypoxic cocaine group (Fig. 24A). In other words, the effect of cocaine on inspiratory time in the control group was abolished by exposure to chronic hypoxia. Although speculative, one may suggest that the effect of cocaine on inspiratory time was abolished by the formation of VAH because the role of the monoamines was changed by the development of VAH. Additionally, in a previous study (Knight, Erb and Reid, unpublished) chronic cocaine treatment also produced an increase in inspiratory time, which lead to a decrease in breathing frequency, at both 6% and 8% CO₂ and was also abolished by chronic hypoxia treatment (Knight, Erb and Reid, unpublished). A similar effect has been demonstrated for glutamate, where microinjections of MK-801 (an NMDA receptor channel blocker) into the NTS (Reid and Powell, unpublished) or intraperitoneally (Reid and Powell, 2006) resulted in the abolition of the HVR in rats that developed VAH (i.e., were exposed to chronic hypoxia) and not in control rats. Therefore it is possible that in the absence of VAH, monoamines function to attenuate the normally occurring increase in breathing frequency, via an increase in inspiratory time, that
accompanies the HCVR but after the changes in neurotransmission that are produced by 
VAH they are no longer able to do so.

**2E. Day 13 Versus Day 15**

During the day 13 acute breathing trials, cocaine was still being administered to 
the animals, whereas it was not during the day 15 breathing trials (as the mini-osmotic 
pumps only function for 14 days). Thus, performing the trials on days 13 and 15 
permitted assessment of the effects of cocaine on breathing both in the presence of 
administration of the drug (day 13) and during a period of time following the acute 
withdrawal of the drug (day 15). For the most part, there were no differences in any 
response on day 13 compared to day 15.

On the other hand, cocaine treatment did produce a significant increase in 
inspiratory time during exposure to 8% CO2, on day 15 in the control group (SC versus 
CC) that was not observed on day 13. However, this difference was not reflected in a 
statistically different change in breathing frequency. This difference, between trial days, 
on inspiratory time was most likely due to the presence of cocaine on day 13 and its 
absence on day 15. The acute effect of cocaine on breathing is a stimulatory one, as 
cocaine acts to increase breathing frequency via a decrease in inspiratory time by 
stimulating the inspiratory/expiratory phase-switching mechanisms within the PRG 
(Richard et al., 1991). Therefore, it is possible that a direct effect of cocaine on day 13 
caused a reduction in TI under these conditions (8% CO2) that was removed once cocaine 
administration had stopped on day 15. On the other hand, one would have also expected a 
difference between TI in the SC and CC groups (on day 13 at 8% CO2) if cocaine were
directly causing a decrease in T1. Clearly this was not observed. The difference between the 8% CO₂ T1 data on day 13 versus day 15 could be due to changes in monoamine receptors (i.e., they could have been down-regulated or desensitised) after the prolonged increase in stimulation with cocaine treatment. This however, remains speculative.

3. Chronic and Intermittent Hypoxia’s Effects on the Cocaine Induced Locomotor Response

We observed that animals given 10 days of chronic continuous exposure to hypoxia, group CH, exhibited a locomotor response to an acute cocaine injection after a 21-day drug-free period that was comparable to that exhibited by animals in the control condition, group CH (Fig. 27D). Thus, chronic continuous exposure to hypoxia did not cross-sensitise to the locomotor-activating effects of an acute cocaine challenge at test. Conversely, animals given 10 days of intermittent exposure to hypoxia, group IH, exhibited a significantly greater locomotor response to an acute cocaine injection at test compared to that exhibited by animals in the control condition, group No IH (Fig. 28D). Thus, intermittent exposure to hypoxia did cross-sensitise to the locomotor-activating effects of an acute cocaine challenge. Importantly, neither the chronic continuous nor intermittent hypoxia regimens produced changes in baseline levels of locomotor activity; that is, animals exposed to hypoxia, whether intermittent or chronic continuous, showed comparable levels of activity to their respective control groups during the habituation sessions (Figs. 27B and 28B). This observation rules out the possibility that the sensitised response to cocaine following intermittent exposure to hypoxia was a result of differences in baseline activity.
Our results of a cross-sensitising effect of intermittent, but not chronic continuous, exposure to hypoxia and cocaine are consistent with previous findings. In particular, there are several reports of a cross-sensitising effect of intermittent exposure to a stressor on a subsequent cocaine-induced response. In one study, daily intermittent exposure of rats to footshock (180 shocks per day, 8 sec intershock intervals, 2.5 mA/shock) for 3 days resulted in sensitised stereotypy to an acute cocaine challenge, when testing occurred 28 hours after termination of the stressor (MacLennan and Maier, 1983). Similarly, intermittent exposure to tail pressure (four 1-min applications of tail clamp pressure per day) for 15 days resulted in a sensitised stereotypy response to an acute amphetamine challenge, when testing occurred one day after termination of the stressor (Antelman et al., 1980). In contrast to these effects of intermittent stress exposure, Specker et al., (1994) reported that continuous exposure to food restriction stress for up to 18 days (maintenance at 75% of free-feeding weight) did not alter the subsequent acquisition of cocaine self-administration behaviour in rats.

Our findings of a cross-sensitising effect of intermittent, but not chronic continuous, exposure to hypoxia on cocaine-induced locomotor activity is also consistent with several findings concerning the sensitising effects of prior exposure to intermittent versus continuous exposure to drugs themselves. For example, intermittent exposure to cocaine (5mg/kg per day, i.p.) is associated with the development of sensitisation to the HPA-activating effects of the drug, whereas constant infusion of the drug (5 or 100mg/kg per day, i.p.) fails to induce such a sensitised response (Torres and Rivier, 1992). Likewise, intermittent exposure to morphine (25mg/kg per day, i.p.) is associated with
the development of a sensitised wheel running response, whereas a more frequent, twice-daily, exposure regimen is not (Shuster et al., 1975).

Although our findings are by and large consistent with the outcome of previous studies concerning the cross-sensitising effects of stress and cocaine, they are not consistent with studies exploring the mechanisms underlying the stress-induced sensitisation of responding to a stress challenge at test. For example, Jedema et al., (2001) reported that chronic continuous exposure to two weeks of cold stress (maintenance at 5°C) resulted in a sensitised electrophysiological response to a subsequent injection of the stress-related neuropeptide, corticotropin-releasing factor. In another study, chronic but not intermittent exposure to cold stress resulted in sensitised noradrenaline release in the prefrontal cortex in response to acute tail shock, whereas intermittent but not continuous exposure to foot shock resulted in a similar sensitised response (Jedema et al., 1999).

Collectively, these studies suggest that the type of stimulus presented during the pre-exposure phase, the regimen of presentation, the nature of the challenge at test, and the response measured at test all contribute to the development and expression of sensitisation.

In our study, the differential effect on sensitisation of the different stressor treatment regimens may be attributed, at least in part, to an element of predictability. Specifically, the intermittent hypoxia regimen, unlike the chronic continuous exposure regimen, served as a predictable form of stress, in that each daily exposure was cued by transfer from the vivarium to the room housing the hypobaric chamber, and placement in and pressurisation of the chamber. From this perspective, it is of interest that there is some evidence that exposure to predictable, but not unpredictable, stress is associated
with the development of cross-sensitisation to the locomotor-activating effects of cocaine. For example, Araujo et al. (2003) reported that one-hour exposures to restraint stress at the same time each day for 14 days resulted in a subsequent sensitised locomotor response to a challenge injection of cocaine. In contrast, animals exposed to variable stressors (e.g., food deprivation, restraint stress) at variable times of day during the pre-exposure phase did not exhibit a sensitised locomotor response to cocaine (Araujo et al., 2003). Thus, predictability may be a pre-requisite for the induction of behavioural sensitisation. In fact, there are numerous reports that conditioning factors (including time of day and environment) can play an important role in the expression of behavioural sensitisation (e.g., Vezina and Stewart, 1984; Vezina et al., 1989).

The ability of intermittent, but not chronic continuous, exposure to a stressor to induce cross-sensitisation to an acute challenge may relate to the differential effects of the exposure regimens on the endocrine stress response, and on other stress-related neurochemical systems, including perhaps most notably, dopamine. Whereas intermittent stress regimens, involving relatively brief daily exposures to stressors, tend to be associated with the sensitisation of stress-related endocrine and catecholaminergic responses, more chronic and prolonged exposure regimens tend to be associated with the habituation of those responses. For example, it has been reported that 4 to 6 hours of daily immobilisation stress leads to a progressive decrease in plasma concentrations of catecholamines (Kvetnansky et al., 1971b), whereas repeated briefer (2.5 hours) periods of stress lead to an augmentation in catecholamine biosynthesis enzyme concentrations (Kvetnansky et al., 1971a). In keeping with this finding, Ostrander et al. (2006) reported that exposure to chronic continuous stress, in the form of presentation of variable
stressors during the day, led to a decrease in the sensitivity of HPA axis to psychogenic stressors at four and seven days post stress. Given that the HPA axis has been implicated in the sensitising effects of exposure to drugs of abuse, including cocaine (Stohr et al., 1999; Przegaliński, 2000), this finding may have important relevance in the interpretation of our findings. In fact, it has been reported that continuous infusion of cocaine does not produce a significant change in plasma concentrations of ACTH or corticosterone, whereas intermittent daily injections of cocaine do (Torres and Rivier, 1991). Thus, it is possible that our findings can be explained, at least in part, by differences in the response of the HPA-axis to cocaine as a consequence of the regimen of prior exposure to hypoxia, with intermittent, but not chronic continuous, exposure leading to a sensitisation of the HPA response.

Given the critical role that the mesolimbic dopamine system plays in the cross-sensitising effects of stress and psychostimulants, and the fact that changes in environmental levels of O$_2$ and CO$_2$ can produce profound alterations in midbrain dopamine function (Brake et al., 1997; Flores et al., 2002), it is very likely that our effects can be explained by neuroadaptations in midbrain dopamine function. From this perspective, it is of interest that intermittent, but not chronic continuous, exposure to cocaine is associated with an increased potency of cocaine in inhibiting the reuptake of dopamine in the nucleus accumbens after a 7-day withdrawal period (Lee et al., 1998). In addition, whereas intermittent daily exposures to cocaine are associated with subsensitivity of the dopamine autoreceptors, continuous exposure to cocaine is associated with a supersensitivity of the autoreceptors (Jones et al., 1996). Thus, to the extent that hypoxia induces neuroadaptations within midbrain dopamine systems that are
similar to those induced by cocaine, our findings may be explained by differential alterations in these systems by the intermittent and continuous treatment regimens we employed. It is possible, that is, that intermittent exposure to hypoxia induced neuroadaptations that made the dopamine transporters more sensitive and autoreceptors less sensitive to the effects of a subsequent cocaine challenge. In contrast, it is also possible that exposure to chronic continuous hypoxia induced neuroadaptations that made the dopamine transporters less sensitive and autoreceptors more sensitive to the effects of a subsequent cocaine challenge.

4. Conclusion

This thesis has demonstrated that chronic cocaine treatment, in the absence of chronic hypoxia, is unable to induce the changes in breathing associated with VAH. These results indicate that either cocaine’s effects on the monoamine system are not sufficient to bring about VAH-like changes in breathing or that cocaine is acting within both the CNS and carotid bodies to produce opposing effects on breathing resulting in no overall change. Additionally, this thesis found that intermittent hypoxia resulted in a sensitisation to the cocaine-induced increase in locomotion whereas chronic continuous hypoxia did not. These results provide support to the theory that intermittent but not chronic continuous exposure to a stressor can produce behavioural sensitisation to cocaine. It is likely that these effects were the result of differential neuroadaptations within the midbrain dopamine system, where intermittent exposure lead to a sensitisation and chronic continuous exposure elicited subsensitivity.
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