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# Peripheral chemoreceptors determine the respiratory sensitivity of central chemoreceptors to $CO_2$: role of carotid body $CO_2$

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**Key Points**

- The influence of specific carotid body (CB) normoxic hypocapnia, hypercapnia and normocapnia on the ventilatory sensitivity of central chemoreceptors to systemic hypercapnia was assessed in seven awake dogs with extracorporeal perfusion of the vascularly isolated CB.
- Chemosensitivity in this preparation was similar to that in the intact animal.
- Separation of CB circulation from that of the brain was confirmed.
- When the isolated CB was hypercapnic *vs.* hypocapnic and when the isolated CB was normocapnic *vs.* hypocapnic, the group mean central $CO_2$ response slopes of minute ventilation ($\dot{V}_I$) ($P \leq 0.01$) and mean inspiratory flow rate ($V_T/T_I$) ($P \leq 0.05$) increased significantly. Tidal volume ($V_T$), breathing frequency ($f_b$) and rate of rise of diaphragm EMG were increased in 6 of 7 dogs but did not achieve statistical significance.
- We propose that hyperaddition is the dominant form of chemoreceptor interaction under conditions of quiet wakefulness in intact animals and over a wide range of CB $P_{CO_2}$ and $P_{O_2}$.

**Abstract** We asked if the type of carotid body (CB) chemoreceptor stimulus influenced the ventilatory gain of the central chemoreceptors to $CO_2$. The effect of CB normoxic hypocapnia, normocapnia and hypercapnia (carotid body $P_{CO_2} \approx 22$, 41 and 68 mmHg, respectively) on the ventilatory $CO_2$ sensitivity of central chemoreceptors was studied in seven awake dogs with vascularly-isolated and extracorporeally-perfused CBs. Chemosensitivity with one CB was similar to that in intact dogs. In four CB-denervated dogs, absence of hyper-/hypoventilatory responses to CB perfusion with $P_{CO_2}$ of 19–75 mmHg confirmed separation of the perfused CB circulation from the brain. The group mean central $CO_2$ response slopes were increased 303% for minute ventilation ($\dot{V}_I$)($P \leq 0.01$) and 251% for mean inspiratory flow rate ($V_T/T_I$) ($P \leq 0.05$) when the CB was hypercapnic *vs.* hypocapnic; central $CO_2$ response slopes for tidal volume ($V_T$), breathing frequency ($f_b$) and rate of rise of the diaphragm EMG increased in 6 of 7 animals but the group mean changes did not reach statistical significance. Group mean central $CO_2$ response slopes were also increased 237% for $\dot{V}_I (P \leq 0.01)$ and 249% for $V_T/T_I (P \leq 0.05)$ when the CB was normocapnic *vs.* hypocapnic, but no significant differences in any of the central ventilatory response indices were found between CB normocapnia and hypercapnia. These hyperadditive effects of CB hyper-/hypocapnia agree with previous findings using CB hyper-/hypoxia. We propose that hyperaddition is the dominant form of chemoreceptor interaction in quiet wakefulness when the chemosensory control system is intact, response gains physiological, and carotid body chemoreceptors are driven by a wide range of $O_2$ and/or $CO_2$.

## Introduction

Peripheral–central chemoreceptor interactive effects on control of breathing have been observed using animal models with isolated perfusion of the carotid body and/or central chemoreceptors in such varied conditions as eupnoea, apnoea, hypercapnia, and hypoxia (Day & Wilson, 2007,2009; Smith *et al.* 2007,2010; Blain *et al.* 2009,2010; Dempsey *et al.* 2012; Fiamma *et al.* 2013). However, the exact nature of these chemoreceptor interactions are controversial with studies in a wide variety of experimental preparations and theoretical models claiming additive, hyperadditive, or hypoadditive effects on the control of breathing (Duffin, 1990; Duffin & Mateika, 2013; Teppema & Smith, 2013; Wilson & Day, 2013). Based on these divergent findings some investigators (Wilson & Day, 2013; Guyenet, 2014) have suggested a 'hybrid' model as a basis for peripheral–central interaction, whereby variations in both the experimental models and in the prevailing physiological conditions and/or chemoreceptor stimuli may markedly alter the nature of the chemoreceptor interactions (also see Discussion).

Accordingly, in the present study we have tested the nature of the peripheral–central interaction under novel experimental conditions consisting of hypercapnic stimulation and hypocapnic inhibition at the level of the isolated CB chemoreceptor. This represents an important advance in addressing the interaction problem for several reasons. First, comparing normoxic hypercapnia/hypocapnia to results from our prior use of hypoxia/hyperoxia at the carotid body (Blain *et al.* 2010) provides a test of the equivalence of the observed hyperadditive interactive effect in the presence of both major peripheral chemoreceptor stimuli, *i.e.* $CO_2$ and $O_2$. Second, perturbations in $P_{aCO_2}$ *per se* have widespread physiological significance in the control of breathing and breathing stability during wakefulness and sleep, which appear to depend critically upon peripheral–central chemoreceptor interactions (Smith *et al.* 2007; Dempsey *et al.* 2012; Fiamma *et al.* 2013). Third, we tested these interactive effects in a unique awake canine preparation which incorporates two essential characteristics for quantifying the nature of these interactions, namely (a) that the preparation's chemoresponsiveness is within the physiological range and close to that in the intact animal, and (b) that central and peripheral chemoreceptors are truly separated both anatomically and functionally. Fourth, although there is no direct evidence we are aware of that the carotid sinus nerve discharge pattern can encode information concerning the nature of the carotid body stimulus, there are some lines of evidence showing that carotid body hypercapnia might have quite different cardiorespiratory influences from carotid body hypoxaemia. For example, in the awake goat, carotid body hypoxia, even for very short periods beyond the acute phase, progressively increased the ventilatory response whereas specific carotid body hypercapnia did not (Bisgard *et al.* 1986). In anesthetized goats short periods of hypoxia sensitized output of the carotid body chemoreceptor (Nielsen *et al.* 1988), whereas hypercapnia did not (Engwall *et al.* 1988). In anaesthetized rats, carotid body denervation prevented the response of $CO_2$ sensitive neurons in the retrotrapezoid nucleus to very brief exposures of reduced $F_{IO_2}$, but had no effect on their response to inhaled $CO_2$ (Mulkey *et al.* 2004). In anaesthetized rats conditioned by exposure to chronic intermittent hypoxia for 10 days, acute intermittent hypoxia elicited long-term facilitation of carotid sinus nerve output whereas acute intermittent hyperoxic hypercapnia did not (Peng *et al.* 2003). Further, in awake humans, acute periods of arterial isocapnic hypoxaemia or asphyxia elicited marked lingering after-effects on muscle sympathetic nerve activity once the stimulus was removed whereas similar periods of arterial normoxic hypercapnia did not (Morgan *et al.* 1995; Xie *et al.* 2000,2001).

We found that, in the awake dog, specific carotid body stimulation/inhibition by means of hyper- or hypocapnia resulted in hyperadditive interaction when the central chemoreceptors were stimulated by means of increased $P_{aCO_2}$. The similarity of these hyperadditive interactions to those caused primarily by means of changes in carotid body $P_{O_2}$ suggest that short-term changes in $P_{O_2}$ and $P_{CO_2}$ at the carotid body have equivalent effects on peripheral–central interaction for a given change in baseline ventilation.

## Methods

### Ethical approval

Experimental protocols and the roles of each investigator involved were approved by the Animal Care and Use

Committee of The University of Wisconsin-Madison School of Medicine and Public Health and were performed with strict adherence to all American Association for the Accreditation of Laboratory Animal Care International (AAALAC) and National Institutes of Health guidelines embodied in the *Guide for the Care and Use of Laboratory Animals*, 8th edition, published by the National Academies Press, 2011.

### Animals

Studies were performed during wakefulness on 13 unanaesthetized, spayed ($n = 12$) or anoestrus ($n = 1$), adult, female, mixed-breed dogs (20–25 kg). The dogs were trained to lie quietly in an air conditioned (19–22°C) sound attenuated chamber. The dogs were housed in an AAALAC accredited animal care facility. Seven of the 13 dogs were used in the main perfusion/interaction portion of the study. Four additional dogs were used to assess the anatomical isolation of our perfusion preparation. Two additional dogs were used to further assess the ventilatory effects of unilateral CB denervation.

### Surgical procedures and chronic instrumentation

Our preparation required two surgical procedures performed under general anaesthesia; identical anaesthetic protocols were used for both procedures. All dogs were pre-medicated with acepromazine (0.04–0.08 $mg \cdot kg^{-1}$ S.C. or I.M.) and buprenorphine (0.01–0.03 $mg \cdot kg^{-1}$ I.V. or I.M.); induced with propofol (3–5 mg $\cdot kg^{-1}$ I.V.); intubated immediately with a 7.5 mm ID cuffed endotracheal tube and maintained with 1–2% isoflurane in $O_2$ with mechanical ventilation. Strict sterile surgical techniques and appropriate postoperative analgesics and antibiotics were used. No agents producing neuromuscular blockade ('paralysing' agents) were used.

In the first procedure, using a ventral abdominal midline approach, 12 of 13 dogs were subjected to ovariectomy and hysterectomy, a chronic indwelling catheter was placed into the abdominal aorta via a branch of the femoral artery, and bipolar electromyogram (EMG) recording electrodes were installed into the costal diaphragm. The catheter and EMG wires were tunnelled subcutaneously to the dorsal aspect of the thorax where they were exteriorized a few centimetres caudal to the scapulae.

After a recovery interval of at least 3 weeks a second procedure was performed in which the left carotid body (CB) was denervated (CBD). The right carotid sinus was equipped with a vascular occluder and catheter to permit extracorporeal perfusion of the reversibly isolated carotid sinus–carotid body. A chronic indwelling catheter was also placed in the cranial vena cava via a branch of the jugular vein. Catheters were tunnelled subcutaneously to the lateral aspect of the dog's neck where they were exteriorized. Dogs recovered for at least 4 days before study.

The instrumentation was protected with a heavy nylon jacket and a padded 'Elizabethan' collar modified to allow normal eating and drinking.

Post-operative analgesia was achieved with buprenorphine (0.005 to 0.02 $mg \cdot kg^{-1}$ I.M. or I.V. q6–12 h; initial dose administered 30 min prior to the end of surgery) for the first 24 h in the first procedure and 12 h in the less invasive second procedure. We also administered one S.C. dose of carprofen (4 $mg \cdot kg^{-1}$; Pfizer, NY, USA) just after induction of anaesthesia. We continued carprofen P.O. (2.2 $mg \cdot kg^{-1}$ q 12 h or 4.4 $mg \cdot kg^{-1}$ q 24 h) for 3–7 days.

A wide-spectrum oral antibiotic (cephalexin, Karalex Pharma, NJ, USA) 20–30 $mg \cdot kg^{-1}$ P.O., BID or cefpodoxime proxetil (Pfizer, NY, USA) 10 $mg \cdot kg^{-1}$, P.O., SID or enrofloxacin (Bayer, KS, USA) 5–10 $mg \cdot kg^{-1}$, P.O., SID) was begun 24 h prior to surgery and was continued for 5–7 days post-operatively for the first procedure and 7–21 days for the second procedure. In addition, cefazolin (Sagent, IL, USA) (20–30 $mg \cdot kg^{-1}$, slow I.V. injection) was administered immediately pre-operatively and every 2 h intra-operatively.

It is important to note that we used spayed female dogs (and one ovaries and uterus-intact dog which was in anoestrus) to avoid problems with ventilatory effects of fluctuating ovarian hormone levels. We do not think this introduces a significant bias; we have discussed this in detail in a previous publication (Blain *et al.* 2009).

### Carotid sinus perfusion

Dogs lay unrestrained on a bed within the sound attenuated chamber. The extracorporeal circuit was primed with ~700 ml of saline, 120 ml of allogenic, packed red cell blood (Animal Blood Resources International, Dixon, CA, USA), and 2000 U of heparin and supplemented with 500–1000 $U \cdot h^{-1}$. $P_{CO_2}$, $P_{O_2}$ and pH in the perfusion circuit were set by adjustment of the gas concentrations supplying the circuit and by addition of $NaHCO_3$. Complete isolation of the carotid body from the systemic circulation and absence of any other peripheral chemosensitivity were confirmed by lack of ventilatory response to systemic intravenous injections of NaCN (20–30 $\mu g \cdot kg^{-1}$) during isolated sinus perfusion ('CB perfusion'). The retrograde perfusion of the carotid sinus region raised blood pressure in the sinus <10 mmHg, a level shown to have no effect on breathing in the unanaesthetized canine (Saupe *et al.* 1995). These techniques have been described in detail in previous publications (Smith *et al.* 1995,2006; Curran *et al.* 2000; Blain *et al.* 2009).

### Experimental set-up and measurements

Ventilation was measured using a tight-fitting muzzle mask connected to a pneumotachograph (model 3700, Hans Rudolph, Kansas City, MO, USA) that was calibrated before each study with four known flows. The dogs were acclimated to the mask during several weeks of training prior to study. Costal diaphragm EMG ($EMG_{di}$) signals were amplified, band-pass filtered (100–1000 Hz), rectified and moving-time-averaged (BMA-931; MA-821RSP, CWE Ardmore, PA, USA). End tidal $P_{O_2}$ and $P_{CO_2}$ were measured using appropriate analysers (Applied Electrochemistry S3-A, Pittsburgh, PA, USA and Sable Systems CA-1B, Las Vegas, NV, USA, respectively).

Arterial and perfusion circuit blood samples ($\sim$0.5–1.0 ml) were analysed for pH, $P_{O_2}$ and $P_{CO_2}$ on a blood gas analyser (ABL-5 or ABL 700, Radiometer, Copenhagen, Denmark). Blood pressure was recorded continuously from the femoral artery (Statham P23XL, Harvard Apparatus, Holliston, MA, USA).

Ventilation and blood pressure signals were digitized (128 Hz sampling frequency) and stored on the hard disk of a PC for subsequent analysis. Key signals were also recorded continuously on a polygraph (AstroMed K2G, West Warwick, RI, USA). All ventilatory data were analysed on a breath-by-breath basis by means of custom analysis software developed in our laboratory.

### Experimental protocol

**Interaction.** Eupnoeic ventilation and blood gases and the ventilatory response to hypercapnia via increased $F_{ICO_2}$ were determined under three sets of conditions: (a) when the carotid bodies were both intact with normal endogenous CB perfusion; (b) after unilateral CB denervation with normal endogenous CB perfusion; and (c) during normal, hypocapnic/normoxic, or hypercapnic/normoxic CB perfusion (see below). In the steady-state of each CB perfusion condition, the ventilatory response to inhaled $CO_2$ was assessed in a progressive, stepwise fashion consisting of 5–7 min intervals of air breathing followed by three increasingly higher levels of $F_{ICO_2}$ ($\sim$ 0.02, 0.04 and 0.06) such that $P_{aCO_2}$ was raised in steps achieving a maximum $P_{aCO_2}$ $\sim$10 mmHg >eupnoea. Arterial blood gases were obtained during 3–7 min of exposure to each level of $F_{ICO_2}$, and ventilation, diaphragm EMG activity and blood pressure were recorded continuously throughout.

For CB perfusion, each test protocol consisted of a 5–7 min control period (eupnoea), during which perfusion of the CB was endogenous, i.e. systemic arterial blood. Two 1 ml blood samples were collected at $\sim$3–7 min for determination of control value for blood gases and pH. Then, CB perfusion was abruptly switched to the extracorporeal circuit. The dogs were perfused from the extracorporeal circuit with blood gases and pH concentrations matching a given dog's eupnoeic values (CB normal), or with hypocapnic ($P_{CBCO_2}$ $\sim$20 mmHg) and normoxic blood, or with hypercapnic ($P_{CBCO_2}$ $\sim$60–70 mmHg) and normoxic blood. The different CB perfusate conditions were presented in random order and usually repeated at least once on a different day. In the steady-state of each CB perfusion condition, the ventilatory response to central hypercapnia was assessed as described above; usually two to four response trials were obtained for each CB perfusion condition. We were able to complete central $CO_2$ response trials in all three CB perfusion conditions in six dogs; in a seventh dog we completed only the CB hypo- and hypercapnic portions of the experiment.

**Assessing contamination of vertebral artery blood by perfusate.** Four dogs were instrumented as described above but underwent bilateral CB denervation. CBD was confirmed by absence of a ventilatory response to intravenous NaCN (20–30 $\mu$g·kg$^{-1}$). We used these dogs to assess the significance of potential CB perfusate contamination of the blood flow to the brain via the vertebral artery. To this end, air-breathing ventilation and blood gases were assessed during carotid sinus region perfusion with hypocapnic and hypercapnic perfusates. In two of these bilaterally carotid body denervated dogs we also tested their ventilatory response to inhaled $CO_2$ (as described above) to verify that these animals were responsive to systemic hypercapnia.

### Killing

Upon completion of the protocol, dogs ($n = 12$) were humanely killed with an overdose of 200 mg propofol into the implanted I.V. catheter followed by 5 ml Beuthanasia-D Special (Schering Plough, NJ, USA) via the same catheter after full unconsciousness was achieved. One dog was enrolled in a special protocol at the University of Wisconsin that allows removal of instrumentation and subsequent adoption after a suitable period of recovery.

### Statistics

Slopes of the $CO_2$ responses were determined by linear regression. Significance of the differences in group mean ventilatory responses to central hypercapnia between the three CB perfusion conditions was determined by means of one-way ANOVA. Significant ANOVAs were followed by Tukey's HSD post hoc analyses with correction for unequal $n$ using the harmonic mean of $n$ (Portney & Watkins, 2009). The significance of the ventilatory effects of unilateral CBD was determined by means of one-way ANOVA. Means are presented as mean $\pm$ SD. Differences were considered significant if $P \leq 0.05$.

**Table 1. Eupnoeic values during air breathing when the dogs were intact *vs.* unilateral CBD(CB not perfused)**

|  | Intact | Unilateral CBD |
|---|---|---|
| $n$ | 7 | 7 |
| $pH_a$ | 7.37 (0.03) | 7.35 (0.03) |
| $P_{aCO_2}$ (mmHg) | 39.15 (2.52) | 43.70 (5.94) |
| $P_{aO_2}$ (mmHg) | 101.80 (3.54) | 100.95 (5.70) |
| $\dot{V}_I$ (L·min$^{-1}$) | 4.50 (0.78) | 3.80 (0.59) |
| $V_T$ (L) | 0.36 (0.05) | 0.37 (0.10) |
| $f_b$ (breaths·min$^{-1}$) | 12.84 (2.91) | 11.12 (3.35) |
| $V_T/T_I$ (L·s$^{-1}$) | 0.21 (0.02) | 0.18 (0.02) |

Data are means (SD).

## Results

### Unilateral carotid body denervation

Table 1 shows the effects of unilateral CBD on key ventilatory components for the seven dogs used in the main interaction portion of this study. On average, there was a modest, variable and non-significant hypoventilation ($P_{aCO_2}$ = +4.5 ± 5.9 mmHg; $P$ > 0.08) following unilateral CBD. There were no significant changes in the other ventilatory components. Including two earlier studies from our laboratory (Curran *et al.* 2000; Blain *et al.* 2010) as well as the present study we now have data from 26 dogs before and within 7–10 days following unilateral CBD. In this larger pool of dogs we found a small but significant ($P$ < 0.01) average $CO_2$ retention compared to intact controls (intact: $P_{aCO_2}$ = 39.5 ± 1.9 mmHg *vs.* unilateral CBD: $P_{aCO_2}$ = 41.9 ± 3.9 mmHg). Fifteen of the 26 dogs hypoventilated more than 1.5 mmHg $P_{aCO_2}$ relative to the intact state; three hyperventilated more than 1.5 mmHg, and the remaining 8 dogs changed less than 1.5 mmHg. Intact *vs.* unilateral CBD ventilatory $CO_2$ response slopes were available in 19 of the 26 dogs. The intact ventilatory response to hypercapnia averaged 0.69 ± 0.28 L·min$^{-1}$·mmHg$^{-1}$ and was not significantly different from the unilateral CBD response, which averaged 0.63 ± 0.22 ($P$ > 0.4).

### Assessing contamination of vertebral artery blood by CB perfusates

In two bilaterally carotid body denervated dogs we obtained normoxic steady-state ventilatory response curves to systemic hypercapnia via increased $F_{ICO_2}$ which were decreased relative to the intact condition by 40% ($\Delta\dot{V}_I$ /$\Delta P_{aCO_2}$, 0.57 to 0.34 L·min$^{-1}$·mmHg$^{-1}$) and 70% ($\Delta\dot{V}_I$ /$\Delta P_{aCO_2}$, 0.81 to 0.25 L·min$^{-1}$·mmHg$^{-1}$). Taken together with our earlier findings in this species (Rodman *et al.* 2001), these data confirm that our awake canine model remains highly responsive to systemic (and therefore central) hypercapnia even after bilateral CBD.

Table 2 shows the changes in steady-state eupnoeic air-breathing $P_{aCO_2}$ in four carotid body denervated dogs (the two dogs mentioned in the preceding paragraph plus two additional dogs) in which the carotid sinus region was perfused for 5–10 min with blood that was hypocapnic ($P_{CBCO_2}$ 19–24 mmHg) or hypercapnic ($P_{CBCO_2}$ 66–75 mmHg). Note that the steady-state changes in $P_{aCO_2}$ and the ventilatory variables are small and not correlated with the $P_{CO_2}$ of the perfusate; nor were any transient changes observed in ventilation or $P_{ETCO_2}$ at any time throughout the 5–10 min period of carotid sinus perfusion. The finding that increases or decreases in carotid sinus perfusate $P_{CO_2}$ did not change ventilation in a systematic, predictable fashion in these carotid body denervated dogs indicates that any contamination of the right vertebral artery blood flow with blood that we perfused into the carotid sinus region was functionally insignificant.

### Effects of CB hypercapnia and hypocapnia on eupnoea

**Time course.** In four dogs (of the 7 used in the main perfusion/interaction portion of the study) we obtained technically acceptable transitions from non-perfused to CB perfusion with hypercapnic blood during room air breathing. All four dogs increased $\dot{V}_I$ (mean $\Delta\dot{V}_I$ = +2.32 L·min$^{-1}$; range 0.36–2.89) reaching a peak $\dot{V}_I$ at 29.7 s (mean; range 10–46 s) after perfusion was initiated. All four dogs achieved partial compensation for the transient hyperventilation such that the steady-state $\Delta\dot{V}_I$ values (obtained in the third to seventh minute of perfusion; see Methods) averaged +0.23 L·min$^{-1}$ (range −0.16 to +0.53).

In five dogs (of the 7 used in the main perfusion/interaction portion of the study) we obtained technically acceptable transitions over 3–7 min periods from non-perfused to CB perfusion with hypocapnic blood during room air breathing. All five dogs decreased $\dot{V}_I$ reaching a nadir value (mean $\Delta\dot{V}_I$ = −1.74 L·min$^{-1}$; range −0.39 to −3.57) at 19.7 s (mean; range = 3–32 s) after hypocapnic perfusion was initiated. All five dogs achieved partial compensation for the transient hypoventilation such that the steady-state $\Delta\dot{V}_I$ values (obtained in the third to seventh minute of perfusion; see Methods) averaged −0.43 L·min$^{-1}$ (range +0.62 to −1.68).

Figure 1 shows examples of transitions in response to the hypercapnic CB perfusion in two representative dogs, one with a brisk ventilatory response to changes in $P_{CBCO_2}$ (dog X) and one with a relatively small response (dog W). These transition data are consistent with previous reports from our laboratory (Smith *et al.* 1995; Blain *et al.* 2009).

**Steady-state.** Table 3 shows the effect on steady-state, air-breathing eupnoea during specific carotid body

**Table 2. Mean changes, relative to non-CB perfused, in steady-state air-breathing in response to hypercapnic and/or hypocapnic CB perfusates in four CBD dogs**

| Dog | $P_{CBCO_2}$ (mmHg) | $\Delta P_{aCO_2}$ (mmHg) | $\Delta \dot{V}_I$ (L·min⁻¹) | $\Delta V_T$ (L) | $\Delta f_b$ (breaths·min⁻¹) | $\Delta V_T/T_I$ (L·s⁻¹) | $\Delta$DiRR (% of control) |
|---|---|---|---|---|---|---|---|
| B | 19 | −0.3 | 0.6 | 0.04 | 0.2 | 0.05 | −0.8 |
| D | 24 | −0.7 | −0.1 | −0.03 | 0.1 | 0.00 | 6.7 |
| D | 66 | −1.1 | −0.2 | 0.03 | −1 | 0.01 | 1.0 |
| N | 75 | 0.3 | 1.2 | −0.32 | 1.8 | 0.01 | −31.9 |
| Q | 24 | −1.6 | 1.2 | 0.11 | −3.4 | 0.09 | −33.7 |
| Q | 68 | 0.5 | −0.4 | 0.02 | −2.8 | −0.01 | −7.9 |

**Table 3. Air breathing eupnoeic values during CB perfusion**

| | CB hypercapnia | CB normocapnia | CB hypocapnia |
|---|---|---|---|
| $n$ | 7 | 6 | 7 |
| $P_{CBCO_2}$ (mmHg) | 68.2 (8.3) | 41.1 (6.1) | 22.3 (2.9) |
| pHa | 7.35 (0.05) | 7.34 (0.03) | 7.33 (0.03) |
| $P_{aCO_2}$ (mmHg) | 41.99 (9.67) | 42.51 (7.65) | 45.61 (6.70) |
| $P_{aO_2}$ (mmHg) | 112.64 (14.51) | 102.40 (5.58) | 94.16 (5.70) |
| $\dot{V}_I$ (L·min⁻¹) | 4.82 (1.04) | 4.21 (0.91) | 4.35 (1.13) |
| $V_T$ (L) | 0.47 (0.15) | 0.42 (0.08) | 0.41 (0.12) |
| $f_b$ (breaths·min⁻¹) | 10.85 (2.75) | 10.28 (2.59) | 10.90 (3.05) |
| $V_T/T_I$ (L·s⁻¹) | 0.23 (0.05) | 0.21 (0.02) | 0.20 (0.02) |
| DiRR (% of control) | 107.17 (24.95) | 95.63 (7.54) | 96.94 (15.55) |

Data are means (SD).

normocapnia, hypercapnia and hypocapnia during CB perfusion. We observed that rather marked changes in CB $P_{CO_2}$ of ±20–30 mmHg were required to elicit measurable steady-state hyper- or hypoventilation during air-breathing in the steady-state. These responses were also highly variable among animals. Thus, comparing the group mean responses to CB hypercapnia vs. CB hypocapnia where all seven dogs are represented there was a non-significant trend toward hyperventilation (42 vs. 45.6 mmHg $P_{aCO_2}$) which is accompanied by non-significant trends toward alkalosis and increased $\dot{V}_I$, $V_T$, $V_T/T_I$, and rate of rise of the diaphragm EMG (DiRR). Eupnoeic $f_b$ was unchanged by either hypercapnic or hypocapnic CB perfusion.

### Effects of CB hypercapnia and hypocapnia on the central ventilatory response to systemic hypercapnia

In the time course response to CB hyper- and hypocapnia shown in Fig. 1, the ventilatory response to progressive increases in $F_{ICO_2}$ and, therefore, central hypercapnia are shown over the final three sections of each panel. It is readily apparent in both animals that the magnitude of the steady-state ventilatory responses to comparable levels of increasing systemic hypercapnia was substantially enhanced in the presence of CB hypercapnia (left panels)

vs. CB hypocapnia (right panels). This hyperadditive effect was observed during both the early transitional and the steady-state phases of the ventilatory responses to central hypercapnia.

Figure 2 illustrates the steady-state responses of five ventilatory components in a representative dog to increased central $P_{aCO_2}$ during CB perfusion with normocapnic, hypercapnic and hypocapnic blood. Note that the peripheral–central ventilatory interaction for $\dot{V}_I$ is hyperadditive (i.e. increased slope of CB hypercapnia vs. CB normocapnia vs. CB hypocapnia). In this dog, the hyperaddition of the $\dot{V}_I$ response between CB hypocapnia and CB hypercapnia was due entirely to hyperadditive responses of $f_b$, $V_T/T_I$, and rate of rise of the diaphragm EMG (DiRR) as the $V_T$ response was additive (i.e. no slope change). The slope of the central ventilatory response to CB normocapnia was intermediate to those for CB hypo- and hypercapnia for most variables except $V_T/T_I$ and $V_T$.

Central $CO_2$ response slopes for all variables during CB normocapnia, hypercapnia and hypocapnia are summarized as individual slopes and group means for all animals in Fig. 3. The central $CO_2$ response slopes for $\dot{V}_I$ and $V_T/T_I$ were significantly increased (0.27 ± 0.11 vs. 0.82 ± 0.24 L min⁻¹·mmHg⁻¹ (means ± SD, $P \le 0.01$) and 0.024 ± 0.006 vs. 0.010 ± 0.009 L·s⁻¹·mmHg⁻¹ ($P \le 0.05$), respectively. When isolated CB hypocapnia was compared to CB hypercapnia. Increases in the central $CO_2$ response for $V_T$, $f_b$, and DiRR between CB hypo- vs. hypercapnia increased in 6 of 7 dogs but did not reach statistical significance ($P > 0.05$; also see Fig. 3). Similarly, the central $CO_2$ responses between CB hypocapnia and CB normocapnia were increased for $\dot{V}_I$ and $V_T/T_I$ (0.27 ± 0.11 vs. 0.64 ± 0.25 L·min⁻¹·mmHg⁻¹ ($P \le 0.01$) and 0.010 ± 0.006 vs. 0.024 ± 0.010 L·s⁻¹·mmHg⁻¹ ($P \le 0.05$) respectively. Increases in the central $CO_2$ response for $V_T$, $f_b$, and DiRR between CB hypocapnia and CB normocapnia increased in 5 of 6 dogs but did not reach statistical significance ($P > 0.05$; also see Fig. 3). Finally, none of the central $CO_2$ response slopes between CB normocapnia and CB hypercapnia changed significantly ($P > 0.05$) although most dogs showed an increased slope with CB hypercapnia.

Figures 4–8 show individual steady-state response slopes to central hypercapnia for five key components of ventilation comparing only the hypercapnic and hypocapnic CB perfusion conditions. These figures make the point that hyperaddition (i.e. hypercapnic CB perfusion ventilatory response slopes steeper than hypocapnic CB perfusion ventilatory response slopes) is a consistent finding in all seven animals for most variables. The slopes of the ventilatory responses to central $P_{aCO_2}$ during CB hypercapnia are higher than those during CB hypocapnia in 7 of 7 dogs for $\dot{V}_I$ and $V_T/T_I$ and in 6 of 7 dogs for $V_T$, $f_b$, and DiRR. While the ventilatory responses to a

$\pm$ 20–30 mmHg change in $P_{CO_2}$ in the CB perfusate elicited steady-state changes in air-breathing $P_{aCO_2}$ only over a range of $\pm$4.3 mmHg, these changes in CB $P_{CO_2}$ were accompanied by $\pm$2- to -4-fold changes in the central CO$_2$ responses. Dogs V and W had the lowest and most variable $\dot{V}_I$ response slopes to central CO$_2$ and their eupnoeic ventilation showed little or no steady-state response to CB hypo- or hypercapnia. However, as with the other five dogs, V and W showed clear hyperadditive effects of CB hypocapnia *vs.* CB hypercapnia for most ventilatory components in response to central hypercapnia.
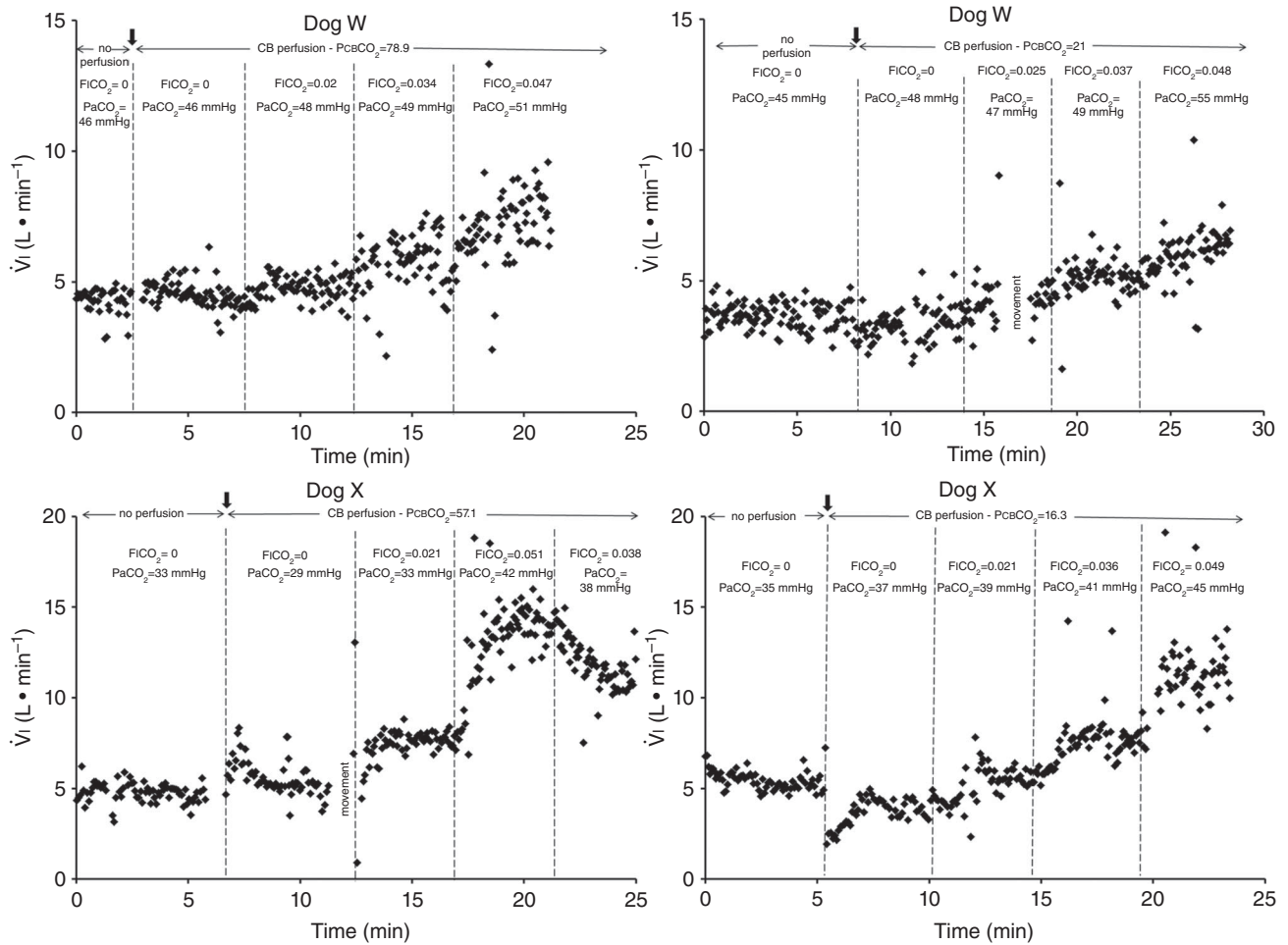


**Figure 1. Breath-by-breath time course plots showing the effects of hypercapnic and hypocapnic CB perfusion on ventilation and the ventilatory response to systemic hypercapnia in two representative dogs**
Key transitions are marked by vertical dashed lines; the initiation of perfusion is indicated by a filled arrow. $P_{CBCO_2}$, $F_{ICO_2}$ and $P_{aCO_2}$ for each condition are indicated above each panel. Dog W (upper panels) showed limited response to CB hypo- or hypercapnic perfusion during air breathing. Dog X (lower panels) showed larger responses to CB hypo- or hypercapnic perfusion during air breathing. Note that in both dogs,(a) there was a transient hyper-or hypoventilation in response to CB hypercapnia and CB hypocapnia, respectively (filled arrow and vertical dashed line),(b) after the transient peak or nadir, partial ventilatory compensation occurred (between first two vertical dashed lines in each panel), and(c) the ventilatory responses to increased $F_{ICO_2}$ and systemic hypercapnia (last 3 sections in each panel) were greater in both dogs during CB hypercapnia *vs.* CB hypocapnia (compare right panel to left panel in each dog after the filled arrow and vertical dashed line).

## Discussion

### Summary of findings

Our study was concerned with the nature of the dependence of the ventilatory response to brain ('central') hypercapnia on $CO_2$-induced inhibition *vs.* stimulation of the vascularly-isolated carotid body chemoreceptor in the canine during quiet wakefulness. Using this preparation we determined that the slopes of the mean responses of $\dot{V}_I$, $V_T$, $f_b$, $V_T/T_I$ and DiRR to increasing steady-state levels of central hypercapnia were increased by 185–462% when the isolated carotid body was hypercapnic relative to when the isolated carotid body was hypocapnic; these changes were statistically significant for $\dot{V}_I$ and $V_T/T_I$. These 2- to 4-fold changes in the central $CO_2$ response occurred despite a relatively small range of changes in air-breathing ventilation ($\Delta P_{aCO_2}$ range −4.3 to +4.3 mmHg) in response to CB hyper-/hypocapnia. These findings demonstrate hyperadditive influences of $CO_2$-induced carotid body stimulation/inhibition on the
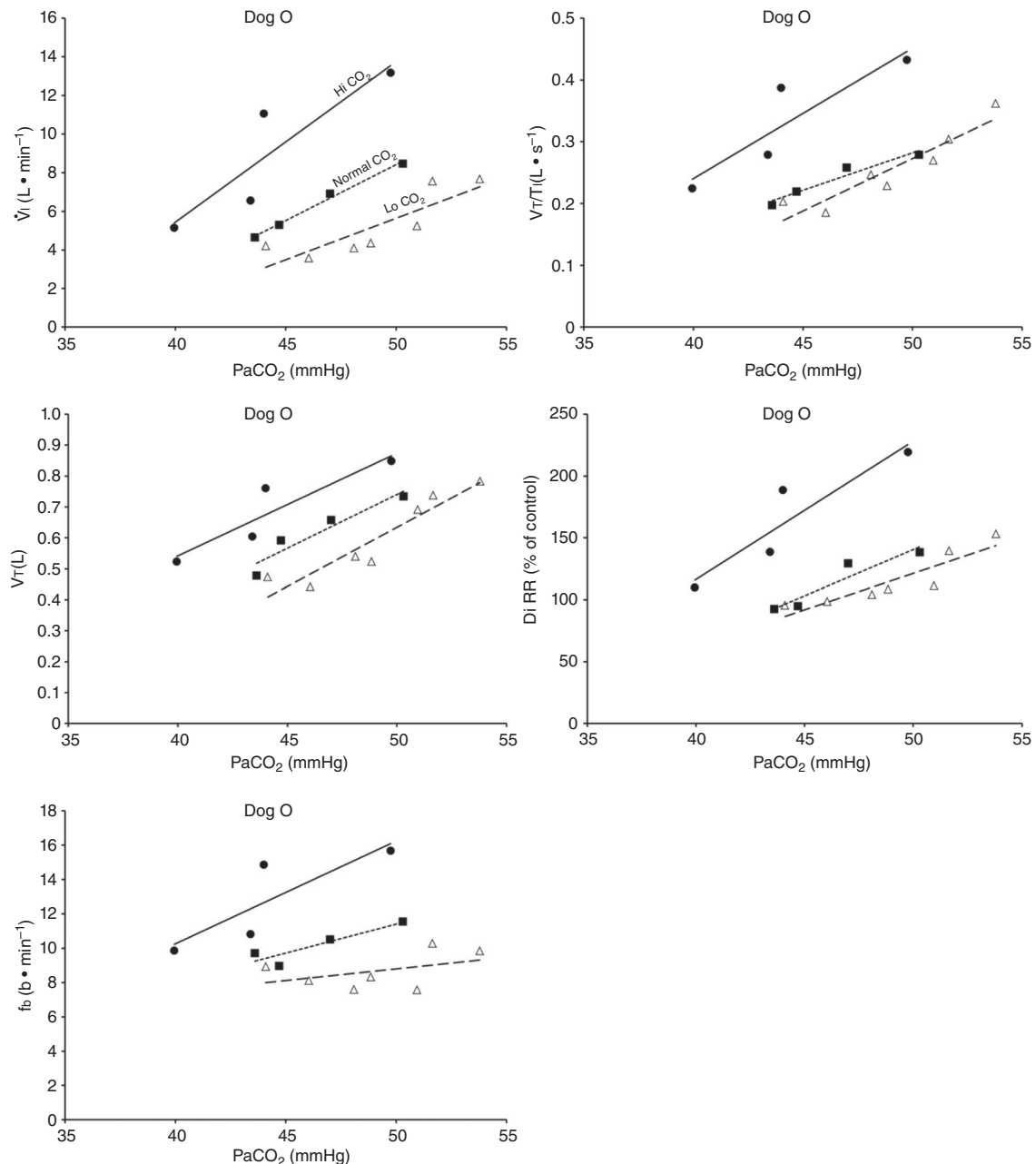


**Figure 2. Steady-state ventilatory responses of five key ventilatory components to increased $FICO_2$**
Data obtained during CB normocapnic ($P_{CBCO_2} = 39.2$ mmHg), CB hypercapnic ($P_{CBCO_2} = 73.4$ mmHg) and CB hypocapnic ($P_{CBCO_2} = 22.3$ mmHg) perfusion in a representative dog. Lines are linear regressions.

central ventilatory response to $CO_2$/pH. Significant but smaller increases in the central $CO_2$ responses also occurred between CB hypocapnia and normocapnia but not between CB normocapnia and hypercapnia. These hyperadditive effects between the peripheral and central chemoreceptors are in the same direction as and of similar magnitude to those previously reported in this canine

preparation for alterations in (primarily) $P_{O_2}$ at the carotid chemoreceptor (Blain *et al.* 2010).

### Limitations of our preparation

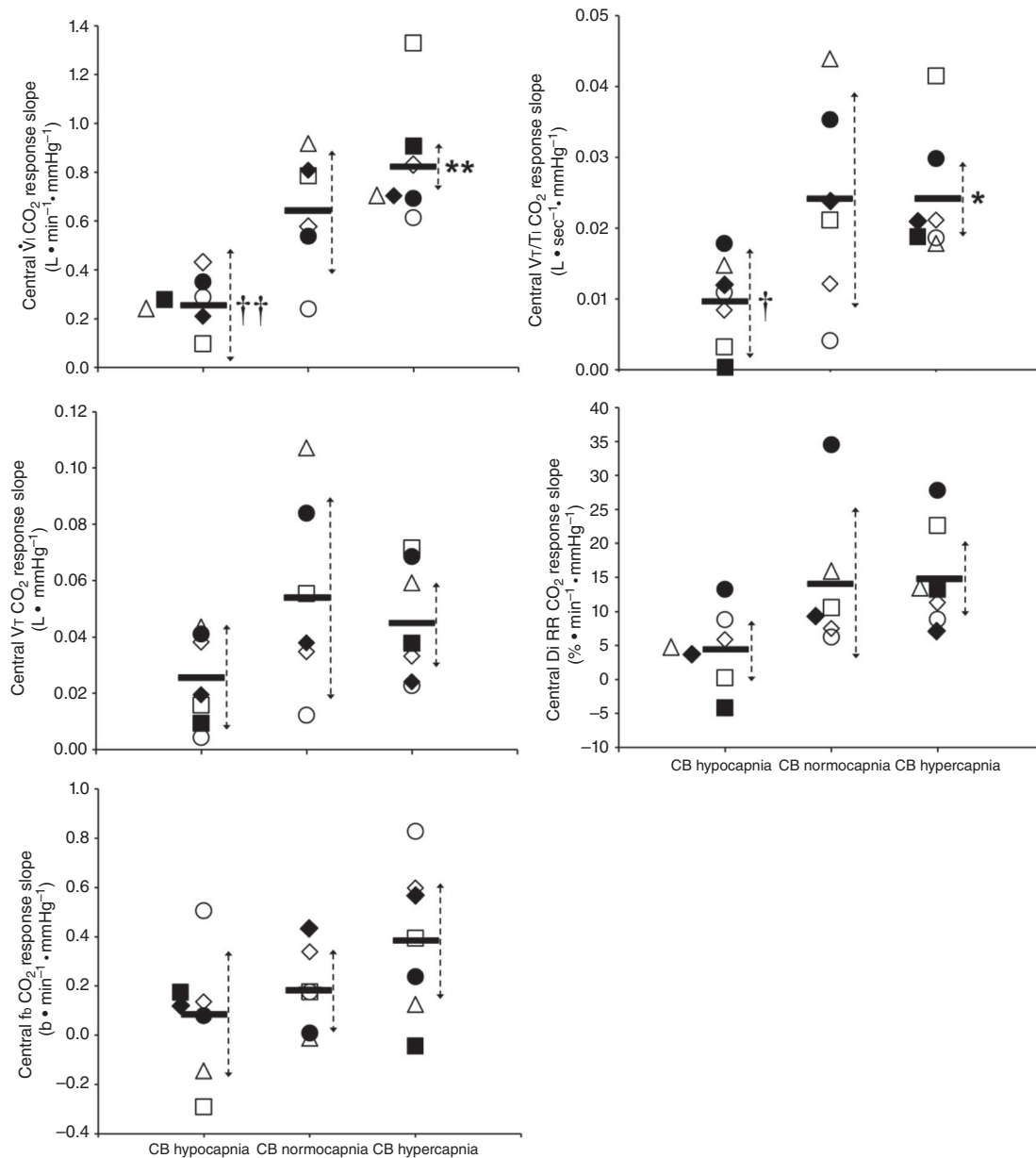**Perfusate contamination of vertebral artery blood.** We have reported previously that our animal preparation has



**Figure 3. Individual and mean ventilatory response slopes to increased systemic $Pa$CO$_2$ grouped by CB perfusate condition**
Each type of symbol represents the responses of one dog; the thick horizontal bars represent the group means; vertical dashed arrows represent ±95% confidence interval. *Mean value significantly different (*$P \leq 0.05$; **$P \leq 0.01$) from hypocapnic mean; †significantly different (†$P \leq 0.05$; ††$P \leq 0.01$) from normocapnic mean. Some symbols have been moved to the left where extensive overlap between points occurred. $n = 7$ for CB hypocapnia ($P_{CBCO_2} = 22.3 \pm 2.9$ mmHg) and CB hypercapnia ($P_{CBCO_2} = 68.2 \pm 8.3$ mmHg), $n = 6$ for CB normocapnia ($P_{CBCO_2} = 41.1 \pm 6.1$ mmHg). (See text for details.)

the potential for a portion of the perfusate of the isolated right carotid sinus region to reach the brain via the right vertebral artery after mixing with systemic blood in the brachiocephalic artery (Blain *et al.* 2010). We note that the right vertebral artery is only one of three major arteries supplying the brain in our preparation; both the left carotid and left vertebral blood flows are not subject to contamination from the carotid body perfusate. We previously established, based on measurements of $\sim 500$ ml · min$^{-1}$ blood flow in the brachiocephalic artery,
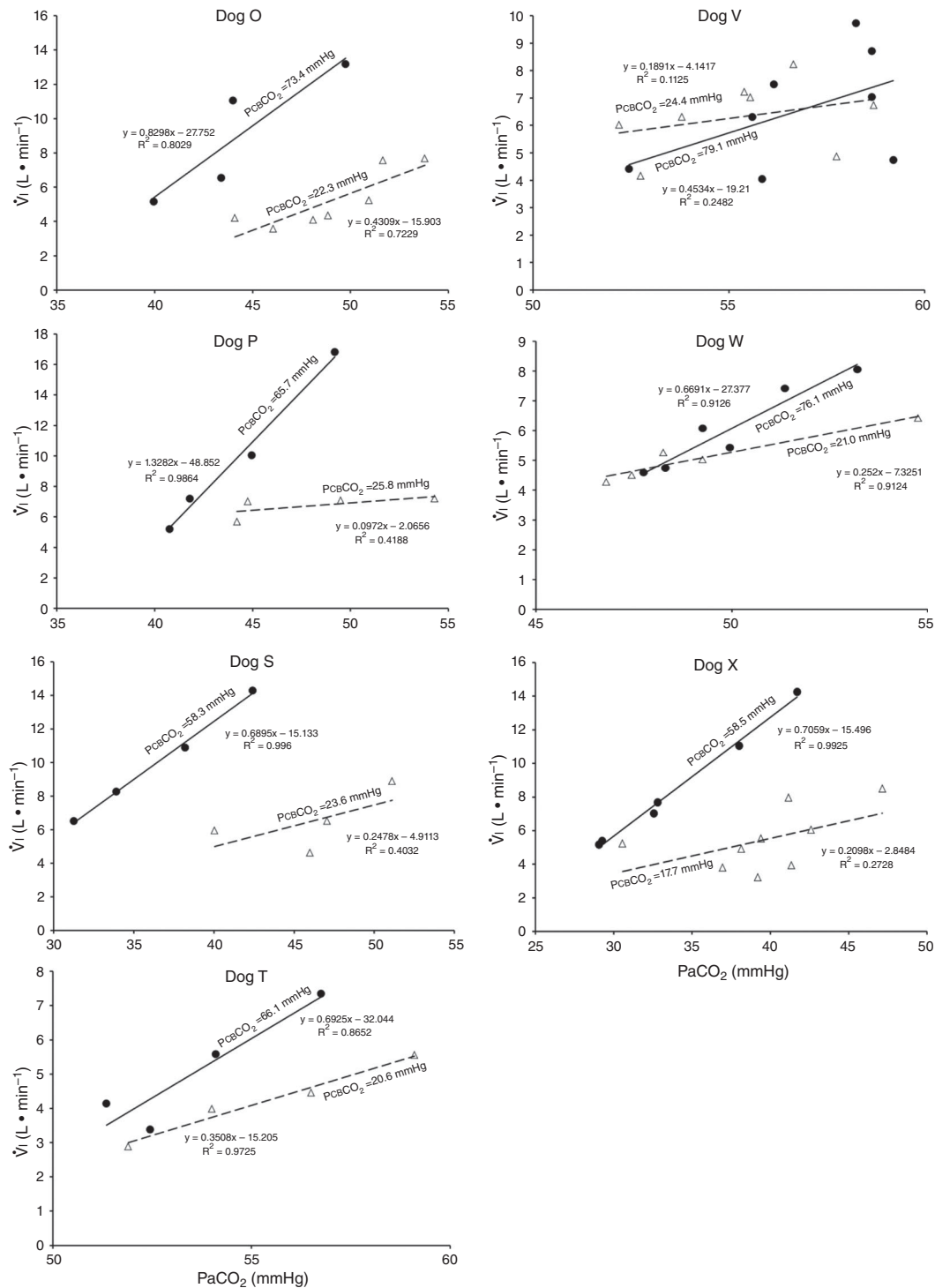


**Figure 4. Ventilatory responses of $\dot{V}_I$ to increased $F_{I}CO_2$ during CB hypercapnic and CB hypocapnic perfusion for each of the seven dogs**
Regression equations and $r^2$ values are adjacent to the regression line they pertain to. (See text for details.)

that our retrograde perfusion of the right carotid artery at a rate of $\sim$40–60 ml·min$^{-1}$ would provide a 5-to10-fold dilution of the perfusate blood available for entry into the right vertebral artery (Blain *et al.* 2010). In the present study we tested the functional consequences of potential contamination of the central blood supply via the CB perfusate by assessing the ventilatory response to perfusion of the isolated carotid sinus region with
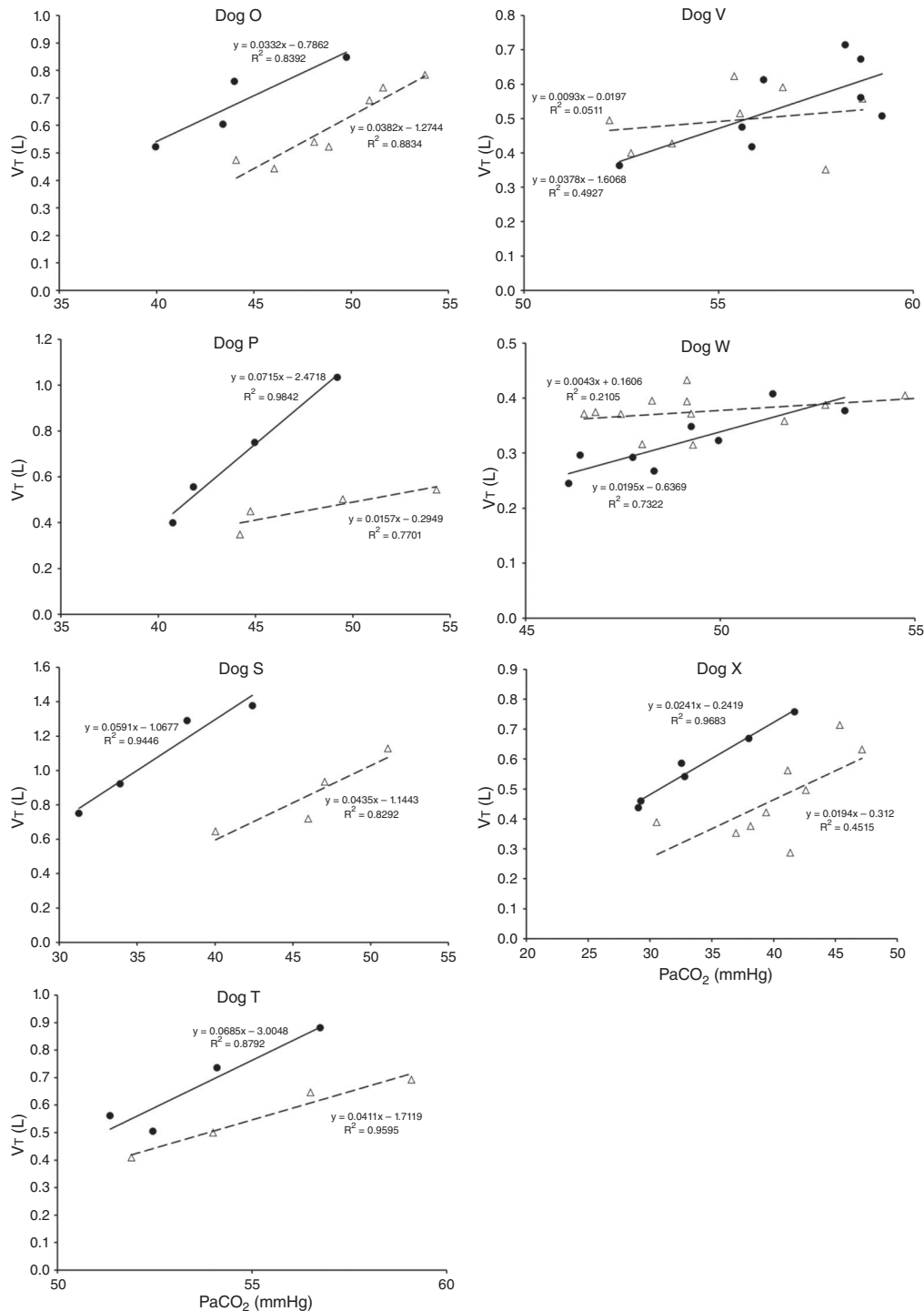


**Figure 5. Ventilatory responses of $V_T$ to increased $F_{ICO_2}$ during CB hypercapnic and CB hypocapnic perfusion for each of the seven dogs**
Regression equations and $r^2$ values are adjacent to the regression line they pertain to (See text for details). Symbols as in Figure 4.

markedly hypocapnic or hypercapnic blood in four carotid body denervated animals. We found no consistent effect of these changes in carotid sinus perfusate $CO_2$ on eupnoeic, air-breathing ventilation, breathing pattern, or diaphragmatic EMG (Table 1). This lack of response to

changes in $P_{CO_2}$ in the carotid sinus perfusate contrasts with the substantial effects of an increased systemic $P_{aCO_2}$ (via increased $F_{ICO_2}$) (Bisgard *et al.* 1980; Pan *et al.* 1998; Rodman *et al.* 2001; Dahan *et al.* 2007) (also see Results) or reductions in systemic $P_{aCO_2}$ (via mechanical ventilation)
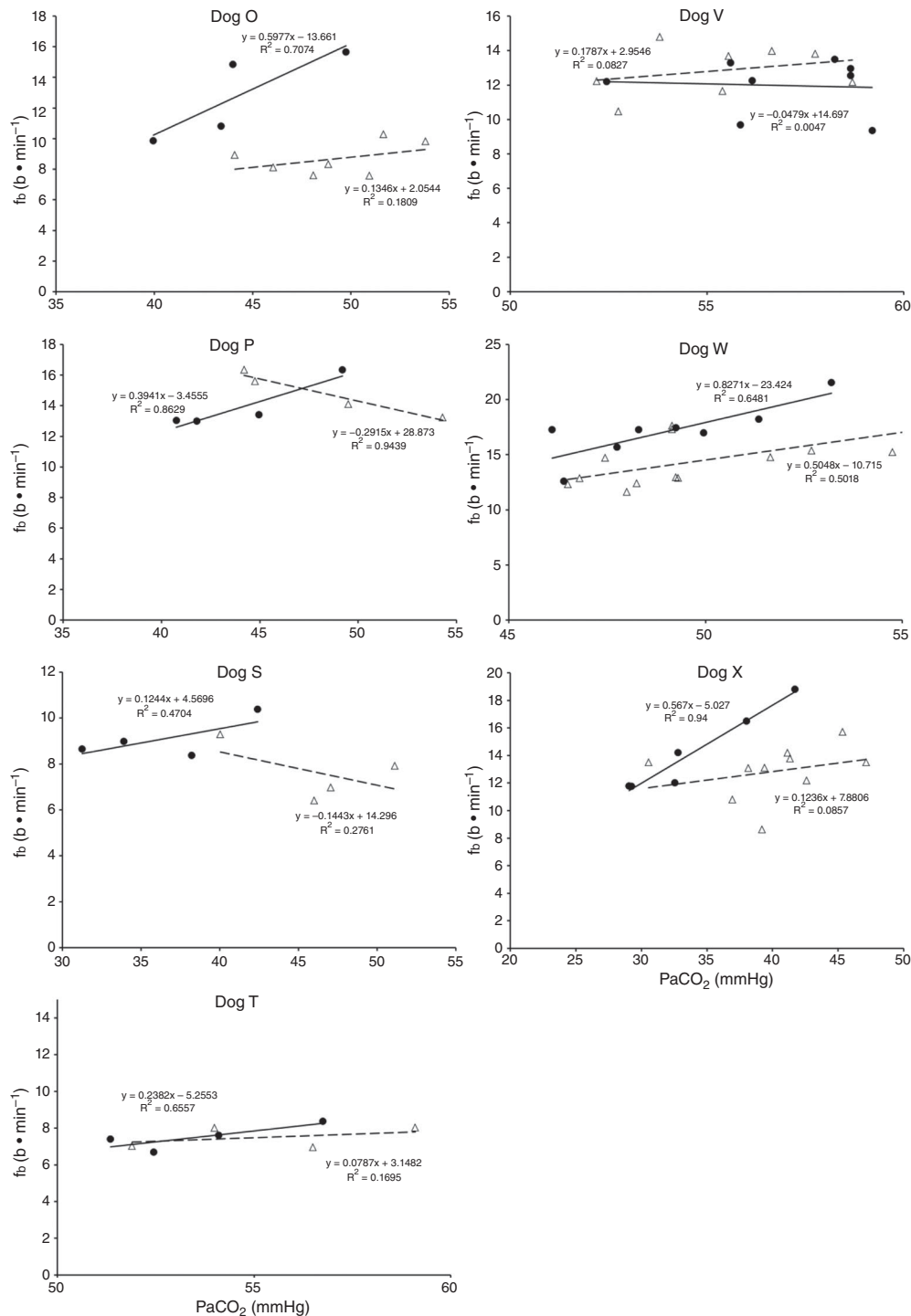


**Figure 6. Ventilatory responses of $f_b$ to increased $F_{ICO_2}$ during CB hypercapnic and CB hypocapnic perfusion for each of the seven dogs**
Regression equations and $r^2$ values are adjacent to the regression line they pertain to (See text for details). Symbols as in Figure 4.

(Nakayama *et al.* 2003) on eupnoeic ventilation in the bilaterally CB denervated dog, goat, or human in which the central $P_{CO_2}$ would clearly have been influenced by changes in the systemic $P_{CO_2}$. Taken together, we interpret these results to mean that any contamination of vertebral artery perfusion of the brain with our carotid sinus perfusates was insufficient to exert discernible functional effects on ventilation.
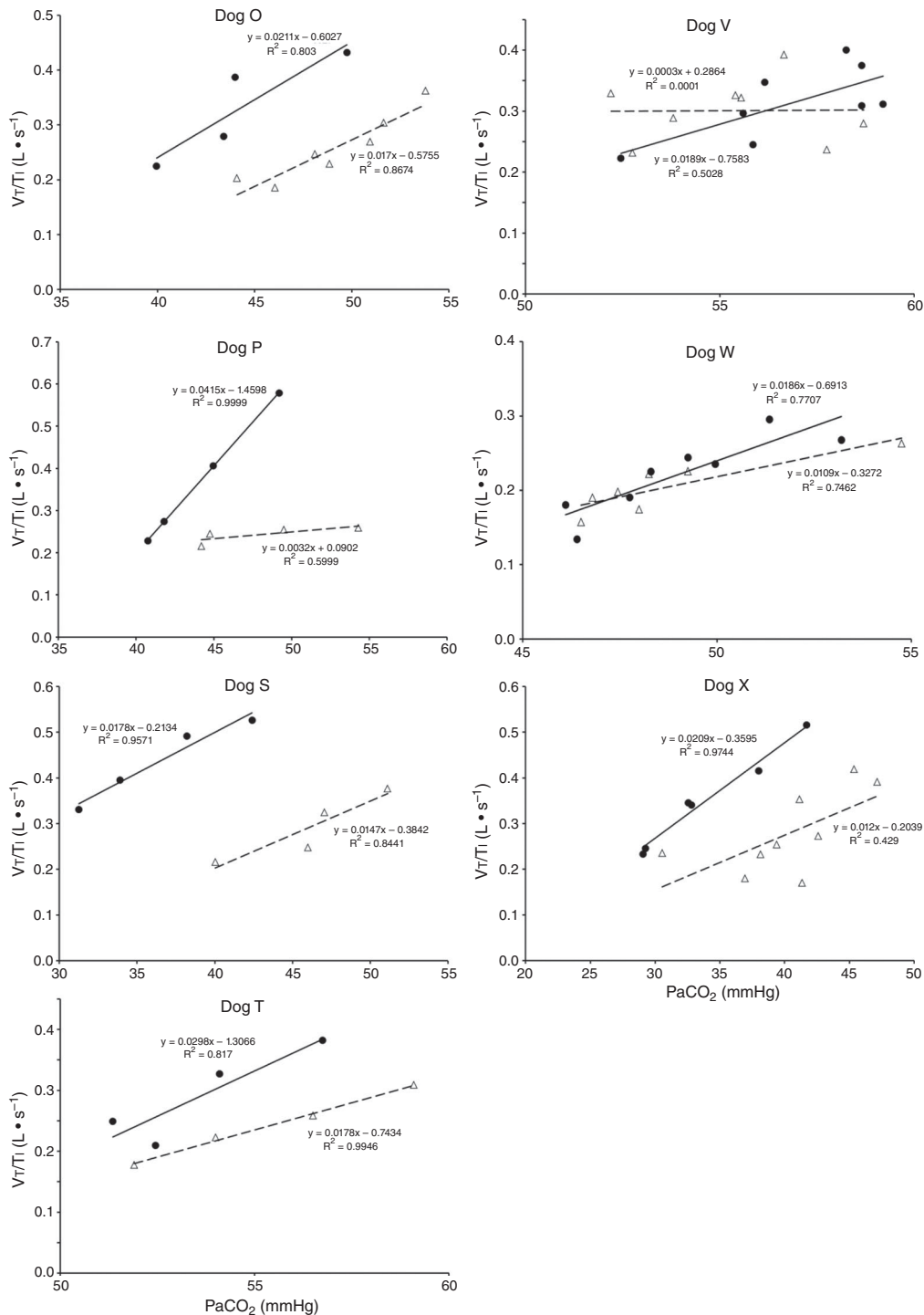


**Figure 7. Ventilatory responses of $V_T/T_I$ to increased $F_{ICO_2}$ during CB hypercapnic and CB hypocapnic perfusion for each of the seven dogs**
Regression equations and $r^2$ values are adjacent to the regression line they pertain to (See text for details). Symbols as in Figure 4.

**Unilateral carotid body denervation.** Our animal preparation is awake and intact except for the required denervation of the left carotid body. So the question remains whether our preparation actually responds similarly to a completely intact, awake preparation with both carotid bodies present. Eldridge *et al.*(1981) showed increased phrenic nerve responses to electrical stimulation of two *vs.* one carotid sinus nerves in anaesthetized cats
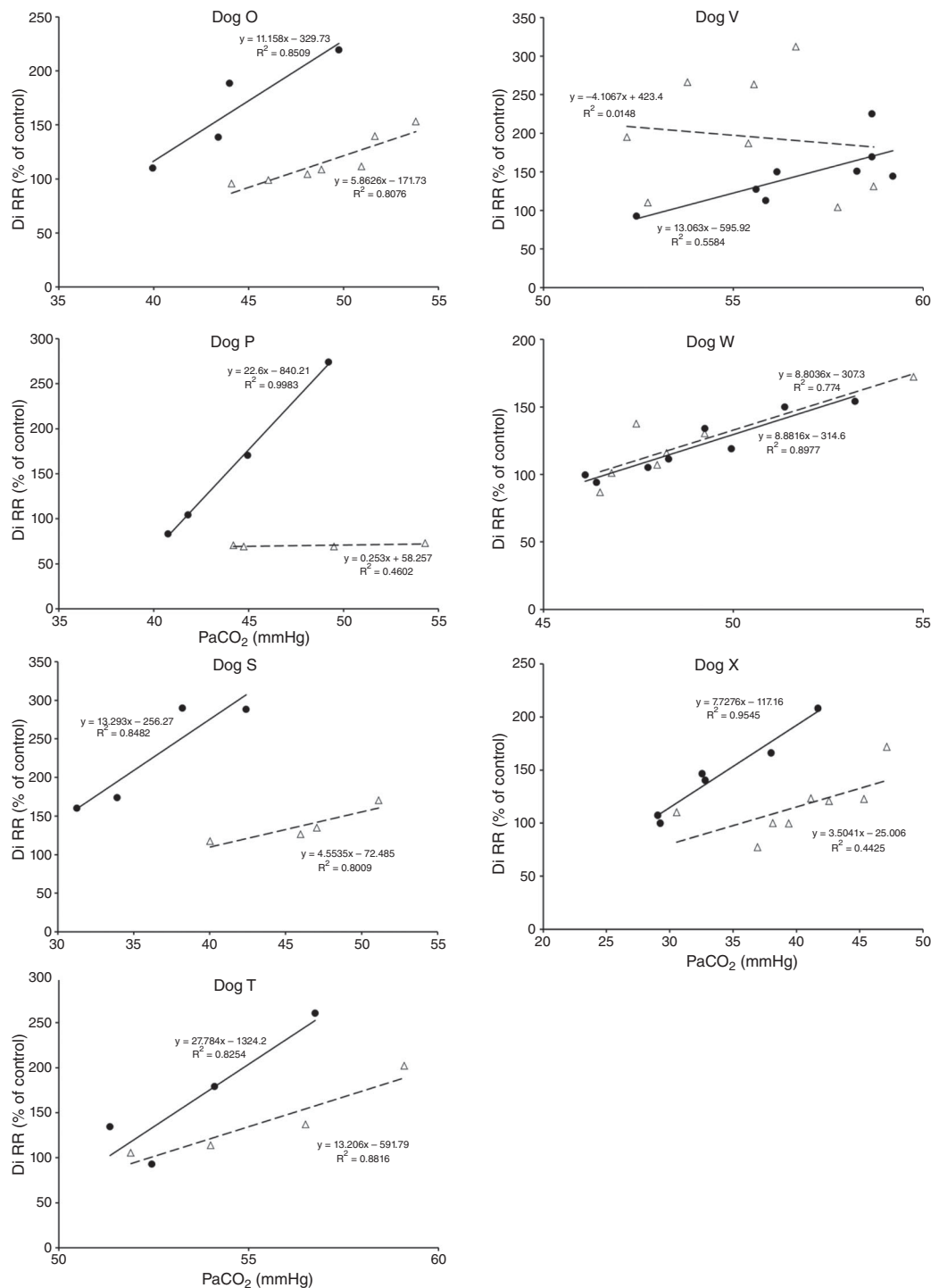


**Figure 8. Ventilatory responses of DiRR to increased $F_{ICO_2}$ during CB hypercapnic and CB hypocapnic perfusion for each of the seven dogs**
Regression equations and $r^2$ values are adjacent to the regression line they pertain to (See text for details). Symbols as in Figure 4.

and Brooks & Tenney (1966) reported reduced ventilatory responses to hypercapnic-hypoxic combinations in two of three goats following unilateral CBD; there was no change in the third goat.

We compared 26 awake dogs before and after unilateral CBD (see detailed findings in Results). During eupnoeic air-breathing, group mean $P_{aCO_2}$ was increased modestly but significantly by $2.4 \pm 3.5$ mmHg following unilateral CBD with no systematic change in the slope of the ventilatory response to increased $F_{ICO_2}$. Similarly, Busch *et al.* (1983) found no difference in the ventilatory response to hypoxia between two different groups of intact *vs.* unilaterally denervated awake goats. We propose that available findings would support the conclusion that most aspects of chemoreceptor-induced ventilatory control in awake animals with one carotid body are very close to those in the intact animal.

**Variability.** On the one hand our use of awake animals ensures that cardiorespiratory response gains to chemoreceptor stimuli are in the physiological range and not subject to 'neural saturation' as might be the case in surgically reduced and/or anaesthetized preparations (Eldridge *et al.* 1981). On the other hand, a limitation of studying the awake animal is that ventilatory responses to hyperpnoeic stimuli are variable – both within and among animals – likely to be attributable at least in part to variable behavioural responses to chemoreceptor and ventilatory stimulation. For example, in 6 of our 7 unilaterally carotid body denervated animals, each with multiple CO$_2$ steady state response tests, we found average coefficients of variation of $24 \pm 17\%$, and $28 \pm 11\%$ for the slopes of the ventilatory response to CO$_2$ for $\dot{V}_I$ and $V_T/T_I$, respectively. Since in our carotid body perfused preparations our CO$_2$ response slopes were obtained from tests conducted over 4–6 days in the same animal, variability would be an important limitation to our ability to detect systematic effects of variations in carotid body $P_{CO_2}$ on the central CO$_2$ response. (Also see the Hyperadditive interaction section below.)

**Effects of changes in systemic $P_{aCO_2}$.** In our isolated carotid body perfused model, as compared to some reduced preparations with isolated perfusions of both carotid body and brain stem (Berkenbosch *et al.* 1984; Day & Wilson, 2007), our increase in $F_{ICO_2}$ will acidify *all* organs exposed to the systemic hypercapnia, not just the ponto-medullary regions. Accordingly, the influence we observed of carotid body $P_{aCO_2}$ on the 'central' ventilatory response to CO$_2$ refers to all CO$_2$-sensitive neurons exposed to the systemic circulation.

**Duration of carotid body chemoreceptor stimulation.** Our current and prior studies demonstrated very similar

hyperadditive effects of CB stimulation/inhibition on central CO$_2$ ventilatory responses for alterations in either CB $P_{O_2}$ or $P_{CO_2}$. However, as summarized in the Introduction, constant or intermittent CB hypoxaemia has been shown to exert quite different effects on ventilation over time from CB hypercapnia. Accordingly, current findings obtained over <30 min of CB hypoxia/hyperoxia or hypercapnia/hypocapnia may not be predictive of their relative influences on central CO$_2$ sensitivity under more chronic conditions.

### Hyper-, hypo- and/or additive interaction of peripheral with central chemoreceptors

The long-standing controversy over the nature of central–peripheral chemoreceptor interaction (whether there is hyper-, hypo- and/or additive interaction of peripheral with central chemoreceptors) was recently reviewed (Forster & Smith, 2010; Smith *et al.* 2010; Guyenet, 2014) and also debated extensively in publications in which three sets of authors defended their positions and suggested new concepts and approaches (Duffin & Mateika, 2013; Teppema & Smith, 2013; Wilson & Day, 2013). Clearly, there are several studies using a wide variety of preparations in animals and also in humans to support additive, hyperadditive, or hypoadditive interactive effects on ventilatory control between peripheral and central chemoreceptors. We now summarize our perception of each of these positions and compare these findings and concepts to our recent and current findings which utilized two different means of stimulation and inhibition of the isolated, perfused carotid sinus region (and, therefore, carotid body chemoreceptor) in the awake canine preparation. We concentrate our analysis on published findings in unanaesthetized preparations and refer the reader to comprehensive recent reviews of this topic (Forster & Smith, 2010; Smith *et al.* 2010; Guyenet, 2014).

**Hypoadditive.** Hypoadditive interactive effects between medullary and peripheral chemoreceptors on ventilatory responses to CO$_2$ have been reported consistently primarily in reduced preparations and perhaps most notably in the rat (Day & Wilson, 2007,2009; Tin *et al.* 2012). The rat preparation has the advantage of including specific, isolated perfusions of the brain stem as well as the carotid chemoreceptor. These preparations have included anaesthesia (Tin *et al.* 2012) and decerebration as well as 'reconfigured' chemosensitive control pathways achieved via removal of vagal, hypothalamic and/or cortical inputs (Day & Wilson, 2007,2009; Guyenet, 2014). In these preparations the ventilatory responses to inhaled CO$_2$ are commonly 10–20% of those in the intact, awake rat of the same strain (Day & Wilson, 2007,2009; Mouradian *et al.*

2012). We also reported hypoadditive effects of an alkaline CSF perfusate which increased the transient carotid chemoreceptor response to NaCN in the awake goat – but interpretation of this augmented transient response was confounded by a concomitant hypoventilation, $CO_2$ retention and systemic respiratory acidosis (Smith *et al.* 1984).

**Additive.** Additive effects between central and peripheral chemoreceptors have been reported primarily (see exception below) in intact humans using methods which rely on separation of the peripheral and central chemoreceptor responses via temporal dissociation of the 'early' (peripheral) and 'late' (central) responses to inhaled $CO_2$ (Clement *et al.* 1992, 1995; St Croix *et al.* 1996; Cui *et al.* 2012). We suggest that any determination of the nature of peripheral–central interaction cannot be adequately quantified in these intact preparations because (a) given that an enhanced or reduced sensory input originating in the carotid chemoreceptors is transduced immediately to central $CO_2$-sensitive neurons (Takakura *et al.* 2006) (i.e. the concept of chemoreceptor interdependence), then precise, complete, temporal separation of the relative responses of the peripheral and central chemoreceptors is not achievable; (b) clear separation of early- and late-phase $CO_2$ responses is not always measurable perhaps because a significant portion of the so-called 'late' response to $CO_2$ might be attributed to peripheral (rather than central) chemoreceptors (Pedersen *et al.* 1999; Dahan *et al.* 2007); and (c) the likelihood that a short-term potentiation of respiratory motor output (Eldridge & Gill-Kumar, 1980) will follow the initial peripheral chemoreceptor responses to $CO_2$ would seriously confound attempts at assigning response gains exclusively to the different sets of receptors. These limitations indicate to us that quantification of the interdependence between peripheral and central chemoreceptors requires their anatomical isolation.

**Hyperadditive.** Hyperadditive effects of peripheral chemoreceptor inhibition/stimulation on the central ventilatory $CO_2$ responses have been demonstrated in two types of awake, cortically and vagally intact preparations. First, in several species including dogs, goats, ponies and humans, bilateral CBD (within the initial few days following CBD) results in significant hypoventilation and $CO_2$ retention during air breathing together with a substantial reduction in the slope of either the normoxic or hyperoxic response to systemic hypercapnia (Bisgard *et al.* 1980; Pan *et al.* 1998; Rodman *et al.* 2001; Fatemian *et al.* 2003; Dahan *et al.* 2007) or to the ventilatory response to focal medullary acidosis (Hodges *et al.* 2005). Rats of several strains appear to be exceptions to this evidence for hyperadditive interaction as bilateral CBD in this species

results in hypoventilation but without any decrement in the slope of the response to systemic hypercapnia (da Silva *et al.* 2011; Mouradian *et al.* 2012; Takakura *et al.* 2014). On the other hand, it is noteworthy that reductions in the slope of the ventilatory response to $CO_2$ and/or reductions in air-breathing eupnoea observed following partial ablation of central chemosensitive regions in the awake rodent was further reduced in a markedly hyperadditive fashion following denervation or hyperoxic inhibition of the carotid body chemoreceptors (da Silva *et al.* 2011; Ramanantsoa *et al.* 2011; Takakura *et al.* 2014).

The second type of awake preparation with findings that support hyperaddition is found in our essentially intact canine model with isolated, perfused carotid bodies. Combining previous and current findings using this preparation revealed hyperadditive effects on the central $CO_2$ response whether the carotid chemoreceptor was exposed to a combination of hyperoxia and hypocapnia *vs.* normocapnic hypoxia (Blain *et al.* 2010) or to normoxic hypercapnia *vs.* normoxic hypocapnia (present study).

Further support for hyperaddition is provided by experiments in the sleeping canine model utilizing transient hypocapnia. Transient hyperventilation-induced systemic hypocapnia in this model resulted in apnoea and periodic breathing with an apnoeic threshold approximately 5 mmHg below eupnoeic $P_{aCO_2}$; following carotid body denervation the absence of apnoea and periodicity in response to transient hypocapnia showed that CBs were required for the apnoeas to occur (Nakayama *et al.* 2003). However, hypocapnia applied only to the isolated CB *per se*, even as much as 10–15 mmHg below eupnoea, did not elicit apnoeas (Smith *et al.* 1995). Further, systemic (i.e. 'central') hypocapnia by itself (achieved by means of mechanical ventilation) when the CB chemoreceptor was maintained at constant normocapnia via extracorporeal perfusion also did not elicit apnoea (Smith *et al.* 2007). Apparently, even a normal 'tonic' sensory input from the CB was sufficient to prevent apnoea in the face of marked central hypocapnia in the sleeping animal. These findings in the sleeping canine are consistent with the hyperadditive effects of CB chemoreceptor hypercapnia/hypocapnia on the steady-state central hypercapnic response slopes shown in the present study and suggest that peripheral–central hyperaddition provides an essential contribution to the development of breathing instabilities/periodicities in non-REM sleep in response to transient systemic hypocapnia.

On the other hand, investigators using a vascularly isolated, perfused carotid body chemoreceptor preparation in the unanaesthetized goat reported additive effects of changes in CB $P_{O_2}$ or $P_{CO_2}$ on the group mean central $CO_2$ response slopes (Daristotle & Bisgard, 1989; Daristotle *et al.* 1990). We are not aware of any specific biological differences between these species (or models) that might account for these differences. To the contrary, the awake

goat – like most other mammals (see above) – does show markedly reduced ventilatory response slopes to systemic hypercapnia following bilateral CBD, *i.e.* evidence which would support a hyperadditive effect of carotid body input on the central $CO_2$ response in this species (Pan *et al.* 1998). So, while we are unable to completely account for these discrepant findings, one potential explanation might be found in the limited ability to detect relatively modest systematic changes in the face of random variations in within-animal $CO_2$ responsiveness as often occurs in awake animals (see Limitations section above). For example, Daristotle & Bisgard (1989) compared their central $CO_2$ response slopes between normoxic *vs.* hypoxic CB perfusions and reported that half of their animals (3 of 6) increased their central $CO_2$ response slopes when the CB was hypoxic compared to when the CB was normoxic, whereas their remaining three animals showed additive effects. Our current study compared normocapnic, hypocapnic and hypercapnic CB effects on central ventilatory responses to $CO_2$. We observed significant differences for $\dot{V}_I$ and $V_T/T_I$ responses between CB hypocapnia and either normocapnia or hypercapnia, but the CB normocapnic *vs.* CB hypercapnic effects were slightly more variable and non-significant. Thus, our experience would suggest that given the variability of responses to chemoreceptor stimuli in awake animals that multiple comparisons across several levels of CB stimluli might offer the best approach to quantifying central/peripheral interaction effects.

**Hybrid models.** Given the divergent types of peripheral–central interactions reported to date across many species and preparations it is not surprising that 'hybrid' models have been proposed to account for these divergent findings (Nuding *et al.* 2009; Wilson & Day, 2013; Guyenet, 2014). The finding that different experimental models, ranging from intact humans and animals to anatomically reduced preparations, are producing these divergent findings is not surprising – especially given the markedly 'reconfigured' chemoreceptor networks and substantial departures from physiological chemosensitivity present in some of these models (see above). On the other hand, a proposal that the nature of peripheral–central interactions may be critically dependent upon changing 'physiological states' and conditions – even within the same experimental preparation – has considerable merit and is especially relevant to interaction effects on ventilatory control (Nuding *et al.* 2009; Wilson & Day, 2013; Guyenet, 2014). For example, Guyenet (2014) predicts that a key determinant of the type of interaction might be found in the resting membrane potentials of the central $CO_2$-sensitive neurons, which in turn would be influenced by the relative strengths of the inhibitory *vs.* excitatory input from the respiratory pattern generator, carotid chemoreceptors, and various links within the

chemoreceptor pathway. Accordingly, it is suggested that the net effect of these inputs might depend upon such factors as the background states, i.e. wakefulness *vs.* sleep *vs.* exercise, prevailing levels of systemic and/or central $CO_2$ and hypoxia, whether ventilatory drive is operating above or below eupnoea, the duration of chemoreceptor stimulation, and the central 'amplification' of chemoreceptor input occurring at one or more sites within the medulla, pons, or hypothalamus (Reddy *et al.* 2005; Schultz & Li, 2007; Nuding *et al.* 2009; Duffin, 2010; Wilkinson *et al.* 2010; Wilson & Day, 2013; Dempsey *et al.* 2014; Guyenet, 2014).

So while future studies may well uncover an effect of one or more of these potential influences on the nature of peripheral chemoreceptor interaction, we propose that current evidence (as described above) is strongly supportive of hyperadditivity as the dominant form of peripheral–central interaction. At present we must limit this proposal to the specific conditions of acute CB inhibition/stimulation ($<30$ min) during quiet wakefulness or sleep, with ventilation operating within a physiological range either above or below eupnoea, and when the multiple links within the chemosensory control system are anatomically intact, chemosensitivity gains are within the physiological range, and sensory inputs from the carotid chemoreceptors are driven by a wide range of $O_2$ and/or $CO_2$. It is unknown if long-term CB inhibition/stimulation via variations in CB $P_{O_2}$ *vs.* $P_{CO_2}$ will have similar effects on central–peripheral chemoreceptor ventilatory interaction; this question awaits further study.

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## Additional information

### Competing interests

None declared.

### Author contributions

C.A.S., G.M.B. and J.A.D. carried out the conception and design of research; C.A.S., G.M.B. and K.S.H. performed the experiments; C.A.S., G.M.B. and K.S.H. analysed the data; C.A.S., G.M.B., K.S.H. and J.A.D. interpreted the results of the experiments; C.A.S. prepared the figures; C.A.S., G.M.B. and J.A.D. drafted the manuscript; C.A.S., G.M.B., K.S.H. and J.A.D. edited and revised the manuscript; C.A.S., G.M.B., K.S.H. and J.A.D. approved the final version of the manuscript.

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