Neural regulation of discontinuous gas exchange in *Periplaneta americana*

James D. Woodman^{a,b,*}, Paul D. Cooper^a, Victoria S. Haritos^b

^a*School of Botany and Zoology, Australian National University, Canberra ACT 0200, Australia*

^b*CSIRO Entomology, GPO Box 1700, Canberra ACT 2601, Australia*

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Abstract

Patterns of gas exchange among terrestrial arthropods are highly variable from continuous to discontinuous with discretely partitioned phases. The underlying initiation and co-ordination of these patterns is relatively poorly understood. Here we present a novel method for the simultaneous measurement of central nervous system (CNS) activity of the metathoracic ganglion and $\dot{V}\text{CO}_2$ in medium to large sized live terrestrial arthropods. Using *Periplaneta americana* at four oxygen levels (40%, 21%, 10% and 2% at 25 °C; $n = 6$ per treatment), we present minimally invasive visualization of nervous output relative to typical resting discontinuous gas exchange (DGE) data for the first time. DGE was maintained when cockroaches were exposed to hyperoxia or moderate hypoxia, but was lost in severe hypoxia. CNS activity was manifested in three signal types: large CNS output coinciding with peak CO_2 production during a burst, moderate CNS output coinciding with CO_2 sawtoothing and fluttering, and minimal CNS activity during the closed phase of DGE in normoxia. Large and moderate CNS outputs were associated with observed abdominal pumping and congruent CO_2 peaks. At 10% oxygen, $\dot{V}\text{CO}_2$ was significantly elevated during the inter-burst period in association with almost constant moderate CNS output between the periodic large CNS output. At 2% oxygen, DGE and large CNS output are lost to continuous CO_2 release and largely continuous moderate CNS output. As previously reported for this species, a central pattern generator for ventilation in the metathoracic ganglion is supported and we infer the presence of localized oxygen chemoreceptors based on clear CNS response to a change in oxygen tension.

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1. Introduction

Efficient gas exchange in most terrestrial arthropods is reliant on the tracheal system and adequate ventilation. Ventilation may be achieved by passive diffusion or may involve active muscular movements and selective opening of the spiracles (Wasserthal, 1996; Lighton, 1996; Slama, 1999; Chown et al., 2006). Patterns of gas exchange vary considerably within and among species relative to factors, including body size and atmospheric oxygen concentration. Whilst various gas exchange patterns have been described, including continuous, cyclic, and discontinuous gas exchange (DGE, e.g. Marais and Chown, 2003), the

underlying generation and co-ordination of these patterns remain relatively poorly understood (Chown et al., 2006).

Among terrestrial insects, a major distinction can be made between ventilation during periods of locomotion or flight, where high-frequency continuous gas exchange is typically observed, and during periods of rest, which may be typified by reduced frequency DGE (Reinhold, 1999). Discontinuous or cyclic ventilation patterns have been recorded for a number of insects during quiescence, whereby CO_2 release bursts are separated by inter-burst periods that may incorporate a closed spiracle phase and a flutter phase, where the spiracles rapidly open and close to regulate tracheal oxygen levels (Lighton, 1996; Chown et al., 2006; Quinlan and Gibbs, 2006). The nervous activity, which initiates and maintains discontinuous ventilation, is of considerable interest as it is likely to provide a clearer understanding of the function of DGE.

*Corresponding author at: School of Botany and Zoology, Australian National University, Canberra ACT 0200, Australia.

Tel.: +61 26 246 4031.

E-mail address: james.woodman@csiro.au (J.D. Woodman).

The motor activity that drives active insect ventilation is dependent on patterned neural control generated by a respiratory pacemaker or central pattern generator (CPG, review: Marder and Bucher, 2001). In locusts, ventilatory rhythm is generated primarily in the metathoracic ganglion of the central nervous system (CNS; Miller, 1960; Bustami and Hustert, 2000). The metathoracic ganglion has also been suggested as a primary CPG in the American cockroach, *Periplaneta americana*, despite evidence for shifting or multiple CPGs (Farley et al., 1967). The co-ordination of ventilatory movements among body segments is achieved by a CPG in one segment imposing a rhythm on other segments via linking interneurons (Lewis et al., 1973; Burrows, 1975, 1982). These interneurons, some of which have been identified in locusts, communicate the rhythm to the abdominal ganglia and subsequently the motor neurons, which innervate the relevant muscles for abdominal pumping and spiracular control.

The ventilatory output from a CPG may vary widely within a species and even within a single individual, depending on many factors (Bustami et al., 2002). For example, oxygen tension has been shown to affect gas exchange for a range of species and more specifically, directly impact on CNS ventilatory output in locusts and dragonflies (Miller, 1960, 1966; Mill and Hughes, 1966). The presence of chemoreceptors sensitive to oxygen concentration, most probably located within the metathoracic ganglion, has been confirmed for the horse lubber grasshopper, *Taeniopoda eques* (Bustami et al., 2002). It was shown that CNS output bursts per minute from the isolated ganglion submerged in saline varied with changes in oxygen tension. When exposed to anoxia, a biphasic response was recorded whereby a dramatic, but transient increase in ventilatory nervous output was followed by total inactivity.

To our knowledge, no previous study has examined live, intact specimens shown to be performing a typical DGE pattern at the time of nervous recording. Previous research has mostly relied on various dissection techniques and the use of isolated sections of the CNS (Myers and Retzlaff, 1963; Farley et al., 1967; Ramirez and Pearson, 1989; Burrows, 1982; Bustami and Hustert, 2000). Whilst isolated sections submerged in saline may provide reliable rhythmic patterns theoretically void of any sensory or behavioral disturbance for up to several hours, it is nevertheless an invasive and damaging technique (Bustami and Hustert, 2000; Zafeiridou and Theophilidis, 2006). The potential for increased noise in the recordings and abnormal ventilation patterns as a result of the dissecting process has long been recognized as an experimental limitation that is difficult to fully reconcile.

Given this background, we recognized potential in being able to describe how DGE is regulated by CNS output in live animals. Specifically, we wanted to describe the ventilatory nervous output from the CNS associated with individual DGE phases and visualize responses to different oxygen tensions. Here we report a novel technique to

address these aims which permits simultaneous measurement of ventilatory nervous activity from the intact CNS and $V\text{CO}_2$ from live specimens. Using *P. americana*, we use this method to gain functional insight into the regulation of DGE in insects and the CNS output associated with ventilation, including CO_2 bursts from the spiracles. We use normoxic as well as hypoxic (2% and 10% oxygen) and hyperoxic (40% oxygen) treatments to report how low and high oxygen levels affect the normal resting gas exchange pattern. Respirometry-only experiments without electrode placement for CNS measurement are used to validate data from the simultaneous CNS measurements trials.

2. Materials and methods

2.1. Study species

The American cockroach, *P. americana*, performs DGE (Kestler, 1985) and has been the subject of previous neurophysiological research identifying the metathoracic ganglion as a respiratory pacemaker (Farley et al., 1967). Adult males were obtained from established breeding colonies maintained at the Australian National University.

2.2. Recording ventilatory activity from the intact CNS

Animals were weighed using an analytical balance (sensitivity 0.1 mg; HR-200, A&D Co., Tokyo, Japan) and cooled to 4 °C to render them immobile. As required, each specimen was transferred onto ice (positioned ventral side up, posterior facing away) for the removal of the second leg from the left side to provide lateral access to the CNS. The animal was then transferred into a custom-built vertical cylindrical screw-top Perspex chamber (internal dimensions: radius 45 mm, height 55 mm, volume 350 cm³), where it was centrally positioned on its back and held in place with a strip of flexible adhesive over the three legs on the right side (Fig. 1). Paired platinum hook electrodes 0.5 mm in diameter were inserted from the side of the chamber on a rigid stalk. The insertion through a rubber seal permitted subsequent fine positioning by micro-manipulators. The electrodes were positioned to the left of the paired nerve cord and curved forceps were used to pierce through the unsclerotized, flexible exoskeleton on each side. From the right, the paired central nerve cords were gently lifted onto the electrodes which were slightly bent upwards at the tip to prevent slipping during a trial. After positioning anteriorly, so as to abut the metathoracic ganglion, the electrodes were raised very slightly to ensure nerve contact and left in place for the duration of the recording. The lid of the chamber was fastened immediately using high vacuum grease to seal the chamber.

The electrodes were connected to an amplifier (Powerlab 4/25T; ADInstruments, Bella Vista, Australia), which was connected to a personal computer. Data acquisition software (Chart v.5.2.2; ADInstruments, Bella Vista, Australia) was used to record electrical output at a

Fig. 1. An annotated diagrammatic representation of the vertical cylindrical respirometry chamber and electrode used for simultaneous measurement of ventilatory CNS activity and gas exchange.

frequency of 10 measurement s^{-1} . Data were subsequently transferred into Microsoft Excel for analysis.

2.3. Recording gas exchange

The recording of CO_2 output for each individual was achieved by the incorporation of the sealed chamber into a flow-through respirometry system using a calibrated Licor 7000 differential infrared gas analyzer (Li-Cor, Lincoln, USA). The input and output tubing (Bev-a-line IV) was connected to opposing gas fittings on the side of the chamber (Fig. 1) and gas was drawn through the system by an Edwards E2M-1.5 high-vacuum pump (BOC-Edwards, BOC group Inc., Sussex, England). Experiments proceeded with either 40%, 21%, 10% or 2% oxygen in nitrogen passing through the chamber at a rate of $250 mL min^{-1}$ (time constant of the chamber = 84 s, see Gray and Bradley, 2006). The experimental gas mixtures were achieved by mixing high-purity oxygen and nitrogen, which passed through an inline moisture and CO_2 removal column (Glass moisture trap, Model 7214; Alltech, Australia). The ratio of each gas used was controlled by a Brooks 5878 mass-flow controller with three Brooks 5850TR mass-flow meters, whose calibrations were checked before use with a calibrated digital flow meter (one each for vacuum, O_2 , and N_2) at $0–500 mL min^{-1}$ (Brooks Instruments, Hatfield, Pennsylvania, USA). The use of mass-flow controllers permitted the gas composition to be instantaneously varied as required without disruption to the gas line.

Raw data from the gas analyzer were logged on to a second personal computer using Licor LI7000 data acquisition software (Version 1.0.1). The lag time between the nervous and CO_2 recordings was negligible due to the simultaneous initiation of both data acquisition programs. The data from each trial were imported into Microsoft Excel for analysis with the CNS data.

2.4. Treatments

Cockroaches were observed periodically during all experiments for visible ventilatory and gross bodily movements and the timing of each was recorded.

Standard patterns of gas exchange and ventilatory nervous output were recorded for *P. americana* at 21% oxygen in nitrogen over a 5 h period. For measuring the effects of different oxygen concentrations, oxygen tensions of 2%, 10% and 40% were used. All trials were conducted under dim light at $25 \pm 1^\circ C$ ($n = 6$ individual animals per treatment). For the three experimental oxygen tensions, each specimen was exposed to 2 h of 21% oxygen in nitrogen, followed by 2 h of the experimental treatment and then a further 1 h of 21%.

To validate the data during CNS measurement we also conducted a series of respirometry-only experiments at each of the previously mentioned oxygen tensions using the same chamber as that used in the CNS measurement experiments. Experiments proceeded under dim light at $25 \pm 1^\circ C$ ($n = 6$ individual animals per treatment) as described for the CNS measurement trials (including the cockroaches being overturned and held in place with the second leg on the left side removed); however, there was no dissection or electrode placement. Exposure to each oxygen tension was maintained for at least 2 h.

2.5. Data analysis and definitions

DGE cycles were selected from the CO_2 output data from each experiment ($n = 2–8$ cycles per animal). The first 30 min of recording in each trial was discarded due to elevated CO_2 output while the animal settled. Periods of gross movement (as opposed to abdominal ventilatory movement) were identified from direct observation as well as perturbation in VCO_2 and excluded from analysis. In the

absence of DGE at 2% oxygen, CO₂ output rates were calculated using a 1 h period for each individual whilst at rest. To be considered as a DGE cycle for inclusion in

analysis, a cycle had to exhibit an inter-burst period, whereby low CO₂ output was maintained, followed by a clearly defined CO₂ burst phase, as typified by DGE cycles

Fig. 2. Representative respirometry-only (without CNS recording) results for *P. americana* at normoxia at 25°C. Animals were overturned and restrained as per the preparation used for simultaneous CNS measurements, but without electrode placement. The gas exchange pattern was separated into a burst phase (B) incorporating sawtoothing (S) and inter-burst phase (I) incorporating the closed (C) and flutter (F) periods.

Fig. 3. Representative results showing ventilatory nervous activity from the CNS (upper trace) and simultaneous measurement of CO₂ output for *P. americana* over 28–40 min (to show 3 DGE cycles) at (a) normoxia—21% oxygen, (b) hyperoxia—40% oxygen, (c) moderate hypoxia—10% oxygen, and (d) severe hypoxia—2% oxygen. Examples of large (L) and moderate (M) CNS output are shown in trace (a).

previously presented for *P. americana* in Kestler (1985) and demonstrated here in Fig. 2.

CNS activity corresponding to $\dot{V}\text{CO}_2$ was analyzed, whereby only clear CNS output that matched the gas exchange data was used, as demonstrated in Fig. 3. The largest CNS activity was defined by the highest amplitude CNS outputs associated with CO_2 bursts during DGE. Moderate CNS activity was defined as a series of outputs of similar amplitude, approximately 20–30% of the amplitude of large CNS output (Fig. 3(a)). Minimal CNS output relative to the baseline was observed only transiently at normoxia during the inter-burst period, when it was assumed that some of the spiracles might be closed and muscular ventilation relaxed (Fig. 3(a)).

3. Results

Measurement of ventilatory CNS activity from the metathoracic ganglion in *P. americana*, whilst simultaneously recording $\dot{V}\text{CO}_2$ for up to 5 h, was consistent and repeatable. Lively specimen condition in excess of 3 days after a trial confirms that there was minimal damage to the cockroaches due to the procedure and indicates that an individual could be repeatedly measured over an extended period if required. We limited the recording of nervous output and $\dot{V}\text{CO}_2$ to 5 h periods due to drying of the contact between the electrode and the ganglion and gradual loss of CNS signal fidelity after that time. By using a flow rate of 250 mL min^{-1} in a relatively large chamber we minimized dehydration of the exposed ganglion, but maintained sufficient CO_2 measurement fidelity to clearly distinguish different gas exchange patterns as well as the phases of the DGE cycle. A stable zero baseline was achieved for both CO_2 and water output before commencing each experiment; however, slightly elevated CO_2 output was observed during the inter-burst period of DGE compared with that previously measured for *P. americana* (Fig. 3; Kestler, 1985). This is most likely associated with either flow rate or the experimental preparation, but does not compromise examination of the correlation between CNS activity and gas exchange. Importantly, after each animal was removed from the chamber, CO_2 and water output measurements consistently returned to a stable zero baseline.

3.1. Ventilation in resting animals

The typical gas exchange pattern of adult *P. americana* when immobile is DGE, featuring three phases (Fig. 2; Kestler, 1985). There is a clearly distinguished CO_2 burst phase, which represents a co-ordinated opening of the spiracles and abdominal pumping to convey a bulk discharge of CO_2 from the body. A closed phase has the lowest CO_2 release of the cycle and is considered a period where the spiracles are mostly closed. The flutter phase follows the closed phase and represents small releases of CO_2 , consistent with rapidly opening and closing the

spiracles to most probably draw air inward to increase oxygen levels (Kestler, 1985; Lighton, 1996). Respirometry-only experiments using an overturned animal held in place, but without dissection or electrode placement, gave a standard three-phase DGE response (Fig. 2) and there was clear congruence of gas exchange pattern with the simultaneous CNS measurement experiments (Fig. 3(a)). The routine establishment of DGE and similar total CO_2 release during CNS recording are contrary to what would be associated with abnormal ventilation. Using ANOVA, mean cycles h^{-1} and CO_2 burst duration were not significantly different between the respirometry-only and CNS experiments during DGE at 40%, 21% and 10% oxygen (d.f. = 1, $F = 0.03$, $p = 0.86$; d.f. = 1, $F = 0.36$, $p = 0.56$). Among all four different oxygen tensions, the mean total CO_2 output rates were also not significantly different between the respirometry-only and CNS experiments (d.f. = 1, $F = 2.16$, $p = 0.17$). Quantitative and qualitative comparisons thus show minimal impact of the dissection and electrode placement on gas exchange patterns.

At normoxia during CNS measurement, ventilation associated with the flutter phase during the inter-burst period and sawtoothing were observed as part of the typical DGE cycle (Figs. 2 and 3(a)). Sawtoothing has been previously described as fluttering during a CO_2 burst by Duncan and Byrne (2000), and here it refers to small spikes of CO_2 release associated with abdominal pumping movements during a burst. DGE was maintained in *P. americana* when cockroaches were exposed to hyperoxia or moderate hypoxia. In 40% oxygen, CO_2 bursts were defined by an initial peak, followed by a drop in $\dot{V}\text{CO}_2$ due to the absence of sawtoothing and then a second peak at the end of the burst (Fig. 3(b)). In 10% oxygen, DGE was maintained, but $\dot{V}\text{CO}_2$ was significantly elevated during the inter-burst period (Fig. 3(c), Table 1). The CO_2 bursts in 10% oxygen usually had two release peaks in close succession at the start of the burst and then protracted CO_2 release for the remainder of the burst. At 2% oxygen, all periodicity of CO_2 release was lost and $\dot{V}\text{CO}_2$ remained relatively constant during exposure (Fig. 3(d)).

Rates of CO_2 output at the four oxygen tensions during simultaneous CNS measurement are presented in Table 1. The total rate of CO_2 output (mL h^{-1}) was not significantly different at any of the oxygen tensions, suggesting the cockroaches maintain their oxygen requirements through ventilation. At 40%, 21% and 10% oxygen, where DGE was employed, CO_2 output increased during the inter-burst period as oxygen tension decreased; however, CO_2 output during a burst, burst period, and burst frequency were not significantly different between oxygen treatments (Table 1).

3.2. Ventilatory CNS output

CNS recordings were coupled with CO_2 output and a range of gas exchange patterns were detected from continuous through to discontinuous cycles depending on

Table 1

The effect of oxygen concentration on gas exchange in *P. americana* ($n = 6$ per treatment) at 25 °C during simultaneous CNS measurement (mean $\pm$ SD)

Oxygen tension (%)	40	21	10	2
Mass (g)	0.984 $\pm$ 0.090	1.100 $\pm$ 0.180	1.121 $\pm$ 0.151	0.958 $\pm$ 0.055
No. of DGE cycles examined	18	23	17	–
Total cycle CO ₂ output (mL h ⁻¹)	0.28 $\pm$ 0.06	0.30 $\pm$ 0.05	0.35 $\pm$ 0.08	0.29 $\pm$ 0.05
Burst CO ₂ output (mL h ⁻¹)	0.45 $\pm$ 0.12	0.47 $\pm$ 0.13	0.44 $\pm$ 0.09	–
Burst period (min)	8.7 $\pm$ 2.4	9.0 $\pm$ 2.5	9.2 $\pm$ 2.5	–
Burst frequency (bursts h ⁻¹)	3.8	3.1	3.6	–
Inter-burst CO ₂ output (mL h ⁻¹)	0.10 $\pm$ 0.06	0.15 $\pm$ 0.07*	0.21 $\pm$ 0.09*	–
Inter-burst period (min)	7.1 $\pm$ 4.4	10.4 $\pm$ 4.9	7.5 $\pm$ 5.1	–
DGE cycle period (min)	15.8 $\pm$ 5.9	19.4 $\pm$ 6.2	16.7 $\pm$ 9.4	–

In the absence of DGE cycles at 2% oxygen, the total cycle CO₂ output value was calculated from a 1 h period when each individual was at rest.* Indicates a significant difference between means in a given row (ANOVA, $p < 0.05$). Fig. 4. A short section of representative CNS recording showing the initial nervous response (upper trace) and corresponding CO₂ release to 2% oxygen after normoxia. The solid vertical line represents the switch to 2% oxygen.

oxygen tension (Fig. 3). Large CNS outputs during DGE cycles were consistently associated with maximal CO₂ bursts (d.f. = 17, $t = 11.23$, $p < 0.001$), where CNS output slightly preceded each CO₂ release peak. At normoxia, large CNS outputs were detected at the beginning of the CO₂ burst (and occasionally also later during the burst) and were followed by consistent, moderate CNS output associated with sawtoothing (Fig. 3(a)). During the inter-burst period, reduced CNS activity was observed immediately after the burst, followed by a return to moderate CNS activity during the flutter phase preceding the next CO₂ burst.

The CNS output pattern was quite different under hyperoxic conditions; large CNS peaks were aligned with observed muscular abdominal pumping and these coincided consistently with the beginning and end of the CO₂ burst (Fig. 3(b)). There were also no periods of moderate CNS output matching with CO₂ sawtoothing or fluttering periods, as seen at normoxia. Other than CO₂ burst-related CNS spikes, moderate CNS output was minimal at 40% oxygen. By contrast, at 10% oxygen, constant moderate CNS output was recorded in between the periodic large

CNS outputs. The large CNS outputs at 10% oxygen coincided with strong abdominal pumping movements and occurred consistently in close succession at the start of the CO₂ burst (Fig. 3(c)). In 2% oxygen, CNS output was largely constant at a moderate level and CNS bursts were not observed after the initial reaction to the gas (Fig. 3(d)). We observed only occasional small muscular ventilation movements during 2% oxygen exposure.

Within 60 s of switching from normoxia to 40%, 10% or 2% oxygen, each animal showed a CNS response to the change in oxygen tension, which coincided with a short series of abdominal movements. The initial CNS ventilatory response to 2% oxygen and the corresponding transient increase in $\dot{V}\text{CO}_2$ is shown in Fig. 4. The CNS response to a switch from normoxia to 40% or 10% oxygen was less intense and typically less than 1 min in duration, with no corresponding peak in CO₂ release.

4. Discussion

Ventilation in insects is controlled by rhythmic nervous innervation that is influenced by metabolic, hormonal,

sensory, neural, and behavioral factors (Bustami and Hustert, 2000; Nation, 2002). The technique used here efficiently records CNS ventilatory activity and is applicable to any medium to large sized terrestrial arthropod (>0.5 g) with ventral access to the nerve cord not requiring large dissection. Whilst *in situ* neurophysiological preparations have been available for some time (e.g. Case, 1961), this present method further minimizes the potential for invasive trauma and confers the ability to concurrently visualize standard resting patterns of $\dot{V}CO_2$. Indeed, for *P. americana*, previous surgical techniques for identifying ventilatory nervous output may have induced CPG shifting and interference with interganglionic control mechanisms (Farley et al., 1967).

For *P. americana*, CO_2 output rates were maintained at all oxygen tensions examined, suggesting, under the assumption of a constant respiratory quotient, that oxygen uptake and metabolic rate are comparable across the range (Table 1). Although DGE was maintained under mild hypoxic conditions, inter-burst CO_2 release was significantly elevated compared with normoxia. This is consistent with that reported for the adult ant, *Camponotus vicinus*, which increased flutter phase $\dot{V}CO_2$ by approximately 37% at 10% oxygen, compared to that at normoxia (Lighton and Garrigan, 1995). For *P. americana* we were unable to consistently distinguish the closed and flutter phases of DGE at each oxygen tension; however, for the combined inter-burst period we observed a similar 40% increase in $\dot{V}CO_2$ at 10% oxygen compared to that at normoxia. Interestingly, DGE cycle duration was not significantly different between normoxia and 10% oxygen in *P. americana*. For *C. vicinus*, hypoxia caused a marked increase in flutter phase duration, which increased DGE cycle duration and therefore decreased cycle frequency (Lighton and Garrigan, 1995). We propose that ventilatory activity during the flutter phase, in addition to oxygen uptake during the CO_2 burst phase, is sufficient to meet oxygen requirements without lengthening of the flutter phase in *P. americana*. DGE was abandoned in *P. americana* at 2% oxygen for continuous gas exchange, as has been reported for another cockroach, *Macropanesthia rhinoceros* (Woodman et al., 2007).

Two extremes of CNS output pattern emerged during gas exchange measured in *P. americana* under different oxygen tensions. Under hyperoxic conditions, large CNS outputs were recorded at the beginning and end of each CO_2 burst (Fig. 3(b)), which we interpret as co-ordinated opening of most of the spiracles and abdominal pumping to facilitate the bulk release of CO_2 . At other times there is minimal moderate nervous activity associated with each gas exchange cycle. By contrast, after the initial reaction (Fig. 4), exposure of *P. americana* to 2% oxygen results in largely continuous moderate CNS output (Fig. 3(d)) that coincided only occasionally with visible muscular ventilation movements. Large CNS output and CO_2 bursts are absent during severe hypoxia and we assume that the

spiracles remain largely open during exposure to maximize oxygen uptake.

At 10% oxygen, moderate CNS activity, punctuated by large CNS outputs at the beginning of each CO_2 burst, is recorded throughout the gas exchange cycle (Fig. 3(c)) consistent with nervous activity to maintain oxygen uptake. Under normoxic conditions, large CNS output signaling the beginning of a CO_2 burst is recorded (occasionally a second large CNS spike was observed later during the burst) with moderate CNS output during the sawtooth and flutter phases of the DGE cycle (Fig. 3(a)).

From these comparisons, it appears that large CNS spikes represent co-ordinated spiracular opening and strong abdominal pumps to affect a CO_2 burst during DGE, and moderate CNS activity is mainly associated with small abdominal movements (sometimes too small to be identified by the naked eye) and spiracular opening and closing during the flutter phase. Moderate CNS output thus most likely results in small-scale abdominal pumping as per the data presented for *P. americana* in Slama (1999) (termed ‘concealed extracardiac pulsations’). Our results support CO_2 bursts (in particular, sawtooth) during DGE at normoxia, that would align with extracardiac pulsations facilitated by the nervous output shown in Fig. 3(a). Future measurement of hemocoelic pressure would be valuable in assigning CNS mediated abdominal movements to CO_2 release for the oxygen tensions other than normoxia in Fig. 3.

As described above, hyperoxia resulted in minimal moderate CNS activity and a loss of CO_2 sawtooth and fluttering compared with that observed in normoxia and mild hypoxia. Sawtooth and fluttering appear to represent important periods of oxygen uptake based on their absence at 40% oxygen and the nearly continuous flutter-type activity at 10% oxygen. We propose that the reduced moderate CNS activity in hyperoxia functions to avoid excessive oxygen uptake, in accordance with the oxygen toxicity hypothesis for DGE (Hetz and Bradley, 2005; Bradley, 2006). Exposure to 40% oxygen results in the elimination of the flutter phase, as oxygen uptake during this period would be unnecessary, given bulk uptake during the burst (Hetz and Bradley, 2005; Woodman et al., 2007). By contrast, we propose that gas exchange at 10% oxygen (Fig. 3(c)) represents an early stage of the degradation of DGE toward the continuous pattern at 2% oxygen, based on constant moderate CNS output during the cycle.

The partial pressure of oxygen in the tracheae is recognized as an important factor regulating the various gas exchange patterns among insects (Burkett and Schneiderman, 1967) and our results support this relative to CNS output from the metathoracic ganglion at different oxygen tensions for *P. americana*. As has been reported for other insects (e.g. Bustami et al., 2002), we infer the existence of localized oxygen receptors in the CNS for *P. americana* based on clear CNS response to changes in oxygen tension

within 60 s of exposure (Fig. 4). Tracheae relay respiratory gases directly into the CNS ganglia (Nation, 2002; Bustami et al., 2002) and therefore, it is interesting that our results show a more immediate response to a change in atmospheric oxygen than that previously reported for a directly exposed isolated grasshopper CNS with the tracheae intact (Bustami et al., 2002). It was suggested that one existing oxygen levels within the tracheae considerably delay the response to a change in atmospheric oxygen. Whilst such an effect is not disputed, our live preparations suggest a lesser delay for *P. americana*, notwithstanding the different tracheal dimensions as a result of the preparation, intact spiracles, and body size. It is possible that a more immediate response to a change in oxygen tension is due to active ventilation in the intact live specimen more rapidly delivering air to the ganglion. Further research to identify the exact location and function of respiratory chemoreceptors would require more invasive and extensive specimen manipulation. For example, Myers and Retzlaff (1963) showed how removal of the cephalic nerves by decapitation immediately disrupted discontinuous ventilation in the Cuban burrowing cockroach. For this species, an oscillatory feedback mechanism between the abdominal ganglia is suppressed by the brain, allowing the CPG to release periodic bursts that result in discontinuous ventilation of the tracheae.

5. Concluding remarks

The technique presented here provides excellent potential for minimally invasive insight into the interactions between the CNS and whole-organism physiology. Specifically, we can determine how patterns of gas exchange are regulated in response to environmental variables such as atmospheres that deviate from normoxia. By real-time switching of experimental treatments and continuous simultaneous recordings, the activity of CPGs for ventilation can be assessed, in addition to inferring the presence of localized oxygen and/or carbon dioxide sensors at different points in the CNS. There is also applied potential in better understanding how different chemicals and drugs may affect the CNS and subsequently, ventilation and spiracular control (Woodman et al., in press).

Whilst insect respiratory physiology has re-emerged as a flourishing and spirited field of research (Quinlan and Gibbs, 2006), an underlying mechanistic understanding behind different modes of gas exchange has remained somewhat left behind. Here we permit for the first time the visualization of ventilatory CNS output relative to typical resting gas exchange regimes at different oxygen tensions.

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