Erratum

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Evolution of air-breathing and central CO_2/H^+ respiratory chemosensitivity: new insights from an old fish?

There is an error in the legend of Fig. 4. The stated conditions (P_{O_2} 675 mmHg; P_{CO_2} 27 mmHg; pH 7.5) for the lower panel are incorrect. The conditions should be: P_{O_2} 270 mmHg; P_{CO_2} 8.5 mmHg; pH 8.01. The correct legend should read:

Fig. 4. Similarities in the ventilatory motor patterns produced by isolated brainstem preparations from the amphibian tadpole and the gar. (A) Integrated nerve root activity from a stage 24 (Taylor and Kollros, 1946) *Rana catesbeiana* tadpole illustrating a bout of lung-inflation bursts (P_{O_2} 675 mmHg; P_{CO_2} 14 mmHg; pH 7.8; 22 °C). Coordinated activity in cranial nerve (CN) VII and spinal nerve (SN) II is a strong and convenient indicator of lung inflation in the tadpole (Gdovin et al., 1998). (B) Integrated nerve root activity from CN V and the medial branch of CN X (CN mX) from an isolated gar brainstem illustrating a bout of putative air-breathing ventilatory (PAV) bursts (P_{O_2} 270 mmHg; P_{CO_2} 8.5 mmHg; pH 8.01; 22 °C). Note that CN X innervates the glottis in both frog and gar (Kogo and Remmers, 1994; Norris, 1925). 1 mmHg=0.133 kPa.

The authors wish to apologise for any inconvenience this error may have caused.

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EVOLUTION OF AIR-BREATHING AND CENTRAL CO_2/H^+ RESPIRATORY CHEMOSENSITIVITY: NEW INSIGHTS FROM AN OLD FISH?

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Summary

While little is known of the origin of air-breathing in vertebrates, primitive air breathers can be found among extant lobe-finned (Sarcopterygii) and ray-finned (Actinopterygii) fish. The descendents of Sarcopterygii, the tetrapods, generate lung ventilation using a central pattern generator, the activity of which is modulated by central and peripheral CO_2/H^+ chemoreception. Air-breathing in Actinopterygii, in contrast, has been considered a 'reflexive' behaviour with little evidence for central CO_2/H^+ respiratory chemoreceptors. Here, we describe experiments using an *in vitro* brainstem preparation of a primitive air-breathing actinopterygian, the longnose gar *Lepisosteus osseus*. Our data suggest (i) that gill and air-breathing motor patterns can be produced autonomously by the isolated brainstem, and (ii) that the frequency of the air-breathing motor pattern is increased by hypercarbia. These results are the first evidence consistent with the presence of an air-breathing central pattern generator with central CO_2/H^+ respiratory chemosensitivity in any primitive actinopterygian fish. We speculate that the origin of the central neuronal controller for air-breathing preceded the divergence of the sarcopterygian and actinopterygian lineages and dates back to a common air-breathing ancestor.

Key words: evolution, longnose gar, *Lepisosteus osseus*, hypoxia, hypercapnia, air-breathing, gill, ventilation, *in vitro* brainstem, bimodal breather.

Introduction

In non-air-breathing fish, from which CO_2 is readily lost to the water ventilating the gills, the need for CO_2/H^+ chemoreceptors is minimal. However, during the evolution of terrestriality, animals were faced with the challenge of eliminating CO_2 in air. Thus, the change in the ventilatory medium from water to air presumably led to the increased importance of CO_2 in the control of respiration and, hence, the need for CO_2/H^+ chemoreceptors (Smatresk, 1990; Milsom, 1995). Air-breathing fish were possibly the first to encounter the need for CO_2/H^+ respiratory chemoreceptors, but the presence of such chemoreceptors in these primitive air breathers remains uncertain (Smatresk, 1990; Milsom, 1995; Graham, 1997; Taylor et al., 1999).

While O_2 is the principal variable determining respiratory drive in all fish (Shelton and Croghan, 1988), many species respond to increased aquatic hypercapnia with mild changes in ventilation (Heisler et al., 1988; Wood et al., 1990; Hughes and Singh, 1970; Smatresk and Cameron, 1982b). Such responses are often attributed to the Bohr and Root effects, whereby increasing $[CO_2]/[H^+]$ causes a reduction in the oxygen-carrying capacity of haemoglobin, thus reducing the oxygen content of the blood and stimulating O_2-sensitive respiratory chemoreceptors (Smith and Jones, 1982). However, not all fish displaying hypercapnic ventilatory responses have Bohr or Root effects, suggesting that some fish species possess mechanisms for detecting changes in $[CO_2]/[H^+]$ that are independent of peripheral O_2 chemoreceptors (Butler and Taylor, 1971). Such mechanisms may involve direct effects of pH and/or CO_2 on the membrane excitability of peripheral and/or central CO_2/H^+-sensitive respiratory chemoreceptors.

The possibility of peripheral CO_2/H^+-sensitive respiratory chemoreceptors in fish has been discussed widely, and some supporting evidence has appeared (for reviews, see Smatresk, 1994; Milsom, 1995; Taylor et al., 1999). Although we consider the existence of these receptors likely, substantive data are sparse (Burleson and Smatresk, 2000). The case for central CO_2/H^+ sensitivity in fish is unresolved. Two pioneering studies in non-air-breathing fish indicate modest increases in the frequency of gill ventilation associated with brain acidosis (Hughes and Shelton, 1962; Rovainen, 1977). However, two more recent studies failed to detect central CO_2/H^+ sensitivity in bowfin *Amia calva*, an air-breathing species closely related to the gar (McKenzie et al., 1991; Hedrick et al., 1991). McKenzie et al. (1991) showed that the response to aquatic hypercapnia was eliminated by making the water hyperoxic. This suggests that hypercapnia affected

ventilation through the Bohr and Root effects, although the authors could not rule out the possibility that a ventilatory response to pH was inhibited by hyperoxia (McKenzie et al., 1991). In the study of Hedrick et al. (1991), altering the P_{CO_2}, pH and P_{O_2} of mock extradural fluid injected into the cranium of conscious intact animals did not change the frequency of air or gill ventilation. Dyes mixed with the mock extradural fluid were deposited on structures around the brainstem, but the actual pH within the brainstem during cranial injections was not determined. Therefore, the possibility exists that the pH and P_{CO_2} within the tissue may have been determined largely by natural vascular perfusion and were little affected by the imposed intracranial injections.

We have re-examined whether fish have central respiratory chemosensitivity using an isolated brainstem preparation from the longnose gar *Lepisosteus osseus*. The gar is a primitive air-breathing actinopterygian, which has a modest ventilatory response to CO_2 (Smatresk and Cameron, 1982b). Not only is the isolated brainstem preparation devoid of the Bohr and Root effects, but it lacks sensory input, including mechanoreceptors, olfactory receptors and peripheral O_2-sensitive chemoreceptors, the dominant source of respiratory drive in fish (Shelton and Croghan, 1988). Free from these confounding factors, such a preparation may be better suited for studying the possible subtle central influence of pH/CO_2 on the respiratory rhythm (Johnson et al., 1998).

Materials and methods

To use the isolated brainstem, we first determined the nature of the ventilatory motor patterns in a semi-intact preparation. Studies of pH/CO_2 sensitivity were then conducted on the isolated preparation. *Lepisosteus osseus* (L.) (body length less than 10 cm; mass approximately 9.5 g) were anaesthetised prior to dissection by immersion in a 1:10 000 solution of tricaine methanesulphonate (MS-222) and decerebrated at the level of the rostral tectum. During dissection, preparations were superfused with mock cerebral spinal fluid (CSF) consisting of (in mmol l^{-1}): NaCl, 104; KCl, 4; $CaCl_2$, 0.6; $MgCl_2$, 1.4; D-glucose, 10; $NaHCO_3$, 25. The concentration of bicarbonate in the mock CSF was higher than that of gar plasma, having a calculated bicarbonate concentration of 10 mequiv l^{-1} (Burleson et al., 1998), but allowed better experimental control of pH and may counteract the limited diffusion of CO_2 out of the tissue (Okada et al., 1993). Dissections were performed in mock CSF equilibrated with an O_2/CO_2 gas mixture with a P_{O_2} of approximately 660 mmHg (1 mmHg=133.32 Pa) and the P_{CO_2} adjusted to give pH 8.0. Dissections were performed at 4 °C to reduce tissue metabolic rate and to improve preparation viability. Experiments were conducted at 22 °C.

Semi-intact preparation

This preparation (N=5) consisted of the body rostral to the pectoral fins, placed ventral side up, with the pharyngeal cavity opened. The remnants of the gut, swim bladder and other extraneous tissues including the heart were removed, sparing the gills, the glottis and their innervation. With the dorsal cranium, the choroid plexus and the cerebellum removed, the fourth ventricle was exposed to superfusate flowing below the preparation. Cranial nerves (CN) V, VII and VIII were cut. The ventral cranium at the level of CN V was removed, so that the stump of CN V was accessible for recording using glass extracellular suction electrodes. A sharp tungsten microelectrode (AM-Systems, Inc., 5–12 MΩ) was inserted through a small incision into the tissues adjacent to the glottis for electromyogram (EMG) recording. The motor output of CN V and glottal EMG signals were recorded using a differential amplifier (model 1700, AM Systems; low and high cut-off 0.3 and 1 kHz, respectively), a moving averager (time constant 100 ms; MA-821, CWE, Inc.) and a computerised data-acquisition system. During these experiments, mock CSF with elevated [Ca^{2+}] (2.4 mmol l^{-1} $CaCl_2$) was used to enhance muscle excitation and thereby help to compensate for muscle 'run-down' in the absence of vascular perfusion. The glottis and gill movements were monitored visually using a dissection microscope (magnification 16×) and event marks and comments were recorded with the digital data.

Preparations were superfused with oxygenated solutions (P_{O_2} 660 mmHg) with [CO_2] adjusted to give pH 8.0 (P_{CO_2} approximately 8.7 mmHg) before and after 20 min challenges with a superfusate having a P_{O_2} of 67 mmHg (measured using Clark-style microelectrodes in the dish; Wilson et al., 1999) at constant pH. Intact gar immersed in water equilibrated with air (P_{O_2} approximately 150 mmHg) have an arterial P_{O_2} of 20–30 mmHg (Smatresk and Cameron, 1982a,c). However, in the superfused semi-intact preparation with its complex geometry, the tissue is probably surrounded by an unstirred layer and, within the tissue, P_{O_2} would be expected to decrease sharply with depth (Okada et al., 1993; Torgerson et al., 1997; Wilson et al., 1999). Thus, a superfusate P_{O_2} of 67 mmHg probably results in hypoxia in non-superficial tissue, including the internally oriented O_2-sensitive chemoreceptors in the gills.

Isolated brainstem preparation

This preparation (N=9) included the brainstem caudal to mid tectum and the spinal cord rostral to spinal nerve (SN) II. The dura and ventral arachnoid were removed, and the preparation was positioned ventral side up in a recording chamber (Wilson et al., 1999). Preparations were left to recover from the dissection for at least 1 h in mock CSF with a P_{O_2} of approximately 660 mmHg and with the P_{CO_2} adjusted to give a pH of 8.0 (P_{CO_2} approximately 8.7 mmHg). Dish pH was measured continuously using a semi-micro pH electrode (model 476156, Corning). Recordings from CN V and CN mX (the mid pre-ganglionic branch of CN X) were made using suction electrodes (with approximate tip diameters of 100 μm) and processed as described above. Hypercapnic challenges consisted of five 20 min periods in which P_{CO_2} was adjusted to give pH values of 8.0 (P_{CO_2} approximately 8.7 mmHg), 8.5 (P_{CO_2} approximately 2.7 mmHg), 7.5 (P_{CO_2} approximately 27.5 mmHg), 8.0 (P_{CO_2} approximately 8.7 mmHg) and 7.5 (P_{CO_2} approximately 27.5 mmHg), respectively.

All data are expressed as means ± S.E.M., unless stated otherwise. Statistical differences were determined for pairwise comparisons using the Mann–Whitney rank sum (MWRS) *t*-test. For multiple comparisons, a one-way repeated-measures analysis of variance (ANOVA) and the Student–Newman–Keuls (SNK) multiple-comparison test were used.

Results

Semi-intact preparation

The semi-intact preparation consisted of the decerebrate brainstem encased in a portion of cranium, both sets of gills, the opercula and the dorsal pharyngeal wall, and included the glottis, a sphincter connecting the pharynx with the air bladder. Cranial nerves (CN) V, VII and VIII were transected, but CN IX and X, innervating the gill arches, glottis and branchial chemoreceptors (Smatresk et al., 1986), were left intact. As hypoxia provides a strong drive for air-breathing in the intact animal (Smatresk and Cameron, 1982a), we used a superfusate with a low P_{O_2} (67 mmHg) to investigate the neuronal correlate of air-breathing. When preparations were superfused with a P_{O_2} of 660 mmHg, we observed the gill arches moving rhythmically with the glottis closed, corresponding to gill ventilation in the intact animal. On exposure to the low-P_{O_2} superfusate, these rhythmic gill movements ceased and, in three out of five preparations, we observed periodic glottal opening, an integral part of the air-breathing behaviour. These responses to low-P_{O_2} superfusate resemble those of the intact animal to hypoxia (Smatresk and Cameron, 1982a; Burleson et al., 1998).

Neuronal correlates of these behaviours were recorded from the root of CN V. Three types of bursts were apparent (Fig. 1; Table 1). (i) Putative gill ventilatory (PGV) bursts, which were persistent, smaller in amplitude than the other burst types and observed visually to be in synchrony with rhythmic movements of the gill arches (0.275±0.05 Hz; mean ± S.D.). These bursts were never associated with glottal opening. (ii) Putative air-breathing ventilatory (PAV) bursts of intermediate amplitude (approximately four times that of PGV bursts) and approximately twice the duration of PGV bursts. PAV bursts were always associated with low-P_{O_2}-induced glottal opening (22/22 observations). (iii) Long-duration (LD) bursts (>20 s), of large amplitude (approximately 12 times that of PGV bursts) and with rapid onsets and followed by a gradual decrease in EMG activity. This last type of burst occurred simultaneously with twitching of the pharyngeal teeth and uncoordinated gill movements. Of 30 LD bursts recorded, 26 (86 %) occurred during exposure to low P_{O_2}, and of the 20 that occurred during visual observation, 11 (55 %) were coincident with glottal opening. While the significance of the LD bursts remains ambiguous, we note that bursts of this type recorded from other reduced brainstem preparations have been interpreted as a pattern akin to mammalian gasping (Kimura et al., 1997; St. John, 1996).

Isolated brainstem preparation

CN V of the isolated brainstem preparation produced all three bursts types observed in the semi-intact preparation (Fig. 2; Table 1). As in the semi-intact preparation, the smallest-amplitude most frequent bursts in CN V (i.e. PGV bursts) were approximately half the duration (1.49±0.64 s; mean ± S.D.) of the less frequent intermediate-amplitude bursts (PAV bursts; 2.64±1.61 s). Unlike PGV bursts, PAV and LD bursts occurred synchronously in CN V and CN mX. All three burst types observed *in vitro* were significantly shorter in duration (MWRS: $t \geqslant 537$, $P<0.01$) and occurred at higher

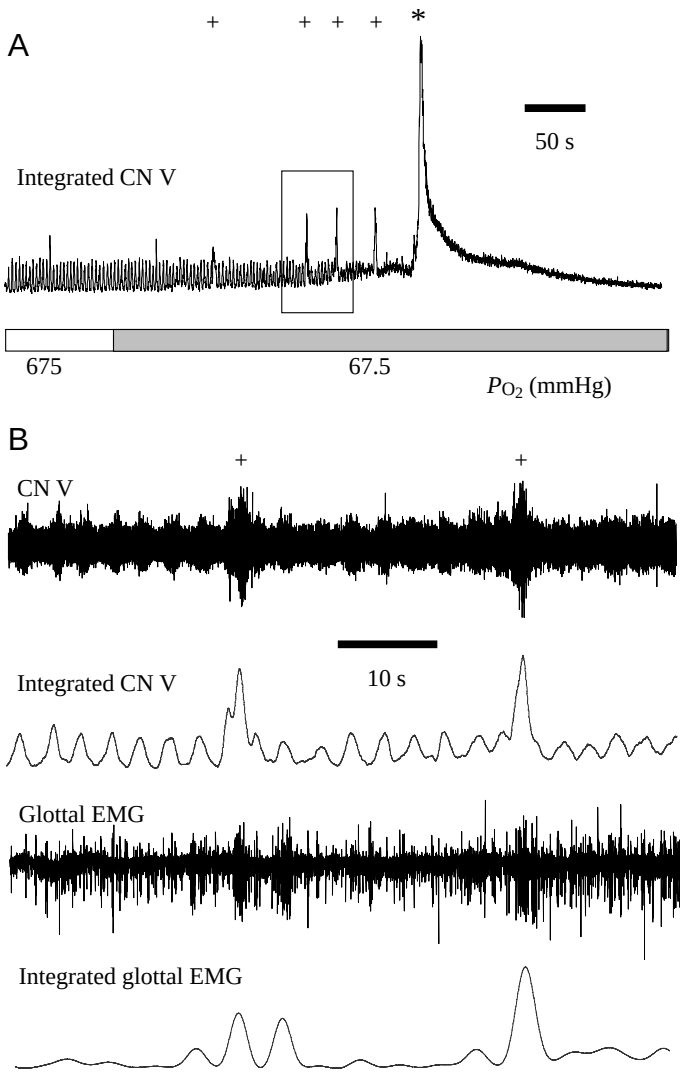

Fig. 1. Ventilatory motor pattern in cranial nerve (CN) V and glottal activity in a semi-intact preparation. (A) Integrated CN V activity illustrating the response to hypoxic exposure (shaded section of lower bar). Three types of motor pattern are apparent: putative gill ventilatory (PGV) bursts of high frequency and low amplitude (not marked), putative air-breathing ventilatory (PAV) bursts (plus signs) and long-duration (LD) bursts (asterisk). The area within the rectangle is expanded in B. (B) Activity in CN V associated with glottal opening (plus signs). Top trace, activity of CN V, recorded extracellularly; second trace, integrated CN V; third trace, glottal electromyographic activity; fourth trace, integrated glottal electromyographic activity (EMG).

Table 1. *Burst duration and amplitude of the three types of motor pattern*

	PGV bursts	PAV bursts	LD bursts
Semi-intact preparation			
Duration (s)	2.22±1.37 (321)	4.69±2.70 (22)	61.67±46.5 (20)
Amplitude (arbitrary units)	1±0.51	3.63±1.80	12.65±8.08
In vitro brainstem preparation			
Duration (s)	1.49±0.64 (658)	2.64±1.61 (245)	36.9±7.8 (19)
Amplitude (arbitrary units)	1±0.33	4.11±2.29	13.67±5.55

Values are means ± 1 S.D., with the number of bursts in parentheses.
PGV, putative gill ventilatory; PAV, putative air-breathing ventilatory; LD, long-duration.

frequencies than was seen in the semi-intact preparation, suggesting that isolating the brainstem leads to an increase in tonic drive and/or the loss of an inhibitory input. The robust nature of the isolated brainstem was demonstrated by its ability to produce these neuronal patterns for at least 9 h.

To test for central respiratory chemosensitivity, we superfused isolated brainstems with mock CSF equilibrated with different levels of CO_2 to generate a pH sequence of 8.0, 8.5, 7.5, 8.0 and 7.5. In seven of the nine preparations having rhythmic PGV bursts, altering pH had no effect on PGV burst frequency (ANOVA: $F_{4,27}$=1.49, N=7, P=0.23). However, decreasing pH increased the frequency of PAV bursts (ANOVA: $F_{4,32}$=3.99, N=9, P<0.01, Fig. 3). At a pH of 8.5 (P_{CO_2} 2.7 mmHg), the frequency of PAV burst was not significantly different from the frequency at pH 8.0 (SNK test: q=0.06, P>0.05). However, when the pH was subsequently decreased to 7.5 (P_{CO_2} 27.5 mmHg), the frequency of PAV bursts doubled (q=4.48, P<0.05). In contrast, decreasing pH decreased the frequency of LD bursts (ANOVA: $F_{4,32}$=4.58, N=9, P<0.01). Thus, the frequency of LD bursts at pH 7.5 was half that at pH 8.5 (q=5.35, P<0.01).

Discussion

Central CO_2/H^+ chemosensitivity in gar

Our results in the gar provide evidence consistent with the presence of a central pattern generator for air-breathing and central CO_2/H^+ respiratory chemosensitivity in this primitive air-breathing fish. Such a central pattern generator for air-breathing does not negate the important influence of peripheral mechanosensory feedback on the motor output in the intact animal. These inputs can affect both the frequency and burst pattern of ventilatory motor acts (Kinkead and Milsom, 1997). Similarly, the presence of central CO_2/H^+ chemosensitivity does not reduce the importance of peripheral chemosensitive mechanisms, which have been documented extensively (Smatresk et al., 1986). Furthermore, while our results illustrate central CO_2/H^+ chemosensitivity, they do not prove

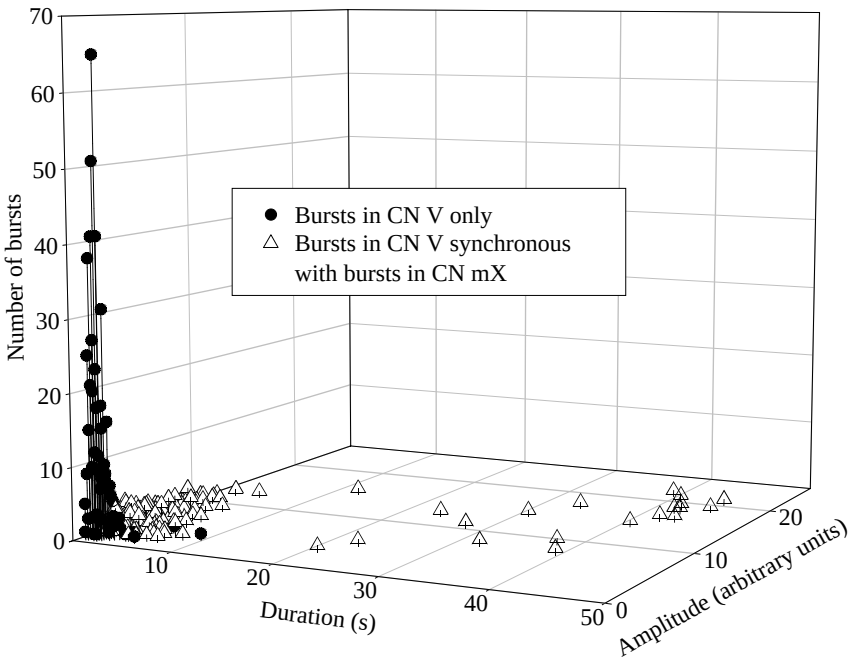

Fig. 2. Illustration of the method used to categorise burst types produced by isolated brainstem preparations into putative gill ventilatory (PGV), putative air-breathing ventilatory (PAV) and long-duration (LD) bursts. From each of the nine preparations used in the study, a 10 min recording period (pH 8–8.5) was chosen to include at least one LD burst. During the selected period, the amplitude and duration of the integrated neurogram for every cranial nerve V (CN V) burst was measured. In addition, CN V bursts were categorised into two types depending on whether or not they were coincident with activity in the medial branch of CN X (CN mX). The results are plotted as a histogram. High-frequency low-amplitude bursts in CN V not coincident with bursts in CN mX were defined as PGV bursts (filled circles). Activity in CN V that coincided with activity in CN mX (open triangles) was defined as either a PAV or a LD burst depending on duration (cut-off 20 s). For the numbers of bursts used to generate this figure and for a direct comparison with the equivalent data from semi-intact preparations, see Table 1.

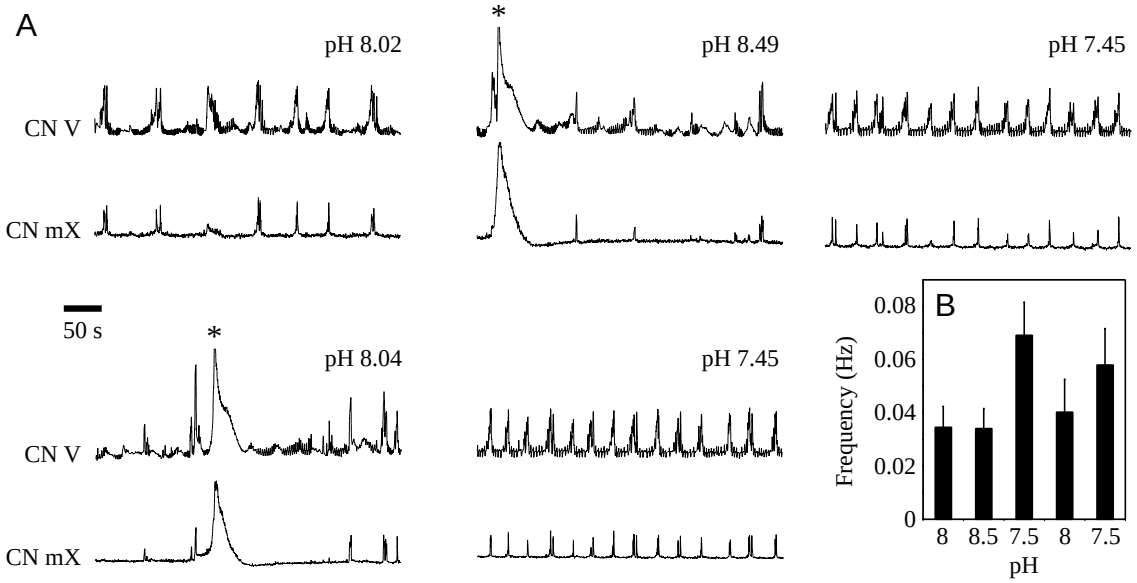

Fig. 3. Effects of hypercapnia on the activity of an isolated gar brainstem. (A) Activity in cranial nerve V (CN V) and the medial branch of cranial nerve X (CN mX) in the isolated brainstem at the end of 20 min sequential exposures to the following pH sequence: 8.02, 8.49, 7.45, 8.04 and 7.45. Asterisks indicate long-duration (LD) bursts. (B) Histogram showing effect of pH on the frequency of putative air-breathing ventilatory (PAV) bursts. Means + S.E.M. from nine preparations.

that such chemosensitivity is of primary importance in the ventilatory behaviour of the intact fish. Thus, our study is subject to a limitation common to all *in vitro* preparations, that one cannot directly extrapolate mechanisms identified *in vitro* to behaviour *in vivo* (see Mitchell, 1993).

Nevertheless, a key advantage of using isolated brainstem preparations to explore aspects of central respiratory control is the lack of possible confounding influences arising from the Bohr and Root effects and from peripheral sensory inputs. Conceivably, for example, strong peripheral signals may mask or override the effects of central CO_2/H^+ chemosensitivity in intact behaving animals. Smatresk (1989) demonstrated the potent role of peripheral inputs in the gar by bilaterally sectioning the branchial branches of CN X in four animals, in three of which the air-breathing response to hypoxic water was 'attenuated'. However, the fact that air-breathing continued (although attenuated) after the surgery suggests that other sites of ventilatory drive exist. Thus, these denervation experiments do not exclude a role for central CO_2/H^+ chemosensitivity.

CO_2 dissolves readily in water and is therefore unlikely to accumulate during gill ventilation when animals are at rest. Even when fish were exposed to aquatic hypoxia (water P_{O_2} below 80 mmHg), which reduces branchial ventilation, CO_2 secretion was primarily *via* the gills, and arterial pH was little affected. However, metabolic and respiratory acidosis does ensue following intense activity and is accompanied by a high air-breathing frequency (Burleson et al., 1998). While the source of the drive for air-breathing following intense activity in intact gar remains to be determined, the blood pH following such activity can fall to 7.2 (Burleson et al., 1998), below that of the mock CSF required by the isolated brainstem to stimulate PAV bursts (see Fig. 3B). Furthermore, the increase in air-breathing frequency following intense activity is of the same magnitude (approximately twofold) as that of the PAV bursts produced by the isolated brainstem following hypercapnic challenge. In comparison with the effects of intense activity, exposing intact gar to aquatic hypercapnia (P_{CO_2} approximately 6 mmHg) is reported to have only a moderate effect on air-breathing frequency, but in those experiments, the decrease in arterial pH was also mild (pH decreased from 7.8 to 7.6). Acidosis, and the need to remove CO_2, is likely to be exacerbated when gill ventilation is compromised fully, such as during emersion or during exposure to contaminated water. The responses of intact gar in these extreme conditions could be of particular importance for survival, but have not been documented.

While response magnitudes were similar, the absolute frequency of PAV bursts produced by the isolated brainstem was considerably greater than that of air-breathing events observed in intact animals. One possible explanation for this discrepancy is that the isolated brainstem may lack inhibitory inputs that suppress air-breathing in intact animals. Sources of inhibitory input might be higher brain centres or peripheral chemoreceptors absent from the *in vitro* brainstem preparation (Milsom et al., 1997; Kinkead and Milsom, 1996; Sakakibara, 1978). Inhibitory signals in the intact animal may also originate from peripheral receptors involved in indicating the presence of water in the buccal cavity or the completion of a successful air breath. Interestingly, glottal openings of gar in air occur at a much more rapid rate than reported previously for air-breathing by intact gar swimming in water (R. J. A. Wilson and M. B. Harris, unpublished data).

Another possible explanation for the high PAV burst frequency of the isolated brainstem is that an acidic core,

inherent to all superfused isolated brainstem preparations (Okada et al., 1993; Torgerson et al., 1997; Wilson et al., 1999), might provide a respiratory drive. For example, the isolated superfused brainstem of the tadpole is 0.4 pH units more acidic at the centre than the surrounding superfusate despite having a high tissue P_{O_2} throughout (>120 mmHg). This acidosis probably results from the build up of CO_2 as a consequence of the diffusional limitations of the tissue (Okada et al., 1993). The isolated gar brainstem preparation, which is of similar dimensions and probably has a comparable metabolic rate to that of the tadpole, is also likely to be mildly acidic with a well-oxygenated core. Ventilatory drive originating from an acidic core not only is consistent with our finding of central CO_2/H^+ chemosensitivity but also implies that the chemosensitive mechanism may be partially saturated in normocapnic superfusate, reducing the possible response of the isolated brainstem to imposed hypercapnic challenge. Thus, central chemosensitivity in the intact animal may have a greater efficacy than we report here.

Evolution of air-breathing

The only other vertebrate that uses bimodal (gill and 'lung') ventilation and in which respiratory sensitivity to central CO_2/H^+ has been reported is the amphibian tadpole *Rana catesbeiana* (Torgerson et al., 1997). While a central pattern generator for air-breathing is generally accepted in amphibians and other tetrapods, air-breathing in fish has previously been considered as a 'reflexive' behaviour triggered by peripheral inputs (e.g. Smatresk, 1994; Brainerd, 1994; Taylor et al., 1999). Our results challenge this view, suggesting that elements present within the *in vitro* brainstem of the gar are sufficient to produce PAV burst activity.

Like the gar, tadpoles use a buccal force pump to breathe (Gans, 1970), and our recordings demonstrate a striking resemblance between the ventilatory motor patterns produced by the isolated brainstems of these two species (Fig. 4). Both commonly produce isolated air ventilatory bursts of similar shape separated by multiple gill ventilatory bursts, but in both species, fictive air ventilatory bursts can also occur in clusters. The resemblance between the outputs of the isolated brainstems was unexpected, given that *in vivo* the sequence of gas transfer to and from their respective air-breathing organs differs (Brainerd, 1994). Importantly, these mechanical differences have led to the hypothesis that the ventilatory pumps of primitive actinopterygians and sarcopterygians evolved separately (Brainerd, 1994). On the basis of the striking similarity between the ventilatory motor patterns produced by the isolated brainstems of the tadpole and gar, we propose that the behavioural differences are the manifestations of a common air-breathing pattern generator differentially modulated by sensory feedback. Future research, comparing the location and mechanisms of central pattern generation in the tadpole and the gar, will be needed to evaluate this hypothesis fully. However, other similarities between breathing in the descendents of the Sarcopterygii and the Actinopterygii have also been documented. For example, both

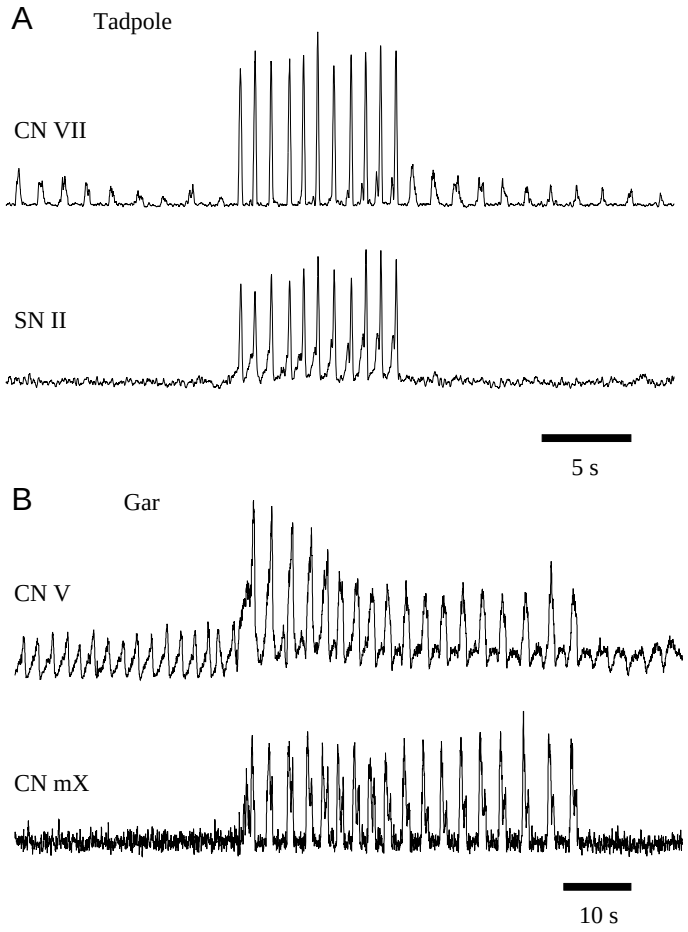

Fig. 4. Similarities in the ventilatory motor patterns produced by isolated brainstem preparations from the amphibian tadpole and the gar. (A) Integrated nerve root activity from a stage 24 (Taylor and Kollros, 1946) *Rana catesbeiana* tadpole illustrating a bout of lung-inflation bursts (P_{O_2} 675 mmHg; P_{CO_2} 14 mmHg; pH 7.8; 22 °C). Coordinated activity in cranial nerve (CN) VII and spinal nerve (SN) II is a strong and convenient indicator of lung inflation in the tadpole (Gdovin et al., 1998). (B) Integrated nerve root activity from CN V and the medial branch of CN X (CN mX) from an isolated gar brainstem illustrating a bout of putative air-breathing ventilatory (PAV) bursts (P_{O_2} 675 mmHg; P_{CO_2} 27 mmHg; pH 7.5; 22 °C). Note that CN X innervates the glottis in both frog and gar (Kogo and Remmers, 1994; Norris, 1925).

lineages have identical mechanoreceptors in their air-breathing organs (Smatresk and Azizi, 1987), exhibit a Hering–Breuer-like reflex (Smatresk and Azizi, 1987; Johansen et al., 1970; Pack et al., 1984) and use similar muscle types to control the glottis (Davies et al., 1993).

In summary, our data obtained with the isolated brainstem of the gar suggest that this actinopterygian fish, like the tetrapods (descendents of Sarcopterygii), has a central air-breathing pattern generator that demonstrates central CO_2/H^+ chemosensitivity. While an important area for future research will be to determine whether these similarities extend to other primitive air-breathing vertebrates, our findings point to

additional parallels between air-breathing in actinopterygian and sarcopterygian lineages. Although any one of these similarities might be the result of homoplasy, together they indicate the potential for a common phylogenetic origin for the neuronal control of air-breathing in primitive fish. Thus, they are consistent with fossil evidence for a common air-breathing ancestor that predated the divergence of the actinopterygian and sarcopterygian lineages (Gans, 1970; Liem, 1988; Perry, 1989).

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