Fictive Gill and Lung Ventilation in the Pre- and Postmetamorphic Tadpole Brain Stem

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Torgerson, C. S., M. J. Gdovin, and J. E. Remmers. Fictive gill and lung ventilation in the tadpole provides a unique opportunity to examine the development of basic neuronal processes that generate and regulate respiratory motor output. The gas exchange needs of very immature bullfrog larvae are met by the passive diffusion of gases across the body surface of the tadpole Rana catesbeiana was examined to characterize fictive gill and lung ventilations during ontogeny. In vitro recordings from cranial nerve (CN) roots V, VII, and X and spinal nerve (SN) root II of premetamorphic tadpoles showed a coordinated sequence of rhythmic bursts occurring in one of two patterns, pattern 1, high-frequency, low-amplitude bursts lacking corresponding activity in SN II and pattern 2, low-frequency, high-amplitude bursts with coincident bursts in SN II. These two patterns corresponded to gill and lung ventilatory burst patterns, respectively, recorded from nerve roots of decerebrate, spontaneously breathing tadpoles. Similar patterns were observed in brain stem preparations from postmetamorphic tadpoles except that they showed a greater frequency of lung bursts and they expressed fictive gill ventilation in SN II. The laryngeal branch of the vagus (Xl) displayed efferent bursts in phase with gill and lung activity, suggesting fictive glottal constriction during gill ventilation and glottal dilation during lung ventilation. The fictive gill ventilatory cycle of pre- and postmetamorphic tadpoles was characterized by a rostral to caudal sequence of CN bursts. The fictive lung ventilatory pattern in the premetamorphic animal was initiated by augmenting CN VII discharge followed by synchronous bursts in CN V, X, SN II, and XI. By contrast, postmetamorphic patterns of fictive lung ventilation were characterized by lung burst activity in SN II that preceded burst onset in CN V and followed the lead burst in CN VII. We conclude that recruitment and timing of pattern 1 and pattern 2 rhythmic bursts recorded in vitro closely resemble that recorded during spontaneous respiratory behavior, indicating that the two patterns are the neural equivalent of gill and lung ventilation, respectively. Further, fictive gill and lung ventilatory patterns in postmetamorphic tadpoles differ in burst onset latency from premetamorphic tadpole patterns and resemble fictive oropharyngeal and pulmonary burst cycles in adult frogs.

INTRODUCTION

The assumption of terrestriality by vertebrates required profound changes in the form and function of the respiratory system associated with the transition from water to air (see reviews by Dejours 1988; Gans 1970; Little 1983; Randall et al. 1981; Shelton et al. 1986). Amphibians, modern descendents of the early tetrapods that migrated from water to land, developed complex respiratory processes and structures in response to differences in the physical properties of air and water. This is particularly apparent in larval forms where gas exchange occurs at multiple sites (skin, gills, and lungs), allowing simultaneous exploitation of both respiratory media. Investigation of the ontogeny of respiratory rhythm generation in the tadpole provides a unique opportunity to examine the development of basic neuronal processes that generate and regulate respiratory motor output. The gas exchange needs of very immature bullfrog larvae are met by the passive diffusion of gases across the body wall (Burggren 1984). As body mass and metabolic demand increase, tadpoles require progressively more efficient gas exchange organs (gills and lungs). The gills are ventilated by a constant, unidirectional stream of water drawn into the mouth, through the oropharyngeal cavity, and out of the opercular spout by rhythmic dorsoventral movement of the buccal floor (DeJongh 1968). By contrast, tidal flow of air into and out of the paired, saclike lungs is accomplished by the same buccal musculature, drawing air in the oropharynx and forcing it into the lungs (Burggren 1984). Lung inflation occurs during the brief opening of the glottis and remains full until the beginning of the next cycle when exhalation, powered by elastic recoil of the respiratory system, drives the air out of the lungs. Before onset of metamorphosis (Taylor-Köllros stage 16), ventilatory patterns appear homologous to those described for lungfish, branchial movements pumping water over the gills and interspersed with irregular lung breaths (McMahon 1969; Smatresk 1990). During metamorphic climax (stage 18–19), the gills and tail degenerate and the lungs become the dominant site for O2 exchange, reaching full maturity in stages 20–25 (Burggren and West 1982). On completion of metamorphosis, the forelimbs develop, the gills and tail are completely reabsorbed, and the branchial cavity is filled with air between breaths (Smatresk 1990).

Investigation of the neural mechanisms responsible for respiratory neuromuscular activity in amphibians was facilitated by the development of superfused in vitro brain stem–spinal cord preparations. The functional significance of the patterns of spontaneous bursting activity observed in cranial nerve (CN) roots of the completely isolated adult frog brain stem preparations (McLean et al. 1995a,b) was elucidated by correlating CN root activity with activity of nerves to respiratory muscles in an in situ preparation (Kirimura et al. 1997) and with neurograms, electromyograms, and mechanical events related to oropharyngeal and pulmonary ventilatory cycles from in vivo preparations (DeJongh and Gans 1969; Ito and Watanabe 1962; Kogo et al. 1994a; Kogo and Remmers 1994b; Sakakibara 1984a,b; West and Jones 1975). Similarly, recent studies of in vitro tadpole brain stem preparations began to characterize the patterns of respiratory activity related to gill and lung ventilation (Galante et al. 1996; Liao et al. 2002).
TABLE 1.  Mean latency and time to peak of pattern 1 and 2 bursts from CN V, VII, X, SN II, and X

<table>
<thead>
<tr>
<th>Nerve</th>
<th>Pattern 1</th>
<th>Pattern 2</th>
<th>Postmetamorphic Stages</th>
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<tbody>
<tr>
<td></td>
<td>Latency</td>
<td>Time-to-peak</td>
<td>Latency</td>
</tr>
<tr>
<td>CN V</td>
<td>5</td>
<td>-481.4 ± 73.7*</td>
<td>367.7 ± 39.9</td>
</tr>
<tr>
<td></td>
<td>CN VII</td>
<td>10</td>
<td>0.0</td>
</tr>
<tr>
<td>CN X</td>
<td>6</td>
<td>454.3 ± 53.8†</td>
<td>472.5 ± 91.6</td>
</tr>
<tr>
<td>SN II</td>
<td>7</td>
<td>NA</td>
<td>324.3 ± 59.5*</td>
</tr>
<tr>
<td>CN XI</td>
<td>3</td>
<td>-90.4 ± 42.5</td>
<td>496.9 ± 142.0</td>
</tr>
</tbody>
</table>

Data shown as means ± SE in ms. Onset latency of pattern 1 and 2 bursts calculated with respect to CN VII. SN II displayed no activity (NA) during fictive gill ventilation in premetamorphic tadpoles. Bursting activity of XI was measured in premetamorphic tadpoles only. * Significantly different from CN VII mean values within both developmental group and type of ventilation (P < 0.05). † Significantly different from CN V mean values within both developmental group and type of ventilation (P < 0.05).

1996; Pack et al. 1993; Torgerson et al. 1997a). However, the separate identification of lung and gill ventilatory activities in these studies must be considered tentative, as an arbitrary CN amplitude criterion alone was used to identify putative gill and lung bursts in CN roots, i.e., large amplitude bursts were assumed to be fictive lung breaths, and small amplitude bursts were assumed to be fictive gill bursts. If the isolated tadpole brain stem preparation is to be utilized as a tool to investigate fundamental questions of rhythmogenesis and central chemoreception, fictive gill and lung ventilatory activity must be separately identified.

The primary purpose of this study is to describe the spatial and temporal patterns of spontaneous bursting activity characteristic of fictive gill and lung ventilation in the in vitro tadpole brain stem. In a separate article (Gdovin et al. 1998), we describe the motor output of the spontaneously breathing larval Rana catesbeiana. In this study, we provide data from the isolated tadpole brain stem preparation that can be correlated with observations from the in vivo study. A second purpose is to describe ontogenetic changes in gill and lung ventilatory motor output. To achieve these two goals, we recorded efferent activity from CN roots, the second spinal nerve (SN) root and the laryngeal branch of the vagus (XI) in pre- and postmetamorphic tadpoles. SN II innervates the hypoglossal muscles and was shown to be a marker of lung breaths in the metamorphic tadpole in vivo (Gdovin et al. 1998). XI can be considered a respiratory nerve because its efferent activity controls the precise opening and closing of the glottal valve during lung ventilation.

METHODS

General

Experiments were performed on 17 larval bullfrog tadpoles (R. catesbeiana) of either sex, obtained from a commercial supplier (Charles D. Sullivan, Nashville, TN). Specimens were assigned to one of two groups based on the criteria of Taylor and Köllros (1946), premetamorphic (stages 4–14, n = 10) and postmetamorphic (stages 20–22, n = 7). During the 5 days preceding experimentation, all tadpoles were housed in aerated, filtered aquariums.
Surgical preparation

Tadpoles were anesthetized in tricaine methane sulfonate (1:10,000) and then weighed. Once unresponsive, the dorsal cranium was removed, and with the aid of a dissecting microscope the cranial and SNs were severed at their respective ostia. After removing the dura and arachnoid dorsally and ventrally, the brain stem was transected just caudal to the level of the fourth SN and just rostral to CN V. In a subset of premetamorphic tadpoles (n = 3), XI, which innervates glottal constrictors and dilators, was dissected and left attached to the brain stem. In these animals, the main trunk of the vagus was freed dorsally by resection of the cartilage surrounding the postotic foramen and by careful excision of the dorsolateral margin of the semicircular canal. Using a ventral approach, XI was exposed by excising the overlying tissue and isolating the entire nerve. Throughout the dissection, the brain stem was superfused with a bicarbonate containing artificial cerebrospinal fluid (CSF) of the following composition (in mM): 104 NaCl, 4 KCl, 1.4 MgCl₂, 10 d-glucose, 25 NaHCO₃, and 24 CaCl₂, bubbled with 98% O₂-2% CO₂, pH 7.8, corresponding to the plasma pH of anuran amphibians (West et al. 1987).

Recording chamber

The brain stem was transferred to a superfusion recording chamber that was described previously (Torgerson et al. 1997b). Briefly, two superimposed disks (2.5-cm OD, 1-mm thickness) with central elliptical holes (2.0 × 0.5 cm) partitioned the chamber into upper and lower compartments. Fine netting (1.0-mm² mesh) spanning the holes was attached to the upper surface of the upper and lower disks, thereby creating a space between the netting for the brain stem. The brain stem was positioned between the two nets, ventral side up, and superfused with artificial CSF (22–24°C) equilibrated with a mixture of CO₂-O₂. The superfusate was conducted from the opposite end of the upper compartment via a paper wick.

Recording

Efferent recordings were obtained from the roots of CN V, VII and X, SN II (hypoglossal in the adult), and XI by using suction electrodes. The pipettes were made from 1-mm OD, thin-walled borosilicate glass and then pulled to a fine tip with a horizontal micropipette puller (Brown-Flaming, model P80). The tip was broken and beveled (Shöhl, Lapp-Technik) to achieve various inner tip diameters ranging from 90 to 350 μm. Action potentials were amplified (AM Systems no.1700, Tektronix AM 502), filtered (100 Hz to 1 kHz), and recorded on videotape with a pulse code modulator (Neurodata DR-890). The signals were simultaneously averaged with a Payerntime averager, displayed on a polygraph (Gould), and digitized and analyzed on a Pentium PC (Datapac II software).

Equilibration of the superfusate with gas having a P CO₂ of 45 and 17 Torr (balance O₂) produced pH values of 7.4 and 7.8. After the brain stem was superfused in the recording chamber for ≥60 min (pH 7.8), stable nerve activities were recorded for 10 min at pH 7.8 and 7.4. Between each 10-min recording, a 5-min equilibration period ensued to allow equalization of tonometer and recording chamber pH and stabilization of the brain stem response to the new superfusate pH.

Analysis

As described in Results, gill and lung bursts were identified by SN II amplitude criteria and by correlations with the SN II recordings described by Gdovin et al. (1998). We define the burst frequency to be the number of bursts per unit time. To evaluate developmental differences in respiratory bursting patterns from CN V, VII, X, SN II, and XI, we measured the time to peak and relative time of onset of fictive gill and lung ventilatory bursts for each 10-min recording at pH 7.4. Mean time to peak was calculated from the onset of the burst to the time of peak amplitude, and mean latency of fictive gill and lung ventilation was determined for each nerve with respect to the onset of the CN VII burst. Mean values of gill and lung motor output for each animal were then used to calculate group means ± SE. Significant differences in the latency and time to peak of fictive gill and lung ventilation within and between each developmental group were examined with a one-way analysis of variance (ANOVA) for repeated measures, with the criterion of statistical significance at P < 0.05. A Student–Newman–Keuls test of pairwise multiple comparisons was used to test significant differences among nerves within treatment groups when ANOVA revealed significant treatment effects.

results

Rhythmic, coordinated bursting patterns were recorded from CN V, VII, X, SN II, and XI in premetamorphic (n = 10) and postmetamorphic (n = 6) larvae when superfused with artificial CSF having a P CO₂ of 45 Torr and pH of 7.4. In raw neurograms, the recruitment of new motor units during pattern 2 is clearly distinguishable. The height of the respiratory bursts was measured by using arbitrary units.
Bursting patterns

As illustrated in Fig. 1, integrated neurograms of CN V, VII, and X from a premetamorphic tadpole revealed two different patterns of rhythmic activity, high burst frequency, low-amplitude bursts (pattern 1) and low burst frequency, high-amplitude bursts (pattern 2). Figure 2 shows both unprocessed and integrated neurograms of CN V, VII, and X in a premetamorphic (stage 4) tadpole, demonstrating patterns 1 and 2. Examination of action potentials in all three nerve records indicates the recruitment of new motor units during pattern 2, as described by Gdovin et al. (1998), further substantiating the distinction between the two motor output patterns.

Commonly, patterns 1 and 2 were not clearly distinguishable from the amplitude of bursts of CN roots. An example of such is illustrated in Fig. 3, where differences between the peak amplitude of patterns 1 and 2 in CN VII were minimal. However, the large amplitude SN II burst during pattern 2 provided an unequivocal marker for this pattern and allowed ready distinction between the two fictive ventilatory patterns. Because of the unequivocal nature of SN II bursts and their direct correlation to lung ventilation, as reported by Gdovin et al. (1998), we used SN II as the marker of pattern 2, whenever possible, in our analysis. Similarly, the Xl neurogram demonstrated pronounced bursting during pattern 2 but only modest activity during pattern 1, as shown in Fig. 4. This distinctive behavior of SN II and Xl neurograms during pattern 2 was consistently observed in all recordings. In 3 of 10 premetamorphic animals and 1 of 6 postmetamorphic larvae, adequate recordings were not available from SN II. Accordingly, an amplitude criterion was used to identify pattern 2 bursts in the analysis shown in Table 1.

The pattern of respiratory motor output was dependent on developmental stage. Figure 5 shows typical simultaneous integrated neurograms of CN VII, X, and SN II from premetamorphic (stage 12) and postmetamorphic (stage 22) tadpole larvae. Premetamorphic fictive ventilation was characterized by pattern 1 bursts observed in CN VII and X, punctuated by sporadic, usually isolated, pattern 2 bursts appearing in all three nerve recordings. Postmetamorphic fictive ventilation, by contrast, was distinguished by an increase in the frequency of pattern 2 bursts in CN VII, X, and SN II.
and SN II, occurring predominantly in clusters, and by the emergence of a pattern 1 in SN II neurograms.

Latency and time-to-peak of bursts

PREMETAMORPHIC ANIMALS. Mean time to peak and latency values of patterns 1 and 2 are displayed in Fig. 6 and listed in Table 1. The pattern 1 ventilatory cycle of premetamorphic tadpoles was characterized by sequential bursts in CN V, VII, and X roots, with activity in Xl occurring in phase with CN VII (Fig. 6A, solid bars). Onset latency of CN V, VII, and X differed significantly \( (P < 0.05) \) from each other as well as the onset of Xl in comparison with CN V and CN X. By contrast, pattern 2 cycles in premetamorphic tadpoles began with slowly augmenting CN VII discharge that burst synchronously with CN V, X, SN II, and Xl (Fig. 6A, open bars). The onset of the pattern 2 bursts in CN V, X, Xl, and SN II significantly lagged behind \( (P < 0.05) \) the onset of lung activity in CN VII. Premetamorphic tadpoles showed no significant difference \( (P > 0.05) \) in the time to peak of cranial and SN bursts when comparisons were made within pattern 1 and 2 ventilatory cycles.

POSTMETAMORPHIC ANIMALS. Pattern 1 in postmetamorphic larvae was distinguished by the emergence of SN II discharge that burst after sequential activation of CN V, VII, and X (Fig. 6B, filled bars). The relative mean onset latency of CN V, VII, X, and SN II bursts all differed significantly \( (P < 0.05) \) with respect to each other. The time to peak of CN VII bursts was significantly greater \( (P < 0.05) \) than the time to peak in CN V, X, and SN II bursts, and the time to peak of SN II burst activity was significantly greater \( (P < 0.05) \) than that of CN V and X. Further, the time to peak of CN VII pattern 1 bursts of postmetamorphic larvae was significantly greater \( (P < 0.05) \) than that of premetamorphic tadpoles.

Like premetamorphic patterns, pattern 2 in postmetamorphic tadpoles was initiated by augmenting activity in CN VII. However, after preliminary CN VII onset, postmetamorphic pattern 2 cycles were characterized by sequential bursting of SN II, followed by simultaneous onset of CN V and X activities (Fig. 6B, open bars). With respect to the mean latency of burst onset, CN VII led CN V, X, and SN II by a significant interval \( (P < 0.05) \), and SN II significantly preceded \( (P < 0.05) \) CN V. The time to peak of pattern 2 in CN VII was significantly longer \( (P < 0.05) \) than that observed in CN V, CN X, and SN II of postmetamorphic larvae.

Discussion

Recordings of efferent activity from the roots of CN V, VII, X, SN II, and from the respiratory nerve Xl in the
isolated brain stem preparation of larval *R. catesbeiana* displayed two types of rhythmic bursting: pattern 1, a high-frequency, low-amplitude oscillation in a rostral-to-caudal sequence, and pattern 2, a low-frequency, high-amplitude rhythm initiated by CN VII and lacking the rostral-to-caudal progression (Figs. 1–5). Pattern 1 was virtually identical in pre- and postmetamorphic animals with the exception that the latter included an oscillation in SN II. Pattern 2 was similar in pre- and postmetamorphic larvae but exhibited distinct differences in the burst latency between nerves. These patterns bear a striking resemblance to those recorded by Gdovin et al. (1998) from CN V, VII, and SN II in the spontaneously breathing decerebrate tadpole, where gill and lung ventilation were mechanically defined. Because of their close correspondence to the in vivo patterns, we classify pattern 1 as fictive gill ventilation and pattern 2 as fictive lung ventilation.

Previous studies used an arbitrary CN amplitude criterion for identifying gill and lung bursts (Galante et al. 1996; Liao et al. 1996; Pack et al. 1993; Torgerson et al. 1997a). The use of such a criterion was not validated, and its sensitivity and specificity are suspect. This study elaborates on the work of Pack et al. (1993) by describing the use of high-amplitude SN II bursts as a sensitive marker of fictive lung ventilation. This study also describes correlates of fictive gill and lung ventilation such as the spatiotemporal characteristics as well as recruitment of Xl bursts during fictive lung ventilation. In our experience, CN burst amplitude is not adequate to identify fictive gill and lung bursts in many cases, but, when used in combination with SN II, any particular burst can be unequivocally identified as pattern 1 or pattern 2.

The overall burst frequencies of pattern 1 and pattern 2 closely resemble those previously reported by Torgerson et al. (1997b) in pre- and postmetamorphic tadpoles. Similarly, the frequency and rostral-to-caudal sequence of pattern 1 observed here closely resembles those reported by Gdovin et al. (1998) for gill ventilation. Comparison of Fig. 6 from Gdovin et al. (1998) and Fig. 6 from this paper show both fictive gill cycles initiated by bursts in CN V followed by activity in CN VII. Further, the frequency of gill bursts in decerebrate metamorphic tadpoles during normoxia (49.3 ± 6.1 min⁻¹) (Gdovin et al. 1998) correlates with gill burst frequency (43.0 ± 2.7 min⁻¹) previously measured in the isolated metamorphic tadpole brain stem preparation when superfused with artificial CSF of pH 7.8 (Torgerson et al. 1997a). This evidence supplements the recruitment and amplitude data discussed above and leads to the conclusion that low-amplitude, high-frequency bursts in vitro represent fictive gill ventilation.

Gdovin et al. (1998) characterized fictive lung ventilation by synchronous burst activity in CN VII and V and by the recruitment of SN II. Similarly, we observed simultaneous bursting in CN V and VII and the appearance of SN II bursts during pattern 2 in vitro, suggesting that high-amplitude, low-frequency bursts represent fictive lung activity. Fictive lung ventilation frequency, previously described in the isolated metamorphic brain stem preparation (0.2 ± 0.1 min⁻¹, artificial CSF, pH 7.8) (Torgerson et al. 1997a), matched measurements reported by Gdovin et al. (1998) in the decerebrate metamorphic tadpole under normoxic conditions (0.3 ± 0.1 min⁻¹). Although the time to peak of gill and lung bursts recorded in vitro ranged between 10 and 100% of in vivo burst time to peak measurements, correlation between both preparations was close, in view of in vitro peripheral deafferentation and gas/pH tissue status (Torgerson et al. 1997b).

Although the spatiotemporal characteristics of pattern 1 and 2 bursts appear similar in pre- and postmetamorphic larvae, several important distinctions can be made. In premetamorphic larvae (<stage 16), respiratory neural output consisted predominantly of gill activity interrupted occasionally by isolated lung bursts. This finding corresponds with respiratory patterns described in intact premetamorphic tadpoles (Burggren and Doyle 1986; Infantino 1992) and confirms previous results from isolated premetamorphic tadpole brain stem preparations (Torgerson et al. 1997a).

Premetamorphic gill motor output cycles in vitro were initiated by CN V activity and followed by sequential bursts of equal time to peak in CN VII and X. This finding agrees with Gradwell's (1972a,b) description of gill irrigation mechanics in premetamorphic tadpoles, showing coordination of mouth opener and closer muscles (innervated by CN V), buccal floor elevators and constrictors (innervated by CN VII), and pharyngeal cavity constrictors and dilators (innervated by CN X). In the isolated premetamorphic tadpole brain stem preparation, we detected no bursting activity in SN II during fictive gill ventilation. Although Gradwell (1972a,b) attributed the activation of mouth opener and pharyngeal constrictor muscles to SN motor output, the contribution of these muscles to gill ventilation was minimal based on electromyographic and pressure recordings. Further, our results agree with Taylor (1985), who reported a similar rostral-to-caudal bursting sequence in CN output during gill ventilation in studies of dogfish. Finally, we observed rhythmic activity in XI during fictive gill ventilation that burst in phase with CN VII. XI innervates dilator and constrictor muscles of the glottis (Sakakibara 1984a) and was previously shown to display bursting activity during fictive oropharyngeal ventilation in vivo (Kogo et al. 1994a,b) and in situ (Kirma et al. 1997) adult bullfrog preparations. XI activity in premetamorphic larvae during fictive gill ventilation may represent fictive glottal closure, ensuring that no water enters the lungs before being forced over the gills.

Fictive lung ventilatory cycles in premetamorphic tadpoles were led by CN VII, demonstrating biphasic activity, with a preliminary slowly augmenting discharge followed by an abrupt increase in activity synchronous with CN V, X, XI, and SN II. Our recordings of XI activity, showing a burst of activity coincident with synchronous lung bursts in CN V, X, and SN II, suggest that laryngeal muscle activity is modulated during gill and lung bursts. This agrees with results reported by Kogo et al. (1994a,b) and Kimura et al. (1997) in the adult frog. Further, exclusive lung burst activity in SN II provides a specific and sensitive marker of fictive lung activity in premetamorphic tadpole, allowing clear differentiation from gill motor output.

Although no study has systematically described the mechanics of lung ventilation in larval amphibians, the basic mechanism, coordinated by buccal and pharyngeal musculature normally used to propel water through the branchial chambers, is thought to resemble lung ventilation described in lung fish (McMahon 1969) and adult frogs (DeJongh and
In the adult frog, McLean et al. (1995a,b), Kogo et al. (1994a,b), and Kimura et al. (1997) recorded efferent activity from specific branches of CN V, X, and SN II innervating buccal and laryngeal muscles and characterized the fictive lung ventilatory cycle by augmenting activity in the sternohyoid branch of SN II followed by synchronous bursting in CN V, X, and the main branch of SN II. This sequential pattern resembles that observed in the postmetamorphic tadpole but contrasts with fictive lung bursts of premetamorphic animals, showing simultaneous activation of CN V, X, XI, and SN II.

In the premetamorphic tadpole brain stem preparation, Galante et al. (1996) established that fictive lung bursts were unaffected by chloride-free superfusate, indicating that postsynaptic inhibition is not essential for fictive lung rhythmogenesis. Hence fictive lung breathing in the premetamorphic tadpole may be generated from a primitive lung central pattern generator (CPG) not requiring postsynaptic inhibition, whereas fictive lung bursts in the adult frog arise from a CPG containing well-developed network properties that include postsynaptic inhibition. Interestingly, Kimura et al. (1997) showed, in the in vitro adult frog brain stem, the dependency of fictive lung ventilation on postsynaptic inhibition; superfusion of the adult brain stem preparation with artificial CSF containing the glycine-receptor blocker strychnine induced a sudden change in the timing and shape of fictive lung bursts from a sequential, augmenting pattern to a synchronous onset burst with decrementing trajectory.

Postmetamorphic (>stage 20) fictive ventilation featured increased frequency (Torgerson et al. 1997a) lung burst activity interspersed with rhythmic gill output. Although clusters of neural lung activity appeared in premetamorphic larvae, they were more frequently observed in postmetamorphic ventilatory patterns (Fig. 5B) and may be correlated with episodes of uniform lung ventilations or lung inflation cycles described in intact adult frogs (DeJongh and Gans 1969; Kogo et al. 1994a,b). This pattern agrees with previous descriptions of postmetamorphic tadpole ventilation in both the isolated brain stem preparation (Torgerson et al. 1997a) and intact, freely swimming animal (Burggren and Doyle 1986; Infantino 1992; West and Burggren 1982).

Fictive gill burst cycles in postmetamorphic larvae were distinguished from premetamorphic patterns by sequential, unequal time to peak bursts in CN V, VII, X, and SN II. Differences in the time to peak of cranial and SN respiratory motor output may be attributed to the shift in respiratory muscle activation with development. In the premetamorphic tadpole, gill irrigation is achieved by the coordination of motor output may be attributed to the shift in respiratory bullfrog. Kogo et al. 1994a,b). This pattern agrees with previous descriptions of postmetamorphic and adult lung ventilatory cycles.

Overall, cranial and SN activity from the isolated brain stem of larval R. catesbeiana closely resembles the pattern of neural activity during gill and lung ventilation in the postmetamorphically breathing decerebrate tadpole. Recordings of SN II bursts improve the accuracy of distinguishing fictive lung from gill ventilation and in many cases are essential for separating these two types of ventilatory motor outputs. Fictive gill and lung ventilatory patterns in postmetamorphic tadpoles differ in burst onset latency from premetamorphic patterns and resemble fictive oropharyngeal and pulmonary burst cycles in adult frogs. Thus, in addition to establishing a descriptive framework for investigating the ontogeny of neural-respiratory control in vitro, we demonstrate a shift in the pattern of gill and lung motor output that accompanies the transition from water to air breathing and provides new insight into the origin and development of the amphibian respiratory CPG.

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References


