

Fictive Lung Ventilation in the Isolated Brainstem Preparation of the Aquatic Frog, *Xenopus Laevis*

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Abstract Aquatic pipid frogs, unlike other anurans, never show sole buccal ventilation cycle, and exhale air from the lung before aspirating air into the buccal cavity. To study the mechanism that pipid frogs lack the buccal cycle, respiratory motor activities were recorded from the isolated brainstem-spinal cord preparation of *Xenopus laevis*. Brainstem preparations of *Xenopus* exhibited the intermittent burst complex (lung bursts) similar to the lung ventilation cycle in vivo. Lung bursts spontaneously occurred in the cranial nerve V, IX and X, and in the hypoglossal and third spinal nerves. Small bursts with regular cycle similar to buccal oscillation in ranid frogs, were observed in the cranial nerve V and X but not in the hypoglossal and the third spinal nerve. These results suggest that *Xenopus* is capable of oscillating buccal rhythm within the brainstem.

1 Introduction

Pipid frogs including *Xenopus laevis* are entirely aquatic and have interesting characteristics from the viewpoint of comparative physiology. First, pipid frogs, unlike other anurans, lack sole buccal ventilation cycle, and have only lung ventilation cycle. Second, they are different from other anurans in the sequence of lung ventilation. Although ranid frogs inspire air into buccal cavity before exhaling air from the lung, pipids fill the buccal cavity with fresh air after exhaling air (Brett and Shelton 1979). During the ventilation cycle, other anurans open nares except when they stuff air into the lung, while the pipids close the nares except when they exhale air from the lung and inhale air into the buccal cavity. Third, pipids have inherent muscles, musculus pulmonum proprius (Mpp) and ventral diaphragm, suspected homologous to the mammalian diaphragm, though the muscles do not separate the pleural cavity from the abdominal cavity (Keith 1905, Snapper et al. 1974). Furthermore, these muscles are innervated with nerve branches of brachial plexus. A recent study suggested that the pipid “diaphragm” might be capable of acting as an esophageal sphincter, like the mammalian rural diaphragm (Pickering

et al. 2004). To study whether adult pipid frogs had lost buccal oscillator itself, the respiratory motor activity was recorded from the isolated brainstem of *Xenopus laevis* without any peripheral reflex loops.

2 Methods

Experiments were performed on nine juvenile and adult (15–35 g, both sexes) African clawed frogs (*Xenopus laevis*). Care of animals and experimental protocols were approved by the Animal Research Committees of the Jikei University School of Medicine. All animals were acquired from commercial suppliers (Watanabe Zoushoku). Each frog was anesthetized in the 0.6% saline containing 0.01% tricaine methane sulfonate until unresponsive to toe pinch. The cranium was opened and the animal decerebrated. The dura and choroid plexus were removed and the brainstem superfused with oxygenated artificial cerebrospinal fluid (aCSF) buffered sodium bicarbonate at 21–23°C. Bicarbonate aCSF (in mM): NaCl, 104; KCl, 4; MgCl₂, 1.4; D-glucose, 10; NaHCO₃, 25; CaCl₂ 2.4; pH 9. Bicarbonate aCSF was equilibrated with 98%O₂ and 2% CO₂ to yield a bath pH of 7.9. The brainstem was isolated with two transections, one just caudal to the cranial nerve III and other caudal to the 4th spinal nerve. Electrical signals were recorded from the roots of cranial and spinal nerves, with some combinations of V, IX, X, hypoglossal nerve (SN2), and the third spinal nerve (SN3), using glass suction electrodes. These signals were amplified ($\times 10,000$) and filtered (1–5 kHz) using differential AC amplifiers (model 1700, A-M Systems), digitized at 400 /s (Powerlab 8sp, ADInstruments) and archived on a HDD. To test the pharmacological properties of the fictive lung ventilation, CNQX (1 μ M), strychnine (1 μ M), and bicuculline (5–10 μ M) were given to the superfusate.

3 Results

The isolated brainstem-spinal cord preparation of *Xenopus laevis* exhibited intermittent bursts (lung bursts) similar to the lung ventilation *in vivo* in all the V, IX, X, SN2 and SN3 recorded. The lung bursts spontaneously occurred as doublet (a pair of bursts), triplet or multiple burst complexes (Fig. 1A). The period of lung bursts are ranging from 10 to 150 s (53 ± 54 s, $n = 9$). Small bursts with relatively regular cycle, low amplitude, short duration and interval (buccal-like bursts) similar to the buccal oscillation in ranid frogs, were observed in the cranial nerve V and X, but not in SN2 and SN3 (Fig. 1B and C). The period of buccal-like bursts are ranging from 1.3 to 3.1 s (2.0 ± 0.7 s, $n = 6$).

Bath application of 1 μ M of CNQX prolonged the interval between the lung bursts without reduction in the amplitude (Fig. 2, $n = 3$). Strychnine (1 μ M) changed the lung bursts into a decrementing shape ($n = 3$), and the burst shape did not re-

cover with washing over 1 hr by aCSF. Lower concentration of bicuculline ($5\ \mu\text{M}$) increased buccal-like bursts (Fig. 2C, $n=3$). Higher concentration of bicuculline ($10\ \mu\text{M}$) decreased buccal-like bursts and changed the lung bursts into decrementing bursts with short duration.

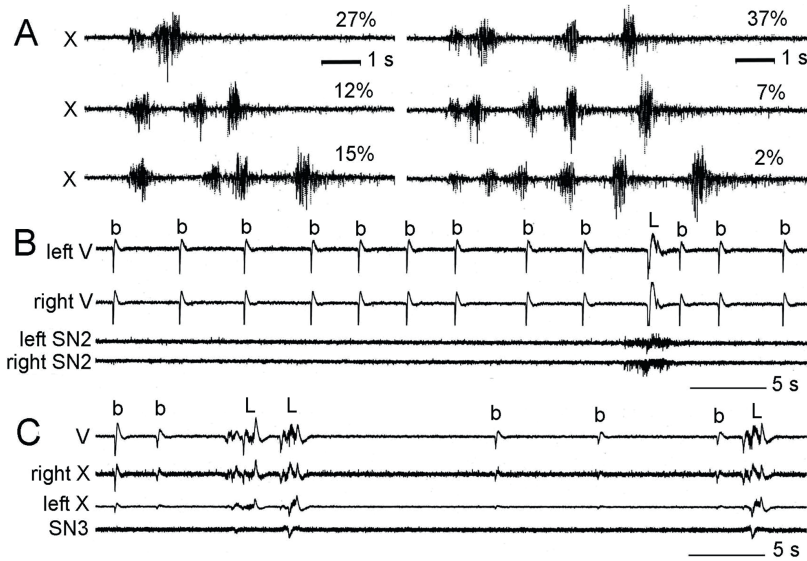


Fig. 1 Lung bursts and buccal-like bursts recorded from the isolated brainstem of *Xenopus laevis*. **A**: Various patterns of lung burst complex in the cranial nerve X. **B** and **C**: Lung bursts (L) and buccal-like bursts (b) in the trigeminal (V) and vagus (X) nerves

4 Discussion

Patterns of the burst complex recorded from the isolated brainstem of *Xenopus laevis* were similar to their lung-ventilation *in vivo*. The activation sequence of motor outputs within a burst complex was consistent with that of the normal ventilation *in vivo*. Similarities in the effects of CNQX, strychnine, and bicuculline on the bursts to ranid frogs are also consistent with their homology (Kimura and Remmers 1994, Kimura et al. 1997, Kawasaki et al. 1995).

Occurrence of buccal-like bursts in the isolated brainstem suggests that Brainstem of adult *Xenopus* is capable of oscillating with buccal cycle. Buccal oscillation of *Xenopus* might be suppressed by some reflex mechanism *in vivo*. However, it is unknown whether buccal-like burst actually functions as some role. Although the reason for lacking buccal-like bursts in SN2 is also unknown, it seems to be convenient not to aspirate water into buccal cavity for their aquatic life. Because of recording nerve rootlet, reciprocally active bursts in SN2 might

mutually bury those activities. To prove this point, it needs recording separately from nerve branches in the in situ brainstem preparation (Kimura et al. 1997).

Absence of buccal-like bursts in SN3 containing branches innervating pipid “diaphragm” may suggest that motor outputs from buccal oscillator had been not supplied to “diaphragm” at the first point in tetrapod evolution.

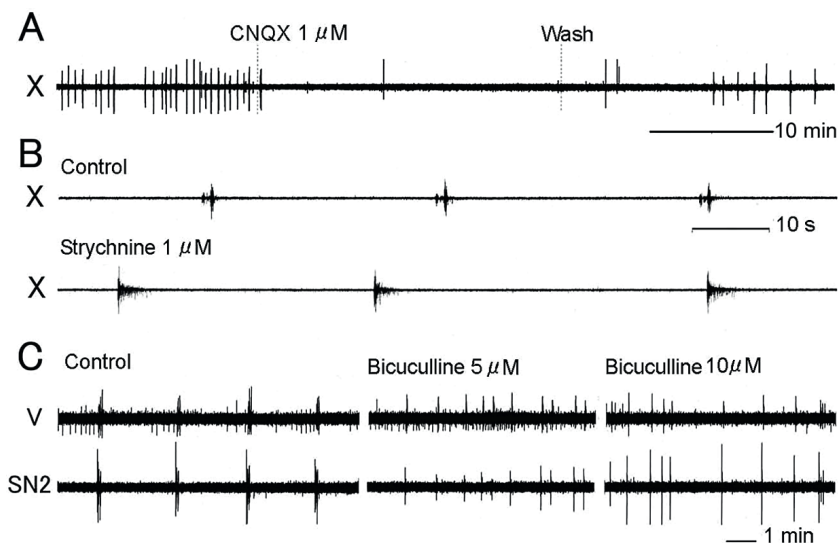


Fig. 2 Effects of CNQX (A), strychnine (B) and bicuculline (C) on lung bursts from the isolated brainstem-spinal cord preparations of *Xenopus laevis*

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