Organization of inputs to motoneurons during fictive respiration in the isolated lamprey brain

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Summary. The intracellular activity of motoneurons during 'fictive' respiration in the isolated lamprey brain was investigated. In association with each respiratory cycle three distinct PSP phases were observed: an early, low amplitude EPSP phase; a large, brief EPSP phase that drove action potentials; and a subsequent IPSP phase (Fig. 1). Selective midline and trigeminal lesions, and trigeminal stimulation, demonstrated that the large excitatory and inhibitory phases were generated by a previously described pair of central pattern generators located in the trigeminal region of the medulla (V) (Figs. 3, 4). Lesion studies further showed that the low amplitude excitatory input could be produced independently of the trigeminal pacemakers (Figs. 3, 5), near the region of the medulla that contains the respiratory motoneurons (VII, IX, and X).

In addition to 'normal' fictive respiration, the isolated brain was found to produce several variations of the respiratory pattern. These motor programs, 'coughs', 'arousal breathing', and 'weak breathing', were distinguished from the normal respiratory pattern by their much longer burst durations, distinctive underlying synaptic input, and separate coordinating mechanism (Figs. 6–8). Activity similar to these motor programs could be independently produced by the caudal medulla after both trigeminal central pattern generators had been removed (Figs. 5, 6). Lesion studies, and the observation that respiratory-related neural activity ceased in the trigeminal region during the production of these long-duration programs, suggest that the caudal medulla also contains paired central pattern generators involved in lamprey respiration (Figs. 5, 9, 10).

Introduction
The isolated lamprey brain spontaneously produces respiratory neural activity in vitro for as long as three days (Rovainen 1977). This activity closely resembles that underlying the respiratory behavior of intact animals, and persists after transections of the brain at the obex and isthmus. Thus, both the pacemakers and motoneurons for respiration are contained within the lamprey medulla (Rovainen 1974). The lamprey brain has been proposed as a model system for the investigation of respiratory pacemaking because of its survival in vitro, the relatively simple organization of the brain along the basic vertebrate plan, and the feasibility of intracellular studies (Kawasaki 1979, 1981; Nieuwenhuys 1972, 1977; Rovainen 1974, 1977, 1979).

At present, the interneurons comprising the respiratory central pattern generator (CPG) have not been identified. However, there is strong evidence from brain lesion and stimulation studies that bilateral CPGs are present within the lateral trigeminal (V) region of the medulla (Rovainen 1983a, b, 1985; Russell 1984). The respiratory motoneurons are situated more caudally in the medulla, in cranial motor nuclei VII, IX, and X which supply the gills. Adult lampreys are tidal breathers, but only one phase of the respiratory cycle is active. Exhalation results from active muscle contraction but the inhalation phase is a completely passive process (Roberts 1950). Similarly, in the isolated brain there are periodic bursts of activity cor-

Abbreviations: CPG central pattern generator; HRP horseradish peroxidase
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responding to exhalation and no antiphasic motor activity is evident.

In the course of investigations undertaken to delimit the respiratory CPGs it was found that certain lesions which eliminated respiratory discharges in cranial nerves failed to eliminate subthreshold respiratory activity in the motoneurons. For example, hemisection caudal to the trigeminal region which always eliminated respiratory nerve bursts on the lesioned side (Rovainen 1983a) did not eliminate rhythmical depolarizations of ipsilateral respiratory motoneurons that occurred in phase with the discharges on the intact side. Furthermore, rhythmical activity was found to be present in some preparations which had received complete transections caudal to the trigeminal pacemaking region.

These results suggested the possible presence of additional pacemaking activity in the caudal medulla separate from the V CPGs. In semi-intact preparations of a different species, it has also been reported that the caudal medulla could independently produce respiratory activity (Kawasaki 1979, 1984). In the present investigation, which utilized isolated brains of two species, obtaining fictive respiratory activity from the isolated caudal medulla was found to be exceedingly difficult. However, fictive "coughs", "arousal breathing", and "weak breathing" were more readily observed. These are additional motor programs which are spontaneously produced by the isolated brain and isolated medulla. A "cough" is defined as a strong bilateral excitation of respiratory motoneurons which is irregular in occurrence and long in duration. It may function to dislodge particles in the gill openings (Rovainen 1974) or to move accumulated blood from the branchial region (Rovainen 1977). "Arousal" and "weak" breathing are described and discussed in the text. "Arousal" breathing occurs spontaneously or after a variety of sensory stimuli and is accompanied by generalized activities in other neural systems (Russell et al. 1985).

In the isolated brain, these motor patterns have distinct underlying synaptic inputs, respond differently to brain lesions and stimulations, and are coordinated via different pathways as compared to normal respiratory activity. The present study suggests that the caudal medulla contains some pacemaking capacity but that it is normally subordinate to the trigeminal centers. The caudal region may be utilized to provide the predominant excitatory drive for the additional forms of respiratory activity that are produced by the lamprey. Part of this work has appeared previously in an abstract (Thompson 1984).

Materials and methods

Adult lampreys, Ichthyomyzon unicuspis and Petromyzon marinus, were used in these studies. The brain and ventral skull were removed from the animal and exposed dorsally. The chordial plexus was dissected away, the roof of the isthmic region was cut at the dorsal midline, and the roof of the obex was cut to allow unobstructed access to the respiratory motor nuclei. Pins through the optic capsules, posterior tectorial cartilage and the notochord stabilized the preparation for intracellular recording. The composition of physiological saline was (mmol/1) 115 NaCl, 2 KCl, 2.6 CaCl2, 2 MgCl2, 2–3 NaHCO3, and 6 glucose. High-Ca fluid contained 20 mmol/1 CaCl2 and 90 mmol/1 NaCl with the other ingredients unchanged. Isolated brains were maintained at 7–12 °C during experiments and were stored in oxygenated normal saline in the refrigerator overnight (4 °C). Some preparations were used for three consecutive days.

Glass suction electrodes were used for extracellular recording and stimulation. Intracellular recordings from motoneurons were obtained with glass microelectrodes filled with 2 mmol/l potassium acetate, resistances of 70–130 MΩ, except for the chloride injection experiments in which case the pipettes were filled with 2 mol/l potassium chloride. Chloride was injected with constant hyperpolarizing current. Respiratory motoneurons were identified by correlating intracellular action potentials with extracellular spikes in the cranial nerves.

In some experiments the brain was lesioned at the midline or behind the trigeminal region, at the level of the Mauthner cell. Lesions were performed with a scalpel fashioned from a minutien pin affixed to the end of a wooden applicator stick. Respiratory activity was monitored extracellularly while lesions were being made. Discharges were kept as close to normal as possible during lesions by halting the scalpel advance when disruption of respiration was detected, and then waiting to finish the lesion until respiratory activity recovered. This procedure seemed to be less likely to cause irreversible loss of activity than lesions made rapidly by the scalpel or scissors.

Records were photographed with an oscilloscope camera, or recorded on a chart recorder (Gould 220).

Results

Figure 1A illustrates the basic external architecture of the lamprey brain and outlines the surface bulges which contain the brain's motoneurons. Respiratory motoneurons are located within the VIIth, IXth and Xth motor nuclei. One respiratory motoneuron has been stained with horseradish peroxidase (HRP). With transillumination, the various landmarks of the lamprey brain are clearly visible in dissected preparations and can be used to guide the placement of the scalpel for precise lesions. The midline is the most obvious landmark, the brain is exceedingly thin at the midline and appears as a bright crease in transilluminated preparations. The motor nuclei prominences appear as darker areas on the floor of the fourth ventricle. The Vth nuclei can usually be seen. The VII, IX, and X motor nuclei are contiguous without