

OXYGEN-SENSITIVE ELEMENTS IN THE BOOK GILLS OF *LIMULUS POLYPHEMUS*

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(Received 21 September 1973)

INTRODUCTION

Rhythmic movements of the gill appendages provide respiratory ventilation of the book gills in *Limulus polyphemus*, the horseshoe crab (Hyde, 1893; Waterman & Travis, 1953). Respiratory movements are driven by contractions of abductor, adductor and branchiothoracic muscles which are controlled by motor centres in the abdominal ganglia (Fournier, Drewes & Pax, 1971; Hyde, 1893, 1906; Knudsen, 1973; Wyse, 1972). The rate and amplitude of these movements are proportional to the oxygen concentration in the animal's environment; all movement ceases under anaerobic conditions (Hyde, 1906; Waterman & Travis, 1953). When oxygen is introduced into the anaerobic sea water, respiratory movements are initiated immediately indicating that this reflex is mediated by external oxygen receptors (Waterman & Travis, 1953). Until recently, attempts to locate these receptors were unsuccessful (Schlein & Barber, 1971; Waterman & Travis, 1953); however, Page (1973) found that the book gills and intercoxal cuticular membranes are responsible for the reflex oxygen sensitivity of the ventilatory system.

Patten & Reddenbaugh (1899) showed that each book-gill lamella is innervated by a branch of the gill nerve ending in sensory cuticular buds.

We have examined oxygen-sensitive units present in the gill nerve. We find three classes of units: those whose activity is increased by oxygen, those whose impulse discharge is depressed by oxygen, and mechanosensitive units whose tactile sensitivity is oxygen-dependent.

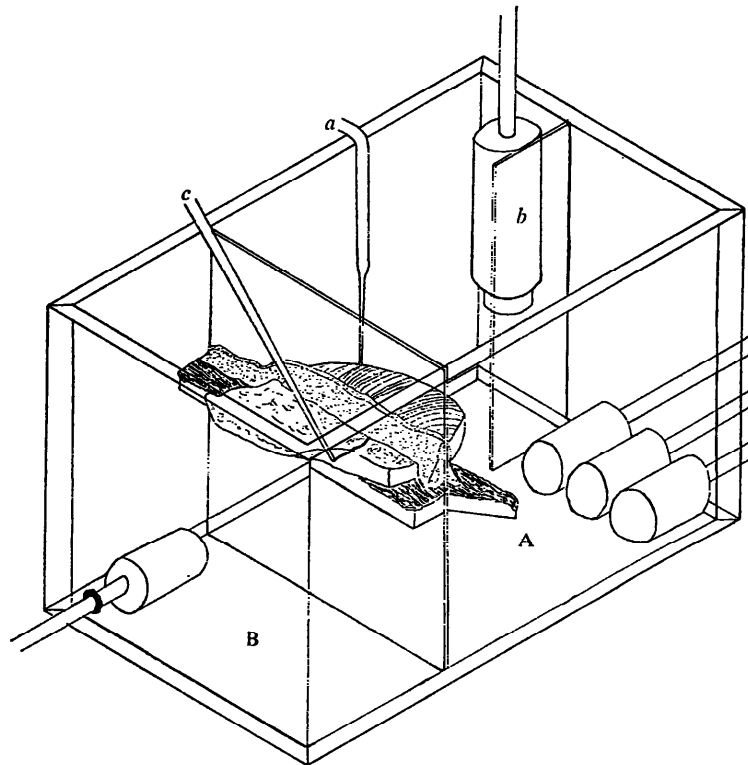
MATERIALS AND METHODS

Adult *Limulus polyphemus* (prosoma widths of 20-25 cm) were purchased from the Marine Biological Laboratory, Woods Hole, Massachusetts. Specimens were fed on beef liver and maintained in a refrigerated circulatory tank filled with synthetic sea water (Dayno). Experimental animals were used within 2 months of delivery.

The first or second left book gill was removed from the animal. The cuticle overlying the musculature of the median lobe was carefully removed exposing the gill nerve medial to its bifurcation at the base of the lamellae.

The posterior branch of the gill nerve was exposed by pulling two successive lamellae apart. The branch was further subdivided with fine needles.

The book gill was placed in a Plexiglass chamber filled with synthetic sea water, as illustrated (Text-fig. 1). A contoured Plexiglass partition was lowered over the book



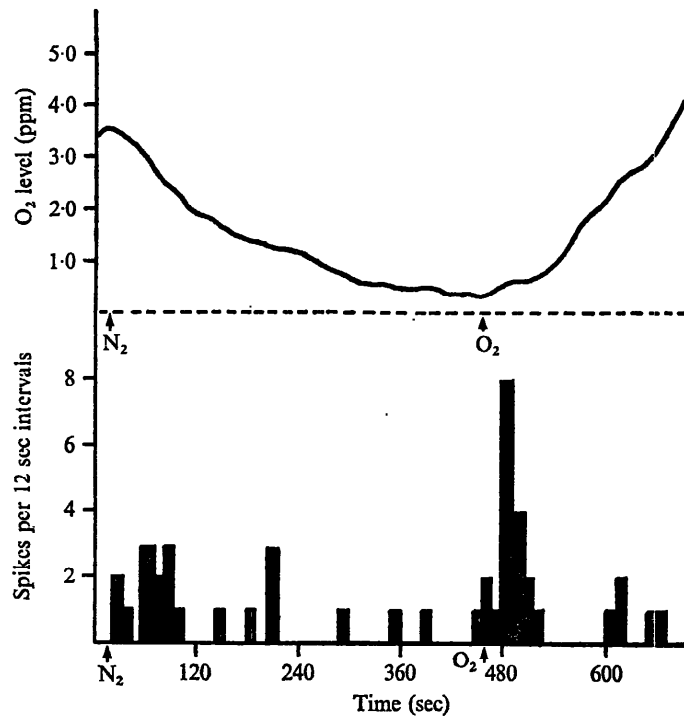
Text-fig. 1. The experimental chamber with partition in place. The gill appendage is positioned so that the book-gill lamellae are on side A of the chamber while the gill nerve is on side B. The taper (*a*) and oxygen probe (*b*) are shown mounted in the chamber; a suction electrode (*c*) is attached to the gill nerve.

gill, subdividing the chamber. Silicone lubricant (Dow Corning) was used to make all joints watertight.

The oxygen tension in the sea water bathing the book gill lamellae could be varied by bubbling nitrogen or air into side A of the chamber. On the opposite side of the chamber (B) the exposed gill nerve was bathed in sea water which was kept at air saturation (5 ppm O₂) by bubbling air through the air stone.

Unit responses to changes in the oxygen level in chamber A were monitored. Dissolved oxygen in side A of the chamber was displaced by bubbling nitrogen into the chamber. To re-introduce oxygen into the anaerobic sea water, the nitrogen was turned off and air was bubbled into the chamber.

Suction-electrode recordings from the gill nerve or subdivided branches were fed into a Grass P15 Pre-amplifier, displayed on a Tektronix 502 Oscilloscope and monitored with an audio monitor. Oxygen concentration in the experimental chamber, measured in parts per million (ppm), corrected for temperature and salinity, was monitored using a Yellow Springs Instrument Company Model 51A Oxygen Meter and probe (YSI no. 5418). The oxygen-meter output was monitored together with the Grass stimulator output on the lower beam of the oscilloscope. Trials were filmed with a Nikon Kohden Kogyo continuous-recording camera.



Text-fig. 2. Histogram of class I unit responses
(Arrows indicate the point of introduction of N₂ or O₂.)

The lamellae received tactile stimulation from a tapered glass rod fastened to a speaker cone. Displacement of the cone was driven by square-wave pulses from a Grass SD5 stimulator amplified with a Heathkit Model AA-18 amplifier. In early experiments a Wavetech Waveform Generator was used without further amplification as a source of square-wave pulses.

The glass rod was lowered until the tip of the probe was directly above the lamellar surface. The probe displacement was just sufficient to elicit a response from touch-sensitive units in the lamellae.

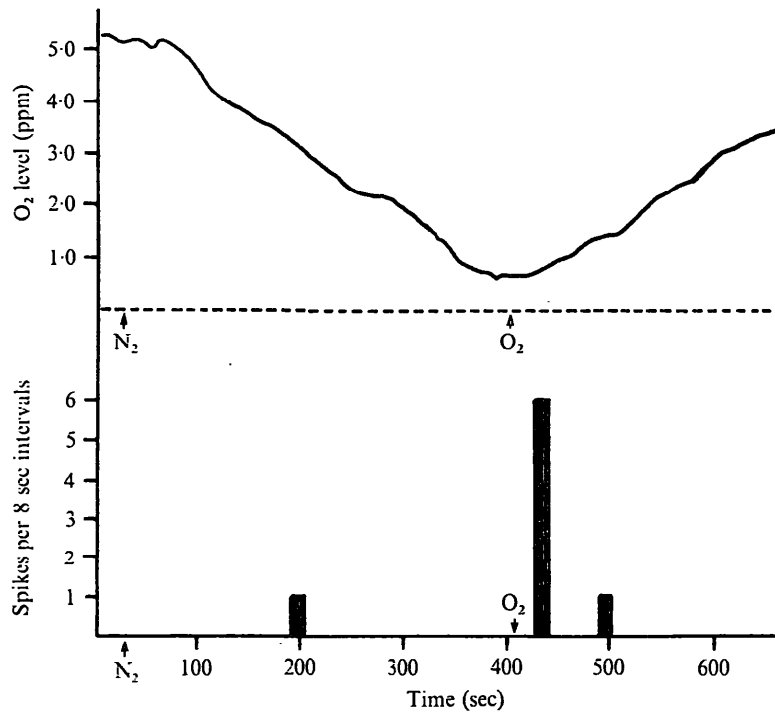
Temperatures for all trials ranged from 19 to 25 °C. Temperature variation in a single trial never exceeded 1 °C.

For morphological examination whole book gills and excised lamellae were placed overnight in 10% solutions of methylene blue in sea water.

RESULTS

In agreement with the findings of Patten & Reddenbaugh (1899) the gill nerve divides into two branches running anteriorly and posteriorly subjacent to the book-gill lamellae. These branches in turn subdivide sending fine fibres to each lamella. Terminal branches from these fibres end in the cuticle.

Book-gill lamellae stained with methylene blue reveal two cuticular structures which may have a sensory function. Lining the lateral edge of each lamella are long slender barbed cuticular hairs and short thickly based spines (Pl. 1). The spines insert along



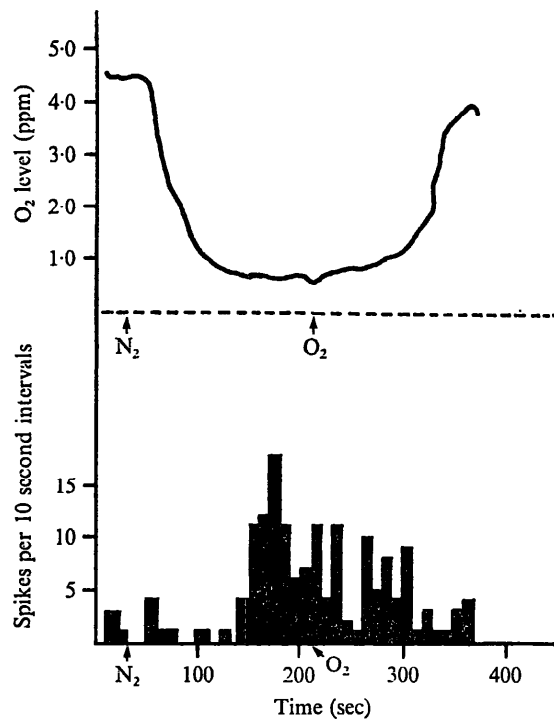
Text-fig. 3. Histogram of class I unit responses.

the edge of the lamella; the hairs insert along the posterior lamellar surface in close proximity to the lateral edge.

Recordings with suction electrodes show that a large number of units are excited by tactile stimulation of the book-gill lamellae. Responses are elicited by stroking the lamellae with a glass probe lengthwise along a lateral edge, by lifting the lamellae or by otherwise distorting the cuticle. Localized tactile stimulation of the hairs with a fine glass needle never produced a neural response; local stimulation or distortion of the cuticle around the spines always elicited a neural response.

Two types of touch-insensitive, oxygen-sensitive gill nerve units were detected. Some units (class I) were excited when air was bubbled into the anaerobic sea water in the test chamber (Text-figs. 2, 3). This was not in response to mechanical disturbance of the sea water since their activities were not affected by turning the nitrogen off and on again. During the initial portion of each trial these units discharged at a low rate despite the relatively high levels of oxygen present. The activity of the unit declined as oxygen was displaced from the sea water by nitrogen. The unit's discharge increased in the interval immediately following the re-introduction of air into the anaerobic chamber. As the sea water approached air-saturation, the unit's activity returned to the initial level. Class I units comprised 50 of 675 touch-insensitive units examined.

In contrast to class I units the discharges of another group of units (class II) increased when oxygen tension decreased in the sea water bathing the book-gill lamellae (Text-fig. 4). These units fired infrequently at the initial high levels of



Text-fig. 4. Histogram of class II unit responses.

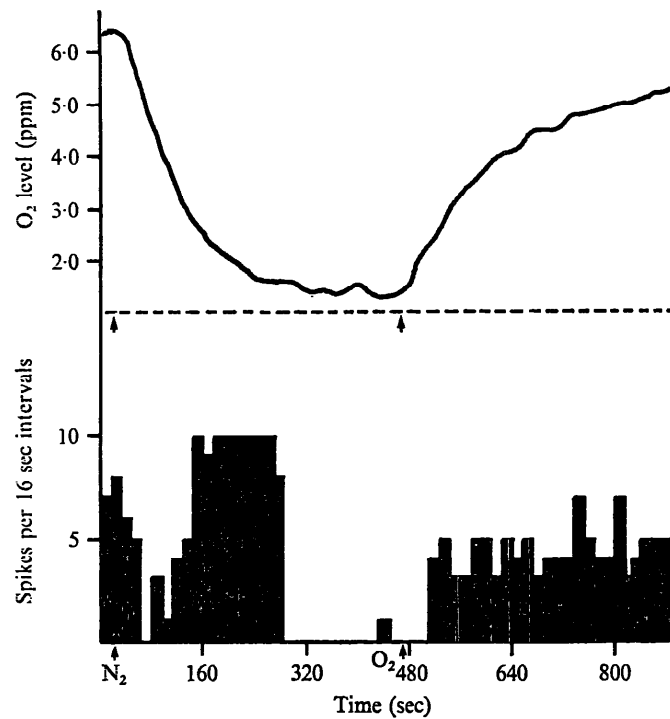
oxygen present at the beginning of each trial; however, as nitrogen displaced the oxygen from the sea water, their discharge rates increased several fold. As oxygen tension was increased to air-saturation, their firing rates diminished to initial low levels. Class II units numbered 36 of 675 touch-insensitive units examined.

As discussed above, many units were found to respond to tactile stimulation of the lamellar cuticle. The responses of about 5% of these touch-sensitive units were oxygen-dependent (Text-figs. 5, 6). These units consistently responded to tactile stimulation at the beginning of a trial when the oxygen tension was near air-saturation. As nitrogen displaced the oxygen in the chamber, the unit ceased to respond consistently to each stimulus. Following the re-introduction of air into the sea water, the unit recovered its touch sensitivity and once again responded to each stimulus. These units comprised 18 of 380 units which responded when the lamellae received tactile stimulation.

DISCUSSION

Unit responses in the gill nerve to varying the oxygen tensions in the sea water bathing the book-gill lamellae indicate that three types of oxygen-sensitive elements are present in the lamellae. Class I units respond to the introduction of oxygen to an anaerobic medium; class II units increase firing rates as the oxygen concentration decreases, while the responsiveness of some tactile units to tactile stimulation is oxygen-dependent.

Earlier studies with *Limulus polyphemus* indicated that the initiation of the rhythmic beating of the book gills when oxygen is introduced into an anaerobic environment is



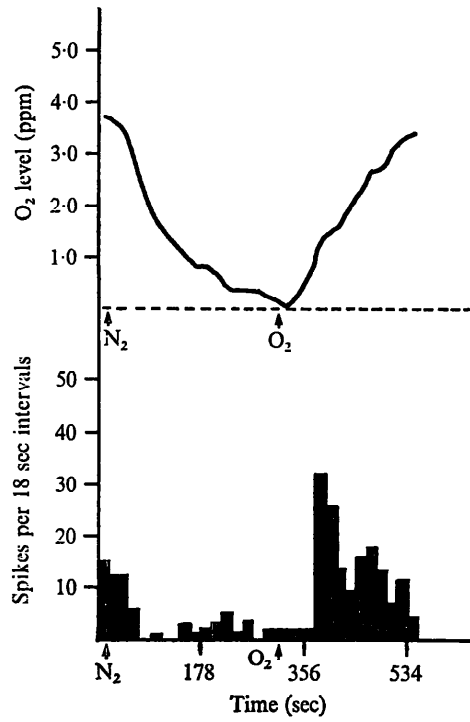
Text-fig. 5. Histogram of tactile oxygen-sensitive units.
Responses to tactile stimulation applied every 1.6 sec.

reflex in nature and dependent upon stimulation of external oxygen sensitive receptors (Hyde, 1906; Page, 1973; Waterman & Travis, 1953).

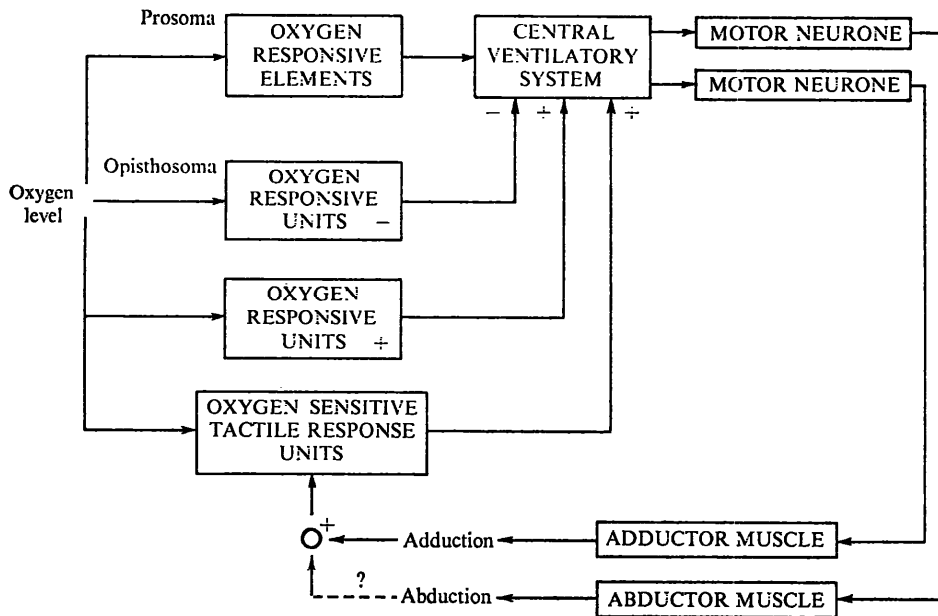
Behavioural observations by Page (1973) indicated that reflex oxygen sensitivity is dependent upon the integrity of the cuticle between the coxa of the prosomal legs and the book-gill lamellae themselves. The present results confirm electrophysiologically that oxygen-sensitive elements are present in the book-gill lamellae. Recent observations while recording from the nerve which innervates the intercoxal cuticle suggest that oxygen-sensitive elements are also present in this cuticle (C. Thompson, unpublished observations).

Book-gill lamellae stained with methylene blue reveal two different classes of cuticular structures which might have a chemoreceptive function: the cuticular hairs and the spines. It is not known which of these classes of elements is oxygen-sensitive. As a result of their apparent touch insensitivity one presumes that the cuticular hairs are sensitive to another sensory modality; some hairs may be oxygen-sensitive. Cuticular spines respond to tactile stimulation; the touch sensitivity of some spines may be oxygen-dependent.

At present it is not known whether *Limulus* is unique in having its oxygen-dependent ventilatory reflexes mediated by external receptive elements. There is no conclusive evidence of which we are aware for the presence of external oxygen receptors in any arthropod other than the horseshoe crab. Available evidence indicates that in insects ventilation is modulated by the direct effects of oxygen upon the central nervous



Text-fig. 6. Histogram of tactile oxygen-sensitive units. Responses to tactile stimulation applied every 1.8 sec.



Text-fig. 7. Model of the organization of the oxygen-dependent ventilatory reflexes.

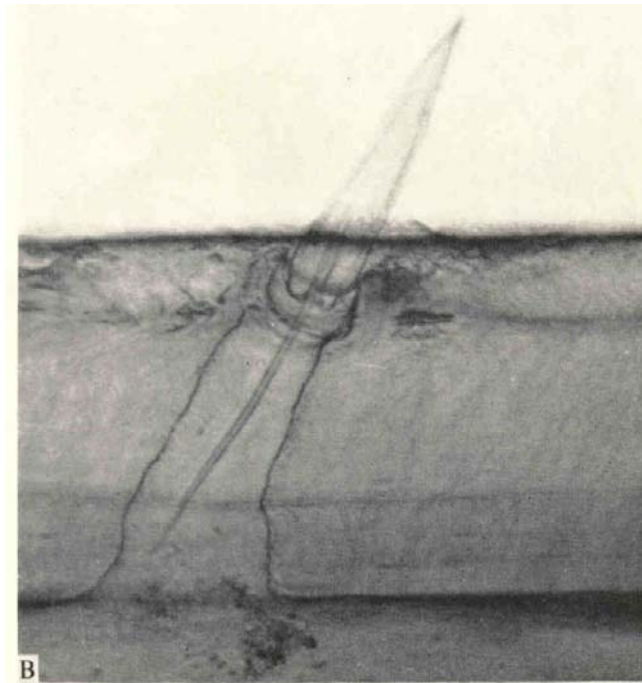
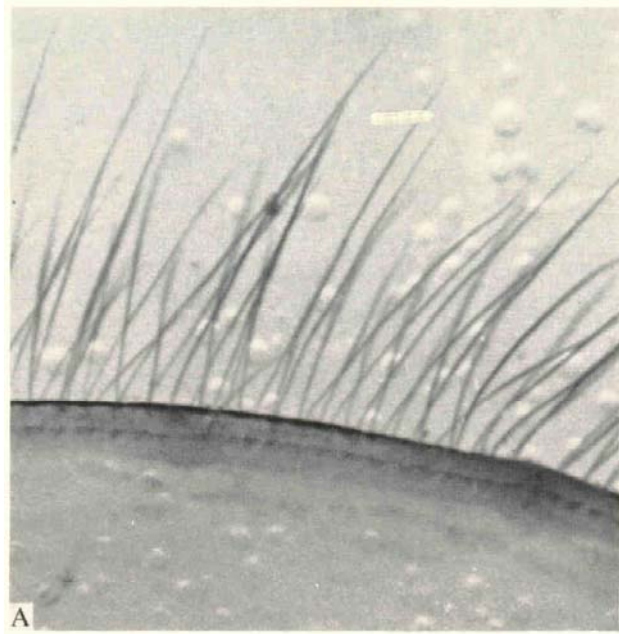
system itself (Miller, 1966). However, Larimer (1964) has concluded that crayfish appear to have receptive elements located in their gill chambers; external oxygen-sensitive elements may therefore be present in some decapod crustaceans.

The organization of the oxygen-dependent ventilatory reflexes in *Limulus* appears to be surprisingly complex; three different classes of oxygen-sensitive units are present in the book-gill lamellae and at least one class of oxygen-sensitive element is presumed from behavioural (Page, 1973) and preliminary electrophysiological observations to be present in the intercoxal cuticle. The only other oxygen-sensitive sensory system which has been examined at the level of the responses of individual sensory units is the chemoreceptors in the carotid body (Eyzaguirre & Lewin, 1961). These units respond to low oxygen tensions in a manner analogous to that of the class II units of *Limulus* book-gill lamellae. That is, as oxygen tension decreases, their rate of spike discharge increases.

A model of the manner in which the known oxygen-sensitive elements may modulate the activity of the central neurones which generate the ventilatory rhythm is shown diagrammatically in Text-fig. 7. When the animal is in an anaerobic environment, all ventilatory movements cease. Under these anaerobic conditions only the class II units are active; presumably the effect of their activity is to suppress the level of excitation in the central ventilatory system thereby blocking the production of a rhythmic ventilatory motor output. When oxygen is introduced into this anaerobic environment, class I units are excited, discharging bursts of impulses, while activity of class II units is inhibited. Class I units are presumed to increase the level of excitation of the central ventilatory system. Therefore the net effect of the resultant excitation of class I and inhibition of class II units is to raise the level of excitation in the central ventilatory system sufficiently to permit the generation of a rhythmic motor output to the adductor and abductor muscles of the gill appendages. Consequent movement of the gill appendages evokes discharge of the oxygen-sensitive tactile units. Maintained ventilation results in part from positive feedback which is established as the result of tactile stimulation of these units by rhythmic ventilatory movement of the gill appendages and the central effect of sensory input from these receptors. It is proposed that activity of the oxygen-sensitive tactile units increases the level of central ventilatory excitation thereby permitting generation of a maintained ventilatory output. The touch sensitivity of these units is oxygen-dependent. Therefore the extent of positive feedback is dependent upon the oxygen tension. The effect of lowering the environmental oxygen tension is that the ventilatory rate decreases and finally under nearly anaerobic conditions ventilation ceases. From the model one would therefore predict that the level of excitation in the central ventilatory system decreases as a result of the increase in the inhibitory effects of activity in class II and the decrease in the excitatory effects of activity in class I units and in the oxygen-sensitive touch-sensitive units.

SUMMARY

1. The unit responses of oxygen-sensitive elements in the book-gill lamellae of *Limulus polyphemus* were examined by recording their unit activities in the gill nerve while varying the oxygen tension in the sea water bathing the lamellae.
2. Three types of oxygen-sensitive units were detected. Some units (class I) were



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excited by oxygen introduced into anaerobic sea water; others (class II) were inhibited by high oxygen levels. Some units responded to tactile stimulation only in the presence of oxygen.

3. It is suggested that the oxygen-dependent ventilatory reflexes result from interaction in the central ventilatory neural system of the excitatory effects of class I units and of touch-sensitive oxygen-dependent units with the inhibitory effects of class II units.

We thank Dr James Wilson for his comments on this manuscript and Mr Philip Smith for aid in preparing Text-fig. 1. This research was supported by a National Institutes of Health Grant (NS 09957 PHY).

REFERENCES

- EYZAGUIRRE, C. & LEWIN, J. (1961). Chemoreceptor activity of the carotid body of the cat. *J. Physiol.* **159**, 222-37.
- FOURTNER, C. R., DREWES, C. D. & PAX, R. A. (1971). Rhythmic motor outputs co-ordinating the respiratory movement of the gill-plates of *Limulus polyphemus*. *Comp. Biochem. Physiol.* **38A**, 751-62.
- HYDE, I. H. (1893). The nervous mechanism of the respiratory movements in *Limulus polyphemus*. *J. Morph.* **9**, 431-48.
- HYDE, I. H. (1906). A reflex respiratory centre. *Am. J. Physiol.* **16**, 368-77.
- KNUDSEN, E. I. (1973). Muscular activity underlying ventilation and swimming in the horseshoe crab, *Limulus polyphemus* (Linnaeus). *Biol. Bull. mar. biol. Lab., Woods Hole* **144**, 355-367.
- LARIMER, J. L. (1964). Sensory induced modifications of ventilation and heart rate in crayfish. *Comp. Biochem. Physiol.* **12**, 25-36.
- MILLER, P. L. (1966). The regulation of breathing in insects. *Adv. Insect. Physiol.* **3**, 279-353.
- PAGE, C. H. (1973). Localization of *Limulus polyphemus* oxygen sensitivity. *Biol. Bull. mar. biol. Lab., Woods Hole* **144**, 383-90.
- PATTEN, W. & REDDENBAUGH, W. A. (1899). *Studies on Limulus*. The nervous system of *Limulus polyphemus* with observations upon the general anatomy. *J. Morph.* **16**, 91-200.
- SCHLEIN, J. M. & BARBER, S. B. (1971). Sensory receptors in the abdominal appendages of *Limulus polyphemus*. *Am. Zool.* **11**, 674.
- WATERMAN, T. H. & TRAVIS, D. F. (1953). Respiratory reflexes and the flabellum of *Limulus*. *J. cell. comp. Physiol.* **41**, 261-90.
- WYSE, G. A. (1972). Intracellular and extracellular motorneuron activity underlying rhythmic respiration in *Limulus*. *J. comp. Physiol.* **81**, 259-76.

EXPLANATION OF PLATE I

(A) Hairs lining lateral edge of a lamella, $\times 15$; (B) single spine, $\times 150$; (C) segment of a hair, $\times 150$