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CENTRAL AND PERIPHERAL NEURONES INVOLVED IN OXYGEN PERCEPTION IN THE PULMONATE SNAIL *LYMNAEA STAGNALIS* (MOLLUSCA, GASTROPODA)

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Abstract—1. Intracellular recordings showed that neurones in the CNS of *Lymnaea* are sensitive to P_{O_2} changes over the mantle area. Some neurones responded with hyperpolarization and decrease of the firing frequency, others with depolarization and increase of the firing frequency.

2. Upon lowering P_{O_2} over the mantle area, peripheral nerves increased their activity.

3. Some neurones sensitive to external P_{O_2} are probably part of a network involved in respiration and control of ionic regulation.

INTRODUCTION

Many animals depend for their life on oxygen and show physiological and behavioural reactions upon changes in the availability of oxygen. Behavioural indications have been obtained that such reactions are mediated by superficial oxygen-sensitive receptors in arthropods (Taylor and Butler, 1973; Taylor, 1982; Page, 1973) and fish (Randall, 1982; Daxboeck and Holeton, 1978; Smith and Jones, 1978).

Molluscs also react to changes in the availability of oxygen. Oxygen is important for respiration behaviour (Jones, 1961), regulation of heart rate (Dieringer *et al.*, 1978), orientation behaviour (Janse, 1981, 1982) and egg-laying behaviour (Timmermans, 1959; ter Maat *et al.*, 1983). The availability of oxygen also affects metabolic activity in molluscs (Ghiretti, 1966; de Zwaan *et al.*, 1976; Wieser, 1981). Moreover, it is known that the respiratory condition is of importance for ionic regulation in these animals (de With *et al.*, 1980; de With and van der Wilt, in preparation). In molluscs virtually nothing is known about the perception of external P_{O_2} or about the integration of P_{O_2} information in the nervous system. Only in the arthropod *Limulus* have electrophysiological responses been obtained from oxygen-sensitive receptors (Crabtree and Page, 1974; Thompson and Page, 1975).

The present study deals with the electrophysiology of oxygen perception in the mollusc *Lymnaea stagnalis*. Responses of central and peripheral neurones are recorded upon changes in the external P_{O_2} . In *Lymnaea* indications already exist that oxygen-sensitive receptors occur in the lung cavity and in the external skin. These receptors appeared to be clearly activated during exposures of the animal to air with different P_{O_2} (Janse, 1981). In the present study, therefore, oxygen sensitivity is investigated by exposing part of the preparations to air with normal and low P_{O_2} . It is shown that individual neurones in the central nervous system (CNS) respond differently to input of superficial oxygen-sensitive receptors. Some of these central neurones are connected to a

neuronal network which is probably involved in the regulation of processes related to respiration and excretion.

MATERIAL AND METHODS

Laboratory-bred snails with a shell length of approximately 30 mm were used. Two types of preparations were made: (1) Preparations used to record from central neurones. They consisted of the mantle (including the lung) connected to the CNS via the visceral and parietal nerves. The lung cavity in these preparations was exposed by a transverse cut in the lung roof. All visceral organs were removed except those situated against the lung wall (kidney, heart and pericard, oothecal gland and bursa copulatrix). The tissue around the pneumostome was left intact (Fig. 1). The preparation was pinned down in a basin consisting of two separate compartments in which mantle and CNS were positioned, respectively. Each compartment had a volume of about 4 ml. The separation between the compartments was air- and water-tight. In the mantle compartment, the nerves were covered with grease. The Ringer solution in the mantle compartment could be removed separately and the compartment could be filled with air or with N_2 gas while it was covered with a glass plate. A vertically placed perspex plate prevented direct gas flow over the mantle. Gas could leave the compartment through an outlet (Fig. 2). Separate P_{O_2} measurements in the mantle compartment (Fig. 3) showed that it took about 30–40 sec to replace the air completely with N_2 gas.

Fig. 1. Diagram of the mantle-CNS preparation: M, mantle; L, lung cavity; H, heart and pericard; P, pneumostome; CNS, central nervous system. See also text.

Fig. 2. Diagram of the set-up used for the recordings from central neurones. Arrows indicate gas flow. P, Perspex plate to prevent direct gas flow over the preparation.

(2) Preparations used to record from peripheral nerves. These preparations consisted of the mantle and the peripheral nerves only. The nerves were cut close to the CNS and the lung cavity was exposed in the same way as described above. Preparations were exposed to N_2 gas or air by placing the recording basin in a large compartment in which the air could be replaced. During exposures to gas the Ringer's solution in the recording basin was partly removed such that the mantle area was exposed to gas while the nerves remained covered with Ringer's solution. P_{O_2} measurements were made during the experiment at the outflow of the gas compartment (Fig. 4). This means that a latency of several seconds existed between the measured and the actual P_{O_2} over the recording basin.

In the experiments on both types of preparations three types of gas exposures were used: (1) Air; in this case only the Ringer solution was removed; (2) Flowing air; pressurized air was blown into the compartment containing the preparation; (3) N_2 gas; pressurized N_2 gas was blown into the compartment. The velocity of gas flow was controlled by the gas pressure at the outlet of the gas containers. Gas pressure was controlled by a valve. During different P_{O_2} measurements in the mantle compartment (first type of preparation) only minor differences occurred.

Intracellular recordings were made with glass micropipettes with a resistance of 10–50 M Ω . Extracellular recordings were made with glass-suction electrodes. Conventional electrophysiological techniques were used for recording, storing and displaying signals. A window discriminator (WPI) was used to select extracellular signals. A digital counter and an interval to frequency converter were used to count action potentials and measure their instantaneous frequency, respectively. Tactile stimuli were delivered with a hand-worked glass probe with a rounded tip (diameter: 0.5 mm). Pneumostome movements were recorded with a movement transducer made in the laboratory. The pneumostome was attached to the transducer with a hook. P_{O_2} measurements were made with a WTW oxy 42 oxygen electrode or with a P_{O_2} -electrode made in the laboratory. The experiments were performed at room temperature with a Ringer's solution of the following composition: NaCl, 30 mM; KCl, 1.5 mM; $MgCl_2$, 2 mM; $CaCl_2$, 4 mM; NaH_2PO_4 , 0.25 mM; $NaHCO_3$, 18 mM.

Fig. 3. Recording of P_{O_2} in the compartment of Fig. 2 (containing the mantle) during replacements of gas. Arrows indicate the onset of gas flow.

Fig. 4. Experimental set-up for the recordings of activity of peripheral nerves during exposures of the mantle to different P_{O_2} . AC, Compartment in which air could be replaced; MC, Compartment in which the P_{O_2} was measured during the recordings; P, Recording chamber in which the preparation was pinned down; E, Suction electrode. Arrows indicate the flow of gas.

RESULTS

In the following experiments preparations were stimulated by exposing the periphery to air or N_2 gas. Mantle preparations were used because the mantle area consists of an area of external skin which is normally exposed to water as well as the lung cavity, which is normally exposed to air. In both structures oxygen-sensitive receptors are probably present (Janse, 1981).

The mantle is innervated by the nerves of the visceral and parietal ganglia (Janse, 1974). Therefore, in the present study attention was primarily focused on the involvement of neurones in these ganglia in O_2 perception. The nomenclature of Wendelaar Bonga (1970), Benjamin and Winlow (1981) and Swindale

Fig. 5. Dorsal view of the CNS of *Lymnaea stagnalis* (after Benjamin and Winlow, 1981 and Slade *et al.*, 1981). LCG and RCG, Left and right cerebral ganglion; LPeG and RPeG, Left and right pleural ganglion; LPiG and RPiG, Left and right parietal ganglion; VG, Visceral ganglion. (1) n. pallialis sinister; (2) n. cutaneous pallialis; (3) n. intestinalis; (4) n. analis; (5) n. genitalis; (6) n. pallialis dexter internus; (7) n. pallialis dexter externus. LPeD₁ and RPeD₁, left and right pedal dorsal cells; VD₁–VD₄, visceral dorsal cells; RPD₁ and RPD₂, right parietal dorsal cells. A, B, F, G, Y, LY: A, B, F, G, yellow, and light yellow cells, respectively, St, statocyst.

Fig. 6A. Responses of VD_1 to mechanical stimulations (horizontal bars) of the pneumostome area (1); mantle edge (2), and the wall of the lung cavity (3). B, C and D. Recordings (B and C continuous) of the activity of VD_1 during exposures of the mantle area to air (Air), N_2 gas (N_2) and flowing air (FA). $\downarrow$ indicates the onset of a particular exposure.

and Benjamin (1976) was used for the different types of cells. Figure 5 gives a diagram of the CNS with the location of the neurones used in the present study. The responses described below for RPD_1 and VD_2/VD_3 and the peripheral afferents were each recorded in at least four to five preparations; the responses in VD_1/RPD_2 and in the G, B and F cells in eight or more preparations.

Responses of central neurones to exposure of the mantle to air or N_2 gas

It is known that neurones can be sensitive to P_{O_2} changes in the extracellular medium which surrounds them directly (Kerkut and York, 1969; Coyer, 1983; Coyer *et al.*, 1983). This may mean that neurones having axons in the peripheral nerves can be affected directly by P_{O_2} changes over the mantle area. In order to obtain indications about the nature of the responses of the neurones to P_{O_2} , the neuronal responses to P_{O_2} changes over the mantle area were compared to mechanical stimulations of different parts of the mantle (and the lung cavity). Possible effects of direct mechanical stimulation by gas flowing over the skin were examined by comparing

the neuronal responses to flowing N_2 gas, to those obtained with similar exposures to flowing air. A neurone was considered to respond to P_{O_2} changes over the mantle when its activity during flowing N_2 gas exposure differed from that during exposure to flowing air.

Neurones were impaled in the dorsal and the ventral parts of the visceral and parietal ganglia. Only neurones in the dorsal part of the visceral and the right parietal ganglia responded to P_{O_2} changes. Apart from the neurones in the visceral and parietal ganglia, recordings were also made from RPD_1 , of the right pedal ganglion, since this neurone has branches in the parietal and right visceral ganglion and has similar inputs with neurones in these ganglia (Benjamin and Winlow, 1981; Haydon and Winlow, 1981).

Responses of identified cells

(a) VD_1 and RPD_2 . VD_1 and RPD_2 are giant neurones (diameter: $\pm 150 \mu m$) located in the visceral and right parietal ganglion, respectively. They are electrotonically coupled and contain an ACTH-like substance. Their axonal branches extend through

Fig. 7A,B,C. Continuous recordings of VD_2/VD_3 during exposures of the mantle area to air (Air), N_2 gas and flowing air (FA) and during mechanical stimulation of the mantle edge (1); pneumostome (2), and wall of the lung cavity (3). The dotted line indicates the resting level of the membrane potential. See also Fig. 6.

the entire CNS (Boer *et al.*, 1979; Benjamin and Winlow, 1981). In isolated CNS preparations the cells have a static firing pattern (Benjamin and Winlow, 1981).

In the semi-intact preparations used in the present study, the cells fired bursts of action potentials which were interrupted by periods of inhibition lasting up to 30 sec (Fig. 6B–D). Our experiments show that the cells received strong excitatory input upon mechanical stimulation of different parts of the mantle and the area near the pneumostome, and weak excitatory input from the skin in the lung cavity (Fig. 6A). Exposure of the periphery to N_2 gas produced, after a latency of about 10 sec, a strong hyperpolarization of approximately 10 mV and a strong decrease in the number of action potentials. Upon exposure of the periphery to flowing air the cells resumed their normal activity (Fig. 6B–D).

(b) VD_2 and VD_3 . These cells are situated in the visceral ganglion near VD_1 . They are electrophysiologically, as well as morphologically indistinguishable from each other (Benjamin and Winlow, 1981). In our preparations the cells were normally silent which agrees with the situation in the isolated CNS (Benjamin and Winlow, 1981). Upon mechanical stimulation of the mantle, pneumostome and lung wall the cells responded with excitatory post-synaptic potentials (EPSPs) (Fig. 7). Exposures of the periphery to N_2 gas evoked, after a latency of approximately 30–40 sec, a slow depolarization of about 8 mV. Re-exposure of the periphery to flowing air gave a restoration of the normal membrane potential (Fig. 7).

(c) $RPeD_1$. $RPeD_1$ is a giant neurone (diameter: $\pm 160 \mu m$) in the right pedal ganglion near the stato-

cyst. As noted by Benjamin and Winlow (1981) in an isolated CNS, the cell had a rather regular firing pattern (Fig. 8B). Mechanical stimulation of the mantle edge produced inhibitory post-synaptic potentials (IPSPs), stimulation of the pneumostome area and the skin of the lung cavity gave EPSPs which occasionally gave rise to an action potential (Fig. 8A). Exposure of the mantle to N_2 gas produce a slight hyperpolarization and a decrease in the firing frequency of the neurone (Fig. 8). The latency of the response was about 50 sec. Exposures to flowing air restored the membrane potential and firing frequency (Fig. 8).

Responses of neurones from identified groups

(a) *Neurones of the B and F group.* The B cells are found in the left anterior part of the right parietal ganglion, the F cells lie in a cluster in the left caudal part of the visceral ganglion (Benjamin and Winlow, 1981). Cells of both groups fired action potentials at a low frequency (0.1–2 Hz). This agrees with the findings of Benjamin and Winlow (1981). In some of our preparations these cells were also found occasionally to be silent. The B and F cells have a common inhibitory synaptic input (Benjamin and Winlow, 1981), which was used as a means for identification. Our experiments show that B and F cells had similar responses to mechanical stimulations as well as to P_{O_2} changes. Mechanical stimulations of the skin in the lung gave IPSPs, whereas stimulation of the pneumostome area and the mantle edge produced EPSPs (Fig. 9A).

About half of the B and F cells (a total of about 30 penetrations were made) did not respond to P_{O_2} changes over the mantle. Responsive as well as

Fig. 8. Recording of RPeD₁ during stimulations of the mantle edge (1); pneumostome area (2), and wall of the lung cavity (3). B–E. Continuous recording of RPeD₁ during exposures to air (Air), N₂ gas (N₂) and flowing air (FA). A and B–E are from different preparations. See also Fig. 6.

non-responsive cells could be found in the same preparations and in the B, as well as in the F, cluster. Probably the clusters are functionally heterogeneous groups of cells. The responsive cells gave a depolarization of approximately 4 mV and an increase in their firing frequency upon exposure of the mantle area to N₂ gas. The latency of the response was about 15 sec. Exposure to flowing air resulted in a restoration of the normal activity of the cells (Fig. 9B–D).

(b) *Neurones of the G group.* The G cluster of cells is located in the dorsal part of the visceral ganglion between the F cluster and the Yellow Cells (Fig. 5). The cells are easily recognizable because they are small and not as white as the surrounding cells. In isolated preparations the cells are silent (Benjamin and Winlow, 1981); in our preparations they fired irregularly (Fig. 10). In the present study it appeared that mechanical stimulation of the mantle edge and

the area around the pneumostome evoked a depolarization and an increased frequency of action potentials, this was (especially in the pneumostome area) followed by a period of inhibition lasting several seconds. Stimulation of the skin of the lung cavity produced a slight inhibition, followed by a burst of action potentials (Fig. 10A,B).

Exposures of the mantle area to N₂ gas produced (after a latency of 20–30 sec) a slight depolarization and a considerable increase of the firing frequency. Exposure to flowing air restored the normal firing rate again (Fig. 10B).

(c) *Neurones which did not respond to P_{O₂} changes over the mantle area*

As mentioned above, a great number of neurones did not respond to P_{O₂} changes over the mantle area. Many of these neurones, however, received synaptic

Fig. 9A. Simultaneous recordings of a B cell (upper) and F cell (lower) during mechanical stimulation of the mantle edge (1); pneumostome area (2), and wall of the lung cavity (3). B, C and D. Recordings of a B cell during exposure of the mantle area to air (Air), N₂ gas (N₂) and flowing air (FA). B and C are continuous recordings. See also Fig. 6.

input upon mechanical stimulation of the mantle. Table 1 shows a summary of identified neurones which did not respond to changes in the external P_{O_2} over the mantle area, but which did respond to mechanical stimulation of the same area. These observations indicate that there are also mechano-receptive elements in the mantle area that are not activated by changes in the P_{O_2} . Moreover, the fact that the LYC and YC did not respond to P_{O_2} changes over the mantle, although they have axons in the mantle nerves (Swindale and Benjamin, 1976; Van Swigchem *et al.*, 1981a), indicates that the P_{O_2} changes used in the present study did not affect axons of central neurones directly. Probably the input on central neurones observed during P_{O_2} changes over

the mantle area originate from specific afferents synapsing on these neurones.

Afferent signals in the nerves during exposure of the mantle area to P_{O_2} changes

The experiments described above indicate that afferent input related to the P_{O_2} over the mantle area enters the CNS via the mantle nerves. In the experiments described in this section the afferent input was studied more directly. The second type of preparation, consisting of the mantle, with opened lung cavity and with peripheral nerves cut close to the CNS, was used. During these experiments the mantle part was successively exposed to air, to N₂ gas and

Fig. 10. Recordings of a G cell. A. During mechanical stimulation of the mantle edge (1); pneumostome area (2), and wall of the lung cavity (3). B. During exposures of the mantle area to air (air), N_2 gas (N_2) and flowing air (FA). A and B are from different preparations. See also Fig. 6.

Table 1. Responses of identified cells or groups of cells (which did not respond to external P_{O_2} changes) to mechanical stimulation of the mantle edge and the pneumostome area

Cell type	Response upon stimulation of		
	Mantle edge	Pneumostome area	Lung wall
YC	H	H	H
LYC*	H	H	H
RPD ₁	E	E	E
A group	E	E	E

*See also Van Swigchem *et al.* (1981b).

YC: yellow cells; LYC: light yellow cells; H: hyperpolarization; E: excitation consisting of depolarization with action potentials.

then flowing air. Activity was recorded in one of the pallial nerves.

Figure 11 shows the recording of the activity of the n. analis during exposure of the mantle area to

different P_{O_2} . Simultaneously the instantaneous frequency of the action potentials with a particular amplitude was measured. The figure shows that during exposure to low P_{O_2} there is an increase of the activity in the nerve. Closer inspection of the nervous activities showed that especially nervous elements with action potentials of a medium amplitude were involved in the response (Fig. 11). Figure 12 shows that there is an increase of the mean frequency of these elements in the second minute of N_2 gas exposure of the mantle area. In still other preparations, the increase in the firing rate was noticeable in the first minute. Similar results were obtained with recordings of the n. pallialis dexter externus. Thus, these experiments indicate that a selective number of nervous elements are activated during exposure of the mantle area to air with low P_{O_2} .

Fig. 11. Activity of the N. analis during exposures of the mantle area to air with low P_{O_2} (A, 8–2.5%) and air with high P_{O_2} (B, 14–20%). Upper traces indicate the instantaneous frequency of the action potentials which were selected by the window (upper and lower levels are indicated by the arrows) in the lower recordings. The recordings are from the experiment of Fig. 12.

Fig. 12. Mean frequency of the action potentials selected by the window in Fig. 11 during changes of the P_{O_2} (lower graph) induced by exposures of the preparation to air (air), N_2 gas and flowing air. Countings were made from the recordings of the experiment illustrated in Fig. 11.

Central connections of neurones sensitive to external P_{O_2} changes

During the search for central connections of neurones sensitive to the external P_{O_2} a white neurone (VD_4 , see also Fig. 5) was found on the dorsal side of the visceral ganglion. The neurone appeared to have connections with neurones in different parts of the CNS including $RPeD_1$ and VD_1/RPD_2 . Moreover, VD_4 is probably involved in the regulation of respiration behaviour as electrical stimulation produced a burst of action potentials which was accompanied by an opening movement of the pneumostome (Fig. 13). Paired impalements of nerve cells (Fig. 14) showed that activity in VD_4 produced inhibition in $RPeD_1$ (A); in VD_1/RPD_2 (B); in Yellow Cells (a group of neurosecretory cells near the right visceroparietal connective, YC in Fig. 5) (C), and in some unidentified cells in the right parietal ganglion (D). Activation of VD_4 produced excitation of $LPeD_1$ (a giant cell in the left pedal ganglion. Figure 5, see also Haydon and Winlow, 1981) (E). VD_4 was inhibited upon activation of $RPeD_1$ (F). This description of synaptic connections of the neurones sensitive to changes in the external P_{O_2} is certainly not complete, but it can be concluded that some of these neurones may be part of a neuronal network with widespread connections in the CNS.

DISCUSSION

The nature of the responses of central neurones to changes of the external P_{O_2}

The present study shows that there are neurones that are sensitive to changes in the P_{O_2} over the mantle and opened lung cavity. Excitatory, as well as inhibitory responses, were found upon lowering the

external P_{O_2} . In this respect the responses of the present study resemble the responses of neurones upon direct exposure to P_{O_2} changes (Kerkut and York, 1969; Steffin, 1975; Coyer, 1983; Coyer *et al.*, 1983). In other aspects there were, however, differences. All neurones of the present study responded in a reversible way and with a relatively short latency. Not all of the responses described by previous authors were reversible; moreover, in their experiments long lasting exposures (1–2 hr) to low P_{O_2} were used.

Several neurones of *Lymnaea* which were shown in the present study to be sensitive to the external P_{O_2} have axons in several mantle nerves. This has been demonstrated for VD_1 and RPD_2 (Boer *et al.*, 1979), for $RPeD_1$ (Haydon and Winlow, 1981) and for the B cells (unpublished results). Yet there are several arguments in favour of the view that the effects of the external P_{O_2} are mediated via special receptors. (1) Recordings from mantle nerves showed that a number of nervous elements responded with increased activity upon exposure of the mantle area to N_2 gas. This indicates that P_{O_2} acts selectively, (2) the present study showed that responses of central neurones upon P_{O_2} changes over the mantle area can differ from responses upon mechanical stimulation of the skin in the mantle and lung area. In a number of cases inhibitory responses were obtained upon exposure to N_2 gas but excitatory responses upon mechanical stimulation of the mantle (e.g. see the responses of VD_1). This indicates that mechano-receptive elements in the mantle area of *Lymnaea* are not responsive to N_2 gas exposures (the nerve recordings showed already that N_2 gas exposure activates nerve elements). (3) Many neurones which receive input upon mechanical stimulation of the mantle did not respond to exposures of the mantle area to different P_{O_2} . This also indicates that the external P_{O_2} acts selectively on particular neurones, and (4) several neurones with axons in mantle nerves did not respond to P_{O_2} changes over the mantle area, indicating that the exposures to air or N_2 gas did not affect these neurones directly.

The conclusion that *Lymnaea* has superficial receptors sensitive to P_{O_2} changes is in agreement with

Fig. 13. Activity of VD_4 (upper record) induced by a depolarizing stimulus and movements of the pneumostome (lower record). The rhythmic movements before and after the burst of VD_4 indicate that still other neurones are involved in the induction of pneumostome movements.

Fig. 14. Simultaneous recordings of VD_4 (lower traces in A-E; upper trace in F), with $RPeD_1$ (A and F), with VD_1 (B), with a yellow cell (C), with an unidentified cell in the left parietal ganglion (D) and with $LPeD_1$ (E). Calibration: 20 mV, 10 sec except in D upper record: 40 mV and F: 5 mV, 2 sec.

results of previous studies in which it has been suggested that *Lymnaea* has O_2 -sensitive receptors in the lung and in the external skin (Janse, 1981). The occurrence of receptors which sense the external P_{O_2} has so far only been reported in arthropods (Page, 1973; Crabtree and Page, 1974; Thompson and Page, 1975) and fish (Bamford, 1974; Daxboeck and Holeton, 1978). The type of response of the O_2 -sensitive

elements in the periphery of *Lymnaea*, consisting of an increase of the nervous activity upon lowering the P_{O_2} , resembles that of a particular class of receptors (Class II) in *Limulus* (Crabtree and Page, 1974), and that of the carotid body of higher vertebrates (Biscoe, 1971). The latency of the response of central neurones to P_{O_2} changes in the skin of *Lymnaea* was 15–50 sec. In the pallial nerves the latency of the response to P_{O_2}

changes was of the same order of magnitude. In *Limulus*, similar latencies were found in the peripheral O₂-sensitive elements (Thompson and Page, 1975).

The function of oxygen perception in Lymnaea

In *Lymnaea* different types of neurones were found to respond to changes in the external P_{O₂}. Among these VD₁ and RPD₂ are remarkable cells. These cells are electrotonically coupled, contain an ACTH-like substance and are probably of a neurosecretory nature. Moreover, they branch extensively all over the CNS (Boer *et al.*, 1979). The properties suggest that VD₁ and RPD₂ have a general regulatory function which is influenced by the availability of O₂ in the environment. The branching patterns of the other types of neurones have yet to be investigated. It is, however, already known that RPeD₁ (Haydon and Winlow, 1981), and the B cells (Janse and van der Roest, unpublished results) branch less extensively.

In *Lymnaea* a number of functions are influenced by O₂. Data are known for respiratory behaviour (Jones, 1961), orientation behaviour (Janse, 1981, 1982), egg-laying behaviour (Timmermans, 1959; ter Maat *et al.*, 1983) and probably also ionic regulation (de With, 1980; de With *et al.*, in preparation). Moreover, it is known that metabolic activity of *Lymnaea* is influenced by the availability of O₂ (Geraerts, 1975; de Zwaan *et al.*, 1976) and that this influence is mediated by a chemical factor in the CNS (Hemminga, 1984). It is conceivable that the neurones described in the present study have a regulatory function in these processes. This is corroborated by the present finding that neurones sensitive to the external P_{O₂} participate in a network with different types of other neurones like VD₄ and the Yellow Cells. Benjamin *et al.* (1983) also described connections between a visceral white cell (VWC) and yellow cells. Recently Benjamin (1984) described this cell (WVI) in more detail. The location and a number of neuronal connections of VWI are similar to those of VD₄. So it may be possible that VD₄ and VWI are the same cell. In the present study it was shown that VD₄ is a neurone related to respiratory behaviour. The yellow cells are supposed to be related to excretory functions (Swindale and Benjamin, 1976; Soffe *et al.*, 1977). Future work will be aimed at the function of this network in the regulation of O₂-dependent processes in *Lymnaea*.

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