RESPIRATORY PUMPING: NEURONAL CONTROL OF A CENTRALLY COMMANDED BEHAVIOR IN APLYSIA

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(Accepted June 30th, 1977)

SUMMARY

Respiratory pumping in Aplysia californica is a relatively stereotyped behavioral pattern with three components: (1) withdrawal of gill, siphon and mantle shelf; (2) closing of parapodia; (3) heart inhibition accompanied by a decrease in vasomotor tone. This phasic behavior is triggered by a central burst-generating network of interneurons in the abdominal ganglion. During respiratory pumping, motor neurons innervating the several effector organs receive a burst of either excitatory or inhibitory synaptic input which has previously been attributed to an unidentified central command cell called Interneuron II. Several of these motor cells are also concomitantly released from tonic synaptic input, which is opposite in sign to that which they receive from Interneuron II. This tonic input has been attributed to an unidentified cell called Interneuron XI. In this paper we identify and describe some of the neurons which contribute to the burst generating network; specifically, we focus on the neurons that produce the synaptic action attributed to Interneurons II and XI. The synaptic actions attributed to Interneuron XI are produced by a single, spontaneously active neuron, cell L24. This cell is a multi-action interneuron: it produces inhibitory synaptic potentials in some follower motor neurons, excitatory synaptic potentials in other follower cells, and a conjoint excitatory–inhibitory synaptic action onto gill motor neuron L7. At low frequency, L24 is excitatory to L7. With high frequency firing of L24, the synaptic potential produced in L7 converts from excitatory to inhibitory. In contrast to Interneuron XI, which is a single cell, the synaptic potentials previously attributed to Interneuron II are actually produced by a cluster of at least 3 respiratory command cells which we call L25, L26 and L27. Each of these cells accounts for only a limited portion of the synaptic input that drives the motor neurons during respiratory pumping. For most motor neurons innervated by both the

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respiratory command cells and Interneuron XI, the two synaptic inputs are opposite in sign. Mutually inhibitory connections between Interneuron XI and some of the central respiratory command cells ensure that the synaptic potentials from these two sources are constrained to occur at different times. Thus, centrally commanded synaptic inhibition or excitation of these motor neurons is made more effective by simultaneous disinhibition or disinhibition of Interneuron XI input. In addition to their role in generating respiratory pumping, L24 and L26 also contribute to the mediation of the defensive gill and siphon withdrawal reflex.

INTRODUCTION

Whereas there is now a fairly good understanding of the neural circuitry underlying simple behaviors, there is as yet little insight into the central pattern generators that control the sequence of complex motor activity. Indeed, in the abdominal ganglion of *Aplysia californica*, identified neurons have been shown to mediate a variety of behaviors. Yet the neuronal control of one of the more complex and frequently occurring behaviors controlled by this ganglion is

Fig. 1. Respiratory pumping behavior. A: lateral view of Aplysia. B1: partially cutaway view showing the normal position of gill, siphon and mantle shelf between the two parapodia. B2: during spontaneous respiratory pumping the gill siphon, mantle shelf and parapodia contract in a characteristic fashion. C: recordings of blood pressure and gill, siphon and parapodia contractions during resting state (C1) and during respiratory pumping (C2).
still unknown. This phasic motor pattern is called 'respiratory pumping'. It has a
duration of about 3-6 sec and occurs at intervals ranging from 20 sec up to a few
hours. As illustrated in Fig. 1, this behavior has three nearly synchronous com-
ponents: (1) contraction and withdrawal of gill, siphon and mantle shelf; (2) closing of
the parapodia; and (3) heart inhibition accompanied by a decrease in vasomotor
tone\textsuperscript{10,19,22,27}. While variations in the amplitudes of these motor effects can occur
within an individual animal, the entire behavior is relatively stereotyped in form, and
tends to occur in an all-or-none fashion (ref. 17, and W. Henng, personal communica-
tion).

Although the function of this pumping movement has not been demonstrated, it
seems likely to be involved in enhancing respiration by causing a rapid turnover of the
blood and seawater within the gill and mantle cavity, respectively. This would result in
a greater diffusion gradient for O\textsubscript{2} and CO\textsubscript{2} across the respiratory exchange surfaces of
the gill. Analogous pumping movements are used by filter-feeding bivalves to increase
the flow of food and oxygenated seawater past their gills\textsuperscript{26,30}. Similar pumping
behavior has also been observed in \textit{Archidoris pseudoargus}\textsuperscript{3} and is used by the
opisthobranch \textit{Notarchus punctata} for respiration as well as for locomotion by jet
propulsion\textsuperscript{2,25}.

The motor neuron pools that mediate various components of respiratory
pumping in \textit{Aplysia} have been well described: the respiratory (gill, siphon and mantle
shelf) and cardiovascular motor neurons are located in the abdominal ganglion\textsuperscript{6,21,22,}
\textsuperscript{21,27,28}, and at least some of the parapodial motor neurons are located in the pedal
ganglia\textsuperscript{10}. During respiratory pumping, the motor neurons that innervate these
effector organs receive bursts of either excitatory or inhibitory synaptic input from an
unidentified central command cell previously called Interneuron II\textsuperscript{14,19,22,28,35}. Inter-
neuron II has been shown to act as a central burst generator, in that the bursts of
synaptic activity attributed to it are not influenced by peripheral feedback\textsuperscript{19}, and occur
in the isolated ganglion. Several of the motor cells which receive Interneuron II
synaptic input are concomitantly released from tonic synaptic input, which is opposite
in sign to that which they receive from Interneuron II (Fig. 10). This tonic input has
been attributed to an unidentified cell called Interneuron XI. Although much is known
about the pattern of synaptic input from Interneurons II and XI onto the motor
neurons, little is known about the source, other than that it originates in the abdominal
ganglion\textsuperscript{10,14,19}. This is so in spite of the fact that synaptic connections in the
abdominal ganglion have been studied intensively by several investigators for several
years, and Interneuron II probably has the most follower cells of any interneuron in
the ganglion.

In order to gain an insight into the mechanism by which the command for
respiratory pumping is triggered, we have searched the abdominal ganglion for
Interneurons II and XI. In this paper we show that the synaptic potentials generated
by Interneuron XI appear to be caused by a single cell.

In contrast, the synaptic actions previously attributed to Interneuron II are
actually produced by a group of at least three cells. In accordance with these results we
have replaced the old 'Interneuron II' nomenclature with the term 'respiratory
command cells’. We use the term ‘command cells’ here to refer to the entire group of higher order interneurons which are involved in generating the synaptic potentials recorded in motor neurons during respiratory pumping. This is not meant to imply that activity in a single individual of this group can release the entire behavior. Additional evidence is required to decide this point. It is clear, however, that each of these cells contributes to some aspect of the respiratory central command which drives the motor neurons during respiratory pumping.

METHODS

*Aplysia californica* weighing 100–250 g were obtained from Pacific Bio-Marine Laboratories (Venice, Calif.). Before use they were maintained in an aquarium filled with artificial sea water (Instant Ocean) at 15 °C. All experiments were performed at 15 °C except those illustrated in Figs. 4 and 5, which were performed at room temperature (20–22 °C). The electrophysiological data shown here are based on 10 successful experiments which were the result of over 200 attempts.

In experiments designed to show the individual behavioral components of respiratory pumping, we recorded from the intact animal (Fig. 1) with a similar preparation used by Pinsker et al. Blood pressure was recorded from a cannula chronically implanted in the gastro-esophageal artery. Gill movement was recorded with a photocell, and siphon and parapodial movements were recorded by two Grass FT03 force-displacement transducers. These were coupled to the animal by 5-0 silk sutures that were maintained taut.

In all other experiments, standard electrophysiological techniques were used to obtain intracellular recordings from neurons in the desheathed abdominal ganglion. Either the isolated ganglion or the ganglion with gill, siphon and mantle shelf attached were used. In some experiments gill movement was recorded with a photocell. The preparation was normally bathed in artificial sea water (ASW), but in some experiments the concentrations of Mg and Ca were elevated 3 times their normal values by substituting the chloride salts of these ions for NaCl (Ca and Mg are normally 11 and 55 mM, respectively, in ASW). Synaptic potentials from one experiment (Fig. 7F) were averaged with a PDP-11 computer (Digital Equipment).

RESULTS

Earlier investigations have shown that many gill, siphon and heart motor neurons receiving the central respiratory command also receive tonic synaptic input of the opposite sign from an unidentified cell, called Interneuron XI. It was inferred that Interneuron XI is a double action cell; it makes inhibitory connections onto a number of motor neurons mediating respiratory and cardiovascular behavior and excitatory connections to most of the cells in the RB cluster (ref. 9 and Fig. 10). When respiratory pumping behavior occurs, the phasic input from the respiratory command cells is accompanied by a silencing of the Interneuron XI input. For example LDH1, a major gill motor neuron, receives tonic inhibitory input from Interneuron XI. During
respiratory pumping the spontaneous inhibitory postsynaptic potentials (IPSPs) are blocked and substituted for by excitatory input (EPSPs) from the respiratory command cells. Our first step in investigating the mechanism underlying the phasing of these two sources of synaptic input was to identify Interneuron XI.

Fig. 2 Map of abdominal ganglion showing most common positions of respiratory command cells (L25, L26 and L27) and Interneuron XI (L24). Other cells, which are included for orientation, are mostly motor neurons to cardiovascular and respiratory organs.
We have found that Interneuron XI, which we now name* cell L24, is a small pigmented cell with a dark rim located on the left ventral surface of the abdominal ganglion, near the commissure and just rostral to the L14 cells (Fig. 2). L24 is identified as Interneuron XI on the basis of data such as that shown in Fig. 3. Cell LDG1 normally receives a tonic IPSP attributed to Interneuron XI. Blocking the spontaneous activity (2–6 spikes/sec) of L24 by hyperpolarization silences the large tonically occurring IPSP in LDG1. These data indicate that the large steadily firing IPSP in LDG1 is due entirely to cell L24. The spontaneous nature of this IPSP appears to be due to endogenous pacemaker discharge of cell L24, since only small EPSP activity is recorded from L24 (Figs. 3, 4 and 6).

We have directly confirmed that cell L24 can account for at least 4 of the connections attributed to Interneuron XI by making intracellular recordings from both cell L24 and the gill motor neurons LDG1, LDG2, and L7, and the siphon motor neuron LBs1. L24 inhibits LDG1 and LDG2, excites LBs1, and produces conjoint excitation–inhibition in L7. Fig. 4 illustrates features of the connections between L24 and the gill motor neurons L7 and LDG1. L24 was kept silent by steady hyperpolarization. In the time between the arrows a depolarizing current was applied to elicit a short train of spikes, which caused a corresponding train of IPSPs in LDG1. The first two PSPs produced in L7 were EPSPs. However, with continued firing, the EPSPs decremented and the response rapidly converted to IPSPs. This conjoint synapse produces EPSPs in L7 as long as the L24 firing frequency is kept low (0.2 Hz). At its normal firing rate (2–6 Hz), L24 produces an IPSP in L7.

The IPSPs produced by L24 in LDG1 and both the excitatory and inhibitory components of the L24 PSPs in L7 occurred without failures with a short and constant

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* We use the definition of interneurons as it is commonly applied to the Aplysia abdominal ganglion: i.e. neurons that are neither motor neurons nor primary sensory cells and that make connections to other nerve cells within the CNS. Roman numerals have previously been used to refer to interneurons in the abdominal ganglion which have been inferred to exist on the basis of synaptic outputs to various follower cells. Some interneurons have also been recorded from directly, and assigned identified cell names, consisting of L or R (left or right of the midline) and an arabic number. Thus, Interneuron I is also known as cell L10*.
Fig 4 Synaptic actions produced by L24 in LDG1 and L7. Simultaneous intracellular recordings from L24 and gill motor neurons LDG1 and L7 while chamber was perfused with a high divalent cation solution to block interneuronal contributions. L24 was kept silent by steady hyperpolarization in the time between the arrows a depolarizing current was applied to elicit a short train of spikes. Each spike in L24 produced an IPSP in LDG1 and a conjoint excitatory-inhibitory PSP in L7.

latency (Fig. 5). They also occurred in the high-divalent cation solution, which reduces interneuronal activity in the ganglion by raising spike threshold. These data strongly suggest that the connections from L24 to these two cells are monosynaptic. L24 was also found to produce a small (1 mV), monosynaptic EPSP in siphon motorneuron LB51. In two cases we examined the connection between L24 and LDG2, another major gill motorneuron activated during respiratory pumping. In one case, interneuron XI produced a small 0.3 mV IPSP in LDG2, while in the second case there was no observable effect.

Fig 5. Evidence for monosynaptic connections between L24 and LDG1 and L7. A. L24 to LDG1 (10 traces superimposed) B. L24 to L7 (5 consecutive traces superimposed). Both sets of data are from the traces shown in Fig. 4, starting with the first spike in that series.
Because most of the identified follower cells of Interneuron XI are on the opposite (dorsal) side of the ganglion from L24, it was difficult to test for more than the 4 connections described above (L24 to LBsb, L7, LDG1 and LDG2). However, the synchrony of postsynaptic potentials attributed to Interneuron XI in various follower cells suggests that L24 projects to at least 20 follower cells (Fig. 10) in both the left and right hemiganglia.

When respiratory pumping movements occur, the spontaneous PSPs from Interneuron XI on its various follower motor cells are abolished. We therefore examined the behavior of cell L24 during a spontaneous respiratory pumping movement and found that cell L24 was inhibited (Fig. 6A). Thus the inhibition of cell L24 during respiratory pumping insures a maximal effectiveness of the respiratory command by removing an opposing source of synaptic input from many of the motor cells. Other experiments showed that during respiratory pumping movements IPSPs in L24 were synchronous with EPSPs in LDG2. IPSPs in cell L24 were also associated with EPSPs in LDG1 (Fig. 6A), but the relationship was not one-to-one. Thus the neuron or neurons that are inhibiting L24 during respiratory pumping are not the same as those directly driving LDG1 (see below and Fig. 8D), but probably are the same as those driving LDG2.

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**Fig. 6.** Synaptic input to L24. A: small spontaneous EPSP burst in LDG1 was associated with respiratory pumping movement of the gill and inhibition of L24. B: weak tactile stimulus to siphon skin (arrows) produced discharge of LDG1 and inhibition of L24. C: data from a different experiment where a single spike produced in an LE mechanoreceptor sensory neuron by intracellular stimulation produces a brief complex EPSP followed by a prolonged inhibition in L24. D: a train of 11 spikes in the same sensory neuron produces a more profound inhibition in L24. The dotted line in C and D represents the resting potential.
Interneuron XI tonically inhibits many of the gill and siphon motor neurons (Fig. 10) and contributes indirectly not only to the centrally commanded respiratory pumping but also to the reflex movements of the gill and siphon in response to tactile stimulation. Tactile stimulation of the siphon not only causes monosynaptic excitation of motor neurons via tactile afferents\(^1\)\(^,\)\(^8\) but also disinhibits the LD and RD motor neurons by inhibiting the action of L24. Fig. 6B illustrates the response of cell L24 and L\(_{DG1}\) to a tactile stimulus delivered to the skin with a von Frey hair (approximately 2 g force) for about 0.5 sec. Cell L24 is inhibited, the spontaneous IPSP in L\(_{DG1}\) is blocked, and L\(_{DG1}\) is excited by afferent input from the LE mechanoreceptor neurons innervating the siphon skin\(^4\). That the firing of these mechanoreceptor neurons contributes to the inhibition of L24 is indicated in other experiments where directly firing individual sensory neurons by intracellular current pulses also produced, after a brief initial excitation, a strong inhibition of L24 (Fig. 6C and D). The connection has polysynaptic components, however, and several spikes from a single sensory neuron are usually required to recruit inhibition in L24. In addition, the excitatory component was labile and not always observed. This fact probably accounts for absence of excitatory input from the skin in the experiment of Fig. 6B.

Antidromic stimulation revealed that cell L24 sends axons out the left and right pleural-abdominal connectives. L24 also receives inhibitory input sometimes preceded by a slight excitation, in response to stimulation of any of the other nerves of the abdominal ganglion.

(B) Respiratory command cells

Whereas a single cell accounts for the synaptic potentials attributed to Interneuron XI, the PSPs previously attributed to Interneuron II are produced by a group of at least three respiratory command cells. The data presented below suggest that the members of this command cell cluster may be divided into two functional groups. Our working hypothesis is that there are two or more closely coupled cells, which we call 'burst generators', that actually trigger the respiratory command. These cells in turn drive a second subgroup of two or more 'relay' interneurons, which make synaptic connections to the motor neurons (Fig. 10).

(1) Burst generators (cell L25)

We have not been able to account convincingly for the complete neural network responsible for generating the burst activity in the respiratory command cells. We have, however, identified one interneuron that appears to account for at least part of the burst generation. This cell, which we call L25, is about 50 \(\mu\)m in diameter and is located on the ventral surface of the abdominal ganglion rostral to cell L10 (Fig. 2). Fig. 7A–D shows simultaneous intracellular recordings from L25 and from L10, a command cell in the cardiovascular control system\(^19\). During respiratory pumping L10 always receives a large, smooth, long-lasting hyperpolarization such as that shown in Fig. 7A. This phasic inhibition plays an important role in respiratory pumping by ensuring that the cardiovascular motor neurons do not receive a conflicting command.
Fig. 7. Cell L25. A: burst of spikes occurs spontaneously in L25 synchronous with central command for respiratory pumping which also produces inhibition of L10. B: firing L25 with an intracellular current pulse (arrows) results in inhibition of L10. C: depolarization of L25 with a sustained intracellular current pulse (arrow) results in spikes plus recruitment of EPSPs onto itself, eventually leading to a high frequency burst. D: starting from a slightly hyperpolarized holding potential, a depolarizing pulse (first arrow) applied to L25 recruits a short burst of spikes plus EPSPs. Although L25 was hyperpolarized at the second arrow, the PSP burst continued to accelerate, and was accompanied by inhibition in L10. E: biphasic PSPs (dots) recorded from L25. Similar PSPs were recorded both during spontaneously occurring bursts and following a depolarizing current pulse that elicited spikes in L25. F: computer average of 35 biphasec PSPs recorded from L25 (Fig. 7E). After sampling, peaks of individual PSPs were aligned and average was computed.

from L10. In extensive experiments recording simultaneously from L10 and from identified motor neurons, this smooth hyperpolarization was found to occur spontaneously only during respiratory pumping. Therefore the data in Fig. 7A show that L25 fires in a short burst during respiratory pumping. That L25 contributes at least
some of this phasic inhibition produced in L10 is illustrated in Fig. 7B, where L25 is fired by a brief intracellular pulse of depolarizing current. Because discrete IPSPs were not produced it is not possible to tell from our data whether this is a monosynaptic connection. The peak hyperpolarization produced in Fig. 7B is somewhat less than that which occurs spontaneously (Fig. 7A), but the maximum spike frequency was slower in the former case. Although L25 may account for much of the respiratory command input to L10, when this cell is fired by intracellular current pulses it has no effect on other Interneuron II followers such as L12, L13 and L14.

L25 is classified as a burst generator because firing a short train of 10–20 spikes in this cell with a depolarizing intracellular current pulse usually recruited excitatory input back onto itself, and this positive feedback sometimes led to a high frequency burst (Fig 7C) The PSPs recruited in this way are shown more clearly in Fig. 7D, where a hyperpolarizing current was applied to L25 after a brief burst of spikes initiated by a 3-sec depolarization. Note that the inhibition of L10 is synchronous with the EPSPs recorded in L25. A plausible interpretation of these data is that activity in L25 triggers a regenerative burst in one or more cells. Inhibitory input to L10 and excitatory input to L25 are generated by at least one of these cells.

The PSPs which are recruited in L25 by intracellular depolarization or which are recorded from this cell during a spontaneous burst have diphasic shapes (Fig. 7E and F) similar to the electrotonic PSPs transmitted between electrically coupled cells in Aplysia. Based on these results we suggest that the generation of the respiratory central command may be due to two or more electrically coupled cells which are mutually excitatory. Activity in one of these coupled cells (e.g. L25) may in some cases be sufficient to trigger the entire network, but the source of excitation to the network which initiates the burst remains unknown. The possibility remains that L25 and other cells involved in generating the burst that drives respiratory pumping may have some endogenous burst-generating properties due to special membrane conductance mechanisms.

(2) Relay neurons
L25 is apparently involved in generating the respiratory pumping central command, but has not been shown to make monosynaptic connections to the identified motor neurons which receive synaptic input during respiratory pumping. A second class of cells has been found which can be shown to make monosynaptic connections to some of these follower motor neurons. Firing members of this second class of cells with intracellular current does not have the regenerative effect of recruiting EPSPs back onto themselves. These intermediate or ‘relay’ interneurons are presumably interposed between the burst generator interneurons and the motor neurons (which include gill, siphon, parapodial and cardiovascular motor cells).

(a) Cell L26. One example of the respiratory command relay neuron type is cell L26, which is located on the left ventral surface of the abdominal ganglion, caudal and lateral to the LE cluster of mechanoreceptor neurons (Fig. 2) Cell L26 is approximately 50 μm in diameter with dark orange pigmentation. It is normally silent with little background synaptic activity. Cell L26 is excited during spontaneous respiratory
pumping and it in turn excites the major gill motor neuron LD_G1. Thus, during a spontaneous respiratory pumping command, motor neuron LD_G1 and L26 both become active (Fig. 8A). Firing L26 with an intracellular current pulse produces EPSPs in LD_G1 (Fig. 8B). When we hyperpolarized L26 during spontaneous respiratory pumping, the excitatory input to LD_G1 was dramatically reduced, indicating that cell L26 produces most of the respiratory pumping command observed in LD_G1 (cf. Fig. 8A and D). The short and constant latency between spikes in L26 and the EPSPs in LD_G1 suggest that the connection between these two cells is monosynaptic (Fig. 8C). Hyperpolarization of L26 just prior to achieving its peak firing rate during a

![Cell L26](image)

Fig. 8. Cell L26. A: spontaneous burst in L26 produced excitatory synaptic activity and firing in LD_G1, which in turn produced a gill contraction. B: depolarization of L26 with intracellular current pulse produces one-for-one EPSPs in LD_G1. The first spike in L26 does not produce a distinct EPSP in LD_G1 due to the simultaneous occurrence of an IPSP from Interneuron XI (cell L24). C: the short and constant latency between the L26 spikes and the EPSPs in LD_G1 suggests that this is a monosynaptic connection. D: hyperpolarization (arrows) of L26 at start of rapid firing phase of respiratory central command blocked the gill contraction and concomitant excitatory synaptic activity and burst in LD_G1. Note that the IPSPs in LD_G1 from Interneuron XI were blocked during the period of excitatory input to L26 and not just during L26 spike activity.
respiratory pumping command (Fig. 8D) uncovers an excitatory synaptic drive in this cell which continues for a few seconds beyond the initiation of the hyperpolarizing current pulse. The excitatory drive is presumably from the respiratory command burst generators.

Cell L26 also produces inhibitory input onto the siphon motor neuron LBs1, which is consistent with the finding that LBs1 is inhibited during respiratory pumping\textsuperscript{22}. Unlike the large contribution of L26 to the LDG1 excitation, the inhibition of LBs1 is small, and probably not monosynaptic, indicating that other interneurons mediate most of the inhibition to LBs1 during the central respiratory command. In addition to its role in mediating respiratory pumping, L26 also acts as an excitatory interneuron during reflex-activated gill withdrawal, since tactile stimulation of the siphon skin produces brisk discharges in L26. We have not examined whether the input is mediated by direct monosynaptic connections from identified mechanoreceptors\textsuperscript{4}, by the burst generators, or by unidentified interneurons.

Directly firing L26 produced EPSPs in LDG1 but it does not inhibit the spontaneous IPSP in LDG1 produced by L24. During spontaneous respiratory pumping, however, this IPSP is blocked by inhibition of L24 (see above and Fig. 6A). Thus, the inhibition of cell L24 is not mediated by cell L26, but instead appears to be a result of connections from one or more other cells which mediate the central respiratory command. Some weak synaptic input from L26 to L24 could, however, go undetected, since we have not made simultaneous recordings from these two cells.

![Image](99)

**Fig. 9** Cell L27. A. spontaneous burst of spikes in L27 associated with typical patterns of respiratory command driven excitation of an RD cell and excitation-inhibition of an RB cell\textsuperscript{19,22,28} B: spontaneous respiratory central command results in spikes in L27 and IPSPs in LCp, a pericardial motor neuron\textsuperscript{18}. C: intracellular current pulse (arrows) fires L27 causing inhibition of LCp. D: faster sweep speed shows that spikes evoked by depolarizing current are associated with one-for-one IPSPs in LCp. At higher sweep speed the latencies were found to be short (10 msec) and constant. **E** synchronous IPSPs in an RD cell and L27 from Interneuron XI, cell L24\textsuperscript{19}.
(b) Cell L27. Cell L27 is another example of a respiratory command relay neuron. This small cell, about 50 μm in diameter, is located on the dorsal surface of the ganglion rostral to L11 (Fig. 2). It is normally silent but fires at a high frequency during the central respiratory command (Fig. 9A and B). It contributes at least a portion of the inhibitory action of the respiratory command on pericardial motor-neuron LCr18, since firing L27 with current pulses produced IPSPs in cell LCr (Fig. 9C and D). The short (10 msec) and constant delay between spikes and PSPs suggest that this is a monosynaptic connection. However, like those of L26, the range of L27’s synaptic connections is limited. It has no effect on RD and RB cells, nor on R15, all of which receive strong synaptic input during the respiratory central command (Fig. 9A and ref. 14). Cell L27 receives spontaneous inhibitory input, which is synchronous with an IPSP in the RD cells that has been attributed to Interneuron XI (Fig. 9E and ref. 19). Spontaneous inhibition from Interneuron XI was not observed in cell L26. It appears that L27 does not cause the inhibition of Interneuron XI seen during Interneuron XI bursts, since firing L27 produced no effect on the frequency of the spontaneous IPSP produced by Interneuron XI in an RD cell.

DISCUSSION

Spontaneous respiratory pumping is one of the most conspicuous and frequently occurring behaviors observed in Aplysia, and has many of the features of a simple fixed-action pattern. During respiratory pumping there is a complex pattern of synaptic activity observed in the motor neuron pool which mediates the behavior, but up to this time little was known about its origin. Our results indicate that the pattern of synaptic input recorded in the motor cells during respiratory pumping is generated by a network of at least 4, and probably more, higher order interneurons. A complete analysis is not yet possible, however, since the interneurons which drive the motor neurons are small, are located on both dorsal and ventral surfaces of the ganglion, and apparently are not always located in the outermost layer of cell bodies that surround the neuropile.

(A) Interneuron XI (cell L24)

L24 is a double-action cell. It mediates inhibition onto gill motor neurons LDG1 and LDG2, excitation onto siphon motor neuron LB1, and conjoint excitation and inhibition onto gill motor neuron L7. These connections appear to be monosynaptic. L24 is also presumed to make the other connections previously attributed to Interneuron XI (Fig. 10).

L24 is one of two identified double-action neurons in the abdominal ganglion; the other is the cholinergic cell L1013,39. Both of these cells make a conjoint synapse onto gill motor neuron L7. These synapses are frequency-dependent, converting from excitation to inhibition with rapid repetitive firing. The available evidence suggests that for L10–L7 the mechanism for this conversion is the desensitization of the excitatory receptor on the L7 membrane39. The conversion mechanism at the L24–L7 synapse is not known. The normal spontaneous rate of firing in L7 (2–6 spikes/sec) is
Fig. 10. Summary wiring diagrams for the neural circuit that drives respiratory pumping. A: connections which were demonstrated in the experiments discussed in the text. Solid lines show connections known to be monosynaptic. B: schematic diagram summarizing known connections between respiratory command cells and Interneurons I and XI, and some of their connections to cardiovascular and respiratory motor neurons. Dark lines show connections which have been demonstrated directly; others were inferred on the basis of PSPs recorded simultaneously in two or more follower cells\(^{18,19,22,28}\). Symbols for synaptic actions are: \(\rightarrow\) electrical; \(\leftarrow\) inhibitory; \(\rightarrow\) excitatory (chemical). Several of the cells shown here represent a class of motor neurons (e.g. LDs 1–3 stands for LDs1, LDs2 and LDs3). With the exception of RB the function of the RB cells is not known. See references\(^6,22,24,27,28\) for details concerning motor neurons. L10 (Interneuron I) also makes connections to many of the motor neurons shown here\(^{18,19}\).
high enough to maintain the PSP onto L24 in the inhibitory mode. We observed an EPSP at this synapse if L24 firing was artificially silenced for about 5 sec by hyperpolarization. We do not yet have enough data to tell whether the typical pause in L24 activity caused by a respiratory command burst (Fig. 6A) is long enough to convert the IPSP in L7 to an EPSP.

(B) Respiratory command cells

The data for cells responsible for producing the respiratory central command onto motor neurons have been presented in the framework of a working hypothesis (Fig. 10). According to this proposal, the cluster of respiratory command cells has at least two levels of organization: two or more mutually excitatory ‘trigger’ or ‘burst-generating’ cells (e.g. L25), which are indirectly coupled to the follower motor neurons by two or more ‘relay’ interneurons (e.g. L26 and L27). Future experiments will be directed towards a more rigorous testing of this hypothesis.

The finding that the respiratory central command may be attributed to three or more cells runs counter to the assumptions of earlier investigators, who attributed this input to a single cell14,27. The experiment reported by Jacklet et al 11 also bears on this point. They recorded from an abdominal ganglion possessing an abnormally long commissure linking the left and right hemiganglia. These authors concluded, from recordings made with the commissure severed, that the cell which drives respiratory pumping is in the right hemiganglion. However, the recordings they obtained from L11 and from the pericardial and genital nerves after cutting the commissure reveal burst activity generated in the left hemiganglion. This activity may also be part of the respiratory central command. Such an interpretation suggests the existence of two respiratory command centers — one on each side of the ganglion. Presumably these two command centers would normally be coupled, but with the commissure severed they could burst independently of each other. Alternatively, it is conceivable that both parts of a cell whose axon has been cut when it crosses the commissure may be capable of generating bursts. That is, an ectopic pacemaker region may arise when a bursting cell is cut into two parts.

Spontaneous respiratory pumping has also been examined in Archidoris3. Here the giant cerebral neurons appear to play a similar command function as the respiratory command cells in Aplysia.

(C) Role of L24 and L26 in defensive gill and siphon withdrawal

While our analysis focuses on the neuronal control of spontaneous respiratory pumping, the results also shed light on the question of how individual neural elements can be involved in the mediation of more than one behavior. For example, spontaneous respiratory pumping utilizes some of the same motor neuron pool as the defensive gill and siphon withdrawal reflex. The results indicate that these two behaviors also share common interneurons. In response to tactile stimulation of the siphon skin, the mechanoreceptors which innervate this region produce monosynaptic EPSPs in several gill and siphon motor neurons. A polysynaptic pathway also exists, via at least two excitatory interneurons interposed between the sensory and motor neurons4,16,22.
(Castellucci, Byrne and Kandel, in preparation) Our results indicate that L24 and L26 also play a role in mediating this reflex. L24 is inhibited by input from the mechanoreceptor cells (Fig 6). Silencing this cell produces disinhibition of the LD and RD gill and siphon motor neurons, and of gill motor neuron L7 (Figs. 6B and 10). Thus, the effectiveness of the excitatory drive onto the motor neurons from the sensory neurons would be enhanced. The actions of L24 may also contribute to habituation of the gill withdrawal reflex. Since all other known PSPs from the mechanoreceptor neurons decrease with repeated stimulation\(^7\)-\(^8\) (Castellucci et al., in preparation), it seems likely that the inhibition of L24 also decreases. This in turn would lead to a decrement of the disinhibition of the motor neurons and thus contribute to the total net decrement of synaptic excitation which underlies habituation. These effects would probably be small, however, compared to the decrement of the direct monosynaptic excitatory component\(^8\). The recent finding of Advocat et al.\(^1\) suggests an additional role for L24. These authors found that siphon withdrawal reflex could be inhibited by feeding animals prior to stimulation. Since L24 is the only identified cell which makes inhibitory connections to many gill and siphon motor neurons, it seems a possible candidate for the neuron mediating the modulation of the reflex behavior.

L26, which is a respiratory command relay cell, also contributes to the defensive gill withdrawal reflex. It is excited by skin stimulation, so it acts as an excitatory interneuron in the reflex pathway to at least one cell, gill motor neuron LD\(_{G1}\). A corollary to this finding is that other interneurons in the reflex pathway may also serve as additional relay cells mediating the respiratory central command. This role of L26 in the defensive reflex suggests an explanation for the proposed functional division of respiratory command cells into burst generators and relay cells. The burst generators are presumably involved in triggering the complete respiratory pumping sequence. The relay cells, however, may also be involved in other behaviors (such as defensive withdrawal), which utilize only a restricted subset of the entire respiratory command motor pool. Thus the general principle of hierarchical organization of sensory and motor cells in Aplysia\(^4\),\(^2\) may also be present at the level of the organization of command interneurons.

(D) Interactions between interneurons

It has previously been shown that two different behavioral patterns controlled by the abdominal ganglion are constrained to occur at separate times because of the mutually inhibitory connections between the command cells which generate them. Thus, the increase in heart rate and decrease in vasomotor tone driven by activity in Interneuron I (cell L10) are prevented from overlapping with the respiratory pumping behavior driven by the group of respiratory command cells\(^{15},\(^{19}\). This principle of mutual inhibition between higher order interneurons in the motor pathway may be extended to the interaction between the respiratory command cells and Interneuron XI (L24). The mutual inhibition between these interneurons was found to be exerted at the level of spike generation in the interneurons: the possibility of presynaptic inhibition of the synapses between interneurons and motor neurons has not been examined.
Many of the motor neurons which receive phasic synaptic input during respiratory pumping also receive at other times synaptic input from Interneuron XI (Figs. 6, 9A and 10). In all but one case (cell L7), these common follower cells receive input of opposite sign. Thus the respiratory command synaptic input to motor cells is made more effective by the simultaneous silencing of the opposing Interneuron XI PSPs.

ACKNOWLEDGEMENTS

We thank T. Carew, V. Castellucci, W. Hening, E. R. Kandel and I. Kupfermann for critically reading an earlier draft of this manuscript, and C. Byrne for computer programming.

This research was supported by N.I.H. Postdoctoral Fellowship NS-03076 and N.I.H. Grant NS 13511 to J.B., I.T. Hirschl Career Development Award and N.S.F. Grant BMS 74-18410 to J.K. and N.I.H. Grants NS-09361 and MH 262102 to E.R. Kandel.

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