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SYNAPTIC BASIS OF SWIM INITIATION IN THE LEECH

III. SYNAPTIC EFFECTS OF SEROTONIN-CONTAINING INTERNEURONES (CELLS 21 AND 61) ON SWIM CPG NEURONES (CELLS 18 AND 208)

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SUMMARY

1. Serotonin-containing cells 21 and 61 strongly excite a swim central pattern generator (CPG) neurone, cell 208, in nearby segmental ganglia in the leech *Macrobdella decora*. This excitatory effect is apparently independent of activity in the swim-initiating neurone cell 204, which monosynaptically excites cell 208 (Weeks, 1982*b*).

2. Cell 208 excites cell 21, apparently directly. This is the first identified direct pathway for feedback from the swim central pattern generator to a swim initiator neurone.

3. Focally applied serotonin has no effect on the soma of cell 208, but causes both excitatory and inhibitory responses in cell 208 when applied to different places within the neuropile.

4. Cell 61 polysynaptically excites distant, posterior cells 208. This excitation is mediated at least in part by the activation of nearby cells 208, which polysynaptically excite posterior cells 208.

5. Cell 208 is dye-coupled intraganglionically to a newly identified pair of neurones, designated cells 18. Cell 208 also excites posterior cells 18, apparently directly. This interaction may be the pathway whereby cell 61 polysynaptically excites posterior cells 208.

6. During swimming, cell 18's membrane potential oscillates in phase with cell 208. Intracellular current injection into cell 18 during swimming perturbs the swim motor pattern. Therefore, cell 18 qualifies as a candidate swim CPG neurone.

INTRODUCTION

Identified neurones activate central pattern generators (CPGs) both by standard synaptic potentials (Rose & Benjamin, 1981*a*; Weeks, 1982*b*; Nusbaum, 1984*b*) and by neuromodulatory mechanisms (Nagy & Dickinson, 1983; Dickinson & Nagy, 1983). In the leech, neurones containing serotonin initiate the swim motor pattern by

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both types of mechanism (Willard, 1981; Kristan & Nusbaum, 1983; Nusbaum & Kristan, 1986). Serotonin released from one of these cells, the Retzius cell, acts as a neurohormone which increases the likelihood of swimming (Willard, 1981). Retzius cell activity causes swimming in an isolated nervous system, but with a long latency (often several minutes) and for a time that greatly outlasts the Retzius cell activity, without causing recognizable postsynaptic potentials (PSPs). In contrast, the serotonin-containing cells 21 and 61 each initiate swimming episodes within seconds (Kristan & Nusbaum, 1983; Nusbaum & Kristan, 1986), and these last only as long as these cells are stimulated, or for a few cycles beyond, suggesting a more direct, synaptic action of these neurones.

Cells 21/61 may normally act in parallel to activate the swim CPG with the previously described swim initiator neurones cells 204 and 205 (Nusbaum & Kristan, 1986). Cells 204 and 205 have been shown to excite some swim CPG neurones, including cell 208, by standard synaptic potentials (Weeks, 1982*a*,*b*; Nusbaum, 1984*b*). In this paper, cells 21 and 61 are shown also to excite synaptically cell 208, as well as a newly identified swim pattern generating neurone, cell 18. The effects on cell 208 of cells 21/61 are compared to those of cells 204/205, and their interactions in swim-initiation are investigated. Some of these results have been previously published (Nusbaum, 1983, 1984*a*; Kristan & Nusbaum, 1983).

MATERIALS AND METHODS

Neurophysiological preparations, electrophysiological recordings and dye-injection techniques were as described in the preceding paper (Nusbaum & Kristan, 1986). Most experiments were performed on the North American hirudinid leech *Macrobdella decora*; some experiments were also performed on the European hirudinid leech *Hirudo medicinalis*. The newly identified neurones in this paper were each assigned an identification number that corresponds both to their typical location in segmental ganglia, and to the previously constructed numbering scheme of Ort, Kristan & Stent (1974) and Muller, Nicholls & Stent (1981).

RESULTS

Interactions of cells 21 and 61 with cell 208

If strong enough, electrical stimulation of cells 21 or 61 activates the swim motor pattern (Kristan & Nusbaum, 1983; Nusbaum & Kristan, 1986). To determine if this effect resulted from direct excitation of identified swim CPG neurones (Friesen, Poon & Stent, 1978; Weeks, 1982*b*; Friesen, 1985), paired intracellular recordings were made of cells 21 or 61 and some of these swim CPG cells. With sufficiently weak stimulation of either cell 21 or cell 61, swimming was not initiated and an excitation of a swim CPG neurone, cell 208, was seen (Fig. 1). This response was a relatively large depolarization, causing either the onset of impulse activity or an increase in frequency. The impulse frequency during the cell 208 response was often equal to or greater than that of the stimulated serotonin-containing cell. Cell 61 excited, with a relatively short latency, only those cells 208 whose somata were in the same ganglion

as the stimulated cell or within two segments of it (Fig. 1C). This matches the intersegmental extent of cell 61 axons (Nusbaum & Kristan, 1986). Stimulating cell 61 had no effect on more distant anterior cells 208 (Fig. 1C), but did cause a longer latency polysynaptic excitation of more distant posterior cells 208 (see Fig. 9). Although not extensively examined, cell 208 was more weakly excited by cells 21 and 61 in *Hirudo medicinalis*.

The excitatory effects of cells 21 and 61 on cell 208 were apparently direct; that is, individual excitatory postsynaptic potentials (EPSPs) were often recorded in cell 208 at a constant latency after each impulse in cells 21 or 61 (Kristan & Nusbaum, 1983). The intraganglionic latency was approximately 12 ms. This synaptic latency is longer than that for many chemical monosynaptic connections in the leech (Nicholls &

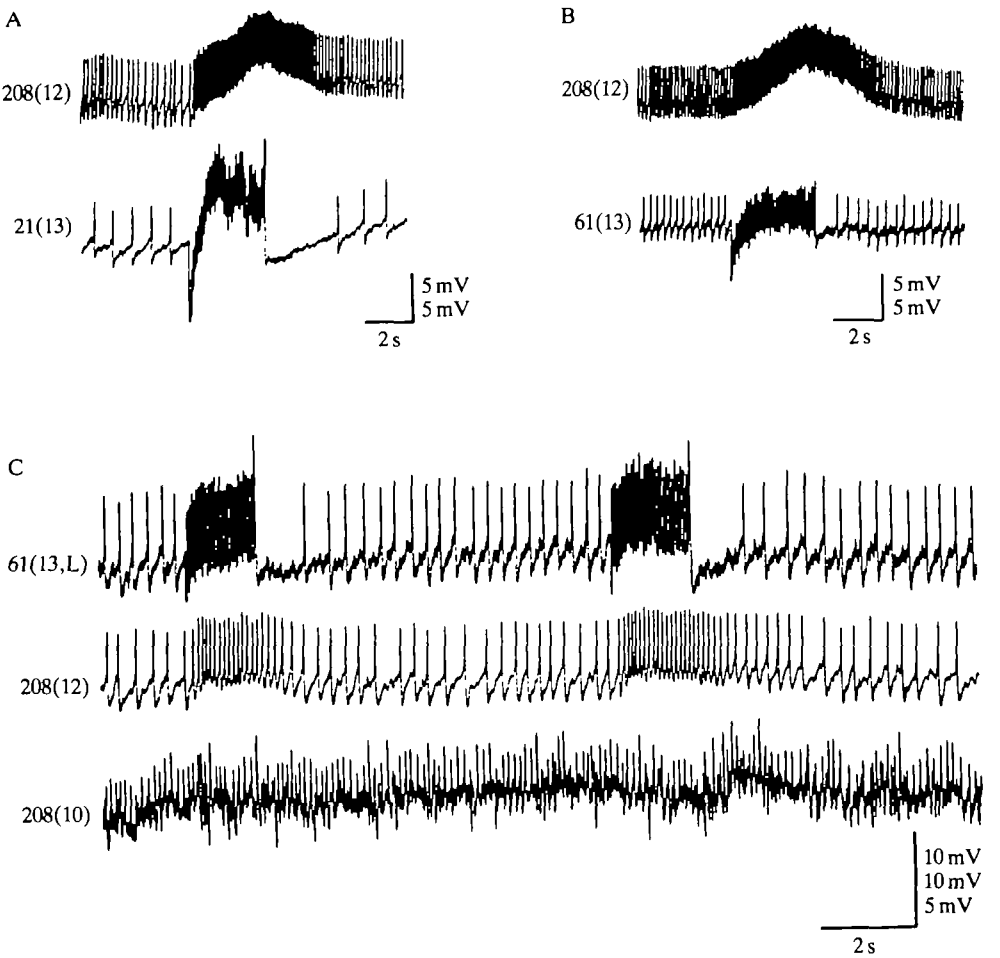

Fig. 1. Intracellular stimulation of the serotonin-containing cells 21 and 61 excites the swim CPG cell 208. Intracellular stimulation (+0·5 nA) of either (A) cell 21 (12 Hz) or (B) cell 61 (13 Hz) in ganglion 13 produced a depolarization and increased firing rate in the cell 208 (22 Hz) in ganglion 12. (C) Intracellular stimulation of cell 61 (+0·5 nA) in ganglion 13 excited cell 208 in ganglion 12 but not in ganglion 10. (Part C is from a different preparation.)

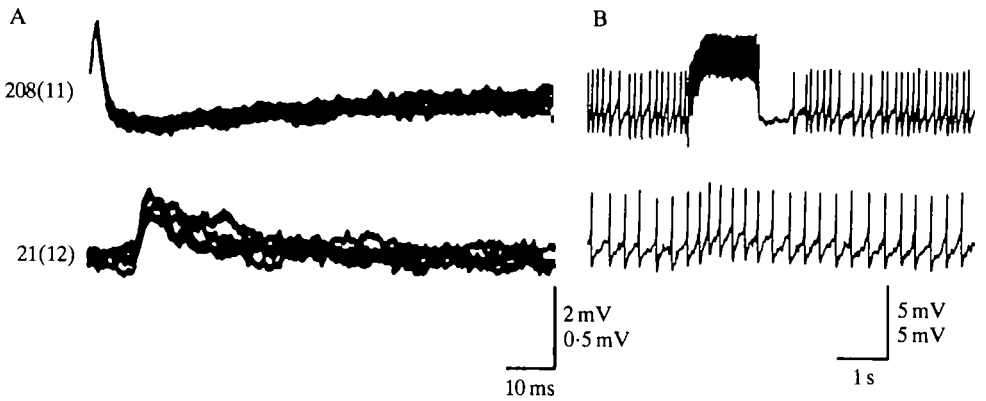

Fig. 2. Cell 208(11) excites cell 21(12). (A) Superimposed oscilloscope sweeps triggered by impulses in cell 208(11) are each followed by a constant latency EPSP in cell 21(12). (B) Intracellular stimulation of cell 208(11) (+1·0 nA) to fire a burst of impulses excites cell 21(12). Parts A and B were from different preparations.

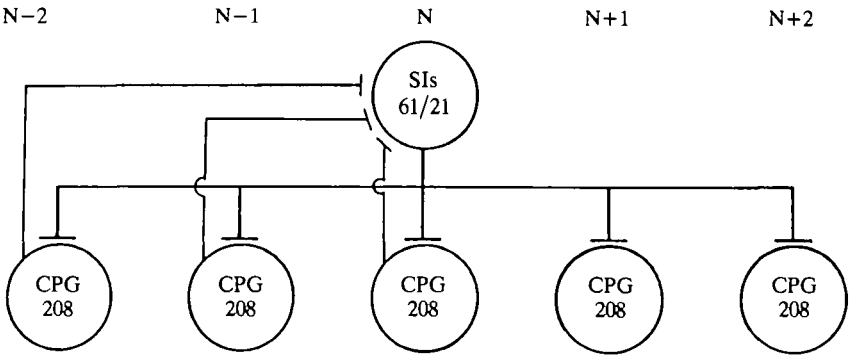

Fig. 3. Schematic diagram of the excitatory interactions between cells 21/61 and cells 208 in nearby ganglia. SIs indicates 'swim initiator neurones', and CPG indicates 'swim central pattern generator neurone'; the designation 'N', 'N+1', etc. indicates the ganglion number in which the neurones of interest are located; 'T' junctions indicate chemical excitatory synaptic connections. The thinner line from cell 208 to cells 21/61 indicates that this interaction is functionally weaker than the excitation of cell 208 by cells 21/61.

Purves, 1970). However, it is comparable to the response of a mechanosensory neurone in the leech to serotonin released by the Retzius cell in cultures of isolated cell pairs (Fuchs, Henderson & Nicholls, 1982). Slow onset synaptic potentials have also been characterized in other systems (Hartzell, 1981).

In turn, cell 208 impulses caused apparently monosynaptic EPSPs in cells 21 in posterior ganglia (Fig. 2A). Each impulse in cell 208 was followed with a constant latency by an EPSP in cell 21, even when cell 208 was firing at impulse frequencies (40 Hz) that were higher than usually seen during swimming. The latency (10 ms) to onset of each EPSP in cell 21 in the next posterior ganglion is the same as that for previously confirmed monosynaptic connections from cell 208 onto identified motor neurones in the next posterior ganglion (Weeks, 1982*b*). This synaptic interaction appears to be functionally weak: intracellular stimulation of cell 208 to fire impulses at 30 Hz, its usual firing frequency during swimming, caused only a slight increase in

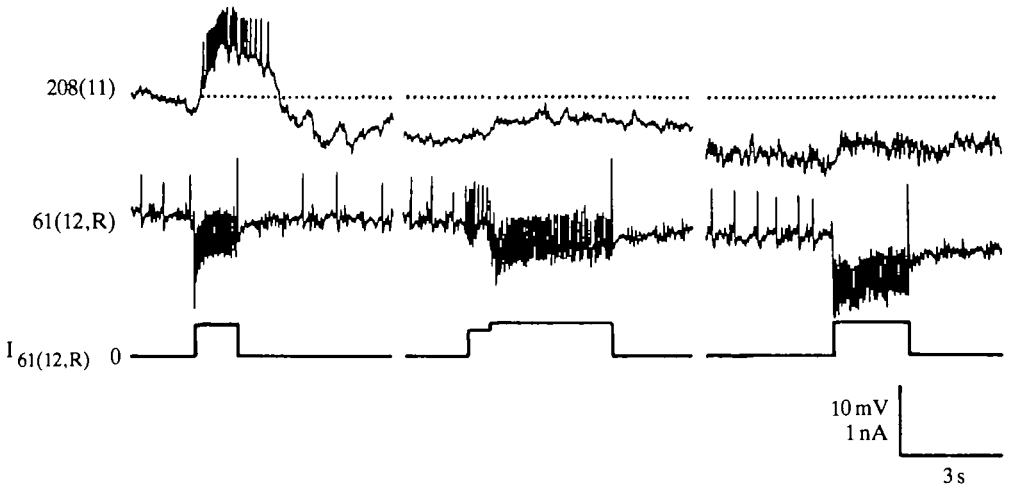

Fig. 4. Voltage sensitivity of the response in cell 208 to impulse bursts in cell 61. Cell 61(12,R) was stimulated intracellularly (+0·5 nA) when cell 208(11) was: at its resting potential (approx. −50 mV; dotted line) (left); hyperpolarized by a hyperpolarizing current injection (−0·5 nA) (middle) and hyperpolarized as a result of a barrage of spontaneously occurring IPSPs (right). In the middle set of recordings, stimulation of cell 61(12,R) occurred in two steps, as indicated by the current monitor trace.

the firing frequency of cell 21 (Fig. 2B). Stimulating cell 208 also caused a weak excitation of cell 61, but no individual EPSPs were apparent. Fig. 3 summarizes the interactions of cells 21/61 and 208.

Individual cell 21/61 EPSPs onto cell 208 became smaller and often disappeared altogether when the cell 208 membrane potential was hyperpolarized from the resting potential (−45 to −50 mV), and depolarizing cell 208 increased the amplitude of these EPSPs (Kristan & Nusbaum, 1983). A similar change was seen in response to an impulse burst in cell 21 or cell 61 (Fig. 4). This excitatory response was usually completely abolished when cell 208 was held at membrane potentials more negative than −65 mV, using separate microelectrodes for recording membrane potential and injecting current in cell 208 (data not shown). No reversal of this response occurred at more hyperpolarized levels. This decreased response with hyperpolarization in cell 208 to cell 21/61 input was also observed when cell 208 received spontaneous inhibitory synaptic potentials (Fig. 4).

These voltage-sensitive characteristics suggested that the excitation of cell 208 was due to the decreased conductance of an ion whose equilibrium potential was either at or more negative than −65 mV. To test this possibility, the input resistance of cell 208 was determined during its excitatory response to stimulation of cell 61, using constant amplitude and duration hyperpolarizing current pulses injected into cell 208. In most cases (seven out of eight), no change in the input resistance was observed; in one case there occurred a slight increase in input resistance. This result could mean that the synaptic input does not operate by changing the conductance. However, it could also mean that the synapses are electrically distant from the soma or that conductance increases associated with impulse activity in cell 208 may

obscure synaptic conductance changes. To reduce impulse activity, ganglia were bathed in slightly elevated Mg^{2+} (8 mmol l^{-1}) saline. In one of four preparations, an increase in the input resistance of cell 208 was observed during stimulation of cell 61 (Kristan & Nusbaum, 1983).

During swimming, the membrane potential of both cell 208 (Weeks, 1982*b*) and cells 21/61 (Nusbaum & Kristan, 1986) undergoes oscillations that are phase-locked to the swim motor pattern. Because cell 208 is sensitive to input from cells 21/61 only while it is not hyperpolarized, the impulses from cells 21/61 would have to occur during the depolarizing part of the cell 208 oscillations; this is confirmed by recording from the two cells simultaneously during swimming (Fig. 5).

Cells 21 and 61 have an additive effect with cell 204 on swim-initiation (Nusbaum & Kristan, 1986). The excitation of cell 208 caused by the simultaneous activation of cells 61 and 204 similarly was found to be greater than the individual effects of these cells (Fig. 6). These experiments also showed that cell 61 had strong effects on cell 208 with minimal activation of cell 204, a documented indirect pathway from cell 61 to cell 208 (Weeks, 1982*b*; Nusbaum & Kristan, 1986). Although possible, it seemed unlikely that the cell 61 effect on cell 208 could be *via* another, unmonitored cell 204, because (1) recordings were made from a number of cells 204 and none of them were strongly excited by the cell 61 activity and (2) cell 204 caused an EPSP in cell 208 which increased with hyperpolarization and decreased with depolarization (Fig. 7); all the EPSPs recorded in cell 208 in response to cell 61 stimulation decreased with hyperpolarization.

Effects of serotonin on cell 208 and other swim-related neurones

To determine whether the synaptic excitation of cell 208 by cells 21 and 61 could be due to serotonin release, exogenous serotonin was applied to cell 208. When serotonin (10^{-4} mol l^{-1}, pH 7·4) was pressure ejected onto the desheathed cell 208 soma, there was usually no response (24 out of 27 cells). In the remaining cases, cell 208 hyperpolarized in response to the first 2–4 pulses of serotonin, after which no further response was elicited. To control for desensitization, the pipette was backed away from the soma for 20 min, then brought back into position for another application of serotonin before it was concluded that there was no effect.

Similar serotonin pulses delivered into the neuropile excited 37 % of the cells 208 tested (7 out of 19) (Fig. 8A). Control ejections of leech saline into the same region had no such effect. The latency to the onset of this excitatory response in different preparations varied from approximately 200 ms to 1 s, possibly due to a slow time course for the onset of the effect of the serotonin. Alternatively, the latencies might have been due to differences in diffusion time from the pipette tip to the responsive cell processes. To determine if this excitatory response by cell 208 was direct, the serotonin applications were repeated while neurotransmitter release was depressed by reducing the concentration of Ca^{2+} (0·45 mmol l^{-1}) and raising the concentration of Mg^{2+} (10 mmol l^{-1}) in the saline bath (Nicholls & Purves, 1970; Nusbaum & Kristan, 1986). Under these conditions serotonin still caused an increase in cell 208 impulse activity (Fig. 8Aii). An indirect effect of serotonin on cell 208

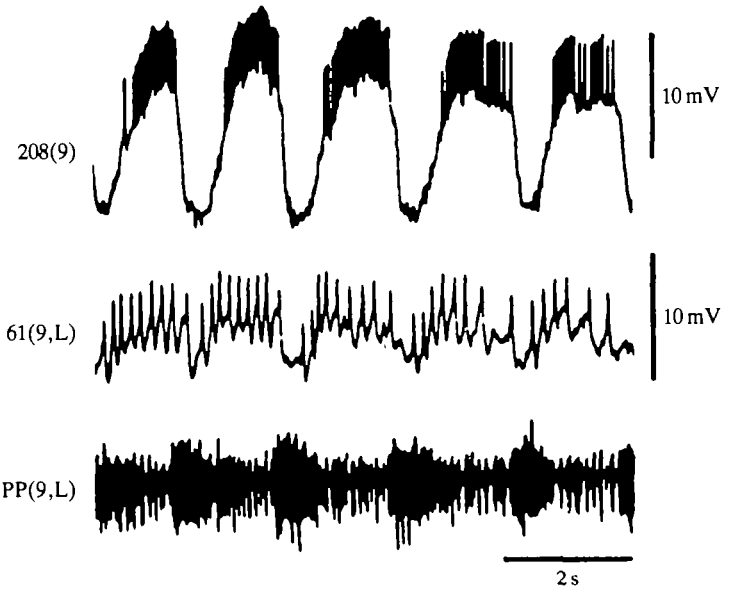

Fig. 5. Activity of cell 61(9,L) and cell 208(9) recorded intracellularly during swimming. Impulse bursts of identified ventral excitor motor neurones (largest units) were simultaneously recorded in the posterior branch of a posterior segmental nerve, PP(9,L). The episode of swimming was initiated by intracellular stimulation of cell 204(12) (not shown).

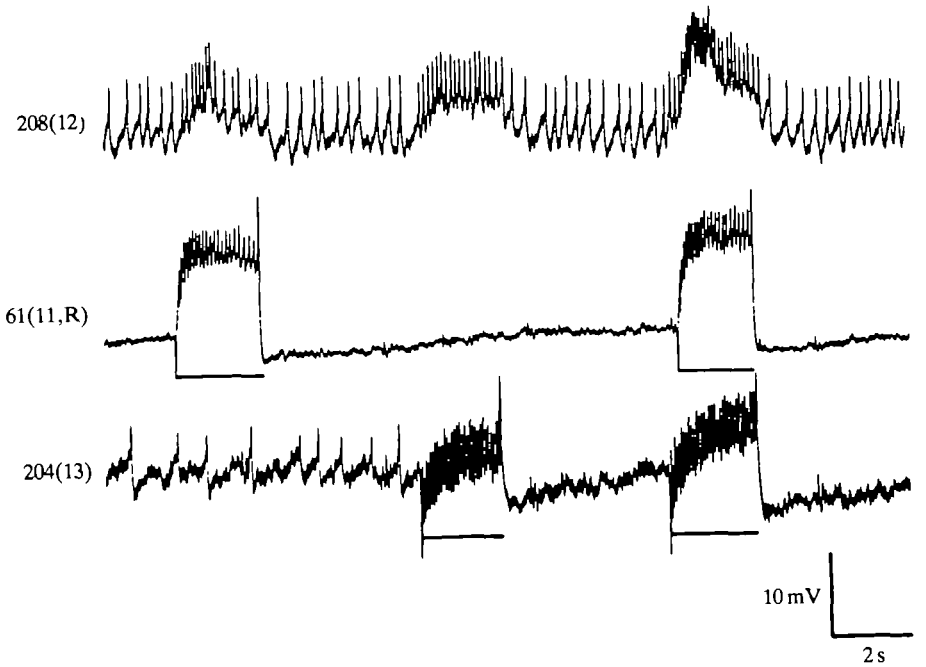

Fig. 6. The response of cell 208 to intracellular stimulation of either cell 61 or cell 204 during simultaneous intracellular recordings. Cells 61(11,R) and 204(13) were each stimulated individually (+0·5 nA; bars) and then at the same time, while recording from cell 208(12).

remains a possibility, however, because some chemical synapses in the leech are only completely suppressed with higher levels of Mg^{2+} (Nicholls & Wallace, 1978). Similar ejection of serotonin into the neuropile either caused no response, or a hyperpolarization in other identified leech neurones, including two neurones (cells 61 and 204) known to excite cell 208 (Fig. 8).

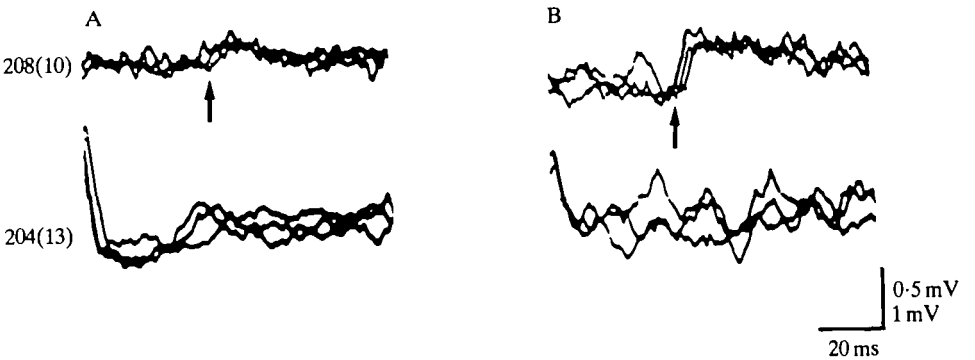

Fig. 7. Impulses in cell 204 cause EPSPs in cell 208 that increase when cell 208 is hyperpolarized. (A) An EPSP was recorded in cell 208(10) (arrow) following each impulse in cell 204(13). (B) The same EPSP (arrow) with −0·5 nA injected into cell 208 through the recording electrode. A and B each show three superimposed traces triggered by impulses in cell 204(13).

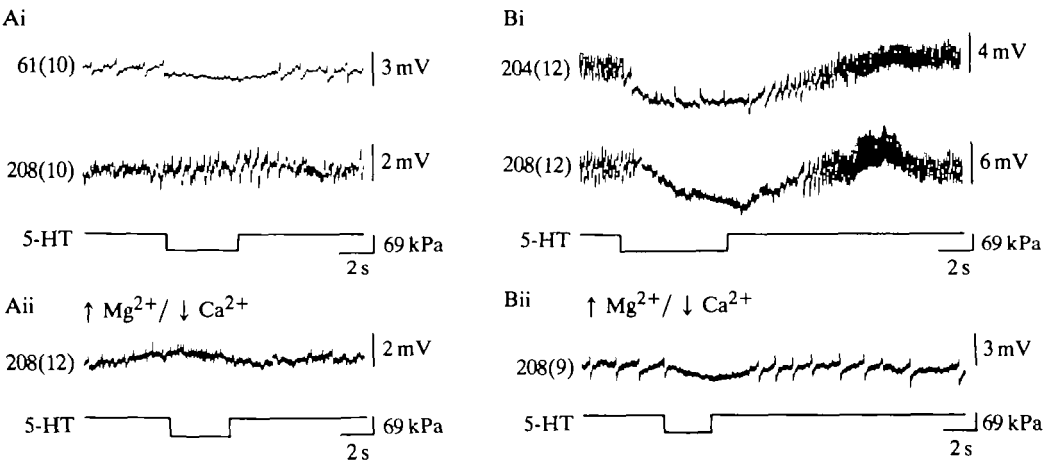

Fig. 8. Effects of serotonin (10^{-4} mol l^{-1}, pH 7·4) (5-HT) pressure ejected into the neuropile on cells 208, 61 and 204. (Ai) The response of cells 208(10) and 61(10) in normal saline, and (Aii) the response of cell 208(12) when the pressure pipette was repositioned (see Bi) in the neuropile with the ganglion bathed in saline with an elevated Mg^{2+} (10 mmol l^{-1}) and a reduced Ca^{2+} (0·45 mmol l^{-1}) concentration, which depresses neurotransmitter release. (Bi) The response of cells 208(12) and 204(12) in normal saline, and (Bii) the response of cell 208(9) when the ganglion was bathed in elevated Mg^{2+} (10 mmol l^{-1}) and reduced Ca^{2+} (0·45 mmol l^{-1}) saline. The recordings of cell 208(12) were from the same preparation. The downward, and upward, inflections of the 5-HT trace indicate the onset and cessation, respectively, of a 69 kPa pulse of serotonin (see Nusbaum & Kristan, 1986).

The other 63% (12 out of 19) of the cells 208 hyperpolarized in response to neuropilar application of serotonin (Fig. 8B). The latency of this response was also usually between 200 ms and 1 s, and it also persisted when neurotransmitter release was depressed by raising the Mg^{2+} and reducing the Ca^{2+} concentrations (Fig. 8Bii). In two additional cells 208, moving the pressure pipette to different regions of the neuropile elicited different responses, as was the case for the cell 208(12) shown in Fig. 8. In many ganglia the serotonin pipette was repositioned in several different locations within the neuropile. In these cases, regions of the anterior, middle and posterior neuropile were tested with no consistent effect observed at any location from ganglion to ganglion. Both excitatory and inhibitory responses in cell 208 to exogenously applied serotonin within the neuropile were also recorded in *Hirudo medicinalis*.

Polysynaptic excitation of cell 208 by cell 61

Evidence for a polysynaptic pathway

Whereas cell 204 directly excites cell 208 in all ganglia (Weeks, 1982*b*), stimulating cell 61 caused only polysynaptic excitation of cells 208 located three or more ganglia posterior to the one in which cell 61 was stimulated (Fig. 9). The strength of this polysynaptic excitation was sensitive to the activity level of cells 208 that were apparently directly excited by cell 61. For example, when cell 208(10) was kept below impulse threshold with hyperpolarizing current (Fig. 9B), the previously strong excitation of cell 208(12) by cell 61(9) was greatly reduced. In general, the direct cell 61 excitation of cell 208 in nearby ganglia was stronger than the indirect excitation of more distant cells 208. The efficacy of this indirect excitation might have been responsible, in part, for whether cells 21/61 initiated a localized or cord-wide swim (Kristan & Nusbaum, 1983; Nusbaum & Kristan, 1986).

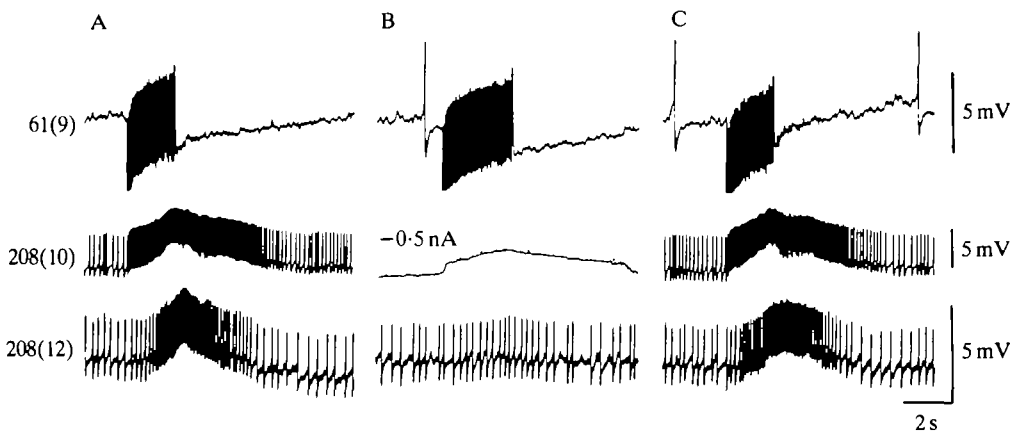

Fig. 9. The effect of cell 61 on cell 208 in distant posterior ganglia. (A),(C) Intracellular stimulation of cell 61(9) (+0·75 nA) with no current injected into either cell 208(10) or cell 208(12) excites both of these cells. (B) Stimulating cell 61(9) (+0·75 nA) with hyperpolarizing current (−0·5 nA) injected into cell 208(10) causes a reduced excitatory response in both cell 208(10) and cell 208(12).

Cell 208 polysynaptically excited posterior cells 208, but weakly inhibited their anterior homologues in *Macrobdella* (Fig. 10). Cell 208 does not excite posterior cells 208, but does inhibit anterior cells 208, in *Hirudo medicinalis* (Weeks, 1982*b*). These interactions between cells 208 serve to reinforce the direct synaptic effects of cell 208 on swim motor neurones.

Cells 18: intermediates in the cell 61 to cell 208 pathway

Intraganglionic connections between cells 18 and 208. A pair of neurones that might mediate this polysynaptic excitation between cells 208 was found; they are designated cells 18 (Muller *et al.* 1981). Both cells 18 were found to be weakly dye-coupled to cell 208 in *Macrobdella* (Fig. 11), but not in *Hirudo*. To detect this dye coupling, it was necessary to fill a cell 208 heavily with Lucifer Yellow and allow the dye to diffuse for approximately 2 h. A similar diffusion time was necessary for Lucifer Yellow injected into cell 18 to pass into cell 208.

Cell 18 extends a single axon out from the ganglion, in the contralateral posterior branch of the posterior segmental nerve (PP nerve) (Fig. 12). The soma of cell 18 is on the opposite (dorsal) surface of the ganglion from that of cell 208 so that direct determination of intraganglionic electrical coupling between these two cell types was not made. Other pairs of dye-coupled cells, however, have been shown to be connected by non-rectifying electrical connections (Stewart, 1981; Muller & Scott, 1981; Margiotta & Walcott, 1983; Piccolino, Neyton & Gerschenfeld, 1984).

The resting potential of cell 18 averaged −45 mV. Like many leech neurones, the impulses appeared to invade the soma passively. The considerably larger diameter of the major neurite close to the soma (Fig. 12) may explain the relatively small impulse amplitude; a signal passively propagated towards the soma would be greatly diminished at this region of increased neurite diameter (Goldstein & Rall, 1974). IPSPs larger than the impulses were routinely recorded (see Fig. 13C), however, suggesting that these synaptic inputs were electrically closer to the soma than was the closest excitable membrane.

Interganglionic interactions from cell 208 to cell 18. Cell 18 received apparently monosynaptic EPSPs from more anterior (Fig. 13A), but not more posterior, cells 208. The fact that cell 208 has only a posteriorly directed axon (Weeks, 1982*b*; Fig. 11) makes a monosynaptic interaction with cell 18 possible. Two other observations further suggest monosynapticity. First, EPSPs in cell 18 followed impulses in cell 208 at a constant latency, even when cell 208 was firing at high frequencies (>40 Hz). Second, the synaptic latency is only 10 ms, much of which must be taken for impulse conduction (Weeks, 1982*b*). Impulse bursts elicited in cell 208 by intracellular stimulation caused a larger depolarization in posterior cells 18 (Fig. 13B) and often an increase in impulse frequency in them (data not shown). Fig. 14 is a schematic summary of the intra- and interganglionic interactions of cells 18 and 208.

Activation of cell 18 by cells 21 and 61. Moderate intracellular stimulation of either cell 21 or cell 61 caused first a depolarization and then a barrage of IPSPs in

cell 18 (Fig. 13Ci). Stronger stimulation of cell 61 increased cell 18's initial barrage of IPSPs (Fig. 13Cii), and made its overall response similar to the membrane potential oscillation that occurs during the onset of swimming (compare Fig. 13Cii to Fig. 15). Even stronger stimulation of cell 61 produced a patterned response in cell 18 similar to a single swim cycle (Fig. 13Ciii). Although the excitatory response in cell 18 had a latency short enough to suggest monosynapticity, impulses in cells 21

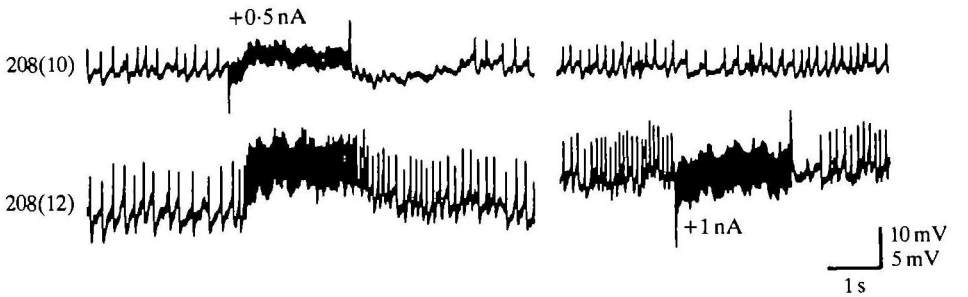

Fig. 10. Interganglionic interactions of cells 208. Paired intracellular recordings of cells 208(10) and 208(12) during which depolarizing current was injected: (left) into cell 208(10) (+0·5 nA), thereby exciting cell 208(12) and (right) into cell 208(12) (+1·0 nA), thereby inhibiting cell 208(10).

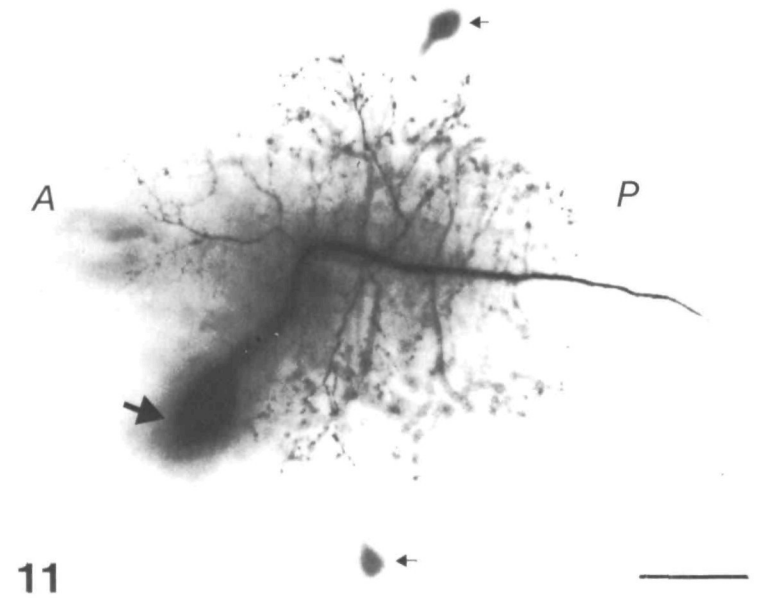

Fig. 11. Cell 208 is dye-coupled to the cell 18 pair. When Lucifer Yellow (Stewart, 1981) was injected into cell 208 (large arrow), the dye also appeared in a pair of laterally located neuronal somata (small arrows). Only the soma and initial segment of the major neurite of each cell 18 is visible at this focal plane. Ganglion viewed in unfixed wholemount. *A*, anterior; *P*, posterior. A negative image of the preparation is shown. Scale bar, 100 μm.

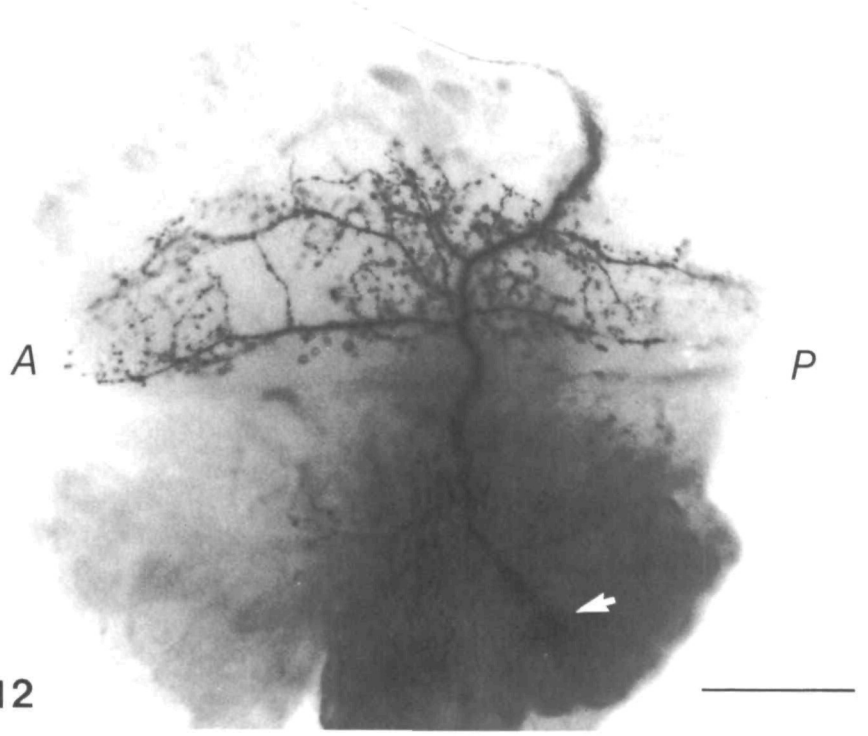

Fig. 12. Morphology of cell 18 determined by Lucifer Yellow injection into cell 18 soma (arrow). The axon of cell 18 leaves the ganglion in the contralateral PP segmental nerve. (In this photograph, the contralateral segmental nerves were crossed and so the axon of cell 18 appears to be leaving in the anterior nerve.) Ganglion viewed in unfixed wholemount. A negative image is shown. *A*, anterior; *P*, posterior. Scale bar, 100 μm.

and 61 did not cause individual EPSPs in cell 18. Cell 204 had similar effects on cell 18 (data not shown).

Cell 18 qualifies as a CPG neurone. Two criteria are used to determine if a neurone is a member of the CPG for a rhythmic motor pattern: (1) the neurone must exhibit rhythmic activity phase-locked to the motor pattern and (2) shortening or lengthening the neurone's activity during the motor pattern must reset cycles of the pattern (Friesen *et al.* 1978). The first criterion was met because cell 18 did undergo membrane potential oscillations during swimming. In fact, its oscillations were cophasic with those of cell 61 and, therefore, with those of cell 208 as well (Fig. 15A). The second criterion was met because prolonged depolarizing (Fig. 15B) or hyperpolarizing current pulses injected into cell 18 during swimming often reset or terminated the swim episode. This effect was not by way of cell 204, whose impulse activity was not affected by current pulses into cell 18 (Fig. 15B).

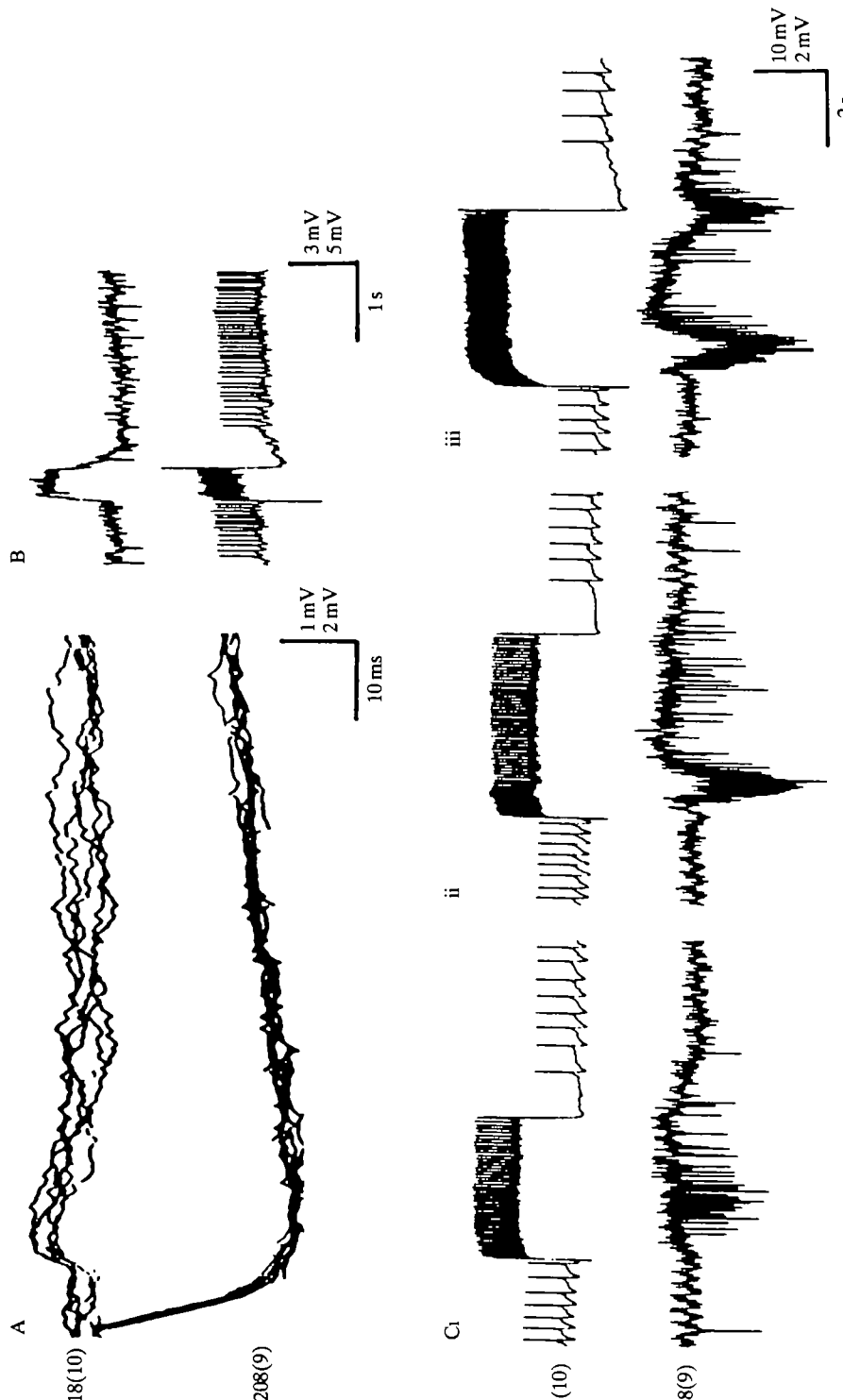

Fig. 13. (A) Cell 208 excites posterior cells 18. Four superimposed oscilloscope sweeps triggered by impulses in cell 208(9) followed by constant latency EPSPs in cell 18(10). (B) The excitatory response of cell 18(10) to intracellular depolarizing current (+1·0 nA) injected into cell 208(9). (C) Cell 61 causes complex responses in cell 18. Panels Ci, ii and iii show the effects of successively stronger stimulation of cell 61(10) on cell 18(9). (Ci, +0·5 nA; Cii, +0·75 nA; Ciii, +1·0 nA.)

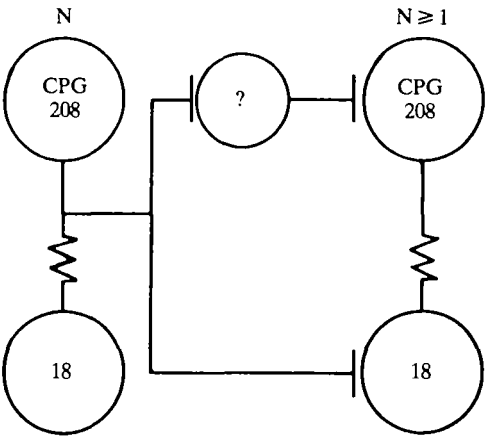

Fig. 14. Schematic diagram of the interactions of cells 18 and 208. The direct excitatory connection from cell 208 (N) to posterior cells 18 is drawn as a chemical excitatory interaction, but whether this connection is chemical or electrical was not examined. The cell labelled '?' indicates that the polysynaptic excitatory pathway from anterior to posterior cells 208 may not be *via* cell 18 but instead may be *via* an unknown cell or cells. The resistor symbol indicates a non-rectifying electrical synaptic interaction. Other labelling as in Fig. 3.

DISCUSSION

Cells 21 and 61 excite cell 208

Cells 21 and 61 initiate swims, at least in part, because of their synaptic excitation of swim central pattern generator neurones (Figs 1, 13; Nusbaum, 1984*b*). The cell 61 excitation of cell 208 appears monosynaptic, but interposed interneurones remain a possibility, because (1) no physiological test can eliminate the possibility of an electrically coupled interneurone (Berry & Pentreath, 1976; Muller & Scott, 1981; Granzow, Friesen & Kristan, 1985) and (2) the levels of Mg^{2+} used in these experiments may not completely block transmitter release (Nicholls & Wallace, 1978).

The lack of a measurable conductance change in cell 208 during excitation from cell 61 might suggest that the interaction is electrical. However, the fact that the PSP amplitude changes with postsynaptic polarization level argues against this. There are alternative possibilities. For instance, the response in cell 208 could result from both a decrease and increase in conductance, due to more than one type of receptor–channel complex being activated, as is the case in the snail *Helix aspersa* (Paupardin-Tritsch, Deterre & Gerschenfeld, 1981) and in individual vertebrate sympathetic ganglion neurones (Kuffler & Sejnowski, 1983; Brown & Selyanko, 1985).

Serotonin effects on cell 208 and swim initiation

The neuronal source and function of the hyperpolarizing response in cell 208 to focally applied serotonin is unknown. Although cell 208 often hyperpolarized in response to exogenously applied serotonin, stimulating cells 21 and 61 always caused a depolarization of cell 208. Such differences in the response to synaptic input and

direct application of the neurotransmitter (histamine) have also been observed in the stomatogastric nervous system of the lobster (Claiborne & Selverston, 1984; J. S. Eisen, personal communication). Multiple types of responses in a single neurone to serotonin application also occur in both vertebrates (Higashi & Nishi, 1982) and other invertebrate systems (Gerschenfeld & Paupardin-Tritsch, 1974; Paupardin-Tritsch *et al.* 1981). The excitatory response to exogenously applied serotonin may

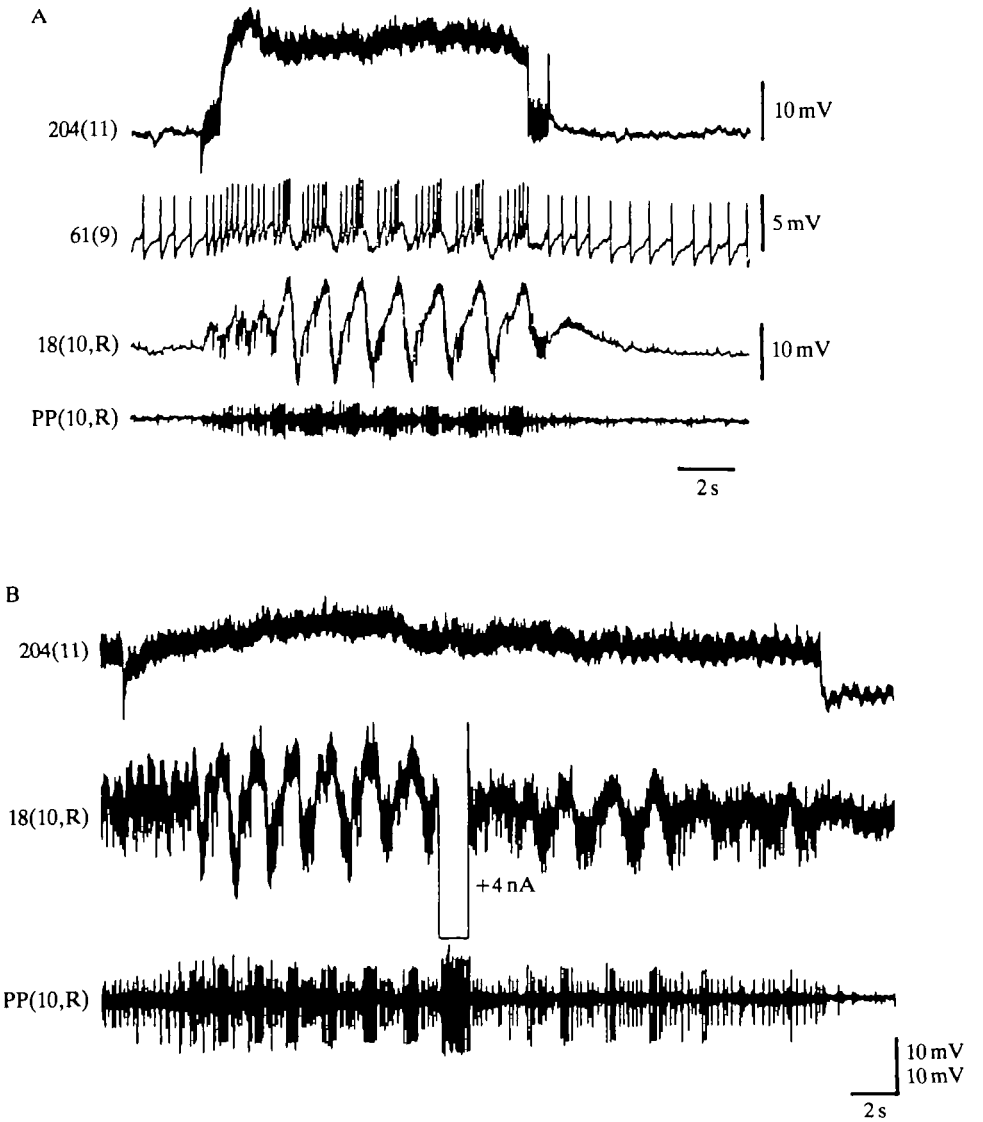

Fig. 15. (A) Activity of cells 61(9) and 18(10) during swimming initiated by intracellular stimulation of cell 204(11) (+1·0 nA). (B) Intracellular stimulation of cell 18(10) (+4·0 nA) during swimming perturbs the swim motor pattern. Swimming was initiated by intracellular stimulation of cell 204(11) (+1·0 nA). In parts A and B, impulse bursts of identified dorsal excitor motor neurones (largest unit) were simultaneously recorded in PP(10,R).

desensitize more readily than the inhibitory response. In the snail *Helix pomatia* an excitatory response on the soma of an identified neurone caused by serotonin ($1{\cdot}5\times10^{-1}$ mol l^{-1}) was reversibly desensitized by bath application of lower concentrations of serotonin (5×10^{-8} mol l^{-1}) which caused no membrane potential change itself (Cottrell & Macon, 1974).

Focal application of serotonin into the neuropile also hyperpolarizes other swim-related neurones in *Macrobdella* (Nusbaum, 1984*b*; Nusbaum & Kristan, 1986). Bath-applied serotonin in *Macrobdella* ($>10^{-6}$ mol l^{-1}) always inhibits these cells as well, which may explain why bath application of these concentrations of serotonin does not initiate swimming in this species as it does in the related leech *Hirudo medicinalis* (Weeks, 1980; Willard, 1981). In *Hirudo*, higher concentrations of serotonin ($>10^{-4}$ mol l^{-1}) inhibit swimming and hyperpolarize swim-related neurones (Nusbaum, 1984*b*). Hence, homologous neurones in these two hirudinid leeches may differ only in the dose dependence of their response to serotonin.

Cell 208 feeds back excitation to cells 21 and 61

The excitatory synaptic effect of cell 208 on cell 21 is the first identified, direct pathway from a swim CPG cell to a swim-initiator cell, as had been proposed to exist by Weeks & Kristan (1978) to help maintain excitation during a swimming episode. This interaction may contribute to the overlapping, phasic activity pattern of cell 208 and the serotonin-containing cells during swimming. A similar feedback has been found from some CPG neurones to the feeding initiator cells in the molluscs *Lymnaea* and *Pleurobranchaea* (Rose & Benjamin, 1981*b*; Gillette, Kovac & Davis, 1982; Kovac, Davis, Matera & Croll, 1983). In both, the cyclic activity of the initiator cells appears to help generate the same behaviour that they initiate.

Role of cell 18

The functional role of cell 18 is not certain. Its anatomy, the phase of the membrane potential oscillations during swimming, and the monosynaptic excitatory input from anterior cells 208 suggest that cell 18 is a dorsal excitor motor neurone (Ort *et al.* 1974; Weeks, 1982*b*; W. B. Kristan & M. P. Nusbaum, unpublished observations). Additionally, Wallace & Gillon (1982) have recently identified a dorsal excitor motor neurone, designated cell 17, that may well be the same cell as cell 18. However, because different aspects of these cells were examined in the two studies, a direct comparison was not possible.

Because of its dye-coupling (Fig. 11) and ability to reset the swimming rhythm (Fig. 15B), cell 18 has electrical contact with the central pattern generator, a property it shares with only one of the previously identified swim motor neurones, cell 1 (which inhibits dorsal longitudinal muscles) (Poon, Friesen & Stent, 1978). However, whether it is truly a pattern generating neurone needs to be further tested. For instance, the amount of current injection needed to reset the swim rhythm may be artifactually high, thereby changing the activity of true CPG neurones by an abnormal means.

Cell 18 may also serve as an interneurone, both from cells 208 to their posterior homologues and from cells 21 and 61 to nearby cells 208. These possibilities could be tested by hyperpolarizing or killing cell 18.

Comparison of the effects of cells 21/61 and 204 on cell 208

Intracellular stimulation of cell 204 initiates swimming more reliably than does stimulation of either cell 21 or cell 61 (Nusbaum & Kristan, 1986). One possible explanation for this is that cell 204 has a greater axonal extent along the nerve cord and hence more extensive access to the segmentally iterated cells of the swim CPG than do cells 21 and 61. As a result of its greater axonal extent, cell 204 directly excites cell 208 in at least 14 ganglia (Weeks, 1982*b*), whereas cells 21 and 61 excite cells 208, apparently directly, in no more than five ganglia. This may also account for the ability of cells 21 and 61 sometimes to initiate the swim pattern in only a localized subset of ganglia (Kristan & Nusbaum, 1983; Nusbaum & Kristan, 1986).

Because of their different types of effects on cell 208, cells 21, 61 and 204 act cooperatively to enhance oscillations in cell 208 during swimming. For example, even the hyperpolarizing phase of these oscillations typically is above cell 208's resting potential, due in part to the tonic excitatory input received from many cells 204 (Weeks, 1981, 1982*b*). This tonic excitation of cell 208 during swimming enhances the excitation from cells 21 and 61. Additionally, because both the serotonin-containing cells and cell 204 are tonically active during the interval between the presentation of a swim-initiating stimulus and swim onset, each of these cells tonically excites cell 208 prior to swim initiation. An augmentation of synaptic excitation by two inputs, one causing a conductance increase-mediated excitation and the other serotonergic pathway causing a conductance decrease-mediated excitation in the postsynaptic cell, has been documented in *Aplysia* (Carew & Kandel, 1976; Klein & Kandel, 1980), and in the mammalian CNS (Wood & Mayer, 1979; Vander Maelen & Aghajanian, 1982).

These results indicate why cells 21 or 61 and 204 have an additive effect upon swim initiation (Nusbaum & Kristan, 1986; see also Nusbaum, 1984*b*). These cells, with the Retzius cells, may well normally act in concert to effect swim initiation in response to naturally occurring stimuli. This hypothesis may be tested by selectively ablating all of the serotonin-containing cells (Glover & Kramer, 1982; Glover, 1984) and observing the effects of stimulation of cell 204 on the swim CPG.

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