Behavioral Function of Glutamatergic Interneurons in the Feeding System of *Lymnaea*: Plateauing Properties and Synaptic Connections with Motor Neurons

MATTHEW J. BRIERLEY, KEVIN STARAS, AND PAUL R. BENJAMIN
Sussex Centre for Neuroscience, School of Biological Sciences, University of Sussex, Brighton, East Sussex BN1 9QG, United Kingdom

Brierley, Matthew J., Kevin Staras, and Paul R. Benjamin. Behavioral function of glutamatergic interneurons in the feeding system of *Lymnaea*: plateauing properties and synaptic connections with motor neurons. *J. Neurophysiol.* 78: 3386–3395, 1997. Intra-cellular recording techniques were used to examine the electrical properties and behavioral function of a novel type of retraction phase interneuron, the N2 ventral (N2v) cells in the feeding network of the snail *Lymnaea*. The N2vs were compared with the previously identified N2 cells that now are renamed the N2 dorsal (N2d) cells. The N2vs are a bilaterally symmetrical pair of electrotonically coupled plateauing interneurons that are located on the ventral surfaces of the buccal ganglia. Their main axons project to the opposite buccal ganglion, but they have an additional fine process in the postbuccal nerve. N2v plateaus that outlast the duration of the stimulus can be triggered by depolarizing current pulses and prematurely terminated by applied hyperpolarizing pulses. Gradually increasing the amplitude of depolarizing pulses reveals a clear threshold for plateau initiation. N2v plateauing persists in a high Mg∗/nominally zero Ca∗ saline that blocks chemical synaptic connections, suggesting an endogenous mechanism for plateau generation. The N2vs fire sustained bursts of action potentials throughout the N2/ retraction phase of the fictive feeding cycle and control the retraction phase feeding motor neurons. The N2vs excite the B3 and B9 feeding motor neurons to fire during the retraction phase of the feeding cycle. They also inhibit the B7 and B8 feeding motor neurons. The B8 cells recover from inhibition and fire during the following swallowing phase. These synaptic connections appear to be monosynaptic as they persist in high Mg∗/high Ca∗ (Hidi) saline that blocks polysynaptic pathways. Strong current-induced plateaus in the N2vs can persist in the absence of extracellular K+ because of the high K+ in the N2v. The plateauing waveform is due to the electrotonic coupling with the N2v cells. The differential synaptic connections of the N2v and N2d cells with retraction phase motor neurons results in a sequence of motor neuron burst activity B9 → B4CL → B8 that produces the full retraction (rasp → swallow) movements of the feeding apparatus (buccal mass). We conclude that the N2v cells are an essential component of the interneuronal network required to produce feeding motor neuron activity.

INTRODUCTION

Rhythmic motor behaviors generally are thought to be generated by networks of interneurons called central pattern generators (CPGs). CPGs have been investigated in both vertebrate and invertebrate model systems where the aim has been to elucidate rhythm generating mechanisms at the level of specific neurons (Getting 1988; Selverston and Moulin 1985). The feeding system of the pulmonate pond snail *Lymnaea* is one example where the individual components of the CPG have been identified and related to rhythmic motor movements involved in food ingestion (Benjamin and Elliott 1989). In this snail, the CPG forms a premotor network distinct from motor neurons. A full understanding of the mechanism by which the CPG generates the cyclical feeding movements requires a detailed knowledge of the numbers and types of CPG interneurons as well as the synaptic connections between CPG interneurons and motor neurons. In *Lymnaea*, motor neurons are driven by CPG neurons to produce a complex triphasic sequence of muscle activity underlying the ingestion of food (Rose and Benjamin 1979).

The discovery of a new type of N2 interneuron the N2 ventral (N2v) cell, active during the retraction phase of the feeding cycle, led us to characterize the firing pattern of the cell and its synaptic connections with motor neurons. We will show that the N2v cells are plateauing neurons that play a crucial role in driving the motor neurons generating the retraction phase (rasp-swallow) of feeding, allowing aspects of the motor mechanisms of motor neuron patterning underlying food ingestion to be understood fully for the first time. Plateauing cells have been found in other molluscan feeding systems (Arshavsky et al. 1988a–c; Quinlan et al. 1995) and are an important feature of CPG organization.

*Lymnaea* is a grazing herbivore that carries out cyclical feeding or biting movements using a toothed radula to scrape food, usually algae, from the surface of floating pond weed or other substrates (Benjamin 1983). The feeding apparatus, the buccal mass, together with the contained odontophore and radula, rotate forward and backward during each feeding cycle and create a characteristic feeding “trail” on an algal substrate. Rose and Benjamin (1979), by combining cinemophotography with muscle recording, recognized that a feeding cycle consisted of protraction of the radula followed by two phases of retraction, called rasp and swallow. Each of these three phases was produced by a single unit of coordinated motor neuron-muscle activity. Specific protraction, rasp and swallow phase motor neurons were identified together with the muscles they innervated (Rose and Benjamin 1979). The motor neurons were driven by identified classes...
of pattern generator interneurons N1, N2, and N3 that fire during only one phase of the feeding cycle (N1, protraction; N2, rasp; N3 swallow). They provided both inhibitory and excitatory inputs, appropriately, to drive the motor neurons (Elliott and Benjamin 1985a; Elliott and Kemenes 1992; Rose and Benjamin 1981a) during the correct phase of the feeding cycle. The synaptic connectivity and endogenous properties of the N1–N3 cells have been interpreted (Elliott and Benjamin 1985a) to produce an informal model of rhythm generation (Benjamin and Elliott 1989). As well as three types of CPG interneurons, there are modulatory interneurons that can initiate and control the frequency of CPG oscillation. One of these is the buccal ganglion slow oscillator (SO) interneuron (Rose and Benjamin 1981b).

It now is realized that there are subtypes within each N cell population of CPG interneurons. This originally was recognized for the N3 cells, where N3t (tonics) and N3p (phasics) were identified (Elliott and Benjamin 1985a). The two types of N3 cells had distinct firing patterns but also different types of synaptic effects on retraction phase motor neurons. They were excitatory on the B3 cells (N3t cells) and inhibitory on B4 and B8 cells (N3p cells). Recently a second type of N1 cell has been described, the N1L (N1 lateral) (Yeoman et al. 1995), that clearly can be distinguished from the original N1 cell type (Elliott and Benjamin 1985a), now called N1M (N1 medial). N1L cells, like the SO and CV1s, drive a high-frequency fictive feeding rhythm in the isolated CNS, but they also have widespread synaptic connectivity with other CPG interneurons. Thus the N1L cells have hybrid properties partly resembling modulatory neurons like the SO, but they also form part of the CPG (Yeoman et al. 1995).

The N2 retraction phase interneurons are the subject of the present paper. The N2v cells are compared with the original N2 cells of Elliott and Benjamin (1985a), now renamed the N2d (N2 dorsal) cells, in their effects on feeding behavior. It will be shown that the plateauing properties of the N2v cells and the differential excitatory and inhibitory synaptic connections of the N2d and N2v cells with retraction phase motor neurons are essential for producing the rasp-swallow phase of the feeding cycle. Together with other interneuronal subtypes of the buccal feeding CPG network, they are required to produce the full pattern of motor neuron activity underlying the ingestion of food in Lymnaea. Evidence presented in the following paper (Brierley et al. 1997a) will suggest that the N2v cells are glutamatergic.

**METHODS**

**Animals**

Wild type *L. stagnalis* (shell length >2 cm) were obtained from Blades Biological, Cowden, Kent, UK, and housed in aquarium tanks containing copper-free Brighton tap water, maintained at 18–20°C. They were subjected to a 12 h light/dark regime and fed lettuce ad libitum.

**Isolated CNS preparation and intracellular recording**

Simultaneous intracellular recordings were made from up to four neurons in the isolated CNS using standard techniques. The isolated preparation consisted of the main ring of periesophageal ganglia together with the paired buccal ganglia, from which most of the recordings were made. Desheathed, enzymically treated ganglia were pinned to the base of a silicone elastomer (Sylgard; Dow Corning)-coated chamber of 1 ml volume. N2-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid-buffered *Lymnaea* saline (Benjamin and Winlow 1981) was perfused continuously through the chamber. Two other salines were used (composition in Yeoman et al. 1993). One, a high Mg<sup>2+</sup>/low (nominally zero) Ca<sup>2+</sup> chemical saline, was used to block chemical synapses. The other, a high Ca<sup>2+</sup>/high Mg<sup>2+</sup> (HiDi) saline, was used to block polysynaptic connections. Glass microelectrodes (15–30 MΩ) were filled with 4 M potassium acetate. For studying the morphology of the N2v neurons, electrodes were filled with Lucifer yellow (3–5% in distilled water) and dye filling achieved by passing hyperpolarizing current pulses for 10–15 min. Fixation, clearing, and reconstruction of cell morphology in whole mounts was carried out as described in Elliott and Benjamin (1985a).

**Twisted preparation**

The newly discovered N2v cells are located on the ventral surface of the buccal ganglia (Fig. 1C), and to record them with the previously described dorsally located buccal neurons, it was necessary to twist one of the paired buccal ganglia through 180° to allow simultaneous viewing of the ventral and dorsal surfaces of the opposite buccal ganglia. As most cells have bilaterally symmetric partners on both buccal ganglia, it then was easy to record equivalent cells on either left or right sides.

**Identification of cells**

The paired N2v cells were identified on the basis of cell body location (Fig. 1C) and their characteristic plateauing potentials (Fig. 2A). They have consistent synaptic connections with feeding motor neurons and CPG interneurons. Recording the N2v cells with at least one of these other cells allowed us to confirm their identity. They also have a characteristic morphology when compared with other *Lymnaea* feeding neurons (Elliott and Benjamin 1985a; Yeoman et al. 1995) as described in RESULTS. The N2d CPG interneurons are a single pair of small cells located just anterior to the N2v cells. They are required to produce the full pattern of motor neuron activity underlying the ingestion of food in Lymnaea. Evidence presented in the following paper (Brierley et al. 1997a) will suggest that the N2v cells are glutamatergic.

**Characterization of N2v cells**

The N2v cells are a pair of bilaterally symmetrically located small white neurons (20-μm diam) the cell bodies of...
which are located in the ventral surfaces of the paired buccal ganglia close to the buccal commissure (Fig. 1C). Lucifer yellow or 5(6)-carboxyfluorescein injection of N2v cells from either left or right buccal ganglia showed that they have similar but mirror-imaged morphology (Fig. 1, A and B). All of the 18 cells that were reconstructed projected to the opposite buccal ganglion neuropile where they terminate with a variable number of branches. Extensive neurite branching occurred in both buccal ganglia, but typically it was more extensive in the ipsilateral buccal neuropile (Fig. 1, A and B). A finer side branch of this main axon in both left and right N2vs projected into the single postbuccal nerve root leaving the buccal commissure (Fig. 1, A and B).

Often, recorded N2v cells were spontaneously rhythmically active, and left and right cells showed striking patterns of synchronous burst activity (Fig. 2A). Bursts of truncated spikes were superimposed on large plateau potentials, the most characteristic feature of these cells. Although plateau potentials occurred in the previously described N2d cell type of Elliott and Benjamin (1985a), the size of the potentials in the N2v cells were much larger and truncated spikes were never seen in the former cell type.

One important reason for the synchronous bursting of the N2v cells was the strong electrotonic coupling between left and right cells. Hyperpolarizing one cell via the recording electrode hyperpolarized the other in a similar manner and coupling was strong in either direction (Fig. 2B). The strength of the DC coupling (ratio of voltage in postsynaptic cell to that in the presynaptic) was ~30% and was sufficient for one cell to drive the other if one of the cells was sufficiently depolarized to plateau (Fig. 2C).

Driving a fictive feeding rhythm in the isolated buccal ganglia, by injection of current into the modulatory cell type known as the SO, showed that the bursting pattern of the N2v could be entrained to the rest of the feeding network. Based on previous analysis of the feeding rhythm (Rose and Benjamin 1979), they fired during the rasp/N2 phase of the feeding cycle. This coincided with periodic hyperpolarization of the SO interneuron and the swallow phase B8 motor neuron recorded at the same time (Fig. 3).

In a SO-driven rhythm, plateau potentials were triggered by synaptic prepotentials (Fig. 3, \(\rightarrow\)) as were spontaneously active N2vs (Fig. 2A). These prepotentials arise from chemical synaptic input from the SO and N1 cells of the CPG feeding network (Brierley et al. 1997b). However, plateau potentials could be evoked by depolarizing current injection in the absence of SO-driven plateauing, and once evoked they could be terminated prematurely by hyperpolarizing current pulses (Fig. 4A, \(n = 10\)). Spontaneously active N2v, where plateaus were triggered synaptically, also were terminated prematurely by application of hyperpolarizing pulses (Fig. 4B). Another feature of plateauing neurons found in other systems (Kiehn 1991) is that plateaus should be triggered at a critical threshold of depolarization. An example of this (\(n = 5\)) is shown in Fig. 4C where gradually increasing the size of depolarizing square current pulses eventually

**FIG. 1.** Location and anatomic characterization of the buccal ganglia N2v cells. Camera lucida drawings of a right (A) and left (B) N2v cell filled with 5(6)-carboxyfluorescein and viewed under ultraviolet light. Main axon projects to the opposite buccal ganglion where it terminates, whereas a 2nd fine axonal process exits via the postbuccal nerve (PBN). Postbuccal nerve in A was twisted during the dissection so it now points anteriorly. Finer neurite branching occurs in both buccal ganglia. C: location of cell bodies on ventral surfaces of the buccal ganglia. A, anterior; CBC, cerebro-buccal connective; DBN, dorso-buccal nerve; L, left; P, posterior; R, right. Scale bars: 250 \(\mu\)m.

**FIG. 2.** Firing pattern and electrotonic coupling of the N2v cells. A: simultaneous intracellular recording of spontaneous activity in left and right cells. Bursts of truncated spikes are superimposed on plateauing waveforms. Complex synaptic prepotentials (\(\rightarrow\)) are present on both cells. B: hyperpolarizing square current pulses were applied to 1 and then the other N2v cell, producing similar but attenuated responses on the cell. C: triggering a plateau by current injection into the right N2v drives a similar plateau potential in the left-side cell.
led to the triggering of a plateau potential that lasted the duration of the injected current. Long-lasting depolarizing current pulses evoked sequences of N2v plateaus that showed a consistent potential for spike triggering (horizontal dashed line in Fig. 4Di). This experiment was carried out in high Mg\(^{2+}\)/nominally zero Ca\(^{2+}\) saline that had blocked chemical synaptic transmission (Fig. 4Dii) in the same preparation. This experiment shows that N2vs are capable of terminating plateaus by a presumably endogenous mechanism. The results shown in Fig. 4 present firm evidence that the N2vs are true plateauing cells.

**N2v excitatory synaptic connections to B3 and B9 motor neurons**

Both the B3 and B9 motor neurons fired due to depolarization during the N2/rasp phase of feeding cycles (Benjamin and Rose 1979; Rose and Benjamin 1981a). Figure 5, Ai and Bi, showed that this depolarization coincided with the firing of the N2vs during spontaneous burst activity in the isolated preparation. This suggested that the N2vs have excitatory synaptic connections to the B3 and B9 cells. This was examined in more detail by evoking bursts of spikes in the N2v and looking at the responses on the motor neurons. Figure 5Aii shows that the N2v evoked a large depolarizing postsynaptic input in a B3 cell, and responses in the B9 cell were of a similar type (Fig. 5Bii), leading to strong bursts of action potentials. These responses could be due to monosynaptic connections. In the case of the B3 cell type, it was likely to be monosynaptic because the response still persisted in HiDi saline (see Fig. 7). Current-injected activation of the N2v inevitably triggered a plateau potential accompanied by high-frequency firing. This made it impossible to determine whether N2v spikes were followed 1:1 by postsynaptic potentials in the B3, B9, and other motor neurons.

**N2v and N2d cells have differential inhibitory synaptic inputs to B4CL, B7, and B8 motor neurons**

All of these cell types were shown previously to receive inhibitory synaptic inputs during the N2/rasp phase of the feeding cycle (Benjamin and Rose 1979; Rose and Benjamin 1981a). They recover from this hyperpolarization and fire bursts of spikes at least partly due to postinhibitory rebound (Benjamin and Rose 1979). However, the duration of the N2 inhibitory input varies depending on the cell type, so the cells recover and fire in a defined sequence rather than exactly synchronously. The B4CL (B4 cluster) cells receive the briefest N2 inhibitory input and recover and fire first during the rasp phase of the feeding cycle defined by Rose and Benjamin (1979). B8 cells received a longer duration hyperpolarizing synaptic input during a N2/rasp phase than the B4CL cells, and consequently they fired later in the feeding cycle than the B4CL cells during the final swallow phase of the feeding cycle (Rose and Benjamin 1979). The B7 cells also receive long-duration N2 inhibition like the B8 cells, but due to their receipt of N3 inhibition and N1 phase excitation, they fire during the protraction phase of the feeding cycle (Rose and Benjamin 1981a). The N2vs are candidates for providing inhibitory synaptic inputs to these various cell types as they fired appropriately during the N2 phase of a fictive feeding pattern (Fig. 6A). This was tested by evoking bursts of spikes in the N2vs. This elicited clear hyperpolarizing responses in B7, B4CL, and B8 cells (Fig. 6, B and C). The responses in the B7 and B8 cells persisted in HiDi saline (Fig. 7), so they were probably monosynaptic. However, the inhibitory responses on the B4CL cells were shorter in duration and delayed compared with those recorded in B8 (Fig. 6C). This suggested that the N2vs were not directly inhibiting the B4CL cells.

Evidence will be presented later to show that the N2v cells are strongly electrotonically coupled to the second type of N2 cell, the N2ds, and can drive them to produce one or two spikes (Fig. 9). Could the N2ds therefore be more directly responsible for the shorter duration inhibitory postsynaptic potentials (IPSPs) on the B4CL cells seen when bursts of spikes are evoked in the N2vs (Fig. 6C)? Direct evidence for this is shown in Fig. 8. Evoking a burst of spikes in the N2d cell briefly inhibited both B4CL cells recorded at the same time, and this was a consistent response in five experiments where the B4CL cells were sampled randomly. Experiments from an earlier paper (Elliott and Benjamin 1985a) also showed short-duration unitary IPSPs accompanying N2d spikes on B4CL cells, consistent with the present result. In some non-SO-driven rhythms, the N2v cells spontaneously plateaued without any obvious activity in other elements of the CPG (Fig. 8B), despite the electrotonic coupling between the N2v and N2d cells. Here N2v bursts are not accompanied by any synaptic input on the B4CL cells (3 were recorded in Fig. 8B). This confirmed the lack of N2v synaptic connections with the B4CL cells, and it appeared that the N2d cells alone provide the inhibitory input to these retractor motor neurons during the N2/rasp phase of the feeding rhythm.

**Electrotomic coupling of N2v and N2d cells**

In twisted preparations, it was possible to record N2v and N2d cells at the same time. In both spontaneous (Fig. 9A) and
Electrotonic coupling between the N2v/N2ds was indicated in Fig. 9C where hyperpolarizing either N2d or N2v cell type with square current pulses via the recording electrode induced similar but attenuated responses in the opposite cell type. For hyperpolarizing pulses of approximately the same size, the ratio of post synaptic voltage to presynaptic (DC coupling ratio) was similar (~30%, Fig. 9C) indicating that the strength of coupling was similar in both directions. Comparison of coupling for depolarizing pulses was more difficult because depolarizing the N2vs evoked plateau potentials (Fig. 9, C and D), whereas this did not occur in

SO-driven fictive rhythms (Fig. 9B), the two cell types fire in the same (N2) phase of the feeding cycle, but the N2d only fires one or two spikes compared with the strong bursts always seen in the N2v. As was previously reported (Elliott and Benjamin 1985a), the N2d cells show a tendency to plateau, particularly in SO-driven rhythm (Fig. 9Bi) but this tendency is much less striking than the N2v cells (Fig. 9, A and Bi). In fact, evidence presented in Fig. 9C suggested that the so-called plateau potentials of the N2d cells may be at least in part electrotonically transmitted coupling potentials due to the plateauing properties of the N2v cells.
CONTROL OF RETRACTION BY SNAIL FEEDING INTERNEURON

FIG. 5. N2v excitatory effects on B3 and B9 motor neurons. 


the N2ds. Indeed, unlike the N2vs, it was impossible to trigger plateau potentials in the N2ds by any form of artificial depolarization even when this was suprathreshold for spiking (Fig. 9D, bottom). Evidence in Fig. 9D indicated that the “plateau potentials” on the N2ds (Elliott and Benjamin 1985a) are in fact electrotonically transmitted potentials with a similar “square” shape to triggered plateaus in the N2v cells. In this experiment, a plateau in the N2v, evoked by current injection, induced a coupling potential in the N2d similar in form to that described by Elliott and Benjamin (1985a) as a “plateau potential”. It appeared that the large depolarizations of the N2v cell due to genuine plateauing are being transformed by electrotonic coupling into the smaller “plateau-like” potential recorded in the N2d cells. In contrast, strong current injection into the N2d in the same experiment, because it did not trigger a N2d plateau, induced only a small depolarization on the N2v and no spikes (Fig. 9D).

Electrotonic coupling and dual component responses on motor neurons

In quiescent preparations, current-induced bursts of N2v spikes often induced dual component responses in motor neurons of the B3, B7, B8 type. In the B7 cell, an initial hyperpolarizing phase (i1) was followed by a second, larger amplitude hyperpolarization (i2) in Fig. 10Ai. In the small number of experiments (n = 3) where it was possible to record a N2v and N2d with the B7 cell, it could be seen that the N2v and N2d responses were delayed suggesting an indirect synaptic connection.
or Fig. 6B (B7 cell). Except in SO-driven rhythms, the N2v cells are always more likely to be active than the N2ds due to their strong tendency to generate plateaus.

Dual responses also are seen in the B3 motor neurons after N2v bursts (Fig. 10B), but here the responses are both depolarizing (e1, e2). However, unlike the B7 cell, the main components of dual response in the B3 cell were not due to the N2d cell. In the experiment shown in Fig. 10B, a burst of artificially evoked N2v spikes caused the initial depolarization of the N2d recorded at the same time. In Fig. 10Bi, this evoked a spike in the N2d but this occurred after the start of the e2 component in B3. Further confirmation that the e1/e2 components were not due to N2d activity is shown in Fig. 10Bii where both components of the B3 response persisted in the absence of any spike activity in the N2d. The N2d cell does in fact have a weak excitatory effect on the B3 cell (Fig. 10C), but in the record shown in Fig. 10Bi, it only causes a slight inflection (arrowed) in the falling phase of the much larger e2 synaptic potential. We interpret this to mean that the N2v is responsible for both e1 and e2 phases of the B3 response because both phases persist in HiDi saline (Fig. 11), a treatment that blocks polysynaptic chemical synapses. However, we still cannot

that the initial component of the inhibitory response is due to spikes in the N2v cell, whereas the delayed component results from the addition of N2d spikes (Fig. 10Ai). The ability of the N2v cell to drive the N2d spikes was presumably due to the electrotic coupling described in Fig. 9. N2d spikes were evoked by a depolarizing waveform from 0.1 to 2 s after the initiation of the plateau in the N2v cell. Confirmation that the second (i2) component of the B7 response is due to the N2d spikes is shown in Fig. 10Aii, from the same experiment. In this record, evoking activity in the N2v cell failed to generate spikes in the N2d cell and the delayed (i2) component was absent. Interestingly, it appeared that only one or two spikes in the N2ds could enhance the already strong hyperpolarization initially produced by the strong burst of N2v spikes. The ability of the N2d cells to further enhance an already large hyperpolarization due to N2v relies on the initial response to N2v because at recorded membrane potential, the N2d only hyperpolarized the B7 by a few millivolts (Fig. 10Aiii). An alternative explanation for i2 is that strong activation of the N2v cells activated a further cell type(s) that also fires during the N2 phase and is at present unknown. As indicated in Fig. 9Aii, evoking bursts of spikes in N2v does not always generate spikes in the N2d cell, and this accounts for the lack of N2d input seen in motor neuron records like Fig. 8B (B4Cl cells).
CONTROL OF RETRACTION BY SNAIL FEEDING INTERNEURON

inputs from protraction phase interneurons like the SO and N1Ms (Elliott and Benjamin 1985a), providing the possibility of modulatory effects. The modulatory effects on the N2d cells by other members of the CPG network will be considered in more detail by Brierley et al. (1997b).

N2v → motor neuron synaptic connections

The N2vs have the appropriate synaptic connections to be important controllers of motor neuron activity. They are dual action interneurons with evidence that they excite (B3, B9) or inhibit (B7, B8) feeding retractor motor neurons (summarized in Fig. 12) via probable monosynaptic connections (HiDi experiments). The only retractor motor neurons that are not directly innervated by the N2vs are cells of the B4CL network.

The N2vs appeared to have dual component responses in B3 cells, with an initial small excitatory postsynaptic potential (EPSP) and a delayed larger amplitude EPSP. The delayed component was likely to be most significant in driving spike activity in the motor neuron. Other cells (e.g., B7) also received dual component synaptic inputs, but the second phase was attributed to the N2ds.

Plateauing neurons in the Lymnaea feeding system

N2v cells are an example of plateauing neurons thought to be important in generating rhythmic motor activity in a number of vertebrate (e.g., Kiehn 1991) and invertebrate systems (e.g., Dickinson and Nagy 1983). In molluscan feeding systems, this property first was described for the N2d cells (Elliott and Benjamin 1985a) of *Lymnaea*, but, as will be discussed later, there is doubt about whether these are really capable of plateauing. Cells with similar plateauing properties are important in the related snails *Planorbis* (Arshavsky et al. 1988a–c) and *Helisoma* (Quinlan et al. 1995). Plateau potentials in the N2v cells can occur spontaneously in isolated buccal ganglia, be driven and entrained to the fictive feeding rhythm by injecting current into the modulatory cell called the SO or triggered by brief depolarizing pulses. The ability to trigger plateaus that outlast the period of stimulation and prematurely terminate them by hyperpolarizing pulses is an important feature of plateauing cells in other motor systems (Kiehn 1991) as is the ability to trigger plateaus at a particular threshold of current injection (Fig. 4). In saline conditions where chemical synapses were blocked, it was still possible to trigger plateaus at particular threshold membrane potentials and they would self-terminate. This suggests that the basic mechanism of plateau production is due to endogenous mechanisms.

Whether the N2d cells are really plateauing cells became an important question in this paper. Simply depolarizing the N2d in a quiescent preparation did not trigger plateau potentials, and the plateau depolarizing waveform could be explained by the strong electrotonic coupling between the N2v and N2d cells. Thus a “depolarizing plateau” could be observed on the N2d cells when a plateau was first evoked by current injection into the N2v. However, these experiments were carried out in quiescent preparations, and we could not rule out the possibility that true plateauing in the N2d cells may depend on release of a modulator from other members of the feeding interneuronal network that would normally be active in fictive feeding rhythms. Under these conditions, plateaus are triggered in the N2d cells by synaptic
N2v/N2d control of the rasp/swallow phases of the feeding cycle in Lymnaea

Retraction of the buccal mass in Lymnaea is divided into two phases, R (retraction) 1 or rasp followed by R2 or swallow (Rose and Benjamin 1979). These follow the initial protraction phase (P) of the Lymnaea triphasic feeding cycle. The main retractor muscle of the buccal mass is the anterior jugalis muscle (ajm), and this is active during both rasp and swallow, together with several other muscles (Rose and Benjamin 1979). During rasp, the lower part of the ajm, together with the radula tensor muscles, are active, and the upper part of ajm contracts later during swallow. A continuous sequence of contractions occurs from lower to upper regions of the ajm, ensuring a smooth backward rotation of the buccal mass and radula during both retraction phases (cinematographic analysis in Rose and Benjamin 1979). This is due to a sequence of motor neuron activity B9 → B4CL → B8 (Fig. 13A). In the full fictive feeding pattern shown in Fig. 13B, the B9 and B4CL neurons fire most strongly during the rasp phase of feeding cycle, whereas the B8 neurons fire during swallow (Rose and Benjamin 1979). These motor neurons also have the correct muscle innervation patterns to control the appropriate phase of buccal mass movements. Thus the B4CL neurons innervate the lower part of the ajm and the radula tensor muscles (3 pairs of these stretch the radula over the odontophore “cartilage”) and thus control rasp. The B8 neurons innervate the upper ajm and therefore control swallow (Rose and Benjamin 1979). The B9 neurons’ innervation pattern has not been investigated, but they probably innervate the lower portion of the anterior jugalis. This would be consistent with their firing pattern, which coincides with the whole of the rasp phase of the feeding cycle (Fig. 13, A and B).

The sequence of motor neuron activity B9 → B4CL → B8 (Fig. 13A) that leads to coordinated rasp → swallow movements during retraction of the radula is explained by the differential synaptic inputs these motor neurons receive from the N2v and N2d retraction phase interneurons (summarized in Fig. 13B). The B9 cells fire first due to the N2v → B9 excitatory synaptic connection. The B4CL cells are inhibited briefly by the N2d cells and recover and fire. Their activity overlaps with the B9 cells because the N2v-induced EPSP on the B9 cell is much longer in duration than the N2d-induced IPSP on the B4CL cells. The B8 cells fire after the B4CL cells because they receive long-duration inhibitory inputs from the N2v cells. They recover and fire a long burst probably due to postinhibitory rebound (PIR), like the B4CL cells (Benjamin and Rose 1979). Thus B9/ B4CL motor neurons cause the initial R1, retraction phase of the buccal mass movement known to be involved in radula rasps and B8 cells the R2, retraction phase necessary for swallow (Rose and Benjamin 1979).

In other pulmonate feeding systems where plateauing retraction phase CPG interneurons have been found (Planorbis: Arshavsky et al. 1988c; Helisoma: Quinlan et al. 1995), only one type of cell has so far been described. The S2 of Helisoma has a similar morphology to the N2v of Lymnaea, but it occurs on the dorsal surface of the buccal ganglia. It may be homologous to the N2d cells of Lymnaea although the glutamate receptor targets of the Lymnaea N2v have different pharmacological properties to those of the S2s of Helisoma (Brierley et al. 1997a). The group 2 neurons of Planorbis mainly occur on the dorsal surface of the buccal ganglia in an approximately similar location to the N2d cells of Lymnaea (Arshavsky et al. 1988a), but they have a different morphology to the Lymnaea N2d and N2v cells (Elliott and Benjamin 1985a; present paper). Only one type of group 2 cell has been found, but this may not be surprising as there appears to be only one phase of retraction compared with the two in Lymnaea. The CPG feeding network of the predatory mollusc Clione also has been studied (Arshavsky et al. 1989) but its interneuronal organization is different and the cellular homologues to the pulmonate retraction phase CPG interneurons difficult to ascertain.

We thank A. Bacon for typing the manuscript and G. Kemenes and M. Yeoman for reading the manuscript. G. Kemenes also helped with the dye-filling experiments.

This work was supported by a graduate studenthip and research grant from the United Kingdom Biotechnology and Biological Sciences Research Council.

Present address of M. J. Brierley: Dept. of Life Sciences, University of Nottingham, University Park, Nottingham NG7 2RD, UK.

Address for reprint requests: P. R. Benjamin, Sussex Centre for Neurosciences, School of Biological Sciences, University of Sussex, Falmer, Brighton, East Sussex BN1 9QG, UK.

Received 29 October 1996; accepted in final form 8 August 1997.
REFERENCES


