The neuronal basis of feeding in the snail, *Helisoma*, with comparisons to selected gastropods

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**Abstract**

Research on identified neurons during the last quarter century was forecast at a conference in 1973 that discussed ‘neuronal mechanisms of coordination in simple systems.’ The focus of the conference was on the neuronal control of simple stereotyped behavioral acts. Participants discussing the future of such research called for a comparative approach; emphasis on structure–function interactions; attention to environmental and behavioral context; and the development of new techniques. Significantly, in some cases amazing progress has been made in these areas. Major conclusions of the last quarter century are that so-called simple behaviors and the neural circuitry underlying them tend to be less simple, more flexible, and more highly modulated than originally imagined. However, the comparative approach has, as yet, failed to reach its potential. Molluscan preparations, along with arthropods and annelids, have always been at the forefront of neuroethological studies. Circuitry underlying feeding has been studied in a handful of species of gastropod molluscs. These studies have contributed substantially to our understanding of sensorimotor organization, the hierarchical control of behavior and coordination of multiple behaviors, and the organization and modulation of central pattern generators. However, direct interspecific comparisons of feeding circuitry and potentially homologous neurons have been lacking. This is unfortunate because much of the vast radiation of the class Gastropoda is associated with variations in feeding behaviors and feeding apparatuses, providing ample substrates for comparative studies including the evolution of defined circuitry. Here, the neural organization of feeding in the snail, *Helisoma*, is examined critically.

Possible direct interspecific comparisons of neural circuitry and potentially homologous neurons are made. A universal model for central pattern generators underlying rasping feeding is proposed. Future comparative studies can be expected to combine behavioral, morphological, electrophysiological, molecular and genetic techniques to identify neurons and define neural circuitry. Digital resources will undoubtedly be exploited to organize and interface databases allowing illumination of the evolution of homologous identified neurons and defined neural circuitry in the context of behavioral change. © 2001 Elsevier Science Ltd. All rights reserved.

**Keywords:** Aplysia; Heterobuccal nerve; Ventrobuccal nerve; Postsynaptic nerve

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**Abbreviations:** A, Aplysia; aj, anterior jugalis; B, buccal; BC, buccal commissure; BCN1, buccocerebral neuron, type 1; C, Clione; CBC, cerebrobuccal connective; con, Contralateral; CPG, central pattern generator; DBN, dorsobuccal nerve; E, excitatory; EPSP, excitatory postsynaptic potential; ET, esophageal trunk; GN, gastric nerve; H, Helisoma; HBN, Heterobuccal nerve; I, inhibitory; in, influential; Ip, ipsilateral; IPSP, inhibitory postsynaptic potential; L, Lymnaea; LBN, laterobuccal nerve; MN, motor neuron; nor, nitric oxide responsive; P, Planorbis; PBN, posteriorbuccal nerve; pj, posterior jugalis; PSP, postsynaptic potential; rad tens, radular tensor; RN, radular nerve; S1, subunit 1; S2, subunit 2; S3, subunit 3; SN, salivary nerve; slrt, supralateral radular tensor; SRT, subradular tissue; VBN, ventrobuccal nerve.

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1. Introduction

There was a remarkable symposium, titled ‘Neuroid and Neuronal mechanisms of Coordination in Simple Systems’, held at the annual meeting of the American Society of Zoologists in December, 1973 (Kammer and Westfall, 1974). The symposium summarized the state of research, a quarter century ago, on mechanisms of coordination in a number of systems of identifiable neurons as well as in non-neural systems. The focus was on the neuronal (and non-neuronal) control of simple ‘stereotyped’ behavioral acts, or, in the ethological language of the time, the neural bases underlying ‘fixed action patterns’ (e.g. Kater, 1974; Kristan, 1974; Lent, 1974; Mayeri et al., 1974; Naitoh, 1974; Selverston, 1974; Spencer, 1974; Stein, 1974). Hierarchical interactions among potential behaviors and the modulation of behavior by learning were addressed (Davis et al., 1974). Participants discussed the future of such research. They called for, a comparative approach; emphasis on structural and functional interactions; attention to the environmental and behavioral context in which the experimental subject normally functions; and the development of new techniques. In addition, the marriage of genetics and neurobiology was presaged at the conference (Hoy, 1974; Ikeda and Kaplan, 1974; Levinthal, 1974), though the recent explosion of molecular biology and its effects on neurobiology could not be anticipated.

In the ensuing quarter century, significant progress has been, and continues, to be made in each of these areas. A consensus has arisen that the behavioral outputs of such simple systems is not so stereotyped as first thought and that even simple neural circuitry is highly modulated (e.g. Harris-Warrick and Marder, 1991; Marder and Calabrese, 1996). Progress in neurogenetics has exploded. New staining and recording techniques and application of molecular biology techniques have illuminated structure–function relationships at levels unimagined in 1973. Perhaps the area of inquiry proposed at the symposium 25 years ago that has fulfilled its potential least is the emphasis on comparative stud-
2. Investigations of oral behaviors and underlying neural mechanisms in gastropod molluscs

Investigations of the neuronal control of feeding in several different opisthobranch and pulmonate gastropod molluscs began in earnest in the early 1970s (e.g. Levitan et al., 1970; Gardner, 1971; Rose, 1971; Berry, 1972; Kater, 1974; Kupfermann, 1974; Siegler et al., 1974). Opisthobranchs and pulmonates have a number of large neurons that are identifiable uniquely in each individual of a species. These uniquely identifiable neurons have facilitated greatly the physiological and anatomical circuit analyses.

In addition, the presence of uniquely identifiable neurons indicates heritable developmental programs that generate these neurons, thus offering the possibility for analyses of the adaptive radiation of feeding circuitry. Though the potential for comparative studies of gastropod feeding circuitry to illuminate aspects of the evolution of homologous neurons and neural circuits was recognized in the early 1970s (cf. last paragraph of Kater, 1974), that potential remains largely unrealized. Feeding behaviors and their neural bases have now been more or less studied intensively in several gastropod species such as Aplysia, Clione, Helix, Helisoma, Incilaria, Limax, Navanax, Planorbis, Planorbarius, Pleurobranchaea and Tritonia (Bullock and Dorsett, 1979; Brace and Quicke, 1981; Cohant and Mptso, 1983; Peters and Altrup, 1984; Arshavsky et al., 1988a,b,c, 1989; Cappell et al., 1989a,b; Delaney and Gelperin, 1990; Kobatake et al., 1992; Church and Lloyd, 1994; Quinlan et al., 1995, 1997). Surprisingly, however, little effort has been made to examine the similarities and differences in the neural organization of feeding in the various gastropods.

This report will focus on the neural bases of feeding and regurgitation in the snail, Helisoma trivolvis (Mollusca, Gastropoda, Pulmonata, Basommatophora, Planorbidae). It also proposes the working hypothesis that the similarities outweigh greatly the differences in the neural organization of feeding in those well-studied grazing gastropods that feed with rasping or grasping radulae (e.g. the opisthobranchs Aplysia, and Tritonia, the basommatophoran snails, Helisoma, Planorbis, Planorbarius, and Lymnaea and stylomatophoran snails and slugs, Helix, Limax,). A model developed to represent the organization of a multifunctional buccal central pattern generator (CPG) in Helisoma is offered as a general model for gastropod buccal CPGs. No generally applicable neural organization underlying gastropod feeding behaviors has been proposed previously. The Helisoma model will provide a reference point for examining the adaptive radiation of buccal circuitry. These gastropod buccal CPGs appear to be comprised of multiple interneuronal subunits, much like the unit CPGs hypothesized to control joint movements during vertebrate locomotion (cf. Grillner and Wallen, 1985). The individual CPG subunits are conditional neuronal oscillators, which can be independently activated. The activities of CPG subunits too can be linked functionally in different combinations and in different temporal sequences to produce different behaviors. Both physiological state and acute sensory stimuli can modulate the organization of the CPG to produce the appropriate behavior.

In an effort to facilitate further comparative and evolutionary studies, similarities in structure, function and pharmacology of certain potentially homologous neurons will be addressed in those species, in which the buccal CPG organization has been analyzed most (Helisoma, Planorbis, Lymnaea and Aplysia). Some of the difficulties involved in interspecific comparisons will be addressed and a plea made for more comparative studies.

2.1. Helisoma trivolvis as a neuroethological model system

About 1970, an extensive survey of the gross anatomy of the buccal ganglia and associated structures involved in feeding in various gastropods was made by S.B. Kater (personal communication). The purpose of this survey was to find the preparation offering the best combination of visual identifiability of buccal neurons and robustness of feeding behavior in semi-intact preparations. An albino strain of Helisoma trivolvis was selected and developed as a neuroethological preparation for examining feeding behavior. Hemoglobin inside glial cells provided a red background contrasting with the white ‘polka dots’ of neuronal somata (cf. Pentreath et al., 1985). Simultaneous intracellular recordings from identifiable neurons and extracellular recordings from muscles could be made while the animal performed oral behaviors (e.g. Kater, 1974).

2.2. Feeding behavior and its anatomical basis in basommatophoran snails

Helisoma and several other basommatophoran snails are omnivorous with a variety of feeding mechanisms although, as a group, the basommatophora are more stereotyped in their feeding behaviors than are the opisthobranchs (cf. Audesirk and Audesirk, 1985; Thomas et al., 1985). The anatomy and/or dynamics of the feeding apparatuses have been studied in a number of species (e.g. Hubendick, 1957; Demian, 1962; Hem...
brow, 1973; Dawkins, 1974; Kater, 1974; Goldschmeding and DeVlieger, 1975; Brace and Quicke, 1981; Smith, 1988, 1990; Arnett, 1996). All of these studies drew heavily on the work of Carriker (1946) on *Lymnaea stagnalis*. In *Helisoma* feeding behaviors and their anatomical bases were initially examined by Kater (1974), who also tried to correlate neural motor patterns generated by the buccal ganglia with feeding behavior. Smith (1988, 1990) extended Kater’s observations on the anatomy and dynamics of the feeding apparatus. Later Arnett (1996) combined videomicroscopy of buccal mass movements with simultaneous extracellular myograms and intracellular recordings from motor neurons during oral behaviors in semi-intact preparations. She focused on the roles of three prominent muscles of the feeding apparatus and their activity patterns, and identified motor neurons innervating these muscles. Specific neural patterns mediating typical feeding, regurgitation, and swallowing behaviors were determined.

The mouth of *Helisoma* opens into a buccal cavity within a muscular pharyngeal buccal mass. The buccal mass consists of approximately 30 muscles, most occurring as bilateral pairs (Smith, 1990). The odontophore (tooth carrier) arises from the floor of the buccal cavity. The odontophore consists of a tongue-like ‘cartilage’ and the attached file-like dentated radula. The chitinous radular teeth are secreted by the subradular membrane, which is continuous with the epithelial lining of the oral cavity. The odontophore cartilage is not true cartilage but is a complex tissue including intrinsic muscle fibers and mechanical support structures.

These snails typically eat microflora by protracting the odontophore forward and downward through the mouth and rasping the radula over the substrate. Adjacent to the mouth are one dorsal and two lateral chitonous mandibles. *Helisoma* cuts small pieces from macrophytes by employing the dorsal lip of the odontophore and the dorsal mandible as ‘scissors’. Using the odontophore as a scoop with a moving radular conveyor belt, these snails will also feed on loose material (e.g. in the surface film of ponds) and they also eat carrion routinely. We have not observed *Helisoma* to use the buccal mass as a pump for ingesting semi-decayed matter without use of the odontophore, but this method was reported for the closely related snail *Biomphalaria* (Thomas et al., 1985).

At rest, the odontophore sits at about a 45° angle. During a typical feeding cycle (Fig. 1), the tip (or dorsal lip) of the odontophore is ‘protracted’ or rotated forward and downward and protruded through the mouth. During the second phase, the odontophore is rasped over the substrate and ‘retracted’ back to the rest position within the oral cavity. The tip of the odontophore is kept in close apposition to the food groove along the midline of the roof of the buccal mass during retraction. In the third phase, the odontophore is hyper-retracted, bringing the tip of the radula near the opening of the esophagus. If the snail is feeding slowly, the odontophore returns to the rest position during the inter-bite interval. During the protrusion stroke, the fulcrum, about which the odontophore rotates, is moved posteriorly such that the tip of the odontophore is not in close apposition to the food groove. One variation of the typical feeding behavior is a sequence of repetitive swallows, during which the dorsal lip of the odontophore is thrust or hyper-retracted repeatedly toward the esophagus without intervening protractions of the odontophore (Arnett, 1996).

3. Neurophysiological correlates of feeding in *Helisoma*

Neurophysiological studies have focused on the neural basis of the rhythmic scraping or rasping movements of the odontophore described above. The pioneering study of Kater (1974) influenced greatly not only the subsequent studies of feeding in *Helisoma*, but also comparative studies of feeding in other gastropods (e.g. Benjamin and Rose, 1979; Bulloch and Dorsett, 1979). This influence had both positive and negative

Fig. 2. A schematic diagram of the original model of the *Helisoma* buccal pattern generator and the putative feeding motor pattern. The motor pattern consisted of alternating bursts in retractor (R) motor neurons and ‘protractor’ (P) motor neurons (now known to be hyper-retractor neurons). This pattern was thought to be generated by a single neuronal oscillator comprised of approximately 20 pairs of electrotonically coupled interneurons, collectively called the ‘cyberchron’ (C). The cyberchron was thought to drive and time the action potential bursts in motor neurons by exciting simultaneously the retractor neurons and inhibiting protractor neurons. The protractor motor neurons would then fire bursts of action potentials from postinhibitory rebound. (After Kater, 1974).

Aspects. A series of ‘mistakes’ by Kater (1974) illustrates generally applicable pitfalls in correlating neuronal activity with corresponding evoked muscle activity in relatively complex neuromuscular systems. A visual interpretation of the feeding behavior, combined with the misidentification of the muscular target of a neuron, led to a miscorrelation of activity in a set of neurons with the retraction phase of the feeding cycle and further led to the conclusion that the feeding cycle and its underlying neural pattern were biphasic rather than triphasic. Therefore, I will explain the reason for and the consequences of, the mistakes made in considerable detail.

During typical rapid rhythmic feeding in *Helisoma*, there is no pause at the ‘rest’ position between the retraction and hyper-retraction phases, and protraction for the subsequent bite cycle commences immediately upon termination of the hyper-retraction phase. Therefore, Kater (1974) separated the feeding cycle into only two phases — protraction, and retraction. Kater recorded a biphasic buccal motor pattern with alternating bursts of action potentials in two sets of motor neurons (e.g. Fig. 2). The bursts of action potentials in one set of motor neurons (represented by neuron B27, see Fig. 3) were correctly correlated visually with the retraction phases of the feeding cycles, and hence, this set of motor neurons was designated ‘retractor motor neurons’ (Kater, 1974). Since there was thought to be only a biphasic pattern, the remaining phase of neural activity recorded was taken to represent protraction, and neurons generating action potentials during this part of the cycle were designated ‘protractor motor neurons’ (e.g. neurons B17, B18, B19, B20, and B21; Kater, 1974).

The correlation of the neural pattern and feeding behavior appeared to be confirmed when extracellular myograms attributed to the posterior jugalis (pj), a major odontophoral protractor muscle, were associated on a one-to-one basis with action potentials in motor neuron B21. However, the pj muscle is very thin and overlies the relatively massive supralateral radular tensor (slrt) muscle. It is difficult to distinguish pj activity from underlying tensor muscle activity when recording from the intact buccal mass (cf. Rose and Benjamin, 1979). Recently, feeding in *Helisoma* has been demonstrated to arise from a triphasic motor pattern (see below, Fig. 3; Arnett, 1996; Quinlan and Murphy, 1996; Quinlan et al., 1995, 1997) corresponding to the protraction, retraction, and hyper-retraction phases of the feeding cycle. The slrt muscle contracts during retraction and even more vigorously during hyper-retraction. The muscle potentials ascribed to the pj protractor muscle and associated with neuron B21 action potentials by Kater (1974) were undoubtedly recorded from the underlying slrt. No morphological or other distinctive characteristics of neuron B21 were published, so it is not uniquely identifiable. However the large neurons B18 and B19 displayed similar patterns of synaptic and action potentials to the neuron designated B21 by Kater (1974). Neurons B18 and B19 innervate the slrt, and during feeding motor activity, they fire

Fig. 3. The triphasic feeding pattern of buccal motor neuron activity in *Helisoma*. Simultaneous intracellular recordings from identified buccal motor neurons demonstrated three discrete phases (1, 2, 3) of excitation in each of the four cycles of feeding activity depicted. Protractor motor neuron B6 was depolarized during phase 1, and inhibited during phase 2. Retractor motor neuron B27 was depolarized during phase 2, and Hyper-retractor motor neuron B19 was inhibited during phase 2 and depolarized during phase 3. Note, that the pattern depicted in Fig. 2 corresponds to phases 2 and 3 of the feeding pattern.
bursts of action potentials during the hyper-retraction phase (Arnett, 1996). Careful dissection of the buccal mass, allowing myograms to be recorded from the pj protractor muscle independently of the slrt, revealed activity in pj myograms occurring after the hyper-retractor activity (e.g. B19 bursts) of the previous feeding cycle and prior to retractor activity in, for instance neuron B27. Subsequently, it was demonstrated that protraction phase neurons B6 and B8 innervate the pj (see below; Arnett, 1996). Thus the bi-phasic electrophysiological pattern described by Kater represents the second and third phases (i.e. retraction and hyper-retraction) of the triphasic feeding pattern (compare Figs. 2 and 3). The activity that Kater associated with protraction actually mediates hyper-retraction of the odontophore to facilitate swallowing.

3.1. The original model of the Helisoma buccal CPG

The retractor motor neurons described by Kater (1974) displayed excitatory postsynaptic potentials (EPSPs) underlying their action potential bursts. Simultaneously, the ‘protractor’ (actually hyper-retractor and radial tensor) motor neurons displayed prominent inhibitory postsynaptic potentials (IPSPs). Electrotonic coupling among synergistic motor neurons was demonstrated. A model of the buccal CPG (Fig. 2) was developed whereby only a single neural oscillator would simultaneously evoke EPSPs in the retractor motor neurons and IPSPs in the ‘protractor’ motor neurons. The protractor motor neurons would subsequently generate a burst of action potentials from post-inhibitory rebound. Indeed, the ability of these motor neurons to generate a vigorous burst of action potentials following termination of a hyperpolarizing pulse was demonstrated.

A set of an estimated 20 or so pairs of electrotonically coupled neurons was thought to drive and time the activity of the motor neurons, and thus, were called ‘cyberchron neurons’. These neurons seemed to meet the criteria for comprising the feeding CPG. These neurons evoked PSPs of the appropriate sign for the retraction phase of the feeding cycle in a number of identified motor neurons. A brief stimulation of a cyberchron neuron could sometimes trigger a number of cycles of rhythmic activity in feeding motor neurons. However, action potential bursts typically were not recorded from cyberchron neurons during sustained rhythmic activity (e.g. Kater, 1974). Cyberchron neurons did, however, display typically the depolarizations coinciding with the synaptic activity in motor neurons during the retraction phase of the cycle. Therefore, it was hypothesized that a few neurons of the electrotonically-coupled network of approximately 20 pairs of cyberchron neurons were generating action potentials during any given feeding cycle and, by chance, the active neurons were not recorded.

Electrotonic coupling and its plasticity were studied extensively in this network (Kaneko et al., 1978; Merickel and Gray, 1980). The potential roles of electrotonic coupling during rhythmic action potential burst generation in a reverberating coupled system were examined by computer modeling (Merickel et al., 1977, 1978). Some members of the cyberchron network were also shown to have ‘bursty’ membrane properties (i.e. a region of negative slope resistance in their I–V curve). Later, however, it was shown that the IPSPs evoked in ‘protractor’ (i.e. hyper-retractor) motor neurons by action potentials in cyberchron neurons reversed at membrane potentials much more depolarized than the reversal potential of the IPSPs evoked by the buccal CPG during phase 2 (i.e. retraction) of the feeding cycle (Murphy, 1991). More recently, it has been shown that activity evoked in some ‘cyberchron neurons’ triggers regurgitation. Thus, this group of neurons has been redesignated the Buccal A Cluster (BAC) neurons (Arnett, 1996) since they are not directly the components of the buccal CPG and they neither time nor drive the activity of motor neurons during feeding.

3.2. A triphasic buccal motor pattern underlies feeding behavior

A triphasic buccal motor pattern (Fig. 3) was described in Helisoma (Quinlan and Murphy, 1991, 1996; Quinlan et al., 1995, 1997). It is quite similar to patterns reported to underly feeding in the basommatophoran snail, Lymnaea (Elliott and Benjamin, 1985a) and the opisthobranch, Tritonia (Bulloch and Dorsett, 1979). Since the Helisoma buccal ganglia can produce a number of different motor patterns, this triphasic pattern was designated the ‘standard pattern’ of buccal motor activity and was hypothesized first (Quinlan and Murphy, 1991) and later, demonstrated to underly the typical feeding (Arnett, 1996). This pattern allowed neurons to be classified as active in one or more specific phase(s) of the feeding pattern. A number of neurons active in each of the three phases has been identified (see Figs. 4 and 14 and Table 1).

Protraction phase motor neurons (or presumptive motor neurons) include neurons B3, B6, B7, and B8 (Fig. 5). These neurons are excited during phase 1 and inhibited during phase 2. They recover slowly from the phase 2 hyperpolarization, remaining inhibited through phase 3. The physiology of neuron B3 differs from that of neurons B6, B7, and B8. In rapid feeding rhythms, it fires bursts of action potentials only during phase 1. However, if there is a slow feeding rhythm, neuron B3 fires a burst of action potentials not only during phase 1, but also in the interphase following phase 3. If there is rhythmic retraction and hyper-retraction phase activ-
Fig. 4. A map of the somata of identified Helisoma buccal neurons. Both the caudal surface and the rostral surface of the animal’s left buccal ganglion are depicted. Each identified neuron would have a mirror image homolog in the right ganglion (not shown). Note that some terminology is altered from previous publications. The rostral and caudal surfaces were previously called ventral and dorsal, respectively; the VBN and LBN were previously designated the HBN and VBN, respectively (Kater, 1974). Neurons on the rostral surface were named formerly VB1-VB10 (to indicate ventral buccal neurons; Lukowiak and Murphy, 1987) and they have been renamed 101–110. The changes were made to conform to the in situ morphology of the structures, and to prior terminology of basommatophoran molluscan morphologists (e.g. Carriker, 1946; Hembrow, 1973), and to facilitate comparisons with other gastropods. BC, buccal commissure; CBC, cerebrobuccal connective; ET, esophageal nerve trunk; LBN, laterobuccal nerve; PBN, posterobuccal nerve; VBN, ventrobuccal nerve.

Fig. 5. Morphology of identified Helisoma phase 1 protractor motor neurons. Photographs of neurons injected with the fluorescent dye, Lucifer Yellow. All of the neuronal somata are on the caudal surfaces of the buccal ganglia. Orientation of ganglia is the same as in Fig. 4. Influential motor neuron B6 (top) has axons in both PBNs and innervates the pj muscle bilaterally. Neuron B7 (middle) has an extensive neuritic arbor in the ipsilateral esophageal nerve trunk (ET) and one of its branches, the dorsobuccal nerve (DBN). Neurons B6 and B8 innervate the pj muscle bilaterally and ipsilaterally, respectively, via the posterobuccal nerves (PBNs). Axons of neuron B7 enter the buccal mass via the ipsilateral ventrobuccal nerve (VBN; previously known as the heterobuccal nerve, see the legend of Fig. 4 for explanation of nomenclature). Neuron B34 is excited in phases

Table 1
The sign of PSPs evoked in identified Helisoma effector neurons by each CPG subunit

<table>
<thead>
<tr>
<th>Neuron</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>Axonal projections</th>
</tr>
</thead>
<tbody>
<tr>
<td>B5</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>Ip and con Ets (GNs, DBNs)</td>
</tr>
<tr>
<td>B4</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>Ip and con Ets (SNs, DBNs)</td>
</tr>
<tr>
<td>B34</td>
<td>E</td>
<td>E</td>
<td>I</td>
<td>Ip LBN</td>
</tr>
<tr>
<td>B3</td>
<td>E</td>
<td>I</td>
<td>I</td>
<td>Ip ET (DBN)</td>
</tr>
<tr>
<td>B6</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>Ip and con. PBNs</td>
</tr>
<tr>
<td>B7</td>
<td>E</td>
<td>I</td>
<td>I</td>
<td>Ip VBN</td>
</tr>
<tr>
<td>B8</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>Ip PBN</td>
</tr>
<tr>
<td>B17</td>
<td>I</td>
<td>E</td>
<td>I</td>
<td>Ip LBN</td>
</tr>
<tr>
<td>B19</td>
<td>I</td>
<td>E</td>
<td>I</td>
<td>Ip and con VBNs, ip LBN</td>
</tr>
<tr>
<td>B18</td>
<td>I/E</td>
<td>E</td>
<td>I</td>
<td>Ip and con VBNs</td>
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<tr>
<td>B26</td>
<td>E</td>
<td>I</td>
<td>I</td>
<td>Ip and con VBNs</td>
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<tr>
<td>B27</td>
<td>E</td>
<td>E</td>
<td>I</td>
<td>Ip and con LBNs</td>
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<tr>
<td>B29</td>
<td>E</td>
<td>I</td>
<td>I</td>
<td>Ip and con PBNs</td>
</tr>
<tr>
<td>B110</td>
<td>E</td>
<td>I</td>
<td>I</td>
<td>Ip and con VBNs and LBNs</td>
</tr>
</tbody>
</table>

* I, inhibitory; E, excitatory; Ip, ipsilateral; con, contralateral; ET, esophageal trunk; GN, gastric nerve; DBN, dorsobuccal nerve; SN, salivary nerve; VBN, ventrobuccal nerve; PBN, posterobuccal nerve; LBN laterobuccal nerve.
1 and 2 and can have its highest spike frequency in either phase, depending on conditions. Neuron B34 has an axon in the ipsilateral LBN.

Retraction (phase 2) motor neurons and presumptive motor neurons include neurons B110 (aka VB10), B26, B27, and B29 (Fig. 6). Neurons B29 and B110 generate bursts of action potentials only during phase 2. Neuron B29 has axon branches in both PBNs that pass through the pj muscle (but do not innervate it) and continue to unidentified targets deeper in the buccal mass. Neuron B110 has bilateral axon branches in both VBNs and LBNs. It innervates the slrt muscle (M. Zoran, personal communication). Neurons B26 and B27 are excited strongly by S2 interneurons and more weakly by S3 interneurons. During slow motor patterns, neuron B27 displays a rapid burst of action potentials in phase 2 and fires more slowly in phase 3 (Quinlan and Murphy, 1996). During a vigorous feeding pattern, neuron B27 generates bursts of action potentials confined almost entirely to phase 2 of the pattern because it displays a postburst hyperpolarization that inhibits its firing during phase 3 (e.g. Fig. 3). Neuron B26 has axons in ipsilateral and contralateral VBNs and innervates the slrt muscle and Neuron B27 has axons in the ipsilateral and contralateral LBNs and innervates the aj muscle.

Hyper-retraction (phase 3) motor neurons include neurons B17, B18, and B19. Neuron B17 has an axon in the ipsilateral LBN but specific muscular targets have not been identified. Neurons B18 and B19 have axons projecting bilaterally to innervate the slrt muscle via the VBNs (aka HBNs). Neuron B19 also innervates the lateral portions of the ipsilateral aj muscle via the ipsilateral LBN, and in some preparations, there is an axon in the contralateral LBN and a bilateral innervation of the aj (Arnett, 1996).

3.3. The current model of the multifunctional buccal CPG

The CPG consists of three semi-independent interneuronal subunits, S1, S2, and S3, each of which is an oscillator capable of generating rhythmic bursts of action potentials or, in the case of S2, rhythmic plateau potentials (Fig. 7 and see Quinlan et al., 1995). Each subunit provides excitation or inhibition to subsets of buccal motor neurons. Some motor neurons are excited (or inhibited) by more than one subunit (Table 1) and, thus, can display action potential bursts, which span all
or part of the duration of more than one phase of the cycle (Fig. 3 and cf. Quinlan and Murphy, 1996). Therefore, (and for additional reasons) a number of motor neurons have action potential bursts that overlap in time and do not fit discretely into only one of the three phases of the feeding cycle. In the feeding mode, S1 interneurons stimulate protraction (phase 1) motor neurons and S2 interneurons. This excitation brings S2 interneurons to threshold for plateau potential generation. S2 interneurons provide feedback inhibition onto S1 interneurons, terminating their activity. Simultaneously, S2 plateau potentials inhibit S3 interneurons. When the inhibition subsides, S3 interneurons fire from post-inhibitory rebound. Thus, feeding results from an S1-S2-S3 sequence of activity. As long as there is tonic excitation to S1 interneurons or an excitatory milieu of neuromodulators (e.g. high dopamine concentrations), rhythmic feeding activity will continue (Quinlan et al., 1997).

### 3.4. Plasticity of organization in a multifunctional CPG

A flexible organization of the multifunctional buccal CPG was suggested by the analyses of many simultaneous intracellular recordings of two to four neurons from a set of 20 or so frequently monitored identified neurons. Sets of PSPs, characteristic in sign and amplitude of PSPs occurring during a given phase of the triphasic feeding motor pattern, were recorded from multiple neurons and often occurred either sporadically, or rhythmically, but in the absence of a full triphasic feeding pattern. These phase-characteristic PSPs were correlated consistently across buccal neurons, suggesting that CPG interneurons could be activated in functional motor patterns other than the triphasic pattern, or perhaps in non-functional degenerate motor patterns.

However, the existence of correlated PSPs, that appear to be the characteristic of those of a particular phase of the standard feeding pattern, does not indicate necessarily that these PSPs arise from feeding CPG interneurons. For example, the BAC neurons (aka cyperchron neurons, Kater, 1974) are not a part of the buccal CPG but can evoke widespread PSPs of the sign appropriate for phase 2 PSPs. Therefore, to identify interneurons definitively as components of a multifunctional buccal CPG and to show that modulation of their activities could result in multiple motor patterns, it was necessary to show, (1) that they were active during a particular phase of the triphasic feeding pattern; (2) that stimulation of putative interneurons in quiescent preparations evoked phase-characteristic postsynaptic potentials (PSPs) in a set of follower neurons; (3) that hyperpolarization of the putative interneurons during a feeding pattern eliminated appropriate phase-specific PSPs; (4) that brief hyperpolarizations of interneurons could reset the phase of motor patterns; and (5) that activity of the putative interneurons during non-feeding motor patterns elicits PSPs similar to the phase-characteristic PSPs evoked by the interneurons during the feeding motor pattern. A number of CPG interneurons have been identified and their activity patterns confirmed the flexible organization of the multifunctional pattern generator (cf. Quinlan and Murphy, 1996).

### 3.5. Identified CPG interneurons and motor neurons

Commonly for invertebrates, it is impossible to pigeon-hole neurons as definitively ‘motor neurons’, ‘sensory neurons’ or ‘interneurons’ since a given neuron may function in multiple roles (e.g. Miller and Selverston, 1985; Evans et al., 1996; Hurwitz et al., 1996). With that caveat, in *Helisoma*, identified neurons active in each of the three phases of feeding could be categorized as pure interneurons, influential neurons, or follower neurons on the basis of function and morphology. Central pattern generator (CPG) elements include pure interneurons and influential neurons (cf. Arshavsky et al., 1988a). Pure interneurons either project axons to the cerebral ganglia (BCN1s) or have their processes confined to the buccal ganglia and the proximal areas of buccal nerve roots (e.g. N1a, N1b, N1c). They do not project axons to peripheral targets. Influential neurons are multifunctional neurons that have peripheral axons with either sensory or motor functions but which also participate in the generation and/or modulation of buccal motor patterns. Influential neurons can play roles in the CPG that are as critical and prominent as those of pure interneurons.

Phase 1 pure interneurons include three pairs of BCN1 neurons, with axons crossing the buccal commissure and traversing the contralateral cerebrobuccal connective with axonal arbors both in the buccal and cerebral ganglia. In addition, neurons N1a, N1b, and N1c have processes confined to the buccal ganglia or proximal areas of buccal nerves (Fig. 8). Neuron B6 is a phase-1 influential neuron that also serves as a motor neuron innervating the pj muscle (Arnett, 1996). Stimulation or hyperpolarization, respectively, of neuron B6 can evoke or inhibit the feeding motor pattern.

Central pattern generator (CPG) interneurons evoking retraction phase (i.e. phase 2) PSPs during feeding are the pair of glutamatergic interneurons, B2 (Quinlan et al., 1995). Interneuron B2 is a phase 2 influential neuron, activity in which appears necessary and sufficient to account for S2-evoked PSPs. Neuron B2 has an axon, which traverses the buccal commissure and forms a loop and returns to the ipsilateral neuropile. Neuritic processes arborize in both buccal ganglia and axon branches also project to the buccal mass via the posterior buccal nerves. Functions of the peripheral axons of
neuron B2 are unclear and could include sensory or motor roles (Quinlan et al., 1995). Neuron B29 is a phase-2 influential neuron, stimulation of which can evoke phase 2-like PSPs in other buccal motor neurons (cf. Figs. 3, 6 and 9). However, hyperpolarization of neuron B29 does not eliminate phase-2 activity in other neurons. No ‘pure interneurons’ with axons confined to the CNS and active in phase 2 have been described in Helisoma. However, Arshavsky and colleagues reported ‘group 2’ interneurons in the snail, Planorbis, (like Helisoma in the family Planorbidae) that were active in phase 2 of the feeding cycle and had no processes projecting from the buccal ganglia (Arshavsky et al., 1988a).

The major elements of subunit 3 of the Helisoma buccal CPG are the pair of pure buccal interneurons N3a (Quinlan and Murphy, 1996). Activity in neurons N3a is necessary and sufficient for phase-3 PSPs during the triphasic feeding pattern and also accounts for phase 3-like PSPs occurring in non-feeding patterns. A cluster of S3 influential neurons (B101–104) that are immunoreactive to small cardioactive peptide B (Fig. 10) have somata on the rostral (aka ventral) surfaces of the buccal ganglia and axons in the posterior buccal nerves (Lukowiak and Murphy, 1987). In addition to their roles as S3 CPG elements, these neurons function as radular mechanoaffecters (unpublished data). They compensate apparently for load on the radula by activating S3 interneurons, and thus, the hyperretraction motor neurons.

3.6. Evidence that the triphasic standard buccal motor pattern mediates the typical feeding behavior

Since several different rhythmic motor patterns were recorded from identified buccal neurons in Helisoma, it was necessary to determine which neural pattern mediated the typical rasping behavior described above. This required semi-intact preparations, in which an incision was made along the dorsal midline of the head region, the body wall was reflected outward, and the esophagus and extrinsic buccal retractor muscles were severed to allow the buccal mass to be tilted forward. This brought the buccal ganglia, situated on the caudal surface of the buccal mass, into a dorsal position. A microplatform placed underneath the buccal ganglia stabilized the preparation for intracellular recordings while the buccal mass was free to perform feeding movements (Arnett, 1996; Arnett and Murphy, 1991, 1992). A reasonably good facsimile of feeding behavior could be evoked by feeding stimulants, with the caveat that incomplete protraction of the odontophore was seen, possibly due to a loss of hydrostatic pressure within the body cavity and within the buccal mass. The triphasic motor pattern (Fig. 3) was simultaneously recorded intracellularly from identified neurons and extracellularly from specific muscles of semi-intact adult snails performing feeding-like movements of the odontophore. However, the position of the odontophore could only be seen during part of the feeding cycle in semi-intact adults. It was, therefore, necessary to define external features of the buccal mass that were diagnostic for the position of the odontophore during each phase of the feeding cycle. Newly hatched snails (about 1 mm in shell diameter) were videotaped while feeding on glass slides or transparent plastic sheets. Such small snails are semi-transparent and the positions of the odontophore were correlated readily with external features of the buccal mass. For any given phase of the feeding cycle, the positions and overall contours of

Fig. 8. Morphology of protraction phase S1 interneurons. Interneurons were stained by intracellular injection with Lucifer Yellow. Tracings were made from sets of Ektachrome slides of each neuron taken at multiple focal planes and superimposed. There are six pairs of identified S1 interneurons. The BCN1 group consists of three pairs of similar neurons that are not readily distinguishable from each other. These neurons send axons across the buccal commissure and traverse the contralateral CBC (upper left) to form a terminal arbor in the cerebral ganglion. Often, a short neuritic process extends into one or more of the esophageal trunks (as shown here) or postero-buccal nerves. Neurons N1a, N1b and N1c do not have axons leaving the buccal ganglia though N1b has processes extending a few hundred micrometers into the CBCs.
Fig. 9. Neuron B29 is a retraction phase influential neuron. Simultaneous intracellular recordings were made from neuron B19, which is inhibited during phase 2 and excited during phase 3 of the feeding pattern. Activity in neuron B29 was recorded with a high resistance unbalanced Lucifer Yellow electrode. At the beginning of the record neuron B29 was held hyperpolarized (0.8 nA) and neuron B19 was firing tonically. Neuron B29 was released periodically from hyperpolarization, which evoked anode break bursts of action potentials in neuron B29 that triggered phase 2-like IPSPs in neuron B19 (labeled ‘2’ in the second series of anode break bursts). Spontaneous bursts in B29 coincided with IPSPs in B19, and a depolarizing pulse ‘D’ injected into neuron B29 evoked a phase 2-like IPSP in neuron B19. Calibrations, 10 s, 40 mV.

the buccal mass, as well as the degrees of protrusion of the radular sac were distinctive. Since similar positions and contours of the buccal mass and radular sac were seen during feeding behaviors of semi-intact adults, it was possible to correlate the stages of the feeding cycle with the appropriate phases of the triphasic neural pattern. The aj, pj, and slrt muscles are prominent muscles of the buccal mass that are visible when the mass is viewed from an external perspective. Their states of contraction or relaxation are major determinants of the positions of the odontophore and of the contours of the buccal mass during the three phases of the feeding cycle (Arnett, 1996).

The pj muscle contracts during protraction of the odontophore and different regions of the aj and slrt muscles contract both during retraction and hyper-retraction. Neurons B6 and B8 innervate the pj, bilaterally and ipsilaterally, respectively, and generate bursts of action potentials during phase 1 of the feeding pattern. Neuron B27 generates action potential bursts primarily during phase 2 and innervates the medial portion of the aj muscle, which contracts during retraction. Neuron B19 displays action potential bursts during phase 3 of the standard buccal motor pattern and innervates the lateral regions of both the aj and slrt muscles, which contract vigorously during hyper-retraction of the odontophore. Thus, identification of motor neurons for the aj, pj, and slrt muscles confirmed both the roles of these muscles during feeding and that the standard triphasic buccal motor pattern mediates the most typical feeding behavior (Arnett, 1996).

3.7. Regurgitation

Regurgitation can be triggered in basommatophoran snails by feeding them noxious materials (Bovbjerg, 1968). We observed serendipitously that Listerine is an effective emetic. This observation was exploited to trigger regurgitation in intact newly hatched snails, in which the dynamics of the buccal mass and movements of the odontophore could be observed (Arnett, 1996; Arnett and Murphy, 1994).

Microscope slides were coated with agar containing watermelon extract, a potent feeding stimulant. Listerine was applied via Pasteur pipettes near the mouths of newly hatched snails feeding vigorously on the watermelon-coated slides. These snails would perform immediately one or several odontophore reversals (i.e. regurgitation strokes). A number of features distinguished regurgitation cycles from feeding cycles. The
power stroke of the odontophore switched from retraction during feeding to protraction during regurgitation. During feeding, the fulcrum of the odontophore (i.e. the point about which the odontophore rotates) is moved forward at the beginning of the retraction stroke. Thus, the dorsal lip of the odontophore is apposed closely to the lining of the oral groove during retraction and food particles are swept posteriorly. During regurgitation, the fulcrum of the odontophore moved forward at the beginning of protraction so that the lip of the odontophore was apposed to the oral groove during retraction. Additionally, the protraction phase (i.e. phase 1) was prolonged during regurgitation. Finally, there was, typically, no hyper-retraction of the odontophore during regurgitation cycles. Only the protraction/retraction phases of the trajectory were displayed.

The physiological correlates of regurgitation included prolonged phase 1 bursts of action potentials (underlying the protraction phase), relative to those seen during feeding, and a suppression of S3 activity, therefore, eliminating hyper-retraction. The neural pattern underlying regurgitation was examined in semi-intact adult snails, in which the esophagus was cannulated with polyethylene tubing connected via a two-way valve to separate syringes containing watermelon extract or 25% listerine (Arnett, 1996; Arnett and Murphy, 1994). Feeding behavior and the triphasic feeding motor pattern could be triggered in quiescent snails by perfusing the oral cavity with watermelon extract. Switching the valve to admit 25% listerine into the oral cavity, triggered a number of strong regurgitation cycles, with vigorous protraction of the odontophore, interspersed with occasional weak regurgitation cycles with only partial protraction of the odontophore. The strong protraction cycles were correlated with the prolonged action potential bursts in S1 interneuron N1a, relative to those seen during feeding. Neither hyper-retraction of the odontophore, nor phase 3 action potential bursts in motor neuron B19 were observed.

3.8. Transmitters of CPG interneurons

Here, we will focus on the neurotransmitters, dopamine, GABA and glutamate because their roles within the Helisoma buccal CPG are best characterized. Exogenous application of 5–10 μM dopamine elicits the triphasic feeding motor pattern in Helisoma reliably. An endogenous source of dopamine is phase 1 interneuron N1a of the buccal CPG (Quinlan et al., 1997). Stimulation of interneuron N1a by current injection evokes the triphasic feeding pattern. N1a is an intrinsic element of the CPG. A brief hyperpolarization of neuron N1a during a feeding pattern can reset the phase. The dopamine antagonist, sulpiride, blocks the effects of both dopamine application and of neuron N1a stimulation. Interneuron N1a also stains with the formaldehyde/glutaraldehyde histochemical stain that is indicative of catecholamines and is highly specific for dopamine in molluscan nerve cells. Application of feeding stimulants (e.g. watermelon extract) triggers activity in neuron N1a and consequent feeding behavior.

GABAergic interneurons are also elements of S1 of the buccal CPG. Two of the three pairs of BCN1 neurons are GABA immunoreactive and application of GABA has a number of effects on identified buccal neurons (Richmond et al., 1991, 1994; Murphy, 1993). GABA depolarizes a number of identified protraction phase neurons that are excited by S1 interneurons (Fig. 11). This effect is mimicked by muscimol and blocked by picrotoxin as one would expect if GABA_A, C receptor agonist depolarized phase 1 motor neuron B6 but did not trigger rhythmic activity in subunit 2. Calibrations 40 mV, 10 s.

Fig. 11. GABA excites protraction phase motor neurons and evokes rhythmic activity in CPG subunit 2 by pharmacologically distinct mechanisms. GABA triggered rhythmic action potential bursts interrupted by phase 2 IPSPs in the influential protractor motor neuron B6. Neuron B6 fired tonically in normal physiological saline (top trace). At the first arrowhead, 100 μM was superfused over the preparation. At the second arrowhead, the GABA was washed out with normal saline. The GABA_A receptor agonist, baclofen, triggered rhythmic S2 activity as indicated by the phase 2 IPSPs in neuron B6, but it had no depolarizing effect on neuron B6. Muscimol, a GABA_A receptor agonist depolarized phase 1 motor neuron B6 but did not trigger rhythmic activity in subunit 2. Calibrations 40 mV, 10 s.
through the oral cavity from mouth to esophagus. The primary phase 2 PSPs arise from glutamatergic interneuron B2 (Quinlan et al., 1995). Interneuron B2 has been co-stained by injection of the dye Lucifer Yellow and by immunocytochemical staining with glutamate antibodies. The phase 2 EPSPs evoked by stimulation of neuron B2 are mimicked by Kainate/AMPA and are blocked by CNQX (Quinlan and Murphy, 1991). The phase 2 IPSPs are mimicked by quisqualate and have no known antagonist. Since glutamate is considered generally an excitatory transmitter, neuron B19 was placed in isolation in acute cell culture to determine if the inhibitory effect of glutamate was direct or indirect. Glutamate or quisqualate applied to the isolated neurons B19 directly evoked hyperpolarizing responses.

Perfusion of the oral cavity with a feeding stimulant (e.g. watermelon extract) via canulation of the proesophagus evoked the triphasic feeding pattern and feeding movements (Arnett, 1996; Quinlan et al., 1997; see above). Feeding was evoked even if there were no neural connections between the buccal ganglia and the rest of the central nervous system. Hence, afferent information must have reached the buccal ganglia via the buccal nerve roots. A number of possibilities could account for the differences in the nature of the motor patterns observed by Horwitz and Senseman (1981), Quinlan et al. (1997). Perhaps a cannula in the mouth triggers mechanooaferents that interfere with the generation of the triphasic feeding pattern. Alternatively, differences in the chemostimuli may have contributed to the differences in observed motor patterns.

3.10. Higher order modulation of the CPG

Two descending modulatory elements have been characterized in Helisoma — the pair of giant serotonergic interneurons C1 (Granzow and Kater, 1977; Granzow and Rowell, 1981; Murphy et al., 1985a), and the inhibitory pleurobuccal interneurons Pl1 (Murphy, 1990). Neuron C1 is excited by feeding stimulants applied to the lips and oral veil. Stimulation of neuron C1, or bath application of 1 μM serotonin, evokes consistently the rhythmic phase-locked activity in S2 and S3 of the buccal CPG. Serotonin can, but usually does not, activate the full triphasic feeding pattern. The effects of serotonin on S1 activity are variable and a number of qualitatively different motor patterns (all displaying linked S2–S3 activity) can be evoked by serotonin application (Quinlan and Murphy, 1996). An S2–S3 pattern without activity in S1 mediates rhythmic swallowing (Arnett, 1996).

Patterned activity of the buccal CPG is inhibited rapidly and completely either by exogenous application of the neuropeptide FMRFamide (Murphy et al., 1985b), or by stimulation of the putatively FMRFamideergic interneuron Pl1 (Murphy, 1990). The somata of paired interneurons Pl1 are on the ventral surfaces of the pleural ganglia. The main axon of neuron Pl1 traverses the pleuropedal commissure, the pedocerebral commissure, and the cerebrobuccal commissure to reach the buccal ganglia. It has neuritic arbor in the parietal, pleural, pedal, cerebral and buccal ganglia. Thus, it is perfectly situated to integrate a variety of stimuli and coordinate activity in the feeding and locomotory CPGs and to activate the whole body withdrawal response. Whether it performs all of these integrative roles remains to be determined (but see below).
4. Comparisons of gastropod neurons and feeding circuitry

Two key related questions need to be addressed. 1. Is the model of the buccal CPG, developed to account for data from Helisoma and discussed above (or, for that matter, any other model), generalizable to provide a universal model for gastropod buccal neuronal organization? 2. Are buccal neurons and their interconnections conserved sufficiently to allow homologies of identified neurons to be ascertained and the evolution of circuitry at the cellular and subcellular levels to be analyzed?

The tentative answer to both questions is yes, for those gastropods with rasping or grasping radular feeding modes. However, more comparative data are needed for a definitive answer.

The key feature of the Helisoma CPG organization is that it is composed of multiple semi-independent unit pattern generators (or CPG subunits) that can be activated or inactivated independently and functionally linked in different temporal sequences. Thus, a number of different motor patterns can be produced. In Helisoma, there are three such subunits. During evolution, other gastropod species may have added an additional interneuronal subunit(s), or lost one, to accommodate different behavioral niches.

Confirmation of cellular homologies is problematic (e.g. Croll, 1987; Breidbach and Kutsch, 1995). Here, we will not absorb ourselves with attempted ‘proofs’ of homology in specific cases. None-the-less, the Darwinian paradigm of relation of species by common descent is generally accepted at present. Analyses of cell lineages and patterns of neuronal differentiation indicate clear homologies of identified neurons in some cases (cf. Comer and Robertson, this volume and included references). Many gastropod neurons have distinguishable unique identities. Comparative morphological, physiological, and neurochemical data provide a strong case for cellular homology for a few gastropod neurons. Below, I will suggest candidate homologies, especially for a number of CPG interneurons (cf. Fig. 14).

Perhaps the best evidence for homologies to date in molluscan feeding circuitries relates to higher order modulatory interneurons and to giant buccal neurons that innervate the gut (e.g. Altrup, 1987; Lloyd et al., 1988; Bulloch and Ridgeway, 1995), or the salivary glands (e.g. Kater et al., 1978; Bahls et al., 1980; Barber, 1983; Bahls et al., 1995). A pair of giant serotonergic neurons has been identified in the cerebral ganglia of numerous gastropods and these neurons display great morphological, physiological, and functional similarities (e.g. Weiss and Kupfermann, 1976; Granzow and Rowell, 1981; Croll, 1987; Bulloch and Ridgeway, 1995). More recently, neurons similar to Helisoma neuron Pl1, which inhibits buccal motor patterns, have been identified in a number of basalstomaphorans and stylomatomorphans pulmonates and in the carnivorous pteropod opisthobranch, Clione (Alania, 1995; Alania and Sakharov, 1996 and M. Alania, personal communication). It was suggested above that neuron Pl1 was ideally situated to coordinate feeding locomotion and defensive withdrawal. The effects of Pl1 on locomotion or withdrawal have yet to be examined in Helisoma. However, increased activity in FMRF-amidergic pleurobuccal neurons in Clione, similar to Helisoma neurons Pl1, not only inhibited feeding but was also correlated positively with both spontaneous and induced accelerations of the locomotor CPG (M. Alania, personal communication).

4.1. Biological and methodological complications for interspecific comparisons of gastropod feeding

There are several real or potential factors that may complicate interspecific comparisons of gastropod feeding motor patterns and underlying neuronal circuitries. (1) Incomplete and non-congruent experimental data sets are available for each of the species that have been examined. (2) Interspecific diversity of feeding behaviors in gastropods. (3) Intraspecific diversity of oral behaviors and the underlying multiplicity of motor patterns in any given species. (4) Incomplete congruence of action potential firing patterns in motor neurons with respect to those seen in the CPG interneurons. Motor neuron firing patterns are more diverse and complex than are those in interneurons, due to multiple interneuronal synaptic inputs, multiple effects of transmitters, and diverse intrinsic membrane properties among motor neurons, and the modulation of all of these. Thus, similar CPG patterns could appear different purely as a function of the motor neurons monitored. All of the above factors can be addressed, as indicated below.

4.1.1. Incomplete data

One major difficulty in revealing the generalities of gastropod feeding has been that studies on different species have focused on many different aspects of feeding. For instance, studies in the stylomatomorphans, Pleurobranchae, have focused primarily on sensory processing, behavioral choice, higher order control of feeding, and its modulation by behavioral state or learning (e.g. London and Gillette, 1984; Mpitsos and Cohan, 1986; Delaney and Gelperin, 1990; Kemenes, 1994; Jing and Gillette, 1995). Thus, relatively little is known about the organization of the buccal CPGs in these species. Even in those species in which the buccal CPGs have been studied extensively (e.g. Aplysia, Helisoma, Lymnaea, Cirbry, 1981; Croll, 1987; Croll,
Planorbis), different aspects of the CPGs have been studied. Concerted efforts at a comparative synthesis of the neuronal organization of feeding behaviors has been lacking.

4.1.2. Interspecific diversity

Mechanisms of feeding in gastropods are diverse (e.g. Audesirk and Audesirk, 1985; Thomas et al., 1985), leaving the question as to what extent neuronal circuitry underlying these behaviors has been conserved phylogenetically. Perhaps the most disconcerting scenario in relation to a comparative synthesis could be that gastropod feeding structures and behaviors may have diverged to such an extent that few commonalities remain in their buccal neuronal organization. This situation may hold for some species with highly specialized feeding, such as Melibe (Hurst, 1968) or Navanax, (Susswein et al., 1987; Cappell et al., 1989a,b) where the need for an odontophore has been eliminated largely or wholly. However, most gastropods that have been subjected to neurophysiological analyses use rhythmic movements of a toothed radula during feeding. These include herbivorous or omnivorous pulmonates (e.g. the basommatophorans Helisoma, Planorbis and Lymnaea and the stylomatophorans Achatina, Helix, Incilaria, Limax) and opisthobranchs that graze on algae or sessile invertebrates (e.g. Aplysia, Tritonia). Even aggressive carnivorous opisthobranchs that specialized structures for capturing prey typically ingest or swallow the prey with rhythmic buccal movements (e.g. Clione, Pleurobranchaea).

Close phylogenetic relatedness, combined with similarities of ecological niches and feeding behaviors, should facilitate the analyses of potentially homologous neuronal organization. Although opisthobranchia and pulmonata represent different subclasses of gastropoda, a consensus suggests that they are much more closely related than are the different subgroups of the highly diverse ‘prosobranch subclass’, a paraphyletic grouping no longer considered systematically useful (cf. Ponder and Lindberg, 1996). Here, a significant degree of homology of identifiable neurons of the feeding circuits of opisthobranchs and pulmonates is hypothesized. Similarities in identified neurons and neuronal organization are indicated below.

4.1.3. Intraspecific diversity

A major biological factor obfuscating interspecific comparisons of ‘feeding motor patterns’ and their neural bases is that each species examined has a plastic multifunctional CPG that generates multiple buccal motor patterns. Some of these patterns (usually monitored with the nervous system in vitro or in a semi-intact preparation) mediate variations of feeding behavior, some mediate regurgitation or other non-feeding oral behaviors and some of the patterns may even be non-functional. Over the years, numerous buccal motor patterns have been asserted or implied in the literature to represent fictive feeding, often on the basis of an electrophysiological recording from a single neuron and without concurrent behavioral data. Buccal motor patterns often change qualitatively on a cycle by cycle basis and multiple qualitatively different motor patterns from identified neurons have been depicted as ‘fictive feeding’ in the same figure. Frequently, the original author is conservative, referring to a recording as a buccal motor pattern, only to have it referenced in subsequent reports as a ‘feeding motor pattern’. How then, amongst such confusion, can meaningful interspecific comparisons of feeding motor patterns and their underlying neuronal circuitry be made?

Fortunately, feeding behaviors and the dynamics of buccal masses and odontophores can be analyzed in intact freely behaving juvenile gastropods by videomicroscopy. Reasonable facsimiles of feeding behavior can be evoked in semi-intact preparations while electrophysiological recordings are made from identifiable neurons and/or muscles (e.g. Arnett, 1996; Drushel et al., 1997). A ‘typical feeding behavior’ can be selected and the standard feeding motor pattern underlying this feeding behavior determined. With effort and luck motor neurons displaying common sets of PSPs and the interneurons mediating these sets of PSPs can be identified uniquely. Sets of phase-linked PSPs in multiple identified motor neurons, suggestive of a common presynaptic interneuron or set of interneurons, may be seen to occur in linked fashion in temporal sequences different from that seen in the standard feeding pattern. Thus, plasticity in the organization of the buccal CPG can be characterized, and sometimes controlled, pharmacologically or by modulatory interneurons. Finally, the interactions among identified modulatory interneurons, CPG interneurons and motor neurons can be studied.

Key features of the organization of the Helisoma buccal CPG, and of patterns of activity recorded from buccal motorneurons, help illustrate difficulties encountered when comparing different motor patterns. Each of the three CPG subunits is a semi-independent conditional oscillator that can generate rhythmic activity that may or may not be linked to activity in the other subunits. Furthermore, the order of activity of particular subunits is variable. For instance, subunit 3 may generate action potential bursts that are linked to S2 activity but the S3 activity can precede that of S2, instead of following it as it does in the typical feeding behavior (cf. Arnett, 1996; Quinlan and Murphy, 1996). A subunit also may be active more than once per cycle. For example, subunit 3 may generate bursts of activity both preceding and following S2 activity (Fig. 12). Another complication relative to CPG analysis is that there are strong gradations in the intensity of S1 and S3
activity (cf. Quinlan and Murphy, 1996; Quinlan et al., 1997). Different interneurons active within the same phase of the feeding pattern can have different onsets for firing and not all interneurons of a given phase need to be active in all motor patterns.

4.1.4. Non-congruence of interneuronal and motor neuronal patterns

Perhaps the most prominent complication in feeding motor pattern analysis across species arises from the fact that action potential burst patterns of many motoneurons will not show an abrupt onset or termination of the bursts at the ‘borders’ between subunit activities, even if a rhythmic S1–S2–S3 standard feeding pattern is being generated (see below). Therefore, some identified motor neurons provide a much clearer reflection of CPG activity than other identified motor neurons. To know for certain which motor neurons provide a reliable monitor of CPG activity, relevant interneurons must be identified, and recorded with specific motor neurons under a variety of conditions (e.g. with different modulators in the bath). In general, more than one neuron will need to be monitored simultaneously to obtain a complete reflection of CPG activity.

In Helisoma, since the onset and duration of bursts of action potentials in different S1 interneurons varies, there will be differences in firing onset in motor neurons innervated by the S1 interneurons. There is, however, an abrupt transition from S1 to S2 interneuronal activity because S1 interneurons excite S2 interneurons to threshold for plateau potential generation and S2 neurons have feedback inhibition to terminate the S1 bursts (Quinlan et al., 1995). In spite of this, many motoneurons receive excitation both from S1 and from S2 interneurons, and thus, may fire action potentials across phases 1 and 2 (or similarly 2 and 3, or even 1, 2, and 3) of the feeding pattern (cf. Table 1). An additional complicating factor is that biphasic synaptic responses may occur in follower neurons. In particular, several neurons that are postsynaptic to S2 interneurons (e.g. neuron B18, which is primarily excited during phase 3) show an initial hyperpolarization followed by a depolarization. A consequence of these neural organizational features is that while each ‘feeding’ motor neuron reflects the activity of one or more subunits of the buccal CPG, no single motor neuron provides a direct mirror image of the activity of the CPG.

One can overcome the above complications in motor pattern analysis by having unequivocally identifiable neurons that can serve as reference monitors of activity in CPG subunits. Helisoma neuron B19, for example, is such a reference neuron that reflects reliably the activity of CPG subunits 2 and 3 (Quinlan and Murphy, 1996). One should monitor synaptic potentials and not just extracellular spiking patterns, because similar spiking patterns can arise from different patterns of synaptic activity. Finally, interneurons must be identified and their activity confirmed to be coincident with CPG subunit activity during feeding patterns. They must be shown to evoke phase-characteristic PSPs in appropriate identified follower neurons during feeding patterns. Hyperpolarization of the identified interneuron should eliminate at least some of the PSPs evoked by the CPG subunit. Similarly, the identified interneuron should be able to evoke phase-characteristic PSPs in identified follower neurons when stimulated in quiescent preparations or in preparations generating non-feeding patterns. These PSPs need not be identical to the PSPs generated during feeding patterns since synaptic responses may be modulated during feeding.

4.2. Specific buccal motor patterns

Triphasic patterns of motor neuron activity, similar to that shown above for Helisoma (Fig. 3) have been reported to underlie feeding in the closely related pulmonate, Lymnaea (Elliott and Benjamin, 1985a) and in the opisthobranch, Tritonia hombergi (Bulloch and Dorsett, 1979). Somata of groups of neurons that are active in equivalent phases of the feeding cycles in several different species have been localized to similar areas of their respective buccal ganglia (Bulloch and Ridgeway, 1995).

Putative feeding cycles with two, three, or more phases have been reported in other gastropods. Biphasic cycles reported for the planorbid basommatophoran snails appear to represent partial characterizations of a
Fig. 13. A triphasic buccal motor pattern in *Aplysia*. A, Simultaneous intracellular recordings from a neuron B4/B5 displayed the characteristic ‘butterfly appearance’ that results from the transition from phase 1 excitation to phase 2 excitation. An unidentified motorneuron (MN-1) displayed phase 1 inhibition, phase 2 excitation and phase 3 inhibition. B, A neuron B8 displayed excitation in phases 1 and 2 of a cycle while an unidentified motor neuron (MN-2) displayed distinctive inhibitory inputs during phases 1 and 2 followed by a phase 3 excitation.

Triphasic feeding cycle. The biphasic pattern of alternating bursting in motor neurons described in *Helisoma* by Kater (1974), as discussed above, corresponded clearly to phases 2 and 3 of the standard feeding motor pattern. Similarly, Arshavsky et al. (1988a) described a biphasic pattern of protraction and retraction, separated by a quiescent interval, in *Planorbis*. The pattern described in *Planorbis* corresponded to phases 1 and 2 of the *Helisoma* triphasic feeding pattern. Phase 3 motor neurons that mediate the hyper-retraction of the radula inexplicably were not characterized in *Planorbis*, even though unlabeled somata of what appear to be neurons homologous to the large neurons B18 and B19 of *Helisoma* were included in the *Planorbis* buccal map. In *Aplysia*, a biphasic pattern subserves protraction (phase 1) and retraction (phase 2) of the radula (Hurwitz et al., 1996). A third phase may be involved in swallowing. Perrins and Weiss (1998) presented a triphasic pattern in *Aplysia* but suggested that the third phase may actually represent a pause between biphasic cycles. However, triphasic buccal motor patterns resembling those of *Helisoma* are recorded readily in *Aplysia* neurons (Fig. 13). Neurons B4/B5 receive excitation from phase 1 (protraction) interneurons and from Phase 2 (retraction) interneurons. The onset of the phase 2 depolarization corresponds to a decrease in action potential amplitude and a resultant butterfly appearance of the recording (Fig. 13A cf. Hurwitz and Susswein, 1996). In the instance shown an unidentified motor neuron of the ventral cluster displayed phase 1 inhibition, phase 2 excitation, and inhibition in a third phase. This suggests that neurons B4/B5 may receive excitation from three different sets of interneurons. Similarly, neuron B8 is known to receive excitation during both phase 1 and 2 (Morton and Chiel, 1993). Another unidentified ventral cluster motor neuron displayed distinctive phase 1 and 2 IPSPs followed by a phase 3 burst of action potentials. These ‘phase 3 effects’ could plausibly be explained by mechanisms not requiring phase 3 interneurons, but they are consistent certainly with and suggestive of a tripartite CPG. It remains to be determined whether this third phase of activity in *Aplysia* arises from interneurons homologous to *Helisoma* interneuron N3a. Biphasic motor patterns in other gastropods such as *Clione* (Arshavsky et al., 1989), *Pleurobronchaea* (Davis et al., 1973), and *Helix* (Peters and Altrup, 1984) too may represent partial characterizations of a triphasic pattern.

Buccal rhythms consisting of four or five phases have been reported and may result from the designation of the quiescent interval between cycles as a ‘phase’ of the cycle. For example, the triphasic pattern of feeding motor neuron activity in *Lymnaea* (Elliott and Benjamin, 1985a) was earlier reported to be a four-phase cycle (cf. Fig. 14 in Benjamin and Rose, 1979). Alternatively, the retraction of the radula from its most protracted position to a ‘rest’ position is sometimes subdivided into separate rasp and retraction phases. However, four or five phase cycles (cf. Fig. 2 in Sossin et al., 1987) are within the capabilities of a tripartite pattern generator with flexible linkages among independent conditional oscillators. For example, in *Helisoma*, a rhythmic four-phase S1–S3–S2–S3 pattern can be generated (unpublished observations).

4.3. Interspecific comparisons of CPG interneurons and feeding motor neurons

Few direct comparative studies of similar motor neurons and interneurons involved in feeding in different gastropod species have been attempted. Though the buccal ganglia of pulmonates and opisthobranchs have a plethora of ‘identifiable’ neurons, there are relatively few buccal neurons in any species that are truly ‘uniquely identified.’ Insufficient physiological and morphological data is published to allow re-identification of most buccal neurons that at first glance would seem to be ‘identified’. For instance, on the map of the caudal (aka dorsal) surface of the buccal ganglia of *Helisoma*
provided by Kater (1974) 23 pairs of neurons are depicted and named. However, this study preceded the availability of the dye, Lucifer Yellow and these neurons were ‘identified’ on the basis of relative soma size and position and patterns of electrophysiological activity, with minimal morphological data from Procion stains. Only five of these 23 pairs of neurons are uniquely re-identifiable today (B4, B5, B17, B18, B19). With the exception of those five neurons plus B27, B101 and B110, (Fig. 4) a combination of physiological recordings and morphological stains is necessary for unique identification of Helisoma buccal neurons.

Similarly, Benjamin and colleagues (Benjamin et al., 1979; Rose and Benjamin, 1979, 1981a,b) identified ten ‘types’ of buccal follower neurons in Lymnaea with criteria similar to those used by Kater (1974). Only four of these ten types (neurons B1–B4) represent uniquely identifiable neurons without using morphological data, which has been provided only sporadically (cf. Staras et al., 1998). Arshavsky et al. (1988a,b,c) identified nine ‘groups’ of buccal neurons in Planorbis, again using similar physiological criteria to those of Kater and of Benjamin, with only sporadic concurrent morphological data. The current author is also not blameless. While Lucifer Yellow is used routinely in microelectrodes, yielding morphological data concurrently with physiological data in this author’s laboratory, much of that data sits in reams of chart recordings and photographic negatives on laboratory shelves. I used these examples not to castigate my colleagues but to illuminate the past and current situations, and to call for more published evolutionarily significant data on identifiable neurons, both in print and on the world wide web (cf. Comer and Robertson, this volume).

In spite of the paucity of direct comparative studies, several analogous and potentially homologous neurons have been identified in the basommatophoran snails, Helisoma, Planorbis and Lymnaea, and in two different opisthobranchs, the sea hare, Aplysia, and the pteropod, Clione. The estimated number of neurons in the buccal ganglia of these species varies by about a factor of two (Boyle et al., 1983). Thus, not every neuron in one species will have a unique homolog in other species. For instance, a common developmental cell lineage in two species might result in a lack of a one-to-one relationship between neurons if there is a difference in the number of cell divisions or in programmed cell death in the two species. None-the-less it seems certain that these related gastropods have homologous neurons in the sense that particular neurons result from similar developmental pathways resulting in similar form and function (cf. Breidbach and Kutsch, 1995). The comparisons below are made in an attempt to spur comparative research. More studies combining morphological, electrophysiological, pharmacological and biochemical characterizations of ‘identified’ neurons are needed for each of the species investigated.

We focused on pure interneurons (i.e. no peripheral processes) and influential neurons (i.e. they participate in motor pattern expression but also have peripheral processes that may have sensory or motor functions). While many phase 1, 2, and 3 motor neurons have been identified in several species (e.g. Church and Lloyd, 1994), many of these have great similarities to other motor neurons in the same species and relatively few have been characterized sufficiently to allow ready interspecific comparisons. Neurons were characterized below on the basis of in which phase of the feeding cycle they are most prominently active. Interneurons and influential neurons with major roles in the buccal CPGs were placed in a ‘composite universal tripartite CPG model’ (Fig. 14). Probable or potentially homologous interneurons were boxed together.

![Fig. 14. A composite model for a universal gastropod tripartite buccal central pattern generator. The model displays three subunits (S1, S2, S3) of the CPG, each of which represents a semi-independent neuronal oscillator that provides the primary excitatory to sets of motor neurons active during corresponding phases of a feeding cycle. Neurons in each of the large boxes are active during one of the three phases. Neurons within the smaller rectangles inside the large boxes represent putatively homologous interneurons based on morphological and physiological characteristics. In some cases, these putative homologs have also been shown to have the same neurotransmitter. Note that the BCN1 group is a heterogeneous set of phase 1 interneurons with apparent variability in number among the different gastropods. They have similar morphologies and are all phase 1 interneurons but some fire early during phase 1 and some later during phase. With the exception of Planorbis group 2, the other designations represent individual identified neurons. The arrows indicate that there are excitatory interactions among interneurons of different types within each subunit but should not suggest that every interneuron within a subunit excites every other interneuron. The bar with a perpendicular bar indicates that S1 interneurons excite S2 interneurons. The bars with filled circles represent inhibition. The darker rectangles within each subunit represent the neurons that seem to be necessary and sufficient to generate a triphasic motor pattern. The question marks indicate that, (1) a phase 1 interneuron with a morphology similar to Helisoma interneuron N1a was described in Clione but not given a name; (2) the effects of Lymnaea interneurons N3p and N3t are inhibitory to those motor neurons that are excited by S3 so in that sense they are not a principal part of subunit 3; and (3) it is not yet known whether Aplysia B21 and B22 activate hypothetical phase 3 interneurons. However, like the Helisoma cluster of influential neurons B101–104, they are small cardioactive peptide immunoreactive radular mechanoafferent neurons on the rostral surfaces of the buccal ganglia. They have similar morphologies and fire action potentials during the latter part of spontaneous buccal motor patterns (i.e. during phase 3). H = Helisoma; P = Planorbis; L = Lymnaea; A = Aplysia; C = Clione.](Image 310x445 to 542x515)
Analogous, possibly homologous motor neurons and influential neurons in *Helisoma* (H.), *Lymnaea* (L.), and *Aplysia* (A.)*

<table>
<thead>
<tr>
<th>Phase 1 (protraction) neurons</th>
<th><em>Helisoma</em></th>
<th><em>Lymnaea</em></th>
<th><em>Aplysia</em></th>
<th>Axonal projections</th>
<th>Muscle targets</th>
</tr>
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<tbody>
<tr>
<td>In, B6</td>
<td>In, B31/32</td>
<td>B61/62</td>
<td>A. SRT mn</td>
<td><em>H.</em> PBNs = A. RN</td>
<td><em>H.</em> pj m = A. l 2 m</td>
</tr>
<tr>
<td>B8</td>
<td>B7anor</td>
<td>B61/62</td>
<td>A. SRT mn</td>
<td><em>H.</em> PBN, A. RN, L. pjN</td>
<td><em>H.</em> and L. pj = A. l 2 m</td>
</tr>
<tr>
<td>B7</td>
<td>B7a</td>
<td></td>
<td>A. SRT mn</td>
<td><em>H.</em> VBN = A. N1; L. VBN or LBN?</td>
<td><em>H.</em>? A. SRT, L. pj?</td>
</tr>
<tr>
<td>B3</td>
<td>B5</td>
<td></td>
<td></td>
<td><em>H.</em> and L. DBN</td>
<td>?</td>
</tr>
</tbody>
</table>

| Phase 2 (retraction) neurons   | In, B29   | In, B10   |          | *H.* and L. PBNs | ? Perhaps rad tens m |

| Phase 3 (hyper-retraction) neurons | B19 | B4 | *H.* and L. VBNs and LBNs | *H.* and L. slrt and aj |

*a* in, Influential; PBN, posterobuccal nerve; RN, radular nerve, PJ, posterior jugular; nor, nitric oxide responsive; SRT, subradular tissue; VBN, ventrobuccal nerve; LBN, laterobuccal nerve; DBN, dorsobuccal nerve; rad tens, radular tensor; slrt, supralateral radular tensor; aj, anterior jugular.

The distinction between influential and motor neurons is somewhat arbitrary. There are gradations in the abilities of motor neurons to influence the buccal CPGs, in large part because of extensive electrotonic coupling not only within but across these groups. It has long been known that molluscan motor neurons can influence interneurons. For instance, it was shown by Granzow (1979) that motor neuron B19 could, in some preparations, influence the buccal CPG. Some potentially homologous motor neurons and influential neurons that were not placed in the composite CPG model are indicated in Table 2.

4.4. Protraction phase interneurons

Very similar, perhaps homologous, phase 1 (protraction) catecholaminergic (almost certainly dopaminergic) interneurons have been characterized in *Helisoma* (Quinlan et al., 1997) and *Aplysia* (Kabotyanski et al., 1998). Dopamine may be a universal initiator of gas-tropod feeding motor patterns (e.g. Wieland and Gelperin, 1983; Kyriakides and McCrohan, 1989; Kabotyanski et al., 1994; Quinlan et al., 1997). Neurons N1a in *Helisoma* and B65 in *Aplysia* are similar strikingly. Their somata are in similar locations near the lateral edges of the caudal surfaces of the buccal ganglia. The main axons cross the buccal commissure but do not exit the buccal ganglia. They have neuritic arbors in each of the paired buccal ganglia. Stimulation of these neurons mimics the effects of bath application of dopamine or DOPA. Both neurons N1a and B65 displayed catecholamine histochemical staining. In *Helisoma*, the dopamine antagonist, sulpiride, blocked the effects of bath application of dopamine and of neuron N1a stimulation.

*Lymnaea* interneuron N1L is also a candidate homolog of *Helisoma* neuron N1a and *Aplysia* neuron B65 and is similar to them in relative soma position, morphology and physiological effects on buccal motor patterns (Yeoman et al., 1995). It has been reported to be non-dopaminergic but the negative evidence is not compelling (Vehovsky and Elliott, 1995). The dopaminergic antagonists fluphenazine and ergometrine failed to block the effects of stimulation of *Lymnaea* interneuron N1L. However, the effectiveness of neither antagonist against exogenous dopamine was tested in this system. Furthermore, somata in the vicinity of that of *Lymnaea* neuron N1L displayed dopamine immunoreactivity (Elekes et al., 1991). Another possible homolog of these neurons is a protraction phase interneuron, which has a similar morphology and soma position in the carnivorous opisthobranch, *Clione* (Arshavsky et al., 1989).

Two other types of phase 1 pure buccal interneurons in *Helisoma*, neurons N1b and N1c, were described above (Fig. 8). Neuron N1b is morphologically, at least superficially, similar to the dopaminergic *Aplysia* interneuron B20 (Teyke et al., 1993). There are somata that display catecholaminergic histochemistry in the vicinity of the somata of *Helisoma* interneurons N1b, but whether these interneurons are dopaminergic remains to be determined. Interneurons N1c have somata located laterally on the dorsal surfaces of the buccal ganglia. Interneuron N1c projects an axon to the contralateral buccal ganglion that forms a loop and returns to the ipsilateral buccal ganglion (Fig. 8). This protraction phase interneuron is putatively a homolog of the SO interneuron of *Lymnaea* studied extensively (Elliott and Benjamin, 1985b; Yeoman et al., 1993). A similar phase 1 interneuron, also designated the SO, was also reported in *Planorbis* (Arshavsky et al., 1988a,c). Interneurons of this type have, thus, far been described only in basommatophoran snails.

A widespread class of protraction phase interneurons are represented by the type 1 bucco-cerebral interneurons (BCN1 neurons) in *Helisoma*. Depolarization of these neurons can drive feeding motor patterns and
their action potentials can evoke one-for-one EPSPs in some protraction phase motor neurons. Neurons of this class have somata near the margins of, or sometimes underneath, the somata of the medial giant buccal neurons (e.g. Helisoma neuron B5, Lymnaea neuron B2) that innervate the esophagi of Helisoma, Planorbis, Lymnaea, Aplysia, and Clione (Arshavsky et al., 1988a, 1989; Richmond et al., 1991). The BCN1 interneurons project an axon across the buccal commissure to traverse the contralateral cerebrobuccal connective and enter the cerebral ganglion. Each has a neuritic arbor in both buccal ganglia and a terminal arbor in the cerebral ganglion contralateral to the BCN1 soma. Retrograde staining of severed CBCs in Helisoma with either CoCl$_2$ or Lucifer Yellow show consistently the three neurons of this morphological type in each buccal ganglion. The three pairs of BCN1 neurons are not yet readily distinguishable from each other, but they are heterogenous. Two of the three pairs of BCN1 neurons in Helisoma are GABA-immunoreactive (Richmond et al., 1991).

The numbers of this class of phase 1 interneurons appear to be different in different gastropods. Planorbis, (family Planorbidae, like Helisoma) was reported to have four pairs of these neurons, based on retrograde backfills of the CBC (Arshavsky et al., 1988a). Originally, based upon physiological recordings and a few Lucifer Yellow injections, there were thought to be about ten pairs of interneurons of this type in Lymnaea (Rose and Benjamin, 1981b; Elliott and Benjamin, 1985a). They were referred to at that time as N1 interneurons. It now appears, based upon retrograde staining of CBCs, that there is only one pair of neurons of this type in Lymnaea (Kemenes and Elliott, 1994). It is called the medial N1 neuron or neuron N1M (Yeoman et al., 1995).

Two protraction phase interneurons, B34 and B63, similar to the Helisoma BCN1 morphological type, have been identified in the opisthobranch Aplysia (Hurwitz et al., 1997). Interestingly, interneuron B63 appears to be active during the protraction phase of all buccal motor patterns that have a protraction phase, whereas B34 appears to be devoted to egestion patterns. The carnivorous opisthobranch, Clione, based upon retrograde staining of the CBC, is thought to have only a single pair of protraction phase interneurons (PIN1) of this morphological type (Arshavsky et al., 1989). Thus, currently, there appears to be a greater number of BCN1 type phase 1 interneurons in the pulmonate basommatophoran planorbid snails than in either the closely related pulmonate basommatophoran lymnaeid snails or the opisthobranchs. However, these relative numbers are based on examinations of very few species, and are based largely upon retrograde staining techniques, which can be quite capricious.

4.5. Motor and influential neurons

Helisoma phase 1 motor neurons B3, B6, B7, and B8 were described above (Fig. 5). Motor neurons B31, B32, B61 and B62 of Aplysia have physiological and morphological similarities to neurons B6 and B8 of Helisoma. Aplysia motor neurons B31 and B32 and B61/B62 innervate the intrinsic-2 (I2) muscle, a protractor muscle that is analogous/homologous to the pj muscle innervated by B6 and B8 of Helisoma (Hurwitz et al., 1994; Arnett, 1996). Neurons B31/B32 of Aplysia and B6 and B8 of Helisoma are strongly influential and can evoke buccal motor patterns. An SRT (subradular tissue) motor neuron in Aplysia appears morphologically and physiologically similar to Helisoma neuron B7 (Fig. 5 and Borovikov et al., 1997), but it is not yet known if Helisoma’s B7 innervates the SRT. These neurons have axons in equivalent buccal nerves (Aplysia N3 and Helisoma VBN, formerly called the HBN). Two phase 1 motor neurons were uniquely identified recently in Lymnaea, B7a and B7nor. Neuron B7a is somewhat similar morphologically to Helisoma B7 and Aplysia’s SRT motor neuron in that it has an axon in either the ipsilateral VBN or the LBN (these nerves were not distinguished). Neuron B7a is also an influential neuron that is electrotomically coupled to neuron N1M (Staras et al., 1998). Lymnaea neuron B7nor is responsive to nitric oxide and innervates the pj muscle via the pj nerve (Park et al., 1998), and may be related to the Helisoma B6, B8 and Aplysia B31/B32, B61/B62 neurons.

4.6. Retraction phase interneurons

Pure phase 2 buccal interneurons with no axons traversing buccal nerve roots include the group 2 neurons of Planorbis, and neuron B64 in Aplysia (Arshavsky et al., 1988b; Hurwitz and Susswein, 1996). The Planorbis group 2 neurons displayed endogenous rhythmic activity when isolated from the buccal ganglia (Arshavsky et al., 1988b). Aplysia interneuron B64 was found to be active consistently during Aplysia buccal motor patterns. However, hyperpolarization of left and right neurons B64 failed to eliminate phase 2 PSPs and failed to disrupt buccal motor patterns. Such hyperpolarization revealed large phase 2 depolarizations resembling the plateau potentials seen in the glutamatergic influential interneurons B2 of Helisoma and N2v of Lymnaea (see below). It appears that Aplysia neuron B64 and the Planorbis group 2 neurons may contribute to CPG subunit 2 rhythmicity and have synaptic effects on subsets of buccal interneurons, but activity in the influential glutamatergic interneurons like B2 of Helisoma and N2v of Lymnaea may be necessary and sufficient to provide the primary output of subunit 2.
Glutamate is the primary phase 2 (retraction phase) neurotransmitter, evoking pharmacologically distinct EPSPs and IPSPs in subsets of buccal motor neurons in Helisoma (Quinlan and Murphy, 1996). The ‘phase 3 interneurons’ of Lymnaea clearly are neither morphologically nor functionally equivalent to interneuron N3a of Helisoma. Two types of phase 3 interneurons, N3 tonic (N3t), and N3 phasic (N3p) have been identified in Lymnaea. If one hypothesizes that both Helisoma and Lymnaea have homologs of each of these phase 3 interneuronal types the relationships among the real and hypothetical neuronal types remain unclear. Effects of eliminating activity in either N3t or N3p during a feeding buccal motor pattern in Lymnaea have not been demonstrated. Both neurons evoke PSPs on buccal interneurons and motor neurons. However, it is particularly unclear why neuron N3t is considered part of the buccal CPG. Interneuron N3t fires tonic action potentials essentially continuously except when it is inhibited by phase 1 or 2 interneurons, hence the appellation ‘tonic’. It is active in quiescent ganglia, and it is active in the ‘inactive interval’ between cycles during slow buccal rhythmic activity. Thus, the PSPs evoked by N3t very much resemble the tonic chloride-dependent PSPs recorded in many Helisoma buccal neurons (e.g. B19) either during, or in the absence of, buccal motor patterns (Kater, 1974). The Lymnaea N3p neuronal activity appears restricted to phase 3 of the Lymnaea feeding pattern. However, its synaptic effects are opposite in sign of those of Helisoma phase 3 interneuron N3a on equivalent classes of motor neurons. For instance, there is a set of similar hyper-retraction (aka swallowing) and radular motor neurons in Helisoma and Lymnaea. The largest of these motor neurons are B19 in Helisoma and B4 in Lymnaea. These neurons have similar positions of their somata, similar morphologies, similar (though not identical) physiological activity (see section on comparisons of motor neurons below), and similar muscular targets. Both neurons innervate the slrt and aj muscles. Helisoma neuron B19 is excited by phase 3 interneuron N3a. In fact, the firing patterns of B19 and of interneuron N3a are almost identical to each other during feeding and other motor patterns (Quinlan and Murphy, 1996). The Lymnaea N3p interneuron, however, evokes IPSPs on Lymnaea radular

As with Helisoma, glutamate is the primary phase 2 neurotransmitter in Lymnaea. Recently, Brierly and colleagues (Brierley et al., 1997a,b,c) described a virtually identical and presumably homologous retraction phase, presumptively glutamatergic, interneuron (N2v) in Lymnaea. The originally identified Lymnaea retraction phase N2 interneurons (now called N2d) were shown by photoablation to be unnecessary for the production of normal buccal motor patterns (Rose and Benjamin, 1981b; Kemenes and Elliott, 1994). The N2d neurons have peripheral axons and may be primarily sensory or motor neurons that are coupled electrotonically to interneuron N2v. Neurons B29 in Helisoma (Figs. 6 and 10) and B10 in Lymnaea (Staras et al., 1998) are morphologically similar, relatively weakly, influential phase 2 neurons. Putative glutamatergic retraction phase interneurons in Aplysia remain to be identified but glutamate mimics some of the retraction phase PSPs in Aplysia (Fig. 15).

4.7. Hyper-retraction phase interneurons

Comparisons of phase 3 interneurons among gastropods are more difficult than comparisons among phase 1 and phase 2 interneurons because of ‘presumably missing data’. In Helisoma, interneurons N3a (Fig. 10) account for the primary excitatory and inhibitory phase 3 PSPs in buccal motor neurons. Stimulation of interneuron N3a evokes phase 3-like PSPs in quiescent preparations, and inhibition of interneurons N3a eliminates phase 3 PSPs in buccal motor patterns (Quinlan and Murphy, 1996). The ‘phase 3 interneurons’ of Lymnaea clearly are neither morphologically nor functionally equivalent to interneuron N3a of Helisoma. Two types of phase 3 interneurons, N3 tonic (N3t), and N3 phasic (N3p) have been identified in Lymnaea. If one hypothesizes that both Helisoma and Lymnaea have homologs of each of these phase 3 interneuronal types the relationships among the real and hypothetical neuronal types remain unclear. Effects of eliminating activity in either N3t or N3p during a feeding buccal motor pattern in Lymnaea have not been demonstrated. Both neurons evoke PSPs on buccal interneurons and motor neurons. However, it is particularly unclear why neuron N3t is considered part of the buccal CPG. Interneuron N3t fires tonic action potentials essentially continuously except when it is inhibited by phase 1 or 2 interneurons, hence the appellation ‘tonic’. It is active in quiescent ganglia, and it is active in the ‘inactive interval’ between cycles during slow buccal rhythmic activity. Thus, the PSPs evoked by N3t very much resemble the tonic chloride-dependent PSPs recorded in many Helisoma buccal neurons (e.g. B19) either during, or in the absence of, buccal motor patterns (Kater, 1974). The Lymnaea N3p neuronal activity appears restricted to phase 3 of the Lymnaea feeding pattern. However, its synaptic effects are opposite in sign of those of Helisoma phase 3 interneuron N3a on equivalent classes of motor neurons. For instance, there is a set of similar hyper-retraction (aka swallowing) and radular motor neurons in Helisoma and Lymnaea. The largest of these motor neurons are B19 in Helisoma and B4 in Lymnaea. These neurons have similar positions of their somata, similar morphologies, similar (though not identical) physiological activity (see section on comparisons of motor neurons below), and similar muscular targets. Both neurons innervate the slrt and aj muscles. Helisoma neuron B19 is excited by phase 3 interneuron N3a. In fact, the firing patterns of B19 and of interneuron N3a are almost identical to each other during feeding and other motor patterns (Quinlan and Murphy, 1996). The Lymnaea N3p interneuron, however, evokes IPSPs on Lymnaea radular

Fig. 15. Glutamate depolarizes at least some Aplysia buccal neurons that are excited during the retraction phase of the feeding cycle. An intracellular recording from an Aplysia neuron B4/B5 showed no spiking activity in Aplysia saline. Glutamate (1 mM) was superfused over the preparation (first arrowhead) and it caused a depolarization of about 10 mV, which resulted in continuous firing of action potentials. Note that the glutamate also triggered some EPSPs onto neuron B4/B5 as indicated by the upward deflection of the baseline and a concomitant decrease in action potential amplitude. At the second arrowhead, normal physiological saline (NS) was superfused over the preparation.
tensor neuron B4 during the action potential burst of neuron B4. Thus, for these tensor motor neurons, interneuron N3p (and N3t) appears to act as a brake on the system. Clearly, they do not drive the action potential bursts that occur in phase 3 motor neurons (e.g. B4). To quote Rose and Benjamin (1981a), “The input which we have called ‘N3’ is more interesting. Although it is difficult to see what function this input performs in inhibiting cells which are already hyperpolarized (5 cells, 7 cells) it is clear that the N3 inhibitory input fractionates 4-group and 8 cell bursts into a series of short duration bursts .... It is difficult to provide a functional explanation of the excitatory N3 input to the 3 cell, since this occurs after the 3 cell burst, and all the e.p.s.p.s are subthreshold”.

The phase 3 bursts in Lymnaea B4 (and four-cluster cells) have been attributed to post-inhibitory rebound (PIR) in the motor neurons, just as phase 3 activity in Helisoma B19 was attributed originally to PIR (Kater, 1974). However, Lymnaea’s neuron B4 shows activity patterns similar to Helisoma’s B19 including rhythmic and sporadic excitation independent of phase 2 inhibition. This is consistent with the presence of an unidentified excitatory interneuron similar to interneuron N3a, which accounts for the primary phase 3 excitation in Helisoma motor neurons (compare Figs. 12 and 16).

There is evidence for phase 3 excitation and inhibition in Aplysia (Fig. 13). However, no Aplysia interneurons similar either to Helisoma neuron N3a or Lymnaea neurons N3t and N3p have been reported. However, Aplysia has a population of SCP-immunoreactive radula mechanioafferents on the rostral surface of the buccal ganglia. These neurons are morphologically similar to the B101–104 (akaVB1–VB4) cluster of Helisoma SCPB-ir radula mechanioafferents. These Helisoma neurons are effectively phase 3 interneurons (see above). In addition, at least some of the Aplysia radular mechanioafferents fire action potentials in the latter part (swallowing phase?) of spontaneous buccal motor patterns (Miller et al., 1994). It remains to be seen whether stimulation of these Aplysia neurons can evoke PSPs, similar to swallowing phase PSPs in buccal motor neurons. Direct comparative studies of hyperretraction/swallowing phase interneurons are especially needed at this time.

5. Concluding remarks

We have entered the fourth decade of research on identified neurons and the circuits which they comprise. It is appropriate to consider what they have taught us and what they might teach us in the near future. Perhaps the single greatest point of consensus to arise during this period is that what were initially considered ‘simple systems’ are not simple. Though not simple, many invertebrate systems have the great attribute of experimental tractability. This experimental tractability of systems with relatively small numbers of identifiable neurons led to another major finding — in general, hard-wired circuit diagrams do not underly particular behaviors. There has been a demise of the concept of ‘stereotypy’ with respect to behaviors and the neural outputs of even ‘relatively simple’ circuits. The well-modulated motor system emerged triumphant. Without the identifiability of unique neurons, it would be virtually impossible to ascertain the degree, to which a single neuron’s membrane characteristics and functional interactions with other neurons could be modulated to fit different situations at different times.

It has also become clear that similar rhythmic motor programs — that is, activity in two or more sets of
motorneurons with bursts of action potentials offset by phase lags — could arise from a wide variety of neural circuits. However, the characterized circuits were all composed using a circumscribed number of discrete ‘building blocks’, intrinsic membrane properties and types of synapses (cf. Getting, 1989). Both intrinsic membrane properties and specific synaptic connections can be modulated on a moment to moment basis so that multiple functional motor patterns can arise from a given group of identified neurons. As indicated throughout this volume, future progress based upon identified neurons will encompass further reductionist and molecular analyses of CPG building blocks and computational analyses of circuit function.

During the past 25 years, some of the experimental disadvantages of small mammalian neurons versus large identifiable invertebrate neurons have been mitigated by technical advances — notably the widespread development of patch clamp techniques and molecular expression systems. Many laboratories working initially with the systems of identified neurons in invertebrates have switched to mammalian systems. In addition, the diversity of circuits with similar outputs, the commonality of circuit components, and the functional modulation of a given circuit to produce diverse outputs, have led to the suggestion that what we have already learned from several invertebrate systems may have effectively brought to an end ‘the search for new CPG circuits’. We feel that suggestion to represent a short-sighted view. Though the technical advantages offered by large identifiable neurons over smaller neurons lacking a unique identity may have decreased, the greatest attributes of identifiable neurons for neurobiologists are their unique identities per se! These unique identities allow short- and long-term analyses of circuit functions and their modulations. Furthermore, “With this compendium of data, we will have the unique opportunity to examine the interspecific diversity of the neuronal mechanisms underlying a specific behavior. This approach will be the reductionistic counterpart to the ethological analysis of phylogenetic ritualization (Lorenz, 1966) and should provide significant insight into the evolution of neuronal circuitry.” This optimistic quotation marks the closing remarks of Kater (1974). We are now poised to realize this opportunity.

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