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# Electrophysiological and Behavioral Analysis of Lip Touch as a Component of the Food Stimulus in the Snail *Lymnaea*

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**Staras, Kevin, György Kemenes, and Paul R. Benjamin.** Electrophysiological and behavioral analysis of lip touch as a component of the food stimulus in the snail *Lymnaea*. *J. Neurophysiol.* 81: 1261–1273, 1999. Electrophysiological and video recording methods were used to investigate the function of lip touch in feeding ingestion behavior of the pond snail *Lymnaea stagnalis*. Although this stimulus was used successfully as a conditioning stimulus (CS) in appetitive learning experiments, the detailed role of lip touch as a component of the sensory stimulus provided by food in unconditioned feeding behavior was never ascertained. Synaptic responses to lip touch in identified feeding motoneurons, central pattern generator interneurons, and modulatory interneurons were recorded by intracellular electrodes in a semi-intact preparation. We showed that touch evoked a complex but characteristic sequence of synaptic inputs on each neuron type. Touch never simply activated feeding cycles but provided different types of synaptic input, determined by the feeding phase in which the neuron was normally active in the rhythmic feeding cycle. The tactile stimulus evoked mainly inhibitory synaptic inputs in protraction-phase neurons and excitation in rasp-phase neurons. Swallow-phase neurons were also excited after some delay, suggesting that touch first reinforces the rasp then swallow phase. Video analysis of freely feeding animals demonstrated that during normal ingestion of a solid food flake the food is drawn across the lips throughout the rasp phase and swallow phase and therefore provides a tactile stimulus during both these retraction phases of the feeding cycle. The tactile component of the food stimulus is strongest during the rasp phase when the lips are actively pressed onto the substrate that is being moved across them by the radula. By using a semi-intact preparation we demonstrated that application of touch to the lips during the rasp phase of a sucrose-driven fictive feeding rhythm increases both the regularity and frequency of rasp-phase motoneuron firing compared with sucrose applied alone.

## INTRODUCTION

The nature and function of sensory pathways to centrally located neuronal networks involved in feeding was examined in a variety of molluscan systems, including *Aplysia* (e.g., Rosen et al. 1982a,b), *Limax* (Delaney and Gelperin 1990), *Lymnaea* (Kemenes et al. 1986), *Pleurobranchaea* (Bicker et al. 1982), and *Tritonia* (Audesirk and Audesirk 1980). A detailed understanding of these pathways is of particular importance when they are activated by stimuli used in conditioning experiments where changes in the strength of synaptic connections within the pathway may represent important mechanisms of plasticity contributing to the learned response. The pond snail *L. stagnalis* is a significant model for studying

cellular mechanisms of learning because the neuronal network underlying feeding is known in considerable detail (for review see Benjamin and Elliott 1989), and it was demonstrated that this animal can undergo reliable appetitive conditioning. This was established at both the behavioral level in intact animals (Audesirk et al. 1982; Kemenes and Benjamin 1989a,b, 1994; Kojima et al. 1996) and at the cellular level in semi-intact preparations (Kemenes et al. 1997; Staras et al. 1998b, 1999). In the most thoroughly investigated of these paradigms the conditioning stimulus (CS), a tactile input applied to the lips, is paired with the unconditioned stimulus (US), sucrose (Kemenes and Benjamin 1989a,b). The effects of the unconditioned chemical stimulus on the feeding network were examined in some detail at both the behavioral and electrophysiological levels (Elphick et al. 1995; Kemenes et al. 1986; Yeoman et al. 1995), but the detailed function of the tactile input and the interactions between these two stimuli in normal unconditioned feeding behavior were not investigated previously. Therefore in this study experiments were first performed to characterize the synaptic inputs to identified motoneurons, central pattern generating (CPG) interneurons, and modulatory interneurons of the feeding system, resulting from tactile stimulation of the lips in the absence of a chemosensory food stimulus. Exactly the same types of tactile stimulus and semi-intact preparation were used as in our recent conditioning experiments (Staras et al. 1998b, 1999). A second type of experiment assessed the role of tactile inputs after feeding was initiated by the chemosensory food stimulus.

Evidence for cross-modality integration of sensory information was mainly obtained in *Aplysia* (Rosen et al. 1982b), where the touch component of food applied to the lips reinforces the effects of the chemical stimulus to increase the biting frequency and regularity. In this animal and other opisthobranch mollusks, e.g., *Tritonia* (Audesirk and Audesirk 1980) and *Pleurobranchaea* (Bicker et al. 1982), mechanical stimuli alone appear insufficient to initiate strong ingestion behavior. This was also thought to be the case in *Lymnaea*, where it was demonstrated that touch to the lips cannot elicit unconditioned feeding responses either in the intact animal or in whole lip, semi-intact preparations where the electrophysiological responses to lip touch were recorded on motoneurons (Staras et al. 1998b). Because sucrose can successfully activate feeding in *Lymnaea* (Kemenes et al. 1986; Staras et al. 1998b), it was assumed that chemical cues were most important in initiating feeding, but a subsidiary role for tactile input in the initiation of feeding could not be definitely excluded. An alternative hypothesis is that the lip touch has no role in the initiation of feeding but may interact with feeding neurons to modulate a

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particular phase of the feeding cycle. The results will show that touch to the lips in a semi-intact preparation produces a complex sequence of inhibitory and excitatory synaptic events in all the known neurons of the feeding circuit. The main functional effect of these inputs is to reinforce rasp- and swallow-phase activity once feeding was initiated by food. However, in the absence of a chemical food stimulus, these inputs never led to the initiation of feeding. This reinforcing rather than initiating function for mechanosensory inputs from the lips was supported by analysis of lip stimulation by the food substrate during feeding in freely moving animals and by experiments testing the contribution of the tactile stimulus to sucrose-driven fictive feeding activity in semi-intact preparations. Taken together the data presented here suggest that the tactile component of food provides a mechanical stimulus to the lip structures during the rasp and swallow phases of feeding and through specific connections with neurons of the feeding network mediates a cycle-by-cycle reinforcement of fast, ongoing feeding.

## METHODS

### General procedures

Wild-type specimens of adult *L. stagnalis* were obtained from suppliers (Blades Biological, Kent) and were maintained in large holding tanks containing  $\text{Cu}^{2+}$ -free water at 18–20°C on a 12 h–12 h light:dark regime and fed lettuce three times a week. Before an experiment animals were moved into smaller tanks in the laboratory.

### Whole lip CNS preparation

The preparation used in these experiments was developed to allow intracellular recordings to be made from identified neurons of the feeding network while simultaneously presenting a tactile stimulus or a chemical stimulus, sucrose, to the lip structures (see Staras et al. 1998b). Animals were dissected under a microscope in a Sylgard-coated dish containing HEPES-buffered snail saline (Benjamin and Winlow 1981). A dorsal body incision was used to expose the CNS, and all peripheral nerves except for the median and superior lip nerves were cut. The buccal mass was excised, but a short piece of esophagus was left attached to the brain to assist in pinning out the preparation. The posterior end of the foot was removed, and the preparation was arranged in a purpose-built Sylgard-coated electrophysiology chamber containing saline. The lips were pinned down so that they were accessible for sensory stimulation, and the buccal ganglia were stretched clear of the head structures over a raised block of Sylgard (to stabilize the preparation) and rotated 180° so that the dorsal side was exposed for intracellular recording (Fig. 1A). On several occasions, where it was necessary to record from ventrally located buccal neurons, one of the buccal ganglia was rotated around the buccal commissure to expose the ventral surface (Fig. 1B). The whole lip CNS preparation also made it possible to make recordings from feeding neurons located in the cerebral ganglia. The outer ganglionic sheath of the cerebral and buccal ganglia was removed with a pair of fine forceps. The second, inner sheath was softened with a nonspecific solid protease (Sigma, XIV, Sigma Chemical, Poole, UK). The enzyme treatment was terminated by washing the preparation with fresh saline.

### Intracellular recording techniques

Glass microelectrodes (2 mm, Clark Electromedical, Redding, UK) were pulled on a Narashigi vertical electrode puller and filled with 4 M potassium acetate to achieve a tip resistance of 10–50 MΩ. The electrode tips were marked with a black etching ink (Rotring, part no.

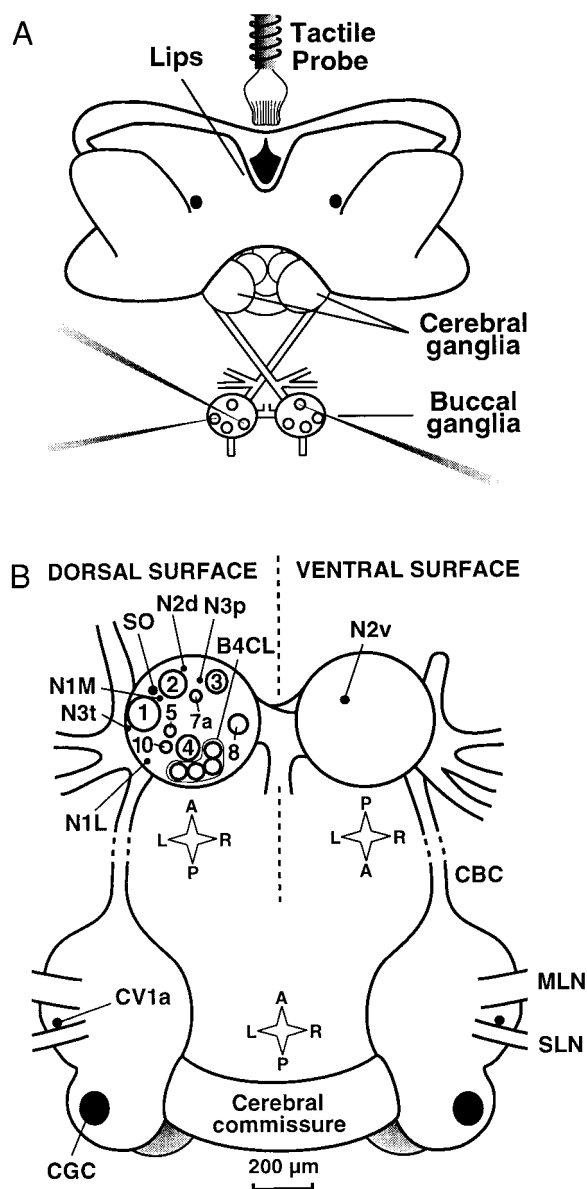


FIG. 1. Semi-intact preparation used to record neurophysiological responses of identified feeding interneurons and motoneurons to a lip tactile stimulus. *A*: schematic diagram showing the arrangement of the preparation. The lip structures, retained as a single unit, are connected to the CNS by peripheral lip nerves. Feeding neurons are located in both the cerebral and buccal ganglia. *B*: positions of identified motoneurons, central pattern generator (CPG) interneurons, and modulatory neurons of the feeding system that were recorded in this study. Buccal ganglia are twisted so that the ventral surface of 1 ganglion and the dorsal surface of the other is exposed. The motoneurons (B1, B2, B3, B4, B4CL, B5, B7a, B8, and B10) are shown as numbered open circles; the CPG interneurons, N1M (N1 medial), N1L (N1 lateral), N2v (N2 ventral), N2d (N2 dorsal), N3t (N3 tonic), and N3p (N3 phasic) and the modulatory interneuron SO (slow oscillator) are all located in the paired buccal ganglia (top). The modulatory neurons CV1 (cerebral ventral 1) and CGC (cerebral giant cell) are located in the cerebral ganglia. All the neurons shown in 1 ganglion have a bilaterally symmetrical homologue in the other, with the exception of the SO. The cerebral and buccal ganglia are linked by the CBC (cerebrobuccal connective). The lips are connected to the cerebral ganglia by the MLN (median lip nerves) and the SLN (superior lip nerves). A: anterior; P: posterior; L: left; R: right.

595617) to improve visualization. The chamber containing the preparation was illuminated from a cold light source and viewed under a zoom microscope. The chamber was connected to a peristaltic pump permitting a rapid exchange of saline.

Micromanipulators with attached headstage preamplifiers (Neurolog, Digitimer, Welwyn Garden City, UK) arranged around the chamber allowed up to four simultaneous intracellular recordings. Signals were fed into amplifiers (NL102G, Digitimer) incorporating a bridge-balance circuit for current injection and then sent to a storage oscilloscope (GOULD 1604, Gould Instrument Systems, Hainault, UK), a chart recorder (GOULD TA240S), and a DAT recorder (BIOLOGIC DTR-1801, Biologic Science Instruments, Claix, France).

### Identification and selection of cell types

The main objective of the electrophysiological experiments was to monitor the synaptic inputs to identified neurons of the feeding network evoked by tactile stimulation to the lips. The feeding network is made up of CPG interneurons, motoneurons, and modulatory interneurons (see Benjamin and Elliott 1989). The three behavioral phases of rhythmic feeding behavior, protraction, rasp, and swallow, are generated by three main types of CPG interneurons known as N1, N2, and N3 (Rose and Benjamin 1981b) (Fig. 1B), each of which have two subtypes. These are the N1 medial (N1M) and N1 lateral (N1L) cells (Yeoman et al. 1995), the N2 dorsal (N2d) and N2 ventral (N2v) cells (Brierley et al. 1997), and the N3 tonic (N3t) and N3 phasic (N3p) cells (Elliott and Benjamin 1985a). All the CPG interneurons occur as bilaterally symmetrical pairs of cells on the dorsal surface of the buccal ganglia, except for the N2v cells, which are on the ventral surface. The identity of the interneurons was established by position, firing activity, and synaptic inputs they receive (summarized by Brierley et al. 1997; Staras et al. 1998a; Yeoman et al. 1995).

The feeding motoneurons recorded in this paper (B1, B2, B3, B4, B4CL, B5, B7a, B8, and B10; Fig. 1B) are located in bilaterally symmetrical pairs on the dorsal surface of the buccal ganglia (Benjamin and Rose 1979; Rose and Benjamin 1979). They receive synaptic inputs from the CPG interneurons and are classified as protraction- (B1, B5, and B7a), rasp- (B3, B4CL, and B10), or swallow- (B2, B4, and B8) phase neurons, defined by the phase in which they become active (see Benjamin and Elliott 1989).

The three modulatory neurons examined were the slow oscillator (SO), the cerebral ventral 1a (CV1a), and the cerebral giant cell (CGC) interneurons (Fig. 1B). The SO is a single unpaired neuron in either the left or right side of the dorsal surface of the buccal ganglia (Rose and Benjamin 1981a). The CV1a cells, a bilaterally symmetrical pair of neurons, are located on the ventral surface of the cerebral ganglia (McCrohan 1984). Both the SO and the CV1a cells can drive active feeding activity in the CPG when they are depolarized, and they both fire in the protraction (N1) phase. The CGCs, a bilaterally symmetrical pair of large neurons in the anterior lobe of the cerebral ganglia, fires tonically showing moderate entrainment to the feeding rhythm (McCrohan and Benjamin 1980).

### Salines

Two modified HEPES-buffered salines were used to investigate the nature of the lip touch synaptic response. High  $Mg^{2+}$ , low  $Ca^{2+}$  (HiLo) saline + EGTA (composition described by Elliott and Benjamin 1989), which contains virtually no  $Ca^{2+}$  and nine times the concentration of  $Mg^{2+}$  present in normal saline, blocks chemical synapses by replacing  $Ca^{2+}$  ions necessary for synaptic transmission with  $Mg^{2+}$  and was used to test for the presence of electrotonic synapses. The monosynaptic nature of chemical synapses was tested by bathing the preparation with high  $Mg^{2+}$ , high  $Ca^{2+}$  (HiDi) saline. This saline is known to increase the threshold of intermediate neurons (Elliott and Benjamin 1989) and block polysynaptic pathways.

### Tactile and chemical stimulation of the lips in semi-intact preparations

An electromagnetic coil-driven mechanical probe was used to deliver the tactile stimulus to the lips of the animal. This was the same

stimulus used in previous experiments to test the survival of a behaviorally conditioned response in semi-intact preparations derived from trained whole animals (Staras et al. 1998b, 1999) and was designed to closely mimic the CS used to train the whole animal (see Kemenes and Benjamin 1989a,b; Staras et al. 1998b, 1999). The end of the probe consisted of a thin wedge of soft flexible plastic. This stimulus, which was of standard duration and intensity, induced a small noise bar (at onset and offset) on the recording equipment so that accurate information about touch response latencies could be gathered.

A sucrose solution (0.01 M) was used to activate feeding in the semi-intact preparations. This stimulus, which is the same as that used in a previous study in *Lymnaea* to activate feeding behavior (Staras et al. 1998b), was delivered from a thin plastic tube positioned at the front of the experimental chamber. Sucrose was released from the end of the tube with a syringe and diffused passively across the lip chemosensory structures. In this way the tactile component of sucrose application could be minimized.

### Behavioral function for lip tactile stimulus

The rasping movements of freely moving whole animals were recorded on videotape as they carried out consummatory feeding behavior in an inverted position at the water surface. This is the feeding position they normally adopt when they feed on floating pond weed in their normal environment or in aquarium tanks in the laboratory. Animals were presented with strips of fish food flake (AQUARIAN herbivore flakes, Pedigree Petfoods, Melton Mowbray, UK), which are presumed to have tactile properties similar to the vegetative diet of algae-covered pond weed or lettuce that they normally consume but had the advantage of being semi-transparent, allowing clear visualization of the lips, radula (rasping organ), and mouth during consummatory behavior. This behavior was recorded with a charge-coupled device camera (DXC-151P, Sony, Japan) mounted onto a dissecting microscope (Leica Wild M420, Heerbrugg, Switzerland), and still frames were captured from the video sequence with computer software.

## RESULTS

### Touch-evoked synaptic responses on identified feeding neurons

The lip touch response in feeding-related neurons was investigated in a semi-intact lip-CNS preparation that consisted of the head of the animal attached to the CNS by two pairs of lip nerves (Fig. 1A). This allowed access to the buccal and cerebral ganglia for intracellular recording and to the lips for tactile stimulation (see METHODS). The tactile stimulus consisted of a brief touch applied to the lip structures anterior to the mouth and most closely resembled the area of the lips accessible for stimulation in the freely behaving animal when conditioning experiments were performed in vivo (Kemenes and Benjamin 1989a,b, 1994; Staras et al. 1998b, 1999). The data will show that a response to lip touch could be recorded on all the identified feeding interneurons and key motoneurons. Each neuron type exhibited a complex but characteristic lip touch synaptic response, the basic form of which remained consistent between preparations. There were clear differences in the overall effects of touch stimulation, depending on where the neurons fire within the behaviorally defined feeding cycle. To emphasize this finding, the lip touch responses on neurons of the feeding system are categorized subsequently in terms of the phase of the feeding cycle in which they fire, e.g., protraction, rasp, or swallow.

**PROTRACTION-PHASE NEURONS.** There are a variety of CPG interneurons and motoneurons (as well as modulatory inter-

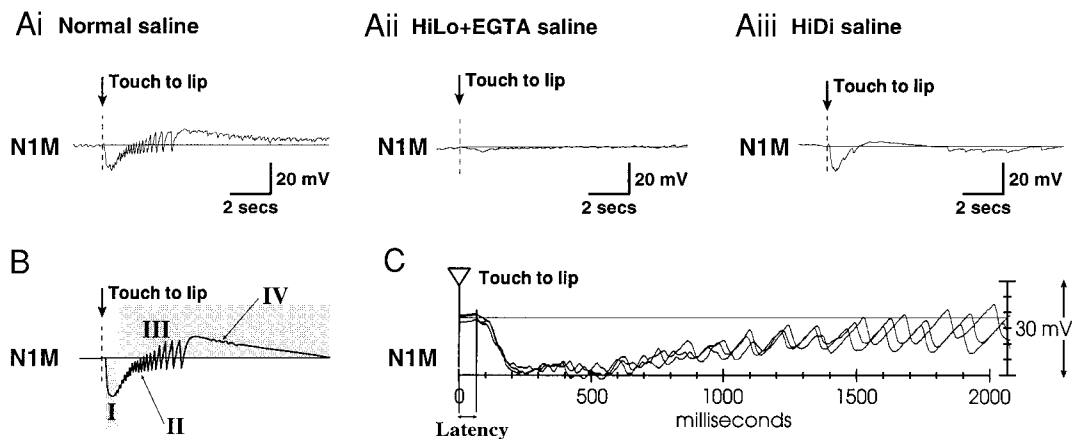


FIG. 2. Lip touch response recorded on the protraction-phase neuron N1M. *Ai*: typical example of the N1M lip touch response recorded in normal saline. The touch evokes a smooth hyperpolarization followed by a slow depolarizing wave. Superimposed on this are large unitary inhibitory postsynaptic potentials (IPSPs) and later a continuous series of small unitary IPSPs. *Aii*: typical response in high  $Mg^{2+}$ , zero  $Ca^{2+}$  saline, which blocks chemical synapses, showing that the touch-evoked synaptic responses are almost completely abolished. *Aiii*: typical response in high  $Mg^{2+}$ , high  $Ca^{2+}$  saline, which enhances monosynaptic inputs and reduces the excitability of polysynaptic pathways, showing that the initial touch-evoked responses are retained, indicative of a monosynaptic pathway (see text for further discussion). *B*: schematic diagram summarizing the 4 main synaptic inputs (I, II, III, and IV) recorded on N1M evoked by lip touch. *C*: 3 superimposed N1M touch responses illustrating the constant latency (75 ms) of the 1st phase of the lip touch response.

neurons) whose activity is largely confined to the N1 or protraction phase of the feeding rhythm. If tactile inputs from the lips were involved in initiating feeding protraction-phase neurons should be excited. One of the most important of these cell types is the CPG interneuron N1M. Its intrinsic excitability and endogenous bursting properties suggest that it is likely to be an important component in making the decision to feed and as such represents a pivotal element in the feeding circuit. For these reasons its synaptic response to tactile stimulation of the lip was used as a model for comparison with touch responses in other interneurons and motoneurons.

**LIP TOUCH RESPONSE ON THE N1M PATTERN-GENERATING INTERNEURON.** The lip touch response recorded in the N1M was complex. At resting membrane potential touch never evoked spike activity or drove fictive feeding rhythms in the N1M ( $n = 50$  cells). Instead it evoked a complex sequence of synaptic events that were mainly inhibitory. A typical example of the N1M lip touch synaptic response is shown in Fig. 2*Ai*. The inputs are summarized in the schematic diagram in Fig. 2*B*. Initially a lip touch evoked a smooth hyperpolarization (input I) with a gradual recovery followed by a long-lasting slow depolarizing wave (input III, Fig. 2*Ai*). Superimposed on the recovery phase were large single inhibitory postsynaptic potentials (IPSPs, input II) and later a continuous series of small unitary IPSPs (input IV) that appear to continue throughout the period of sustained depolarization (input III).

The onset of the initial synaptic component (input I) after the touch is delivered to the lips was  $70 \pm 11$  ms ( $n = 9$ ; Fig. 2*C*). In view of the physical distance separating the lips and buccal ganglia ( $\sim 5$  mm) this is a short latency suggesting that the mechanosensory  $\rightarrow$  N1M pathway is fairly direct. The unitary IPSP (input II) and the secondary depolarization (input III) were far more variable in both onset and duration, which is good evidence for a polysynaptic pathway of activation.

The chemical nature of the synaptic input underlying the N1M lip touch response was investigated in the modified HiLo + EGTA saline, which blocks chemical synapses. In

the preparations tested ( $n = 3$  cells) the majority of N1M responses was rapidly abolished after 50 min, indicating that chemical synapses were involved (Fig. 2*Aii*). The polysynaptic nature of the touch response was examined by bathing the preparation for 50 min in HiDi saline. The amplitude of the initial inhibitory synaptic component (input I) was unchanged in all the preparations tested ( $n = 4$  cells, Fig. 2*Aiii*). Given that HiDi saline hyperpolarizes *Lymnaea* neurons (Elliott and Benjamin 1989) and would tend to reduce the reliability of polysynaptic pathways, this suggests that input I arises from a reasonably direct pathway from the mechanoreceptors in the lips, although not necessarily monosynaptic. Inhibitory input II and excitatory input III were considerably reduced in amplitude, suggesting a more complex, polysynaptic pathway was involved.

In conclusion, the lip touch response recorded on the protraction-phase interneuron N1M was mainly inhibitory, and despite the potential excitatory effects of the input III depolarization touch never resulted in the firing of the N1M cells at resting membrane potential. The inhibitory inputs could have been provided by other CPG feeding interneurons such as N2 or N3, which have previously been shown to have strong inhibitory synapses with the N1M (Elliott and Benjamin 1985a). The subsequent longer latency synaptic inputs could also arise from other CPG or modulatory neurons. These possible sources for the tactile inputs recorded on N1M were considered subsequently.

**LIP TOUCH RESPONSE ON OTHER PROTRACTION-PHASE INTERNEURONS (SO, NIL, AND CV1a).** The SO is a modulatory protraction-phase interneuron that like the N1M is capable of driving the feeding CPG (Elliott and Benjamin 1985b). As was the case with the N1M, the SO received an initial inhibitory touch-evoked input ( $n = 20$  cells) with a comparable latency but of a smaller amplitude (Fig. 3*A*). In the SO, there was also evidence for the presence of the delayed subthreshold depolarizing synaptic components seen on the N1M as well as small unitary IPSPs (arrowed in Fig. 3*A*).

The NIL, another identified protraction-phase CPG inter-

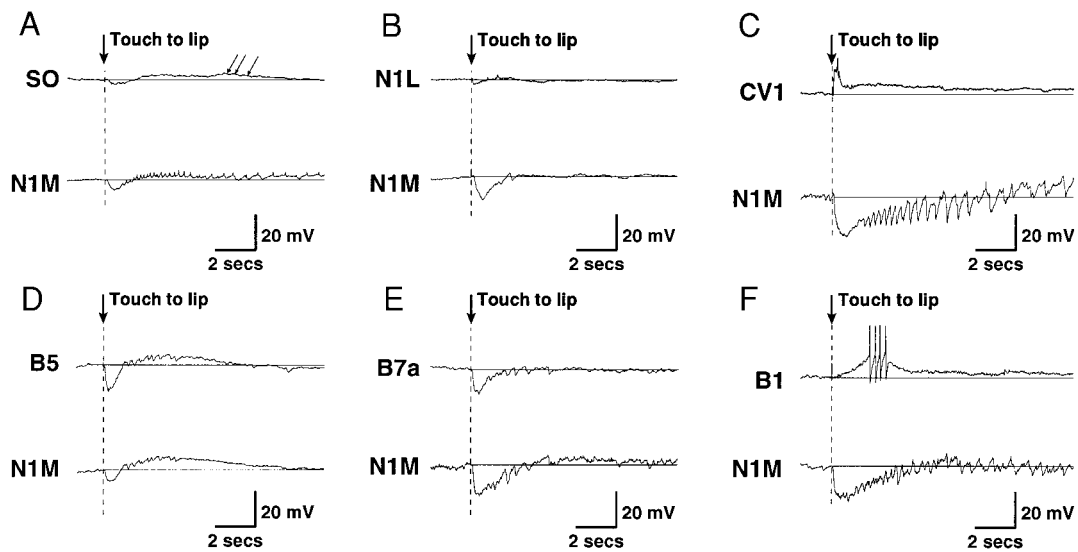


FIG. 3. Lip touch response recorded on the protraction-phase interneurons and motoneurons of the feeding system are similar to the response in the N1M CPG interneuron, apart from the B1 cell whose responses are depolarizing and simpler in waveform than N1M: dual recording from the N1M and A: SO, modulatory interneuron; B: N1L, CPG interneuron; C: CV1, modulatory interneuron; D: B5 motoneuron; E: B7a motoneuron; F: B1 motoneuron.

neuron, fires exclusively in the protraction phase of the feeding cycle. The N1L lip touch response ( $n = 4$  cells) had an onset latency and shape that were similar to those recorded in the N1M, but its amplitude, like that of the response in SO, was smaller (Fig. 3B).

The CV1a is a protraction-phase modulatory interneuron located in the cerebral ganglia that, like the SO, is able to drive fictive rhythmic activity in the feeding CPG (McCrohan 1984). On the basis of the responses recorded in other protraction-phase interneurons one might predict that the CV1a would exhibit a characteristic inhibition to lip touch. However, the reverse was true, and the CV1a showed an overall depolarizing response to touch ( $n = 4$  cells), which during the early phase of the response led to truncated spikes (Fig. 3C). The latency of this response was shorter than in any of the other identified neurons described ( $46 \pm 10$  ms,  $n = 3$ ). This is presumably explained by the fact that the cerebral ganglion-located CV1a is closer to the lip structures along the lip-CNS sensory pathway than the buccal neurons. A second clear depolarizing component is also present on CV1a corresponding to input III on N1M, and a series of weak excitatory postsynaptic potentials (EPSPs) superimposed on this may also correspond with unitary IPSPs (input IV) recorded on the N1M. That the CV1a contradicts the pattern of responses found in the other protraction-phase neurons suggests that it may have a different, unique role to play. Given that the various phases of touch-induced synaptic response are depolarizing, it could theoretically lead to excitation of the whole feeding pattern via its connection with the N1M (McCrohan and Kyriakides 1989). However, in the type of preparation used here, touch never led to sustained activity characteristic of a fictive feeding rhythm. These experiments show that the protraction-phase interneurons are not activated to spiking in response to touch, suggesting that the lip touch sensory pathway is not involved in activating the feeding rhythm. Furthermore, it is clear that none of these interneurons is responsible for producing input I, which must therefore be arising from activity in other (unknown) cells.

LIP TOUCH RESPONSE ON PROTRACTION-PHASE MOTONEURONS (B5, B7a, AND B1). In addition to CPG and modulatory interneurons several identified motoneurons are known to fire in the protraction phase of the fictive feeding rhythm. Motoneurons B5 and B7a, as well as being involved in activating buccal musculature, are also electrotonically coupled to the N1M (Elliott and Kemenes 1992; Staras et al. 1998a), and their activity closely follows that recorded in this CPG interneuron. Theoretically, both cells could activate a fictive feeding rhythm via the electrotonic connection with the N1M cells. In fact, both show a lip touch response (B5,  $n = 3$  cells; B7a,  $n = 4$  cells) in which the initial inhibition and subsequent mixed excitatory and inhibitory components seen on the N1M appear to be present, although they can be variable in amplitude (Fig. 3, D and E). It is unlikely that the shared synaptic inputs could be due to electrotonic coupling alone as this is insufficiently strong (unpublished observations;  $k \sim 10\text{--}15\%$ ) to generate similar amplitude subthreshold synaptic events in all the coupled cells.

The B1 motoneuron, provisionally classified as a salivary gland motoneuron because of its morphology, shows weak activation during the protraction phase of a feeding rhythm. A lip touch produced a weak but delayed depolarizing response ( $n = 3$  cells) that could trigger action potentials 1–2 s after the touch (Fig. 3F). One possibility is that the B1 is only receiving the depolarizing synaptic input III seen on the other protraction-phase neurons because no obvious inhibitory or excitatory inputs I and II were seen on B1 after touch. The latency of this response was difficult to measure because of the slow rise time of the depolarization. The B1 cell appears to be unique among protraction-phase motoneurons in that it alone showed a strong excitatory response to lip touch. However, it has no reciprocal synaptic connections with CPG neurons (Staras et al. 1998a) and so cannot play a role in the initiation of a fictive feeding rhythm.

#### Rasp-phase neurons

The main effects of lip touch on the rasp-phase CPG interneurons were depolarizing, the opposite to the protraction-

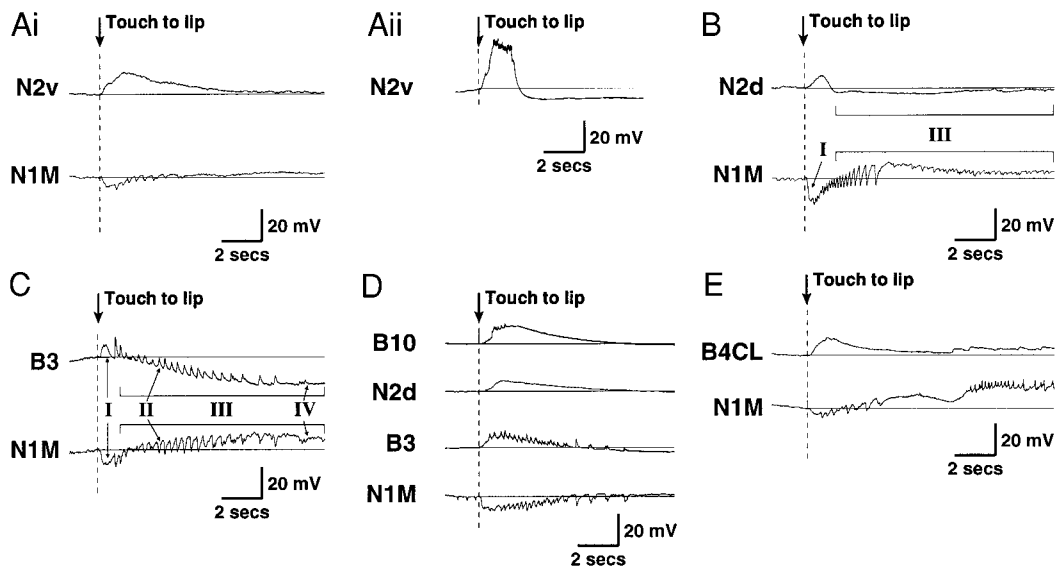


FIG. 4. Lip touch response recorded on the rasp-phase interneurons and motoneurons of the feeding system. *Ai*: dual recording of the CPG interneuron N2v and the protraction-phase CPG interneuron N1M. The lip touch response on N2v consists of a single, normally subthreshold, depolarizing component with the same onset latency as the first input in N1M. *Aii*: example of a lip touch response that evokes a plateau potential on the N2v. *B*: dual recording of the CPG interneuron N2d and N1M. The lip touch response on N2d consists of a depolarizing followed by a hyperpolarizing input. *C*: dual recording of the B3 motoneuron and the N1M. Lip touch evokes a series of inputs (I, II, III, and IV) on the B3 that is identical in latency and waveform but opposite in sign to the response on N1M. *D*: simultaneous recordings from the B10 motoneuron, N2d, B3, and N1M. *E*: dual recording of the B4CL motoneuron and N1M.

phase neurons. Although the responses were usually subthreshold for spike initiation, they could still be important in reinforcing the retraction phase of feeding.

**LIP TOUCH RESPONSE ON RASP-PHASE CPG INTERNEURONS (N2v AND N2d).** The N2v, a recently identified rasp-phase CPG interneuron, has endogenous plateauing properties (Brierley et al. 1997). The N2v lip touch response consisted of a single depolarizing input, the latency to onset of which was similar to the first phase of inhibition (input I) recorded in the N1-phase neurons (Fig. 4*Ai*). In most cells this depolarizing wave on the N2 versus was subthreshold ( $n = 8$  cells, Fig. 4*Ai*), but in one preparation it was sufficient to consistently trigger a full-sized plateau with superimposed truncated spike-like events characteristic of this cell type (Brierley et al. 1997) (Fig. 4*Aii*). The N2d, a second type of rasp-phase CPG interneuron, also exhibited an immediate depolarization in response to touch (Fig. 4*B*). Usually this event had a slower rise time than that observed in the N2v, which made the latency of its onset difficult to assess. The touch input was never sufficient to trigger spikes in N2d ( $n = 9$  cells). The N2d appeared to be hyperpolarized after the initial depolarization, which often caused the membrane potential to fall below the pretouch resting potential. This apparent inhibition had a very slow waveform and had a similar duration to the input III depolarizing wave on the N1M. Most of the depolarizing responses recorded on cells were subthreshold for spike initiation so that they could not be responsible for the synaptic inputs occurring on other cells such as the N1Ms.

**LIP TOUCH RESPONSE ON RASP-PHASE MOTONEURONS (B3, B10, AND B4CL).** The B3 motoneurons receive characteristic synaptic inputs after lip touch ( $n = 50$  cells) that are identical in latency and waveform but are of opposite sign to those recorded in the N1M (Fig. 4*C*). The initial N1M inhibitory input I (Fig. 4*C*) appeared as an equal amplitude depolarizing com-

ponent on B3, usually subthreshold for spike initiation. The unitary IPSPs recorded in the N1M were apparent as unitary EPSPs on the B3 (II, Fig. 4*C*) as were the smaller IPSPs (IV) occurring later during the long-duration hyperpolarization (Fig. 4*D*). The synaptic depolarizing input III in N1M (Fig. 4*C*) was seen as a mirror-image, long-duration IPSP in the B3, and the amplitude and time course were identical.

The B10 cell is a motoneuron of the radular tensor muscle (Staras et al. 1998a). It is weakly active in late protraction and strongly active throughout the rasp phase. It is electrotonically coupled to both rasp-phase interneurons N2d and N2v (Staras et al. 1998a). This coupling could partly explain the similar waveform of the touch response in both N2d and B10 cells seen in Fig. 4*D*, but the depolarizing response is larger in B10 than in N2d, suggesting an independent input. The B4CLs are a group of  $\sim 12$  retraction-phase motoneurons of the anterior jugalis muscle that are active during the late rasp and early swallow phases of feeding. The initial excitatory responses seen in the other rasp-phase interneurons and motoneurons to lip touch are also apparent on the B4CL neurons (Fig. 4*E*,  $n = 4$  cells). The details of this B4CL cell response will be considered in the next section along with those of the B4 and B8 motoneurons. This is convenient because the three cell types are functionally related. They all innervate different parts of the anterior jugalis muscle, the main muscle involved in the retraction of the buccal mass (Rose and Benjamin 1979).

#### Swallow-phase neurons

Evidence from responses recorded in N1/protraction- and N2/rasp-phase neurons indicates that possible roles for the lip touch pathway are the reinforcement of the rasp phase and the inhibition of the protraction phase of the feeding cycle. In a feeding rhythm, the rasp phase is followed by activity in the swallow-phase neurons. Touch-evoked activity causes delayed

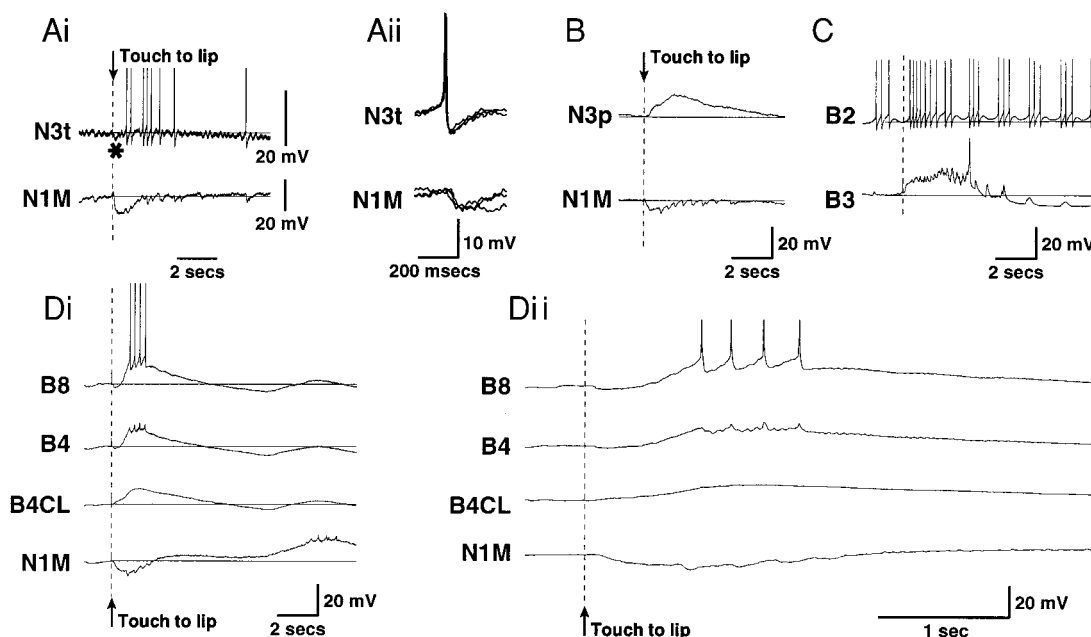


FIG. 5. Lip touch response recorded on the swallow-phase interneurons and motoneurons of the feeding system. *Ai*: dual recording of the CPG interneuron N3t and the protraction-phase CPG interneuron N1M. The lip touch response evokes a small hyperpolarization (\*) followed by a series of spikes on N3t. These spikes are responsible for the unitary IPSPs recorded on the N1M. *Aii*: 3 superimposed N3t spikes and the unitary IPSPs they evoke in N1M. *B*: dual recording of the CPG interneuron N3p and N1M. The lip touch response on N2v consists of a single, subthreshold depolarizing input. *C*: dual recording of the B2 and B3 motoneurons. Touch evokes an extra burst of spikes in the tonically active B2. *Di*: simultaneous recordings from the B8, B4, and B4CL motoneurons and the N1M. Lip touch evokes a similar response in the coupled B8 and B4 motoneurons consisting of an initial hyperpolarizing component followed by a depolarization, which triggers spikes and electrotonic potentials. In B4CL the initial hyperpolarizing component is absent, and the depolarization begins earlier. *Dii*: traces shown in *Di* on a faster time base.

excitation of swallow-phase neurons, which suggested that both retraction phases of the feeding cycle (rasp and swallow) were reinforced by lip touch.

**LIP TOUCH RESPONSE ON SWALLOW-PHASE CPG INTERNEURONS (N3p AND N3t).** The N3t fires a strong burst of spikes throughout the swallow phase of the cycle (Elliott and Benjamin 1985a). Initially, the lip touch response ( $n = 4$  cells) causes a brief, weak inhibition, after which the cell fires a series of spikes (Fig. 5*Ai*). No obvious depolarizing input can account for this delayed spike activity, and it may be due to postinhibitory rebound, a property that has previously been shown to be present in the N3ts (Elliott and Benjamin 1985a) with hyperpolarizing stimuli that were similarly weak and brief as the touch-evoked inhibitory input seen in this study.

Dual recordings of the N3t with the N1M interneurons shows that N3t spikes are responsible for the unitary IPSPs (input II) seen in the N1M touch response. The monosynaptic inhibitory connection between N3t and N1M was described previously (Elliott and Benjamin 1985a) and is demonstrated here on a fast time-base recording in response to touch (Fig. 5*Aii*). The touch-evoked IPSPs recorded on other neurons such as the SO, B5, and B7a and the unitary EPSPs seen on the B3 are also likely to arise from spike activity in the N3t on the basis of previous published data on synaptic connectivity between N3t and these neurons (Elliott and Benjamin 1985a). Activation of the N3t by touch causes large unitary IPSPs on the N1M, and this prolongs the initial period of strong inhibition recorded in response to the lip CS. This type of prolonged inhibitory input would make it highly unlikely that the N1M cells could be excited by touch despite the delayed depolarizing wave (input III) also induced by touch. During fictive

feeding the N3t has a strong influence on the feeding pattern via the  $N3t \rightarrow N1M$  inhibitory connection, and this prevents the N1M cells from firing during swallow. This would be reinforced by the effects of touch to the lips because this stimulus occurred during the rasp/N2 phase of the feeding cycle in the intact snail.

The N3p was originally classified as a purely swallow-phase interneuron, although in many fictive feeding rhythms it fires earlier (Yeoman et al. 1995) and is active during both the late rasp as well as the early swallow phase. On the basis of this firing pattern, one might predict that the N3p response would share much in common with the touch response recorded in the N2 interneuron types rather than the N3t cell. Indeed, the touch response in N3p ( $n = 5$  cells) was very comparable with the N2d response with a similar slow depolarizing input followed by a long-lasting hyperpolarization (Fig. 5*B*). The N3p is never driven to firing by the application of touch, so it cannot be contributing to the later phase of touch-induced synaptic inputs recorded on other cells, such as the N1Ms.

**LIP TOUCH RESPONSE ON SWALLOW-PHASE MOTONEURONS (B2, B4, AND B8).** The B2 motoneuron, involved with gut contraction, exhibits only weak entrainment to the feeding rhythm firing throughout the swallow phase. In most recordings ( $n = 4$  cells) the B2 bursts spontaneously, and in these instances a lip touch triggers an extra burst of spikes (Fig. 5*C*). The B4 and B8 are the main swallow-phase motoneurons. They receive a prolonged inhibition during the N2 phase of feeding and so fire later than the B4CLs throughout the swallow phase of the feeding cycle. Their activity closely follows that in the N3t interneurons to which they are electrotonically coupled (Staras et al. 1998a). The lip touch responses on the B4 and B8

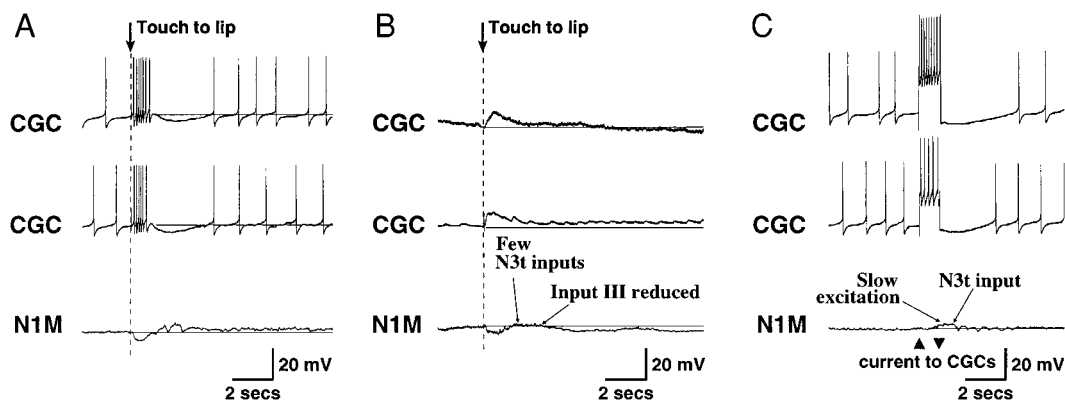


FIG. 6. Lip touch response recorded on the CGCs. *A*: dual recording from the left and right CGCs and the protraction-phase CPG interneuron N1M. Lip touch evokes a burst of spikes in both the left and right CGCs, followed by a recovery period during which no action potentials occur. *B*: when both CGCs are suppressed by injecting hyperpolarizing current, touch leads to subthreshold depolarization of both cells. The characteristic inputs II and input III on the N1M are reduced (compare with *A*). *C*: components similar to input II and III on the N1M can be evoked by injecting depolarizing current in both CGCs to trigger a burst of spikes.

neurons compared with the B4CL neurons reflect these functional differences (Fig. 5*Di*). The B4 and B8 neurons, like the N3ts, initially show a brief inhibition, and this is followed by a rebound excitation ( $n = 20$  cells) often leading to full spikes. This contrasts with the B4CL neurons discussed previously, which are depolarized earlier and show no initial phase of inhibition. This is shown more clearly in the expanded time-base recording in Fig. 5*Dii*. One striking feature of the response to touch is that the B8 cells always fire during the delayed depolarizing response to touch. Electrotonic EPSPs occur on the B4 recorded at the same time, but often they show no full spikes (Fig. 5*Dii*). These results suggest that as well as depolarizing the N2 cells and promoting rasp the touch CS also excites the swallow-phase motoneurons after a delay. This suggests that touch during feeding would tend to strengthen the rasp followed by swallow sequence of activity and would help to coordinate the sequence.

#### Lip touch response on the modulatory CGCs

The CGCs are two large coupled serotonergic neurons, one present in each anterior lobe of the cerebral ganglia (McCrohan and Benjamin 1980). During fictive feeding they fire tonically with a tendency to be excited during N1/protraction and inhibited during N2/rasp. They have complex synaptic connections with most of the CPG interneurons and motoneurons and are known to be important in modulating the feeding network (Tuersley and McCrohan 1989; Yeoman et al. 1996). In response to lip touch ( $n = 10$  cells) they show a burst of spikes of  $\sim 1$  s in duration, which is followed by a recovery period during which no action potentials occur (Fig. 6*A*). The onset of this response is comparable with the latency recorded on most buccal interneurons. Although it is clear from the relatively slow activation that the CGCs are not responsible for the earliest response to touch recorded on other feeding neurons, suppression experiments in which both CGCs are hyperpolarized suggest that they may play some role in the generation of secondary components of the touch-evoked activity in buccal neurons. For example, Fig. 6*B* shows that the suppression of CGC spike activity (in the same preparation as in Fig. 6*A*,  $n = 2$  cells) does not abolish the initial inhibitory component recorded on the N1M, but the subsequent N3t

inputs (input II) are markedly reduced in amplitude. CGC suppression also apparently removes some of the depolarizing component (input III) of the N1M touch-evoked response. This is reduced to the point where the remaining input III inhibitory inputs hyperpolarize the N1M cell below the potential seen before stimulation (Fig. 6*B*). Together these findings indicate that the normal burst of touch-evoked activity in CGCs may assist the N3ts recovery from inhibition to trigger spikes and also assist the recovery of the N1M from its primary touch-evoked inhibition. These conclusions are consistent with previous work demonstrating that CGCs have a monosynaptic excitatory connection with the N3t interneurons and a slow excitatory connection with the N1M (Yeoman et al. 1996). The ability of the CGCs to generate both unitary input II inhibition via the CGC  $\rightarrow$  N3t  $\rightarrow$  N1M pathway and slow input III depolarization via the CGC  $\rightarrow$  N1M excitatory connection is confirmed in Fig. 6*C*, where instead of touch current induced bursts of CGC spikes evoked these types of input (compare with Fig. 6*A*). CGCs also have synaptic connectivity to other CPG interneurons, SO, and many motoneurons and so may also shape the general lip touch-evoked response in all these cell types. This remains to be investigated.

#### Behavioral function for the lip touch pathway

The electrophysiological data presented previously show that the touch stimulus plays a role in reinforcing the rasp phase of the feeding cycle, but whether touch to the lip as a component of a solid food stimulus could provide a sensory input to the feeding system during normal ingestion of food was unclear. This was tested by videotaping four freely behaving animals that were feeding on solid fish food flake in an inverted position at the water surface. An example of one ingestion sequence is shown in Fig. 7. The sequence of seven frames (approximately every 200 ms) shows one complete cycle of feeding with protraction (P), rasp (R), and swallow (S) marked on each frame. Several cycles of feeding already occurred before this sequence, so part of the food piece was already inside the mouth. In frames 1–3, the mouth opens during the first part of a new feeding cycle, and this is known to be accompanied by protraction of the radula (see Rose and Benjamin 1979). The food does not move during this phase.

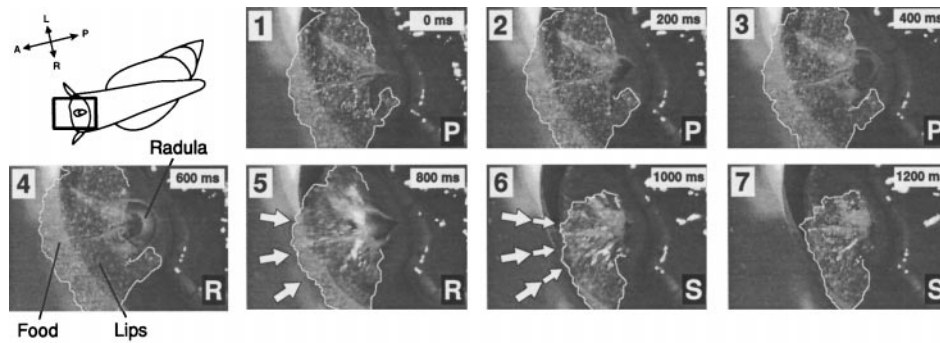


FIG. 7. Tactile stimulation of the lips by solid food in a freely feeding animal. Seven successive video frames of a single feeding cycle (P: protraction, R: rasp, S: swallow) viewed from beneath the animal (see cartoon inset). The animal was feeding on a fish food flake (outlined in white to improve visualization) in an inverted position at the water surface (its preferred feeding position). Several cycles of feeding already occurred before this sequence, so part of the food piece was already inside the mouth. During the rasp and swallow phases, as the animal pushes the radula forward, food is pulled (see arrows in frames 5 and 6) across the lip surface anterior to the mouth (labeled in frame 4). In addition, in the rasp phase the lips are pressed down on the food substrate, and we propose that this together with the movement of food across the lip surface provides a component of tactile stimulation analogous to the  $\sim 400$ -ms lip stimulation we present with the mechanical probe (see text).

However, during rasp (frames 4–5) the food is moved further into the mouth. The first forward, then backward, and upward rotating movement of the radula pulls food across the area of lip anterior to the mouth (see Fig. 7, arrows), which provides a mechanical stimulus to the lip. This particular location on the lip is the same one used for application of the mechanical probe in the semi-intact preparation. During swallow (Fig. 7, S, frames 6–7), the tactile stimulus is maintained as the food is being pulled even further into the mouth. This sequence of feeding behavior was typical of all four animals examined. Although food moving across the lip structures is likely to be the primary source of lip tactile stimulation during feeding, the maximal extrusion of the radula during late protraction and rasp pushes the lips forward onto the food substrate (not shown), and this would probably provide a second component of mechanical stimulation. This observation is supported by early work of Hubendick (1956), who examined the mechanics of feeding in *Lymnaea*. He demonstrated clearly that the lip structures are pushed onto the substrate during the rasp phase. This together with the movement of food across the lips results in the tactile stimulation being strongest for  $\sim 400$  ms in the rasp phase of the feeding cycle.

#### *Interaction between tactile and chemical stimuli in fictive feeding rhythms*

The food piece used in the behavioral experiments of Fig. 7 presumably also contained a chemical as well as a mechanical stimulus. This chemical component of the food stimulus must have been essential for initiating ingestion as simply stroking the lips with a mechanical probe never activates maintained sequences of feeding movements in the whole animal. The relative roles of chemosensory and mechanical stimuli in generating ingestion proved difficult to study quantitatively in the intact snail by using food pieces, so electrophysiological experiments were carried out instead with the semi-intact preparation (Fig. 1A). This type of preparation allowed both types of stimuli to be applied separately or together. By using a sucrose solution as a chemostimulant (see Kemenes et al. 1986; Staras et al. 1998b) rather than solid food, it was possible to minimize the normal tactile component. The lip tactile stimulus could be made explicit by applying it with the same soft plastic mechanical probe that was used to map the tactile inputs in

quiescent preparations. The duration of the stimulus was  $\sim 400$  ms, the same as the duration of the strongest tactile stimulation during feeding in intact animals. In these experiments, a rasp-phase motoneuron (B3) was used to monitor fictive feeding activity in a sucrose-driven rhythm, and the effects of additional tactile stimulation were tested.

Purely sucrose-driven rhythms were often slow, and this is a common observation in semi-intact preparations (Staras et al. 1998b). In the preparation shown in Fig. 8A, patterned CPG synaptic input was obvious with sucrose stimulation, but the B3 cell did not fire consistently. However, with the addition of touch to the lip during the rasp phase, the cell fired in a much more regular manner and the frequency of the rhythm was higher (Fig. 8B). The brief ( $\sim 400$  ms) tactile stimulus was provided at the onset of each rasp phase during continuous sucrose stimulation to mimic the natural stimulating effect of food revealed in the video sequence. An expanded cycle from Fig. 8B (dashed box) is shown in Fig. 8D, illustrating that the touch stimulus is applied after the onset of the rasp phase and so is contributing to the reinforcement of rasp, not its initiation. The ineffectiveness of lip touch stimulation alone in initiating and maintaining a fictive feeding rhythm was confirmed in the same preparation (Fig. 8C). Periodic application of touch alone produced the expected weak depolarizing response on B3, but this was never strong enough to evoke bursts of spikes.

We also tested for the effects of touch stimuli applied during interburst intervals rather than during bursts and for long-lasting effects of touch that might continue beyond the duration of a single feeding cycle. To achieve this we applied touch at various points during sucrose-activated fictive feeding. When touch stimuli were applied in the intervals between rasp-phase bursts, this had no effect on the duration of ongoing bursts or the frequency of the rhythm (Fig. 8Ei). In contrast, repeated application of touch during every other B3 burst increased the overall frequency of the rhythm. The first effect of the touch underlying this frequency increase was a reduction of the duration of the touch-stimulated bursts only (Fig. 8Eii). These were significantly shorter ( $3.7 \pm 0.2$  SE s,  $n = 3$  bursts) compared with subsequent nontouch-stimulated bursts in the same rhythm ( $11.5 \pm 0.3$  s,  $n = 3$  bursts) (paired  $t$ -test:  $df = 2$ ,  $t = 17.8$ ,  $P < 0.001$ ). This first effect of the touch therefore did not last longer than a single cycle. The second effect of the

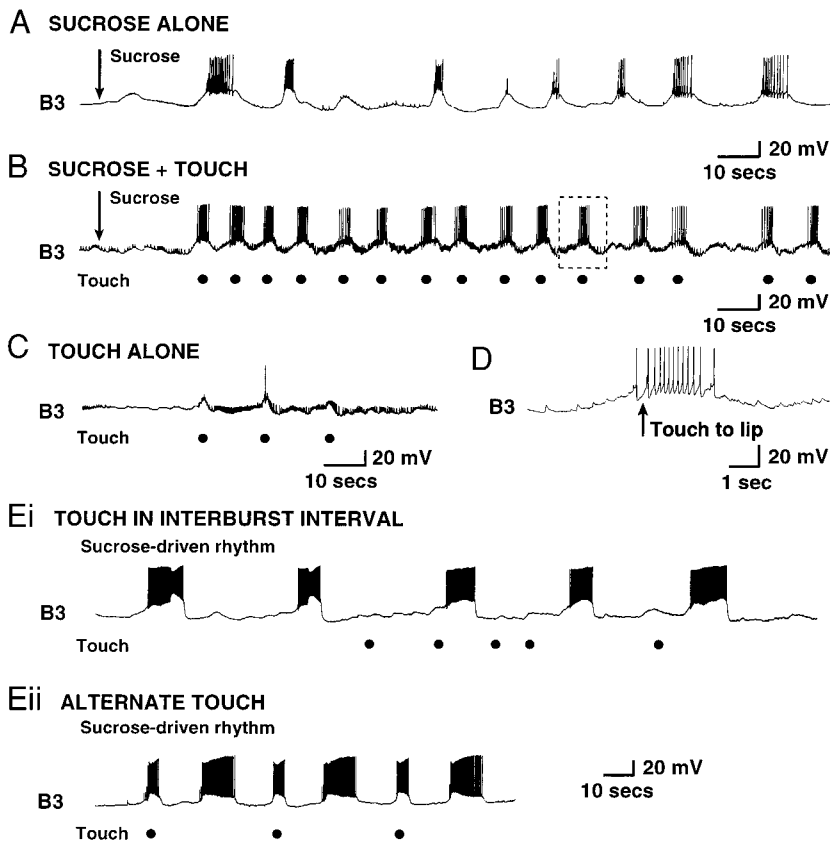


FIG. 8. Functional role for the lip touch pathway in fictive feeding behavior. *A*: typical sucrose-evoked fictive feeding response recorded from the B3 motoneuron in a semi-intact whole lip preparation. Cycles of CPG-driven fictive feeding activity are seen as bursts of spikes (during rasp phase) on the B3. The semi-intact preparation receives no tactile stimulation of the lip structures. *B*: fictive feeding response recorded in the same preparation as in *A* when a touch stimulus is given during each B3 burst (rasp phase) of a sucrose-driven rhythm. The fictive feeding rhythm becomes more regular, and the frequency of CPG-driven bursts is increased (see text and Fig. 9 for detailed quantitative analysis). *C*: response of B3 in the same preparation as *A* and *B* when 3 tactile stimuli are given in the absence of sucrose. *D*: B3 burst from *B* (dashed box) on a faster time base showing that the lip touch is presented after the first spike. *E*: effect of touch stimuli applied in the intervals between rasp-phase bursts of the feeding cycle (*Ei*) and at the onset of every other rasp-phase burst in B3 (*Eii*). *Ei*: touch applied in intervals between rasp-phase bursts have no effect on sucrose-evoked fictive feeding. In this experiment, application of the sucrose stimulus to the lips led to a tonic depolarization of the B3 membrane by  $\sim 20$  mV, and therefore the excitatory postsynaptic potential responses to touch appear smaller in amplitude than in *C*, which shows touch responses of a B3 cell at resting potential. *Eii*: touch applied in every other rasp-phase burst in B3 increases the frequency of fictive feeding when compared with *Ei*. It only influences the duration of the burst that is accompanied by touch and not the subsequent one but decreases all interburst intervals (for detailed statistics, see RESULTS).

touch was that the intervals that followed each burst (mean: 13.5 s, SE  $\pm 0.2$ ,  $n = 6$  bursts), including nonstimulated ones ( $13.0 \pm 0.6$  s,  $n = 3$  bursts; Fig. 8*Eii*), were shorter when compared with the rhythm shown in Fig. 8*Ei*, in which touch was applied in the intervals between bursts or not applied at all ( $38.8 \pm 2.6$  s,  $n = 5$  intervals; unpaired  $t$ -test:  $df = 6$ ,  $t = 8.06$ ,  $P < 0.001$ ). The mechanism for the complex changes occurring during the touch stimulation of alternate B3 bursts and affecting interburst intervals is unclear but must be longer lasting than a single cycle.

The experiments comparing sucrose-driven fictive feeding rhythms with those where touch stimuli were used to reinforce each rasp-phase burst suggested that the addition of touch in a sucrose-driven rhythm increased both the frequency and regularity of the fictive feeding rhythm. The effect on frequency was analyzed quantitatively by comparing the effects of sucrose alone and sucrose + touch in six animals. The number of feeding cycles in the 2 min after the first cycle after sucrose presentation was counted with and without tactile stimulation. When analyzed statistically, the number of cycles with sucrose + touch (median: 12.5, interquartile interval: 10.5–14.0) was found to be significantly higher than with sucrose alone (median: 8.0, interquartile range: 5.5–10, Wilcoxon matched-pairs rank test:  $Z = -0.02$ ,  $P < 0.04$ ), as illustrated in Fig. 9*A*.

The effect of touch on the regularity of sucrose-driven fictive feeding cycles was examined in a frequency histogram (Fig. 9*B*). The frequency of occurrence of cycle periods as a percentage of the total observations for sucrose alone and sucrose + touch was compared in the same six preparations. In the sucrose + touch rhythms, 54.7% of cycle periods were within the 5- to 10-s bin, whereas with sucrose alone cycle periods were much more widely distributed with 90% spread

over four bins between 5 and 25 s (Fig. 9*B*). Statistical analysis ( $F$  test) showed that in sucrose-alone rhythms the fictive feeding rate was significantly more variable than in sucrose + touch rhythms ( $F = 5.17$ ,  $P < 0.001$ ).

## DISCUSSION

### Lip tactile responses in feeding neurons

One of the main objectives of this paper was to characterize and functionalize the lip touch CS pathway in terms of the responses recorded on identified neurons of the feeding network. One possible role for the lip touch would be as part of the mechanism for initiation of the feeding response to potential food items. If this were the case, touch to the lips would be expected to excite protraction-phase neurons, such as the SO or the N1M cells. These are capable of driving a fictive feeding pattern of  $N1 \rightarrow N2 \rightarrow N3$  activity in isolated preparations (Elliott and Benjamin 1985b; Rose and Benjamin 1981a). An alternative notion is that the lips may be stimulated as the animal contacts the food substrate, and in this case the tactile pathway may be more important in modulating ongoing chemically driven feeding activity, particularly in the phase of feeding where the lips receive most tactile stimulation. These alternative hypotheses were examined by intracellularly recording identified neurons in the feeding network and testing the touch responses in each (summarized in Fig. 10).

The data presented in this paper show that the protraction-phase CPG interneurons (N1M and N1L) and motoneurons (B5 and B7a) received mainly inhibitory inputs (input I, II, and IV, Fig. 10, P), and despite the presence of a delayed depolarizing wave (input III) they were never depolarized enough by the

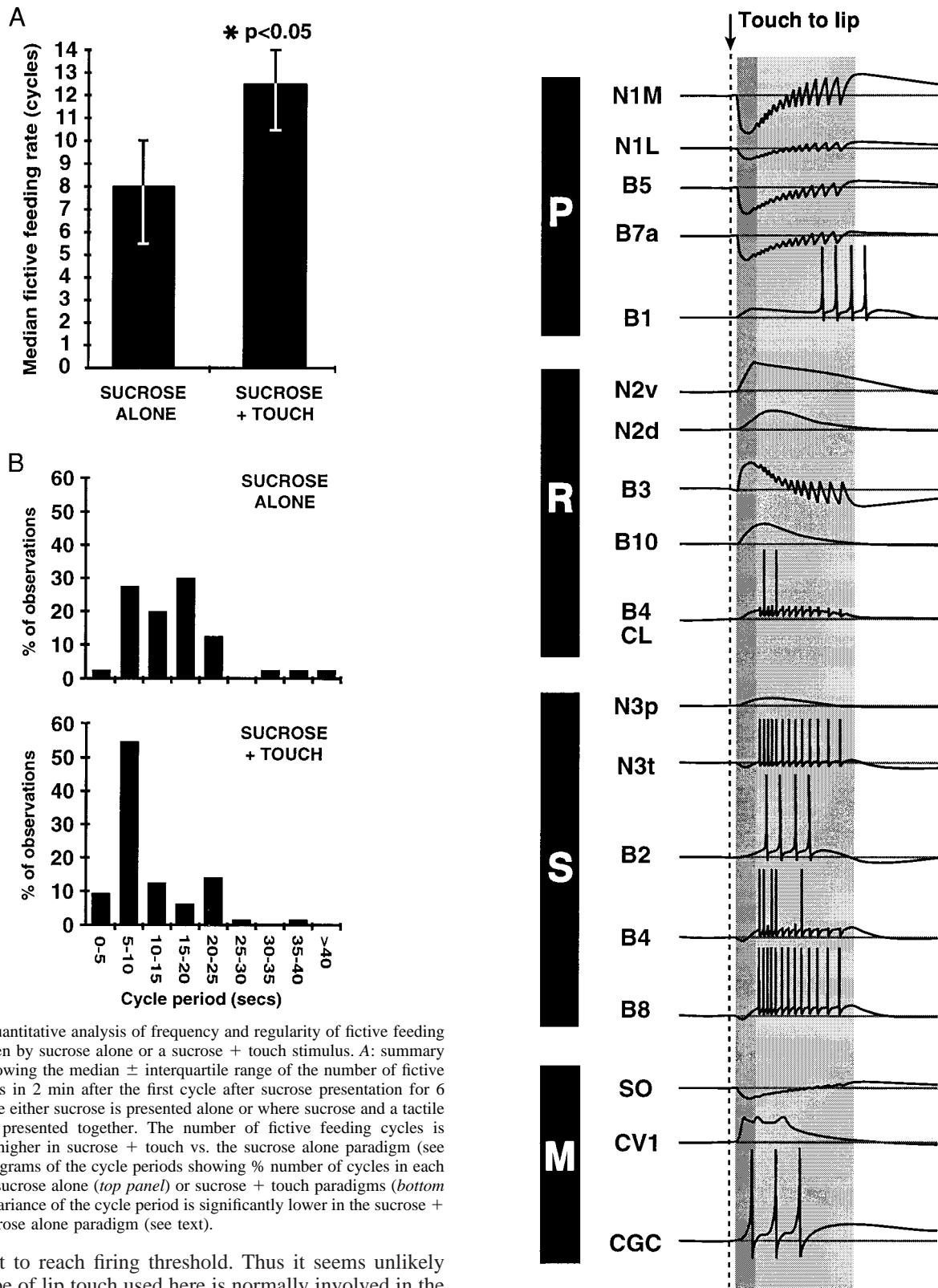


FIG. 9. Quantitative analysis of frequency and regularity of fictive feeding rhythms driven by sucrose alone or a sucrose + touch stimulus. *A*: summary histogram showing the median  $\pm$  interquartile range of the number of fictive feeding cycles in 2 min after the first cycle after sucrose presentation for 6 animals where either sucrose is presented alone or where sucrose and a tactile stimulus are presented together. The number of fictive feeding cycles is significantly higher in sucrose + touch vs. the sucrose alone paradigm (see text). *B*: histograms of the cycle periods showing % number of cycles in each bin (5 s) for sucrose alone (*top panel*) or sucrose + touch paradigms (*bottom panel*). The variance of the cycle period is significantly lower in the sucrose + touch vs. sucrose alone paradigm (see text).

touch input to reach firing threshold. Thus it seems unlikely that the type of lip touch used here is normally involved in the sensory initiation of feeding. Previous experiments with a different, split-lip preparation showed that over one-half of them gave brief fictive feeding responses to touch, consisting of one to three cycles of activity (Kemenes et al. 1997), but these never occurred in the current whole lip preparation. The reason for the difference in the responses in these two preparations is unclear. It may be explained by a number of different

FIG. 10. Summary of the lip touch responses in feeding interneurons and motoneurons of the feeding system, divided into protraction (P), rasp (R), swallow (S), and modulatory (M) cell types. The shaded bands show the 2 main phases of synaptic inputs on the feeding neurons. The first phase (darker band) is inhibitory on most of the protraction- and swallow-phase neurons and excitatory on the rasp-phase neurons. The second phase (lighter band) produces a more complex synaptic response with both excitatory and inhibitory synaptic inputs on many of the neuron types (see text).

factors such as the absence of contralateral connections in the split-lip preparation, which may be potentially important in the final shaping of sensory signals arriving at the brain. However, in both whole and split-lip preparations a maintained fictive feeding response to touch could only be evoked after a lip touch CS was repeatedly paired with a US, which was either the stimulation of an interneuron driving the feeding CPG (Kemenes et al. 1997) or a food stimulus (Staras et al. 1998b). In whole lip preparations, only the B1 protraction-phase motoneurons were excited by unconditioned touch, and as these are probably salivary gland motoneurons and play no role in pattern generation this is unlikely to be of major significance in the sensorimotor organization of the buccal mass movements. Rasp-phase interneurons (N2d and N2v) and motoneurons (B3, B10, and B4CL) all showed depolarizing responses to touch (Fig. 10, R) although this rarely evoked action potentials. However, during a CPG-driven rhythm, touch arriving during the rasp phase of the feeding cycle would tend to further depolarize the rasp-phase neurons, which were already depolarized by the appropriate synaptic inputs and in this way reinforce ongoing activity in both interneurons and motoneurons.

Swallow-phase interneurons (N3p and N3t) and motoneurons (B4, B8, and B2) all showed initial inhibition followed by depolarization that was often sufficient to drive them into spike activity (Fig. 10, S). Assuming that touch mainly occurs during rasp this would tend to prevent activity during the protraction phase of the feeding rhythm and then promote swallow. The overall effect of touch on the retraction phase of the feeding cycle in the intact snail would be to reinforce rasp-swallow neuronal activity in the correct sequence and inhibit protraction.

Touch-induced responses also occurred on modulatory neurons (Fig. 10, M). These were complex and not always easy to interpret from the functional point of view. The SO was inhibited by touch, and given that it is a protraction-phase neuron that can contribute to the activation of feeding (Yeoman et al. 1995) it was not surprising that like the N1M it showed this response. However, contrary to expectation was the touch-induced depolarization of the CV1a, another protraction-phase modulatory neuron. This effect was always subthreshold but still interesting. It suggests that the CV1a may play a different role in feeding to the SO, a neuron with which it is often compared. The weak depolarizing tactile input to the CV1a may also be potentially important because it may be strengthened during appetitive training by using touch as the CS (Kemenes and Benjamin 1989; Staras et al. 1998b, 1999). The CGCs, another modulatory neuron type, which play an important role in the feeding system as gating neurons as well as having effects on frequency control of the feeding CPG (Yeoman et al. 1994a,b), were also excited by touch. Activation of the CGCs would promote activation of feeding in general, but in this context they were most interesting because they were at least partly responsible for the long depolarizing wave (input III) recorded on the N1M cells. This suggests that they could be responsible for similar inputs on other cells recorded in this paper, although this was not tested directly.

#### *Functional role for lip tactile response in feeding neurons*

The nature of the touch responses recorded electrophysiologically on feeding neurons supports the notion that this

pathway may be important first in contributing to the rasp phase of feeding and then in activating neurons associated with the swallow phase. Although several neurons in the feeding network contribute to the touch response on other neurons through previously reported synaptic connections (e.g., N3ts and CGCs), none of the identified feeding neurons appears to be responsible for the primary synaptic response, the rasp-reinforcing component, which indicates that this probably arises from neurons in as-yet unidentified lip mechanosensory pathways.

The hypothesis that the touch CS pathway reinforced retraction-phase activity was supported by video analysis of feeding behavior showing that the lips received the strongest tactile stimulation during the rasp and swallow phases as solid food was taken into the mouth. The duration of the period while this strong tactile stimulation was maintained was  $\sim 400$  ms, the same as the duration of the tactile stimulus used in the semi-intact preparations. By using a semi-intact whole lip-CNS preparation we were able to separate the tactile and chemical components of a food stimulus and test the effects of lip touch stimulation in sucrose-driven fictive feeding rhythms. We demonstrated that, when a lip touch equivalent to the proposed behaviorally relevant input was provided during each rasp phase, it led to more rhythmical rasp-phase bursts in retraction-phase motoneurons and a higher frequency of fictive feeding than sucrose alone, providing evidence that the lip stimulus is important both in reinforcing a particular phase of the rhythm and also as a general stimulus for maintaining a high-frequency rhythm. The mechanism by which touch increases the frequency of fictive feeding is unclear but seems to be based on a simultaneous shortening of both the duration of rasp-phase bursts and the intervals between them (see Fig. 8).

The current work relates to a previous study on the opisthobranch mollusk *A. californica*, which provided evidence to show that integration of tactile and chemical cues was most effective in achieving high-frequency consummatory feeding behavior (Rosen et al. 1982b). In the presence of a tactile and chemical stimulus, feeding was more regular and occurred at a higher frequency than when a chemical stimulus was applied alone. In *T. diomedea*, chemical stimulation alone is important in eliciting repeated biting movements (Audesirk and Audesirk 1979). However, mechanical stimulation of the cavity of the buccal mass in the presence of a chemostimulus was demonstrated to bring about inhibition of biting responses and promotion of the swallow phase of feeding (Audesirk and Audesirk 1979). In this mollusk, mechanosensory neurons with receptive fields in the buccal mass that have the appropriate synaptic connectivity to bring about the initiation of swallow phase were also identified (Audesirk 1979). It is likely that mechanosensory neurons are present in the lip structures of *Lymnaea*, which also have phase-dependent effects. This results in the inhibition of protraction and the promotion of the rasp and swallow phases of feeding. The integration of chemical and tactile information in feeding at the cellular level was not analyzed in detail in any system, although higher-order neurons that may be sites of convergence of both chemical and tactile cues were identified in several systems such as the cerebral-to-buccal interneurons in *Aplysia* (Rosen et al. 1991), the cerebral to buccal interneurons in *Limax maximus* (Delaney and Gelperin 1990), and complex mechanoreceptors in *Tritonia* (Audesirk and Audesirk 1980). As we demonstrate in the present paper, in *Lymnaea* the initial synaptic inputs arising

from the lip tactile stimulus can be recorded on the majority of feeding motoneurons, CPG, and modulatory interneurons with approximately the same latency, suggesting that convergence of tactile and chemical may occur on all the feeding neuron types together rather than in a more hierarchical arrangement where higher-order neurons act as sites of sensory integration.

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## REFERENCES

- AUDESIRK, G. AND AUDESIRK, T. E. Complex mechanoreceptors in *Tritonia diomedea*. I. Responses to mechanical and chemical stimuli. *J. Comp. Physiol. [A]* 141: 101–109, 1980.
- AUDESIRK, T. E. Oral mechanoreceptors in *Tritonia diomedea*. I. Electrophysiological properties and location of receptive fields. *J. Comp. Physiol. [A]* 130: 71–78, 1979.
- AUDESIRK, T. E., ALEXANDER, J. E., AUDESIRK, G. J., AND MOYER, C. M. Rapid, nonaversive conditioning in a freshwater gastropod. I. Effects of age and motivation. *Behav. Neural Biol.* 36: 379–390, 1982.
- AUDESIRK, T. E. AND AUDESIRK, G. Oral mechanoreceptors in *Tritonia diomedea*. II. Role in feeding. *J. Comp. Physiol. [A]* 130: 79–86, 1979.
- BENJAMIN, P. R. AND ELLIOTT, C.J.H. Snail feeding oscillator: the central pattern generator and its control by modulatory interneurons. In: *Neuronal and Cellular Oscillators*, edited by J. Jacklet. New York: Dekker, 1989, p. 173–214.
- BENJAMIN, P. R. AND ROSE, R. M. Central generation of bursting in the feeding system of the snail, *Lymnaea stagnalis*. *J. Exp. Biol.* 80: 93–118, 1979.
- BENJAMIN, P. R. AND WINLOW, W. The distribution of three wide-acting inputs to identified neurons in the isolated brain of *Lymnaea stagnalis*. *Comp. Biochem. Physiol. A Physiol.* 70: 293–307, 1981.
- BICKER, G., DAVIS, W. J., MATERA, E. M., KOVAC, M. P., AND STORMOGIPSON, D. J. Chemoreception and mechanoreception in the gastropod mollusc *Pleurobranchaea californica*. II. Neuroanatomical and intracellular analysis of afferent pathways. *J. Comp. Physiol. [A]* 149: 235–250, 1982.
- BRIERLEY, M. J., STARAS, K., AND BENJAMIN, P. R. Behavioural function of glutamatergic interneurons in the feeding system of *Lymnaea*: plateauing properties and synaptic connections with motor neurons. *J. Neurophysiol.* 78: 3386–3395, 1997.
- DELANEY, K. AND GELPERIN, A. Cerebral interneurons controlling fictive feeding in *Limax maximus*. III. Integration of sensory inputs. *J. Comp. Physiol. [A]* 166: 327–343, 1990.
- ELLIOTT, C.J.H. AND BENJAMIN, P. R. Interactions of pattern-generating interneurons controlling feeding in *Lymnaea stagnalis*. *J. Neurophysiol.* 54: 1396–1411, 1985a.
- ELLIOTT, C.J.H. AND BENJAMIN, P. R. Interactions of the slow oscillator interneuron with feeding pattern-generating interneurons in *Lymnaea stagnalis*. *J. Neurophysiol.* 54: 1412–1421, 1985b.
- ELLIOTT, C.J.H. AND BENJAMIN, P. R. Esophageal mechanoreceptors in the feeding system of the pond snail *Lymnaea stagnalis*. *J. Neurophysiol.* 61: 727–736, 1989.
- ELLIOTT, C.J.H. AND KEMENES, G. Cholinergic interneurons in the feeding system of the pond snail *Lymnaea stagnalis*. II. N1 interneurons make cholinergic synapses with feeding motor neurons. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 336: 167–180, 1992.
- ELPHICK, M. R., KEMENES, G., STARAS, K., AND O'SHEA, M. Behavioural role for nitric oxide in chemosensory activation of feeding in a mollusc. *J. Neurosci.* 15: 11: 7653–7664, 1995.
- HUBENDICK, B. The eating function in *Lymnaea stagnalis* (L.). *Ark. För Zool.* 10: 511–521, 1956.
- KEMENES, G. AND BENJAMIN, P. R. Appetitive learning in snails shows characteristics of conditioning in vertebrates. *Brain Res.* 489: 163–166, 1989a.
- KEMENES, G. AND BENJAMIN, P. R. Goal-tracking behaviour in the pond snail *Lymnaea stagnalis*. *Behav. Neural Biol.* 52: 260–270, 1989b.
- KEMENES, G. AND BENJAMIN, P. R. Training in a novel environment improves the appetitive learning performance of the snail. *Lymnaea stagnalis*. *Behav. Neural Biol.* 61: 139–149, 1994.
- KEMENES, G., ELLIOTT, C.J.H., AND BENJAMIN, P. R. Chemical and tactile inputs to the *Lymnaea* feeding system: effects on behaviour and neural circuitry. *J. Exp. Biol.* 122: 113–137, 1986.
- KEMENES, G., STARAS, K., AND BENJAMIN, P. R. In vitro appetitive classical conditioning of the feeding response in the pond snail *Lymnaea stagnalis*. *J. Neurophysiol.* 78: 2351–2362, 1997.
- KOJIMA, S., YAMANAKA, M., FUJITO, Y., AND ITO, E. Differential neuroethological effects of aversive and appetitive reinforcing stimuli on associative learning in *Lymnaea stagnalis*. *Zool. Sci.* 13: 803–812, 1996.
- MCCROHAN, C. R. Initiation of feeding motor output by an identified interneuron in the snail *Lymnaea stagnalis*. *J. Exp. Biol.* 113: 351–366, 1984.
- MCCROHAN, C. R. AND BENJAMIN, P. R. Patterns of activity and axonal projections of the cerebral giant cells of the snail *Lymnaea stagnalis*. *J. Exp. Biol.* 85: 149–168, 1980.
- MCCROHAN, C. R. AND KYRIAKIDES, M. A. Cerebral interneurons controlling feeding motor output in the snail *Lymnaea stagnalis*. *J. Exp. Biol.* 147: 361–374, 1989.
- ROSE, R. M. AND BENJAMIN, P. R. The relationship of the central motor pattern of the feeding cycle of *Lymnaea stagnalis*. *J. Exp. Biol.* 80: 137–163, 1979.
- ROSE, R. M. AND BENJAMIN, P. R. Interneuronal control of feeding in *Lymnaea stagnalis*. I. Initiation of feeding by a single buccal interneuron. *J. Exp. Biol.* 92: 187–201, 1981a.
- ROSE, R. M. AND BENJAMIN, P. R. Interneuronal control of feeding in *Lymnaea stagnalis*. II. The interneuronal mechanisms generating feeding cycles. *J. Exp. Biol.* 92: 203–228, 1981b.
- ROSEN, S. C., TEYKE, T., MILLER, M. W., WEISS, K. R., AND KUPFERMANN, I. Identification and characterization of cerebral-to-buccal interneurons implicated in the control of motor programs associated with feeding in *Aplysia*. *J. Neurosci.* 11: 3630–3655, 1991.
- ROSEN, S. C., WEISS, K. R., COHEN, J. L., AND KUPFERMANN, I. Interganglionic cerebral-buccal mechanoafferents of *Aplysia*: receptive fields and synaptic connections to different classes of neurons involved in feeding behaviour. *J. Neurophysiol.* 48: 271–288, 1982a.
- ROSEN, S. C., WEISS, K. R., AND KUPFERMANN, I. Cross-modality sensory integration in the control of feeding in *Aplysia*. *Behav. Neural Biol.* 35: 56–63, 1982b.
- STARAS, K., KEMENES, G., AND BENJAMIN, P. R. Pattern-generating role for motoneurons in a rhythmically active neuronal network. *J. Neurosci.* 18: 3669–3688, 1998a.
- STARAS, K., KEMENES, G., AND BENJAMIN, P. R. Neurophysiological correlates of unconditioned and conditioned feeding behavior in the pond snail *Lymnaea stagnalis*. *J. Neurophysiol.* 79: 3030–3040, 1998b.
- STARAS, K., KEMENES, G., AND BENJAMIN, P. R. Cellular traces of behavioral classical conditioning can be recorded at several specific sites in a simple nervous system. *J. Neurosci.* 19: 347–357, 1999.
- TUERSLEY, M. D. AND MCCROHAN, C. R. Postsynaptic actions of serotonergic cerebral giant cells on buccal motoneurons in the snail *Lymnaea stagnalis*. *Comp. Biochem. Physiol. C Pharmacol. Toxicol.* 92: 377–383, 1989.
- YEOMAN, M. S., BRIERLEY, M. J., AND BENJAMIN, P. R. Central pattern generator interneurons are targets for the modulatory serotonergic cerebral giant cells in the feeding system of *Lymnaea*. *J. Neurophysiol.* 75: 11–25, 1996.
- YEOMAN, M. S., KEMENES, G., BENJAMIN, P. R., AND ELLIOTT, C.J.H. Modulatory role for the serotonergic cerebral giant cells in the feeding system of the snail, *Lymnaea*. II. Photoinactivation. *J. Neurophysiol.* 72: 1372–1382, 1994a.
- YEOMAN, M. S., PIENEMAN, A. W., FERGUSON, G. P., TER MAAT, A., AND BENJAMIN, P. R. Modulatory role for the serotonergic cerebral giant cells in the feeding system of the snail, *Lymnaea*. I. Fine wire recording in the intact animal and pharmacology. *J. Neurophysiol.* 71: 1357–1371, 1994b.
- YEOMAN, M. S., VEHOVSZKY, A., KEMENES, G., ELLIOTT, C.J.H., AND BENJAMIN, P. R. Novel interneuron having hybrid modulatory-central pattern generator-properties in the feeding system of the snail, *Lymnaea stagnalis*. *J. Neurophysiol.* 73: 112–124, 1995.