A Disynaptic Sensorimotor Pathway in the Lobster Stomatogastric System

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SUMMARY AND CONCLUSIONS
1. In the lobster Homarus, muscle gm 1 that causes protraction of the medial tooth of the gastric mill system is innervated via a dorsal branch of the anterior gastric nerve by motoneurons (GM) arising in the stomatogastric ganglion (STG) (Fig. 1).
2. A ventral branch of the anterior gastric nerve (VAGN) contains a single unit that is mechanosensitive, responds to gentle pressure on the stomach wall in the vicinity of gm 1, and evokes reflex activation of GM motoneurons (Fig. 2).
3. This mechanoreceptor neuron (called anterior gastric receptor, AGR) has been identified morphologically (Fig. 3) and electrophysiologically (Figs. 4 and 5). The bipolar cell body is located in the dorsal ventricular nerve immediately posterior to the STG. It sends out long peripheral processes in the left and right VAGNs to ramify bilaterally in the epidermis of the stomach wall underlying muscle gm 1. The axon of the AGR runs anteriorly through the STG and projects to the left and right commissural ganglia (CoGs) via the stomatogastric (STN) and inferior esophageal nerves.
4. AGR activation of GM motoneurons disappears after cutting the STN, indicating that the reflex is mediated by an axonal pathway involving rostral ganglia (Fig. 6).
5. Electrophysiological (Fig. 7) and morphological (Fig. 8) methods were used to identify an interneuron (commissural gastric neuron, CG) located in each CoG and intercalated between AGR and GM. Axons of the two CGs project to the STG via the superior esophageal nerves and the STN.
6. Simultaneous intracellular recordings from the three cell types demonstrate that AGR excites CG, which in turn excites GM; in each case excitatory postsynaptic potentials follow presynaptic impulses one for one and at constant latency (Fig. 9). Raising the threshold for spiking with saline containing high divalent cation concentrations further indicates that both excitatory connections are monosynaptic and confirms that AGR does not directly excite GM motoneurons (Fig. 10).
7. The input/output properties of AGR in this disynaptic excitatory pathway (Fig. 11) are discussed as also are the functional implications of such a long-loop pathway for sensorimotor integration.

INTRODUCTION
The role of sensory information, especially proprioceptive feedback, in the organization of rhythmic motor output has been studied extensively in a variety of different preparations, invertebrates and vertebrates alike (3). In most cases these investigations analyze the relationship between input (sensory) and output (motor) with the intervening central nervous system regarded more or less as a black box. This approach has been necessary largely due to the complexity and/or lack of knowledge, especially at the cellular and synaptic level, of the central nervous circuits that process sensorimotor information and of how sensory inputs interact with these circuits.
There are, however, several invertebrate motor systems, for example the flight system of insects (21, 22) and crustacean locomotion (28, 31), where the central circuit and its output pattern have been established from
in intracellular studies on the nervous system in isolation (9), and the in vitro investigation then expanded to include sensory pathways normally associated with the motor network in the intact animal. A rhythmic motor system that has been widely studied as a model of a central pattern generator (CPG) is that controlling digestive movements of the gastric mill in the foregut of decapod crustacea. The central organization of this motor behavior has been studied extensively (see 25, 27) with in vitro preparations of the stomatogastric nervous system: gastric pattern generation occurs in a small network of neurons located in the stomatogastric ganglion (STG) and derives mainly from synaptic connections between motoneurons innervating the gastric teeth muscles. Although the gastric CPG can continue to operate in the stomatogastric nervous system devoid of proprioceptive inputs, a rich variety of sensory receptors have access to this CPG in the intact animal (8). In the first of these two papers, we identify and characterize one such afferent pathway to the gastric motor network of the lobster, Homarus gammarus. Consistent with the parsimony of invertebrate nervous systems, the input element consists of a single primary mechanoreceptor neuron that has access to the gastric motor network via two (bilateral) interneurons. In the following paper (30) we begin to examine the complex influence of this “simple” proprioceptive pathway on gastric motor output and to demonstrate that a considerable integrative ability resides with special membrane properties of the intercalated interneurons.

A preliminary report of these results has been published elsewhere (29).

METHODS

Adult specimens of the European lobster H. gammarus were used in all experiments. Animals were obtained from fisherman supply and housed in tanks of fresh running seawater (12°C).

Electrophysiology

SEMI-INTACT PREPARATIONS. Early experiments (n = 7) were performed in vivo on animals restrained in a tank of chilled and aerated artificial seawater containing (in mM) 400 Na+, 9.8 K+, 10.1 Ca++, 52.6 Mg++, 28 SO42-, and 535 Cl that was buffered to pH 7.45 with 2.5 mM NaHCO3. A window was opened in the dorsal carapace immediately above the gm 1 muscles and STG (see Fig. 1A). Polyethylene suction electrodes were used to stimulate or record en passant from the ventral anterior gastric nerve (VAGN), which contains the peripheral branch of the sensory anterior gastric receptor (AGR) neuron, and from the dorsal anterior gastric nerve (DAGN), which contains the axons of motoneurons (GM) innervating gm 1 (see Fig. 2). Mechanical stimulation was applied by probing the stomach wall in the region of the insertions of gm 1 (Fig. 1, B and C) with an electromechanical pulser.

IN VITRO PREPARATIONS. For subsequent experiments (n = 28), the standard preparation used was a modified version of the “combined” stomatogastric preparation of Silverston et al. (27). In all cases, the isolated preparation included the STG, the esophageal ganglion (OG), the bilateral commissural ganglia (CoG) along with a short section of each circumsophageal connective (CC), and their interconnecting nerves ( stomatogastric nerve, STN; inferior esophageal nerves, IONs; superior esophageal nerves, SONs; see Fig. 1A). The preparation was superfused continuously with oxygenated artificial seawater maintained at 15–18°C with a thermoelectric cooling system (Midland Ross). In several experiments the stomach wall oscillette, which carries the insertion of gm 1, was cut free from the rest of the stomach and along with the muscle then transferred to the Petri dish still attached to the stomatogastric nervous system (see Fig. 4).

The initial section of the dorsal ventricular nerve (DVN) and both CoGs were desheathed for access with microelectrodes (10–12 MΩ) to the somata of GM motoneurons (in the STG), AGR (in the DVN), and the CG interneuron (in the CoG).

Standard electrophysiological techniques were used for intracellular recording and stimulation. Extracellular recordings were made with platinum wire electrodes placed against appropriate nerve tracts and isolated electrically with Vaseline. Intracellular electrodes were filled with 3 M KCl. As a test for monosynaptic coupling, the normal bathing saline was replaced with a high divalent cation solution (2.5 times normal, i.e., 25 mM Ca++ and 125 mM Mg++) (4). Conventional techniques were used throughout for display, storage, and transcription of recorded data.

Anatomy

To characterize penetrated neurons morphologically the tip of microelectrodes were filled with 3% lucifer yellow (in distilled water), then the stem was backfilled with 5% LiCl (32). Standard procedures were used for subsequent intracellular dye injection, tissue treatment, and visualization of stained cells (Fig. 8).
The central projections and cell body location of the AGR sensory neuron were determined by migration of cobalt chloride (8.5% in distilled water) from the cut end of the VAGN toward the center (Fig. 3A). Peripheral projections of AGR were determined using the same method but with migration from the STN or from the peripheral cut end of the VAGN (Fig. 3B). Migration occurred for 36–48 h at 4°C; then preparations were reacted with 2% ammonium sulfide, fixed for 1 h in 2.5% glutaraldehyde, dehydrated, and cleared in methyl salicylate.

Transverse sections of the peripheral branch of AGR were obtained by fixing freshly dissected VAGN in a formaldehyde-glutaraldehyde mixture, post-fixing with 1% osmium tetroxide, and embedding in epon araldite. One-micrometer sections were stained with toluidine blue and then viewed and photographed under light microscopy.

RESULTS

Background: medial tooth of the gastric mill

In large decapod crustaceans such as Homarus, the gastric mill region of the stomach carries three calcified teeth, two lateral teeth, and a single medial tooth. These teeth are involved in a four-phase rhythmic behavior, which serves to chew ingested food (13). The present paper concentrates on the motor activity of the medial tooth system, which is suspended sagittally from the dorsoposterior wall of the stomach (Fig. 1A) and whose rhythmic movements of retraction (Fig. 1B) and projection (Fig. 1C) push food anteriorly and grind it between the two lateral teeth. As shown in Fig. 1, cyclic motion of the medial tooth is caused by antagonistic

FIG. 1. Gastric medial tooth system in the stomach of Homarus gammarus. A: anterodorsal view of the foregut showing the stomatogastric nervous system in situ and the muscles controlling movements of the medial tooth. B and C: semidiagrammatic drawings of a "para" sagittal section through the dorsal stomach wall showing the mechanical basis for medial tooth retraction by contraction of muscle gm 4 (B) and tooth projection caused by simultaneous contraction of muscles gm 1 and gm 2 (C). Anterior is to the right. Horizontal bars: 5 mm. CC, circumoesophageal connective; CoG, commissural ganglion; OG, esophageal ganglion; STG, stomatogastric ganglion; AGN, anterior gastric nerve; DAGN, dorsal anterior gastric nerve; DVN, dorsal ventricular nerve; gm 1, anterior protractor muscle of the medial tooth; gm 2, posterior protractor of the medial tooth; gm 4, retractor of the medial tooth; ION, inferior esophageal nerve; LVN, lateral ventricular nerve; MT, medial tooth; OES, esophagus; SON, superior esophageal nerve; STN stomatogastric nerve; VAGN, ventral anterior gastric nerve.
muscles acting on a leverlike system of ossicles: retraction results from contraction of the intrinsic gm 4 muscle (Fig. 1, A and B), while simultaneous contraction of the bilateral extrinsic muscles gm 1 and gm 2 causes the tooth to protract (Fig. 1, A and C).

In Homarus (24), like Panulirus (26), muscle gm 4 is innervated by a single dorsal gastric (DG) motoneuron, whereas muscles gm 1 and gm 2 receive several common GM motoneurons (4 in Panulirus and at least 7 in Homarus; J. Simmers and F. Nagy, unpublished observations). DG projects from the STG to gm 4 via the DVN and the lateral ventricular nerves (LVNs). The multi-branched axons of GM motoneurons innervate both left and right members of gm 1 via the anterior gastric nerves (AGNs), and gm 2 via the DVN and LVNs (see Fig. 1A). Thus a penetrated soma in the STG can be readily identified as GM by recording simultaneously from its axon in AGN and the LVN.

A further important diagnostic feature of GM in Homarus is that these cells receive a strong excitatory input from an identified interneuron (commissural gastric, CG) located in each commissural ganglion (see below; 23, 24).

Although in vitro combined preparations of the stomatogastric nervous system in Homarus can continue to generate rhythmic gastric output, the results presented here were obtained from preparations that were not expressing gastric cycling. This condition was preferred, since it allowed extrinsically evoked synaptic events to be examined more easily in the absence of activity derived from intrinsic network rhythmicity.

Characterization of a mechanosensitive unit in VAGN

During a preliminary search for sensory inputs to the gastric system in semi-intact preparations, a ventral branch of the AGN that carries innervation to the gm 1 muscles, was found to contain a unit that is clearly not motor in function. This small branch is subsequently referred to as the VAGN, whereas the main branch with axons of GM motoneurons is termed the DAGN (see Fig. 1A).

Several features characterize the unit in VAGN. First, this cell is unique to VAGN: of the 35 electrophysiological experiments reported here, none have been observed to have the cell in any branch of the DAGN and, moreover, it is the only unit recorded in VAGN (e.g., Fig. 2). Second, the cell is always spontaneously active in en passant recordings from VAGN in semi-intact preparations. This activity consists of continuous tonic firing at extremely constant frequency (although varying from 3 to 12 Hz in different preparations) throughout the course of an experiment (up to 12 h or more). Third, the cell responds to mechanical stimulation of the stomach wall. Gentle pressure exerted externally or internally (with a probe inserted into the stomach via the esophagus) on the gastric ossole on which gm 1 is attached (Fig. 1, B and C) provokes an increase in firing frequency whose intensity depends on the strength (displacement of 1 to several mm) and duration of the stimulus (Fig. 2A). The response shows little or no adaptation when the stimulus is maintained or repeated, and it is typically followed by postexcitatory depression, giving rise to a silent period before resumption of spontaneous spiking activity. The unit shows no response to imposed movement of the stomach wall other than in the region of the attachment of gm 1.

A clue to the functional role of the cell is shown in the experiment of Fig. 2, B-E, where the axonal activity of GM motoneurons was monitored in DAGN. Mechanical stimulation of the gastric ossole evokes a stimulus-dependent response in the VAGN unit and a burst of spikes in GM motoneurons (Fig. 2B). A similar motor response is obtained with electrical stimulation of the VAGN (Fig. 2D). However, cutting the whole AGN close to its emergence from the DVN (see Fig. 1A) abolishes activation of GM motoneurons either by mechanical stimulation of the gastric ossole (Fig. 2C) or by electrical stimulation of the VAGN (Fig. 2E), although the responsiveness of the VAGN unit remains unaffected (Fig. 2C). This argues against the possibility that the mechanical or electrical stimuli are affecting GM motoneuron directly, but rather strongly suggests that the single unit contained in VAGN is a mechanoreceptive element that reflexly excites the motoneurons. This element, which was named AGR, is characterized further in the following section.

Identification of AGR

MORPHOLOGICAL IDENTIFICATION. Only one cell is ever stained in cobalt backfills (to-
Fig. 2. A mechanosensitive unit in VAGN that reflexly excites GM motoneurons. A: suction electrode recording from VAGN (top) in a semi-intact preparation showing a single unit that fires spontaneously. Mechanical stimulation of the stomach wall (near the insertion of muscle gm 1) changes the unit's tonic activity as a function of the imposed movement (mvt, bottom). B: repetitive stimulation in a different preparation evokes similar increases in the firing of the unit in VAGN (middle) and activates GM motoneurons recorded extracellularly in the ipsilateral DAGR. C: this effect on GM motoneurons seen in DAGR disappears when AGN is cut centrally near the DVN, but the mechanosensitive response of the cell in VAGN remains. D and E: activation of GM motoneurons by direct electrical stimulation (25 Hz via suction electrode) of VAGN (D, cf. B) is also abolished after cutting AGN (E, cf. C). Horizontal bar: 1 s. See legend of Fig. 1 for definitions of abbreviations.

ward the STG) of the cut proximal end of either or both VAGNs (Fig. 3A). That the VAGN carries AGR alone is further evident from the single fiber profile seen in transverse sections of VAGN after its branch point with DAGR (data not shown), and is consistent with the purely unitary activity observed in all extracellular recordings from this nerve (e.g., Fig. 2).

The central geometry of AGR shows a
FIG. 3. Morphological identification of AGR. A: backfilling VAGN (right) with cobalt chloride reveals a single bilateral neuron (AGR) whose bipolar cell body lies in the DVN. The cell's axon runs through the STG into the STN while posteriorly, a medial dendritic process gives rise to bilateral branches in the AGNs. B: peripheral processes of AGR. Camera lucida drawing of the mechanoreceptor stained with cobalt from its axon in the STN. The dendritic projections of AGR are bilaterally symmetrical, ramifying in soft connective tissue underlying the insertions (outlines shown) of the left and right gm 1 muscles. Anterior is to the top and right in A and B, respectively. Horizontal bars: 100 μm (A) and 1 mm (B). See legend of Fig. 1 for definitions of abbreviations.

A large bipolar cell body (35 μm at its widest point and up to 100 μm long) located in the DVN immediately posterior to the STG (Fig. 3A). There is some positional variation of the cell body within the DVN, although only rarely (n = 3) has it been observed within the STG itself. The axon runs forward from the soma into the STN, passing directly through the STG without apparent ramification. A single wide dendritic process runs posteriorly for a short distance in the DVN, then bifurcates to send branches in left and right AGNs and finally into the VAGN.

The peripheral morphology of AGR was determined by cobalt staining where migration occurred from the distal cut ends of a
VAGN or from the STN in preparations where the nerve branch was dissected out along with the appropriate region of the dorsal stomach wall (Fig. 3B). Again, only one cell in VAGN is ever stained. On either side, AGR projects laterally as a single process running down through fiber bundles of muscles gm 1 until it penetrates the epidermis of the stomach wall. Thereafter it branches extensively giving of secondary and tertiary branches distributed over a well-defined area (3–4 mm²) that never extends beyond the region of attachment of muscle gm 1. Small end bulbs are sometimes evident on terminal processes that do not appear to be associated with any discrete cuticular structure. As seen in the bilateral “hil” of Fig. 3B obtained from the cut end of the AGR axon in the STN, a considerable distance (6–8 mm) separates the two dendritic fields that are remarkably similar in shape and distribution. The cell, therefore, has a morphology appropriate to responding bilaterally to any mechanical deformation of its presumed stretch-sensitive dendrites in the vicinity of the attachment of muscle gm 1 (Fig. 2). No evidence, morphological or physiological, has been found for more than one AGR cell type, either with unilateral or bilateral projections, in this region of the stomatogastric system.

**ELECTROPHYSIOLOGICAL IDENTIFICATION.** An initial identification procedure with in vitro recordings from AGR (see METHODS) was done to compare properties of the cell in the isolated system with those of the mechanosensitive unit observed in the VAGN in vivo (cf. Figs. 2 and 4). Intracellular recordings from a large bipolar cell body close to the STG in the DVN (Fig. 4.1) identify it as AGR on the following basis. First, the cell fires autogenetically at low constant tonic frequency throughout impalement (Fig. 4B). The large intracellular spikes are correlated 1:1 with impulses recorded extracellularly from the only unit active in VAGN. Second, in isolated preparations where the distal terminals of VAGN are left attached to the appropriate region of the stomach wall (Fig. 4A), mechanical stimulation of this region while recording from the cell body evokes changes in intrasomatic spiking (Fig. 4C) similar to the unitary responses recorded from the VAGN in vivo (Fig. 2A). Third, similar to the increase in firing rate produced by mechanical stimulation in vivo (Fig. 2B), increasing the firing rate of AGR by direct depolarization of its cell body causes activation of motoneurons innervating muscle gm 1 (Fig. 4D), in this case firing in a GM motoneuron penetrated in the STG and discharge.

**FIG. 4.** Electrophysiological confirmation that AGR is the mechanosensitive unit in VAGN. A: diagram of the in vitro preparation recorded in B–D. Note a portion of the stomach wall underlying gm 1 was left attached via VAGN to the otherwise isolated nervous system. B: spontaneous spikes in the unit of VAGN correspond 1:1 with intracellular impulses recorded in the cell body of AGR. C: intrasomatic response to probing the piece of stomach wall (+) (cf. Fig. 2A). D: increasing AGR firing by depolarizing current injection (bottom) excites a penetrated GM motoneuron (middle) and other GM cells recorded with an extracellular electrode on the DGN (top) (cf. Fig. 2B). Horizontal bars: 1 s, vertical bars: 20 mV (AGR), 10 mV (GM), and 1 mm (movement monitor). See legend of Fig. 1 for definitions of abbreviations.
in other GM cells monitored extracellularly in DAGN.

A first step to understanding the reflex effect of AGR on the GM motoneurons was to trace AGR's dendritic and axonal projections throughout the isolated stomatogastric nervous system using multiple-electrode recordings (Fig. 5A). Spontaneous or evoked action potentials in the cell body of AGR are matched 1:1 with spikes in both the left and right VAGNs and the IONs (Fig. 5B). Evidence that these impulses are all occurring in different regions of the same cell is shown in the oscilloscope sweeps of Fig. 5. C and D. In Fig. 5C, "orthodromic" stimulation (by single electrical shocks to the left VAGN) gives rise successively, without failure, and at constant latencies, to spikes in the AGR cell body (2), the contralateral VAGN (3), the STN (4), and the left ION (5). Conversely, "antidromic" stimulation (Fig. 5D) with shocks to the IONs elicits impulses 1:1 again.

FIG. 5. Electrophysiological characterization of AGR. A: preparation used for recording intracellularly from AGR (2), and extracellularly from the left (1) and right (3) VAGNs, the STN (4), and an ION (5). B: 1:1 correlation between spikes occurring spontaneously or evoked by current injection (1) in the cell body of AGR and impulses in the 2 VAGNs (1 and 3) and the ION (5). C: "orthodromic" stimulation of AGR. Five superimposed oscilloscope sweeps triggered by brief electrical shocks to the left VAGN (1). Each stimulus is followed by a spike in the AGR soma (2), the right VAGN (3), STN (4), and ION (5). D: "antidromic" stimulation of AGR: as in C but with oscilloscope sweeps triggered by stimuli applied to the ION (5). Spikes now occur in the descending sequence of STN (4), soma (2), right (3), and left (1) VAGNs. Horizontal bars: 500 ms (B) and 10 ms (C and D); vertical bars: 20 mV. See legend of Fig. 1 for definitions of abbreviations.
at constant latencies, in the direction STN (4), soma (2), and finally the left (3) and right (1) VAGNs. Delays for impulse conduction between appropriate electrode pairs were similar in both directions, with the conduction velocity of the cell's axon occurring at \( \approx 2.5 \) m/s. Thus, after convergence of the AGR bilateral dendrites onto a central soma and axon, the latter runs through the STG (cf. Fig. 3A), ascends in the STN, traverses the esophageal ganglion, then bifurcates into the left and right IONS in the direction of each CoG (Fig. 5A).

AGR activation of GM via a long-loop pathway

Stimulation of the mechanoreceptor AGR, either mechanically (Fig. 2B) or by depolarizing its soma (Fig. 4D), excites GM motoneurons. What pathway mediates this sensorimotor relationship? One possibility is direct collateral (mono- or polysynaptic) connections from AGR onto GM in the STG. In the experiment shown in Fig. 6, A and B, for example, spontaneous discharge (12 Hz) in AGR is associated with a high level of synaptic activity and firing in a penetrated GM cell. Injection of hyperpolarizing current to reduce the AGR firing rate causes GM to hyperpolarize abruptly to a smooth base line except for large individual EPSPs correlated 1:1 with impulses still occurring in AGR. Prestimulus levels of activity return immediately in both neurons when current injection ceases. Discrete, constant-latency EPSPs in GM, especially evident when AGR is firing at low frequency (see also Figs. 9B and 10D), fit with strong coupling between the two cells. Important to note, however, is that these observations were obtained from “combined” in vitro preparations (Fig. 4A) in which the STG remained attached to the OG and CoG. If AGR-GM coupling is confined to the STG, then cutting the STN (and therefore the axon of AGR) should have little or no effect on the reflex pathway. That this

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**Fig. 6.** AGR activates GM motoneurons indirectly. A: “combined” experimental preparation including STG, esophageal (OG), and CoG ganglia. B: hyperpolarization of AGR to reduce spontaneous discharge decreases synaptic excitation of a GM motoneuron, which hyperpolarizes and stops firing. Large individual excitatory postsynaptic potentials are still evident in GM as a result of continued firing in AGR. C: AGR no longer excites GM motoneurons after cutting the stomatogastric nerve (4L). Note same experiment as in Fig. 4D before STN section. D: spikes descending in the STN associated with ascending AGR impulses. Oscilloscope sweeps triggered by AGR soma spikes show impulses following in the STN; the two IONS, and eventually a second group of smaller impulses occurring in the STN. Horizontal bars: 1 s (B and C) and 10 ms (D); vertical bar: 10 mV. See legend of Fig. 1 for definitions of abbreviations.
FIG. 7. Electrophysiological identification of the CG interneuron. A: combined preparation used for recording simultaneously from CG, AGR, and GM. B: typical spontaneous intracellular activity in the CG neurons of the left (L) and right (R) CoG and a GM neuron in the STG. Small potentials in both CGs are decremental axon spikes, whereas the larger events, occurring synchronously in the 2 cells, are excitatory postsynaptic potentials (EPSPs) giving rise to 1 or 2 impulses. Hyperpolarizing both cells to stop spontaneous firing abolishes synaptic activity in GM, except for EPSPs resulting from spikes that continue to arise from the common EPSPs in the interneurons. C: the large EPSP in CG originates in AGR. Superimposed impulses recorded in the cell body of AGR, and its axon in the left ION, give rise at constant latency to large EPSPs in left CG and spikes recorded en route to the STG in the left SON. Horizontal bar: 200 μA and 10 ms (C); vertical bars: 10 mV. See legend of Fig. 1 for definitions of abbreviations.
smaller potential that is not bilaterally synchronized (Fig. 7B). Manipulation of either cell's membrane potential indicates that the smaller of these events is an attenuated axonal spike, since its discharge can be decreased or eradicated completely by soma hyperpolarization (second part of recordings in Fig. 7B). By contrast the larger event is evidently an EPSP, its frequency remaining unaffected by membrane polarization of CG and its amplitude increasing with hyperpolarization.

That CG causes synaptic excitation of GM motoneurons in the STG (24) is also evident in Fig. 7B. When the two interneurons are hyperpolarized simultaneously to prevent spontaneous firing, there is a dramatic reduction in synaptic activity in a penetrated GM. Under these conditions, the only EPSPs still occurring in GM are synchronized to the common EPSPs in the two CGs. These bilateral synaptic potentials continue to provoke sufficient depolarization to trigger one (and sometimes two) axonal spike(s) in each CG neuron, which then give rise to a summated EPSP in GM.

The source of the large common synaptic potentials in CG interneurons is the mecha-noreceptor AGR. This is seen in Fig. 7C, where oscilloscope sweeps triggered by spontaneous impulses ascending from the cell body of AGR via its axon (ION) to the left CoG, show large time-locked EPSPs in a CG soma that give rise, in turn, to spikes seen descending in the latter's axon (in the SON) toward the STG. In addition to their size, a feature of AGR-evoked EPSPs in CG is their long duration (~40 ms), reaching peak amplitude after some 2-3 ms then decaying relatively slowly (see also Fig. 9B). During this long decay, depolarization may remain sufficient to trigger a second spike in the interneuron. Whereas the first impulse, which arises directly out of the peak of the EPSP, is strictly correlated with each presynaptic spike in AGR (see SON record), subsequent CG spikes are not, occurring at variable times during the EPSP's slow repolarizing phase.

MORPHOLOGICAL IDENTIFICATION. Injection of lucifer yellow into the cell body of CG reveals an unusual central morphology, even for arthropod neurons (Fig. 8). The cell body (diam. 30-40 μm) is located dorsally in the posterior quadrant of the commissural ganglion. From the soma a long (~500 μm) thin neurite loops down through the neuropile to join the initial segment of the axon. Consistent with electrophysiological recordings (Fig. 7C), the axon of CG then exits the ganglion in the ipsilateral SON to descend in the STN to the STG. A characteristic feature of CG is a very large (diameter greater than the

Fig. 8. Morphological identification of CG interneuron. Lateral view of left CG after injection with lucifer yellow. The dashed lines denote the upper and lower surfaces of the left commissural ganglion as it appears from the midline of the stomatogastric nervous system in vitro. The SON (containing the CG axon) and ION ascend from the plane of view toward the left and right, respectively. Horizontal bars: 100 μm. See legend of Fig. 1 for definitions of abbreviations.
soma) central integrating segment that forms a distinct T junction with the soma neurite and the axon (Fig. 8). This teldike process projects upward in the neuropile before branching into a fine dendritic plexus. Such a geometry provides a morphological substrate for the unusual size relationship between EPSP and axon spike observed in CG soma recordings (cf. Fig. 7B). The wide integrating segment would provide a powerful shunt to reflected axon spikes as they spread electrotonically to the soma from their trigger zone along the initial axon segment. In contrast, synaptic potentials arising on the dendritic tree would be attenuated minimally as they descend and pass out of the integrating segment into the narrow soma neurite.

Synaptic pathway from AGR to GM

Together the above results indicate that AGR has access to GM motoneurons via a (bilateral) long-loop pathway involving the CG interneurons in the CoG. This excitatory pathway can be directly observed by simultaneous intracellular recordings from the somata of the three cell types (Figs. 9 and 10). In the experiment of Fig. 9A, for example, spontaneous action potentials in AGR are correlated with large potentials (EPSP + spike) in a penetrated CG and EPSPs in a GM neuron. Depolarizing AGR to increase its discharge rate causes steady depolarization and a similar increase in the firing of CG. This results, subsequently, in suprathreshold excitation of the impaled GM motoneuron and other GM cells recorded extracellularly in DAGN.

Although the functional excitatory relationship between AGR, CG, and GM are evident in Fig. 9A, it remains to demonstrate, first, that the connections between AGR and CG, and between CG and GM, are monosynaptic and second, to confirm that AGR has no direct access to GM in the STG. These questions are critical to the analysis of the integrative properties of the sensorimotor pathway (see Ref. 30) and are examined in the following experiments.

A first test to establish the monosynaptic nature of the cellular relationships is shown in the experiment of Fig. 9B where the left and right CG interneurons were penetrated along with AGR and a GM. Action potentials at all discharge frequencies (0–60 Hz) tested in AGR are followed without failure and at constant latency by simultaneous EPSPs in the two CGs (see also Fig. 7C). Spikes arising on these EPSPs are themselves always followed 1:1, again at constant la-
tenacy, by EPSPs in the impaled GM motoneuron. This strict sequence of postsynaptic events in response to AGR impulses is therefore consistent with monosynaptic connections between AGR and the bilateral CGs, and in turn between the CGs and GM.

A second test for monosynapticity was to compare the synaptic relationships between the three cell types (Fig. 10A) observed in normal saline (Fig. 10, left) with those apparent under modified saline containing 2.5 × normal Ca++ and Mg++ concentrations (Fig. 10, right). Raising the divalent cation concentrations raises spike threshold without significantly altering synaptic transmission, and thereby increases the likelihood of blocking (and thus revealing) a polysynaptic pathway (4, 11). The pairwise recordings in

![Diagram of synaptic relationships between AGR, GM, and CG neurons.](image)

**FIG. 10.** Tests for monosynaptic relationships in the AGR-GM pathway. A: experimental preparation including the STG and right commissural ganglion. B1–D1 were recorded under normal saline, whereas those on the right (B2–D2) were obtained in saline modified to raise the spike threshold of neurons (see text). The oscilloscope sweeps were triggered by impulses either in AGR (B, D) or in CG (C). B: monosynaptic excitatory connection from AGR to CG. In normal saline (B1), excitatory postsynaptic potentials (EPSPs) and spikes in CG are associated 1:1 with impulses in AGR. In modified saline (B2) the CG EPSPs continue to follow AGR 1:1, but spikes now arise at variable times on the synaptic potential or may fail to occur. C: monosynaptic excitatory connection from CG to GM. In both normal (C1) and modified (C2) saline, CG impulses always evoke EPSPs in GM. D: lack of direct connections between AGR and GM. Whereas in normal saline AGR reliably evokes EPSPs in GM (D1), in modified saline (D2), the latencies vary or the PSPs fail altogether. These changes are directly due to the modified spiking ability of intercalated CG (see B2). Horizontal bar: 20 ms; vertical bar: 10 mV. See legend of Fig. 1 for definitions of abbreviations.
Fig. 10, B and C show that the timing and occurrence, first, of EPSPs in CG evoked by impulses in AGR (cf. Fig. 10B, I and 2) and second, of EPSPs in GM evoked by spikes in CG (cf. Fig. 10C, I and 2) remain unaltered by high cation saline. Under these conditions, such strict 1:1 following of AGR by CG, and of CG by GM, would not be expected if an additional element(s), with its spike threshold now raised, was intercalated between either cell pair. Moreover, the pre- and postsynaptic responses observed in Fig. 10, B and C now explain the input/output relationships between AGR and GM evident in Figs. 10D. Whereas in normal saline the presence of intervening CG is not apparent (Fig. 10D1) due to its 1:1 spiking with AGR (Fig. 10B1), in modified saline the latencies of the GM EPSPs become variable or they may fail to occur (Fig. 10D2). This change in access of AGR to GM is directly due to an increase in spike threshold of CG (Fig. 10B2), whereby the ability of AGR-evoked synaptic potentials to trigger impulses in CG decreases, the latter occurring at variable times after peak potential or failing altogether. We conclude therefore that AGR is coupled disynaptically with GM motoneurons via the CG interneurons in the CoG. Furthermore, the lack of synaptic potentials in GM other than those responses occurring at relatively long latency (Fig. 10D1), in addition to the effects of modified saline (Fig. 10D2), confirms that no additional direct relationship exists between the receptor and motoneurons in the STG.

Does AGR have the same access via CG to all motoneurons \( (n > 7) \) in the GM population? Although this has not been tested with systematic study of individual ganglia, several relevant observations can be drawn from 40 or so penetrations of the GM cell type mostly in different preparations. As in other stomatogastric preparations (26, 27), GM neurons in Homarus are electrically coupled and indistinguishable from one another using intracellular criteria (see also 24). In addition to receiving identical synaptic inputs within the same ganglion, GM cells in the present study never failed to manifest uniformly large, discrete EPSPs arising from impulses in the two CG interneurons; there is no evidence to suggest that these excitatory connections are other than direct onto each and every GM motoneuron. Thus, notwithstanding individual differences in firing threshold, impulse patterning and other inherent properties, we regard GM neurons as members of a homogenous cell population that provides a common target for disynaptic input from the mechanoreceptor AGR.

**DISCUSSION**

The cells and their synaptic connections described in this study provide a discrete input-output pathway for mechanoreceptor information through the stomatogastric system of Homarus. The neural pathway found is summarized in Fig. 11. A striking feature is its extreme numerical simplicity, even for invertebrates, employing just a single mechanoreceptor neuron (AGR), a bilateral pair of higher-order interneurons (CG), and a small population of functionally homologous motoneurons (GM). No other neural elements are directly implicated in the pathway; all synaptic connections appear to be excitatory.

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**Fig. 11.** Summary diagram of the sensorimotor circuit involving AGR-CG-GM. All synaptic connections are excitatory and monosynaptic between the 3 neuronal levels. Mechanical activation of AGR is presumed due to contraction of the bilateral gm1 muscle. The circle enclosing GM corresponds to at least 7 electrically coupled motoneurons (in Homarus), which are considered equivalent and all postsynaptic to CG. See legend of Fig. 1 for definitions of abbreviations.
chemically mediated, and monosynaptic on either side of the CG interneurons. The small number of neurons and their evidently strong synaptic couplings suggest that a quantitative description of the circuit is feasible.

**Precedents for AGR in the crustacean stomatogastric nervous system**

Twenty years ago, Larimer and Kennedy (16) described a new "unusual" type of mechanoreceptor neuron in the stomatogastric system of the crayfish, *Procambarus*. On the basis of extracellular recordings from semi-intact and isolated preparations, they concluded that there exists a unique multi-branched cell with bilateral terminal processes in the left and right AGN and a bifurcating axon that projects to the CoG. The unit responded to mechanical stimulation of the anterior stomach wall close to its presumed receptor terminals and, furthermore, each dendrite was found to spike autogenically. Larimer and Kennedy (16) also noted the association between their physiological findings and a single bipolar neuron discovered many years earlier in methylene blue-stained preparations of the STG of *Homarus* and *Astacus* (1, 18). Recent ultrastructural studies and cobalt staining have also revealed a single bipolar neuron of unknown function in or near the STG of *Panulirus* (14, 15). These cells are most likely the homologues of AGR in *H. gammarus*.

AGR seems to correspond most closely to the "hypodermal cutaneous receptor" type in the classification of Bush and Laverack (5) for mechanoreceptors in decapod crustacea. This class of receptor cell is typically bipolar with multiterminal dendrites that do not penetrate cuticle but terminate in underlying epidermis. Other similarities between AGR and this cell type, as for example the abdominal cutaneous receptor at the base of crayfish swimmerets (19), include 1) relatively central soma location generally in the proximal region of the appropriate ganglionic nerve root, 2) long stretch-sensitive dendrites that branch extensively to innervate widely separated areas of soft cuticle, and 3) dendritic spiking with impulses initiated in a given terminal branch to invade all others.

**Input/output function of AGR**

In any sensory neuron constructed like AGR it is not too surprising to find impulse generation as the means of communication within and between the cell’s distant receptive regions and its soma and central axon. As suggested for the mechanoreceptor neuron described by Larimer and Kennedy (16), one role of AGR could be to serve as a mixing circuit that balances accidental asymmetries in the action of the left and right gm 1 muscles.

Although the precise mode of natural stimulation of AGR in the intact animal is currently unknown, activation of the receptor by gm 1 contraction is favored on the basis of AGR’s close anatomical relationship with this muscle, and the regional specificity and nature of its response to mechanical stimulation in semi-intact and isolated preparations. In this scheme, without excluding other possibilities, a clue to the output role of AGR derives from the finding that its discharge causes strong (albeit disynaptic) excitation of motoneurons (GM) that innervate gm 1, the muscle from which the receptor originates. The analogy with an “assistance” type reflex is therefore appealing; gm 1 contraction (which causes the medial tooth to protract) stretching and activating AGR with the resultant positive feedback reflex serving to reinforce the ongoing movement.

What is the functional significance of such positive feedback in the medial tooth system? The gm 1 muscle and its motoneurons participate in a centrally generated motor program that drives the grinding motion of the medial tooth (13). As in other rhythmic motor systems, cyclic movements of the medial tooth can be divided into alternating phases of powerstroke (protraction) and returnstroke (retraction). In locomotor systems of crustacea, for example, positive feedback reflexes are involved in compensating motor output to powerstroke muscles under variable loads (2, 12, 31). Positive feedback from mechanoreceptors also reinforces the powerstroke of locomotion in both the cockroach and the cat (20). Similarly, compensation and reinforcement of medial tooth protraction during powerstroke in the face of variable quantities and sizes of food particles could be accomplished by AGR in the stomach of crustacea.

**Functional basis of long-loop reflex pathway**

There are no indications physiological or morphological that AGR makes any direct
synaptic connections with GM motoneurons in the STG. Rather the sensorimotor reflex is mediated entirely by a long projection pathway via the two CG interneurons, one in each CoG. Why involve these distant bilateral interneurons? As first noted by Otlov (18), the CoG are the major coordination centers of the foregut. They receive inputs from a variety of sources, including the brain and the thoracic and abdominal nervous systems. They also serve to integrate neural information arising from within the stomatogastric nervous system itself. Indeed most foregut sense organs influencing motor output from the STG project first to the CoGs (8). In the role of higher-order sensory integration, the CG interneurons appear to be no exception. In addition to synaptic excitation from AGR, the CGs receive strong excitatory and inhibitory inputs from another gastric mechanoreceptor system, the paired multunit posterior stomach receptors that monitor movements of the posterior part of the gastric mill and also function in the reflex control of gastric motor output (6, 7). As for AGR, this control is mediated by convergence onto the two CG interneurons (17), again indicating their importance as primary integrators of gastric mechanosensory information.

A further consideration of the CG role in the AGR to GM sensorimotor pathway is that there does not appear to be any direct bilateral interactions between the left and right interneurons themselves. No evidence has been found in this study or previously (24) for mono- or polysynaptic pathways that could couple their activity patterns and ensure coordinated output to the STGs. Bilateral coupling does derive from AGR, however, since clearly the latter’s excitatory influence is sufficiently strong to synchronize spiking activity in its two CG followers. In terms of reflex control, moreover, bilateral synchronization would have the functional advantage of causing a twofold gain increase in AGR effects at the level of the GM motoneurons.

The properties of the sensorimotor pathway described in this paper indicate the CG role as a relatively passive “through pathway” for sensory information to gastric motoneurons. As a simple relay, CG appears to perform well, transforming excitatory synaptic input from AGR into spiking activity and transmitting this information with little failure, and with the same sign, to GM motoneurons. In this scheme, and despite the above functional considerations, the purpose of routing AGR input through such a tightly coupled circuit is not immediately obvious. The properties of this disynaptic reflex seem more analogous to the efficient monosynaptic muscle stretch reflex of vertebrates, for example, than to a typical multisynaptic reflex. In the latter, reflex enhancement suppression and even switching of sign can result from gating different interneuronal relays in or out of the pathway (10, 22). As seen in the following paper (30), however, the “simple” hard-wired AGR-CG-GM reflex is also capable of such integrative flexibility.

ACKNOWLEDGMENTS

We thank Dr. A. Selverston for his comments on an early draft of the manuscript.

This study was supported by le Ministère de la Recherche et de la Technologie Grant 85.C.1152.

Received 19 May 1987; accepted in final form 20 October 1987.

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