12-1-1995

The contributions of motor neuronal and muscle modulation to behavioral flexibility in the stomatogastric system

Patsy S. Dickinson
Bowdoin College

AMER. ZOOL., 35:548–555 (1995)

The Contributions of Motor Neuronal and Muscle Modulation to Behavioral Flexibility in the Stomatogastric System[1]

PATSY S. DICKINSON

Department of Biology, Bowdoin College, Brunswick, Maine 04011

SYNOPSIS. The stomatogastric nervous system of crustaceans, which controls the four parts of the foregut, is subject to modulation at all levels, sensory, central and motor. Modulation of the central pattern generators, which are themselves made up largely of motor neurons, provides for increased behavioral flexibility in a variety of ways. First, each of the pattern generators can be reconfigured to give multiple outputs. Second, the "boundaries" of the different pattern generators are in fact somewhat fluid, so that the neuronal composition of the pattern generators can be altered. For example, neurons can switch from one pattern generator to another, or two or more pattern generators can fuse to generate an entirely new pattern and thereby produce a new behavior. The mechanisms responsible for many of these modulations include alterations of both intrinsic properties and synaptic interactions between neurons. In addition, the alteration of membrane properties contributes more directly to the behavioral output by changing action potential frequency. Finally, the muscles of the stomatogastric system can themselves be modulated, with the cpv1 muscle, for example, becoming an endogenous oscillator in the presence of either dopamine or the peptide FMRFamide.

INTRODUCTION

Behavioral flexibility and variability can be generated on a number of levels, as is evidenced by a number of recent studies demonstrating neuromodulation at the sensory, central and motor levels of organization in circuits controlling defined behaviors (see Marder 1987 for reviews). One of the systems that has proven most useful for the study of modulation is the stomatogastric nervous system of crustaceans. Modulation has been seen at all levels in the stomatogastric system, but has been particularly well studied in the central pattern generators that control the various parts of the foregut (Harris-Warrick *et al.*, 1992). Unlike many pattern generators, those of the stomatogastric system are made up largely of motor neurons, so that modulation of the pattern generators directly affects motor output. Thus, the division between central and peripheral mechanisms of modulation is blurred in this system.

The stomatogastric system itself controls the four parts of the foregut in crustaceans: the oesophagus; the cardiac sac, which serves largely for food storage; the gastric mill, where food is shredded by the single medial and two lateral teeth; and the pylorus, where food is filtered and sent on for further digestion in the midgut or returned to the gastric mill for further shredding (Fig 1A). Each of these regions of the foregut contracts with a characteristic period and pattern, and each is controlled by a pattern generator located within the four ganglia of the stomatogastric nervous system (Fig 1B). The pattern generators for both the pyloric pattern (period 0.5–2 sec) and the gastric mill pattern (period 5–10 sec) are located largely in the stomatogastric ganglion (STG), a ganglion that contains only about 30 neurons; in consequence, these pattern generators have been very well studied (Harris-Warrick *et al.*, 1992). The pattern generator for the oesophageal network (period 5–10 sec) is least understood, but is thought to involve com-

[1] From the Symposium *Invertebrate Neuromuscular Organization: Peripheral Contributions to Behavioral Variability* presented at the Annual Meeting of the American Society of Zoologists, 27–30 December 1993, at Los Angeles, California.

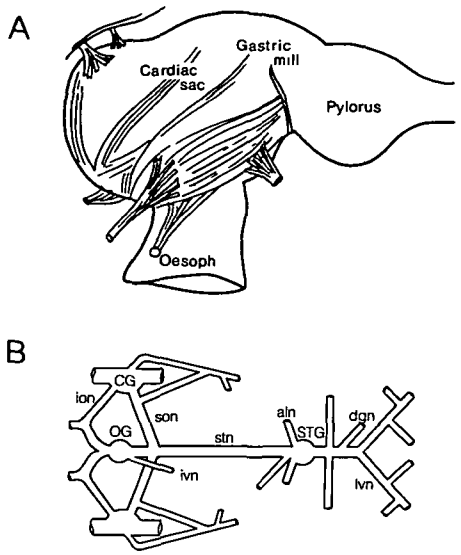

FIG. 1. The lobster foregut (A) and stomatogastric nervous system (B). Abbreviations: aln, anterior lateral nerve; CG, commissural ganglion; dgn, dorsal gastric nerve; ion, inferior oesophageal nerve; ivn, inferior ventricular nerve, lvn, lateral ventricular nerve; OG, oesophageal ganglion; son, superior oesophageal nerve; STG, stomatogastric ganglion; stn, stomatogastric nerve.

ponents in both of the paired commissural ganglia (CG) as well as the unpaired oesophageal ganglion (OG); that for the cardiac sac pattern (period 15 sec–2 min) is widely distributed throughout the stomatogastric system, involving components in all four ganglia (Dickinson *et al.*, 1993).

MODULATION OF THE STOMATOGASTRIC PATTERN GENERATORS

Network reconfiguration

The output of each of the stomatogastric pattern generators is plastic, so that the behavior of the stomach as a whole can be altered. In large part, this behavioral flexibility derives from changes in synaptic connectivity and intrinsic neuronal properties that result in what Getting termed a "polymorphic network" (Getting and Dekin, 1985). Thus, the network can be reconfigured to give multiple outputs, in effect making a number of functional networks. Such network reconfiguration can be seen both in entire networks and within smaller subsections of each network. For example, the lateral gastric (LG) and medial gastric (MG) neurons are electrically coupled to one another and are connected by reciprocal inhibitory synapses. In addition, they receive common synaptic input from the commissural gastric (CG) neuron (Fig. 2A, C). Their firing patterns result from the conflicting connections that tend to make them fire in phase (electrical coupling, common input) or out of phase (reciprocal inhibition; Nagy

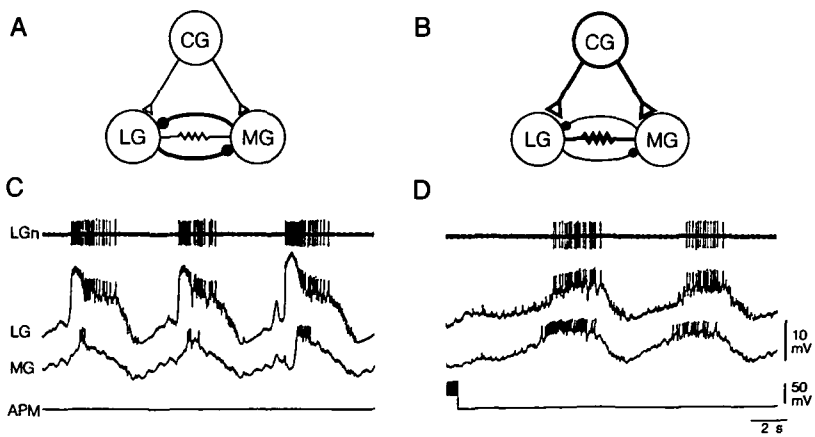

FIG. 2. Modulation of mixed chemical/electrical synapses between the LG and MG neurons of the gastric mill reconfigures the circuit. A, B. When modulatory neuron APM is silent (A) or active (B), the effective circuit changes, so that different synapses, shown by darker lines, are more important in determining the timing of LG and MG firing, in the two cases. C, D. Firing patterns and phase relationships that result when APM is silent (C) or active (D) differ. When APM is silent, reciprocal inhibition dominates (C) and LG starts firing before MG. When APM is active, inputs from the Commissural Gastric (CG) neurons and electrical coupling predominate, so that LG and MG fire nearly synchronously (D). Modified from Nagy *et al.* (1988).

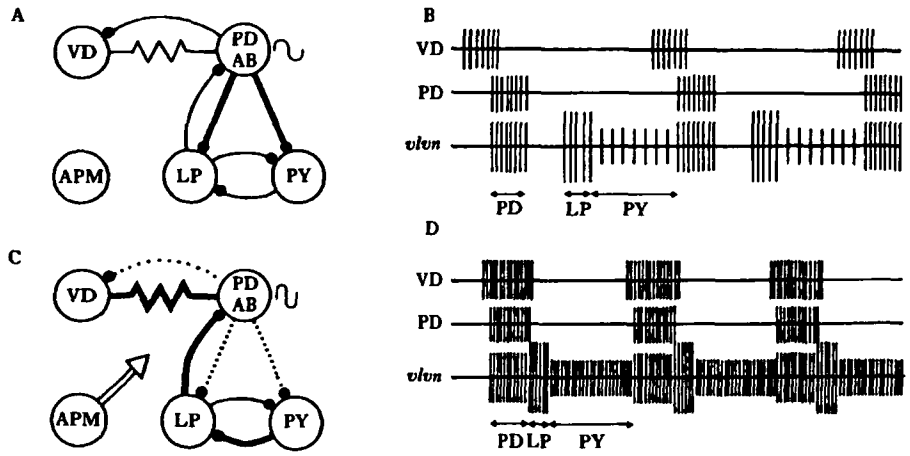

FIG. 3. Modulation of the entire pyloric network by AMP. A, B. When APM is silent, the inhibitory chemical synapses from AB/PD (dark lines) are important in determining the pattern, which is shown in B. C, D. When APM is active, the functional importance of some synapses (shown by dotted lines) decreases, whereas that of other synapses (darker lines) increases. The pattern seen when APM fires is shown diagrammatically in D. Firing frequency of all neurons increases, and phase relationships change. From Nagy and Dickinson (1983).

et al., 1988). Modulatory inputs, including the Anterior Pyloric Modulator neuron (APM), alter the balance between the two types of connections by altering the membrane properties of the LG neuron. Thus, when the gastric mill rhythm is active, but APM is silent, LG tends to fire before MG, its strong burst inhibiting MG and preventing it from firing until later during the LG burst (Fig. 2A, B). However, after APM has fired, the two neurons fire nearly synchronously, the common input and the electrical coupling now being the stronger forces (Fig. 2C, D; Nagy *et al.*, 1988).

Modulators can also induce larger scale reconfigurations. Activity in APM, for example, alters the entire pyloric network, as is summarized in Fig. 3. Pyloric cycle frequency increases, as does action potential frequency in all neurons of the network. In addition, phase relationships are altered: the lateral pyloric (LP) neuron fires earlier, and the ventricular dilator (VD) and PD neurons fire in phase when APM is activated (compare Figs. 3B, 3D).

Modulators can also activate specific behavioral patterns by selecting only a subset of the neurons in a given pattern generator. Thus, not only the interactions between the neurons in a pattern generator, but also the functional composition of a network can be changed by modulators. For example, in the presence of dopamine, only 4 of the 6 neuronal types that make up the pyloric pattern generator are active (Flamm and Harris-Warrick, 1986*a*, *b*). Proctolin likewise produces a pattern involving 4 neuronal types, but the specific subset of 4 differs from that seen in dopamine (Hooper and Marder, 1987). Thus, each modulatory compound configures the network in a different way by activating or silencing certain neurons, by altering the membrane properties of specific neurons, and by altering the synaptic interactions between specific neurons within the pattern generator. The motor output of these functionally different circuits is then transformed into movement, but it is important to recognize that this transformation may not be linear. It is also important to recognize that even neurons that are not actively participating in the motor pattern may contribute to the behavior. Certain muscles will not be activated, which in itself alters movements. In addition, silent neurons that are electrically coupled to oscillatory neurons can increase or decrease the frequency and amplitude of those oscillations (Hooper and Marder, 1987; Kepler *et al.*, 1990).

Changes in the boundaries or membership of pattern generators

It is clear from the discussion of network

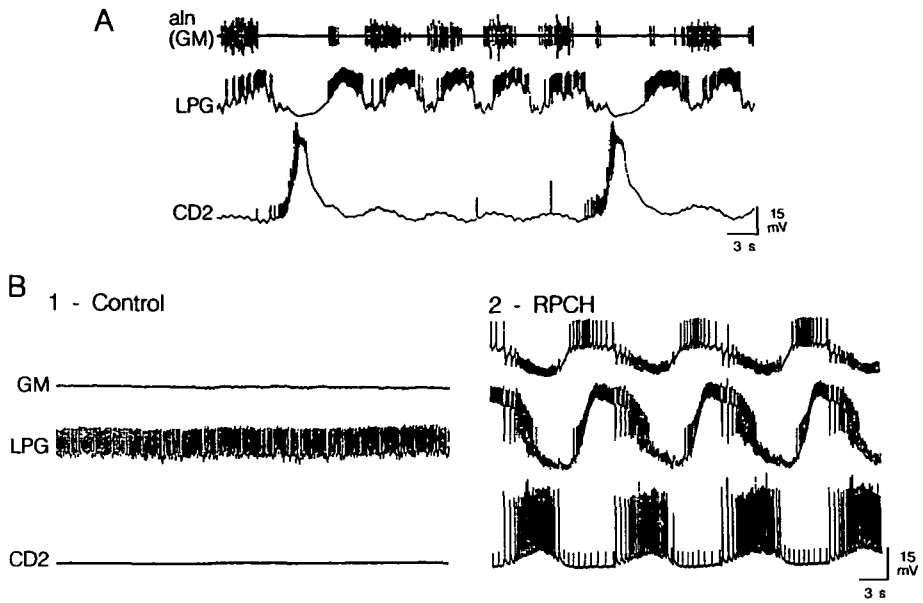

FIG. 4. The gastric mill and cardiac sac networks fuse to produce a novel pattern when RPCH is bath-applied to the isolated STG. (A) When the *stn* is intact and both cardiac sac (CD2) and gastric mill (*aln*, LPG) rhythms are active, the two patterns show dramatically different periods. There are slight interruptions of gastric mill activity during cardiac sac dilator bursts. (B1) When the *stn* is blocked, rhythmic activity in both systems stops, suggesting that such rhythmicity is dependent on modulatory inputs that enter the STG via the *stn*. (B2) When RPCH (10^{-6} M) is bath applied, both gastric mill and cardiac sac neurons become rhythmically active in a single, conjoint pattern that differs from either of the two individual patterns. Modified from Dickinson *et al.* (1990).

reconfiguration above that all the neurons in a pattern generator are not always active members of that pattern generator. Recent work has shown that neurons can participate in more than one pattern generator, and that entire pattern generators can merge. Thus, the boundaries between the 4 pattern generators of the stomatogastric system are not fixed, but are instead fluid, so that neurons can move from one to another, singly or *en masse*. For example, Hooper and Moulins (1989) showed that the ventricular dilator (VD) neuron, which is normally a member of the pyloric pattern generator, begins to fire in cardiac sac time when a defined sensory input is stimulated. At this time, it no longer fires the short, frequent bursts that characterise the pyloric pattern, but instead fires in longer, less frequent bursts that are typical of cardiac sac patterns.

The boundaries between two (or more) of the stomatogastric pattern generators can also be virtually eliminated, as occurs when the peptide red pigment concentrating hormone (RPCH) is applied to the isolated STG. In the intact system, the neurons of the gastric mill pattern generator, such as the gastric mill (GM) and lateral posterior gastric (LPG) fire with a period of 5–10 sec, whereas cardiac sac neurons such as the cardiac sac dilator 2 (CD2), fire with a period of minutes (Fig. 4A). Blocking the single input nerve to the STG, the stomatogastric nerve, blocks all rhythmic activity in both pattern generators, pointing to the importance of modulatory inputs in not only altering but also maintaining rhythmic activity (Fig. 4B). When the STG is then bathed with RPCH, both the gastric mill and cardiac sac neurons again fire rhythmically. However, all of the neurons fire in a single pattern, which is different from either of the original patterns (Fig. 4C).

Interactions of neuromodulators

The stomatogastric nervous system contains a plethora of neurotransmitters and

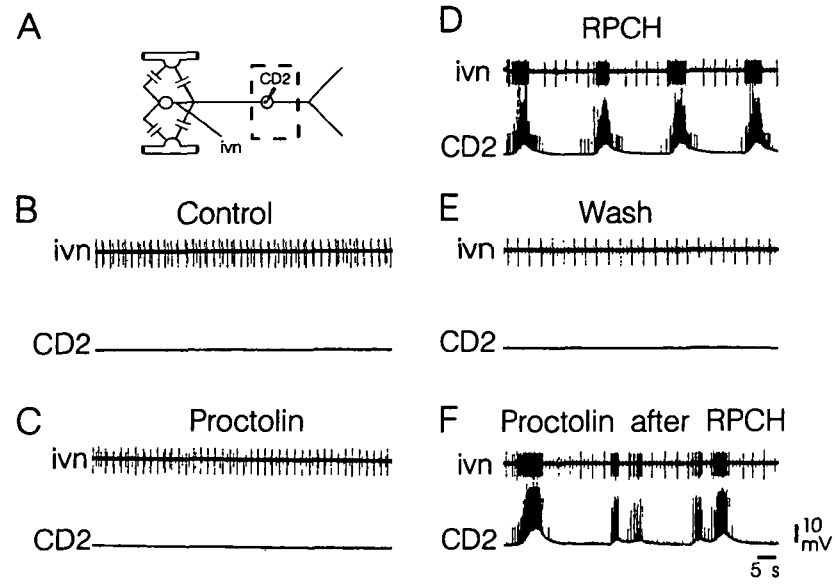

FIG. 5. Bath application of RPCH before application of proctolin alters the response of the cardiac sac pattern generator to proctolin. (A) Diagrammatic view of the stomatogastric nervous system, showing the sites of recording (*ivn* extracellular, CD2 intracellular) and of superfusion with proctolin or RPCH (dashed box). (B) In the control (*sons* and *ions* cut), there is no cardiac sac motor pattern. (C) Bath application of proctolin (10^{-6} M) does not induce rhythmic activity in either the *ivn* or CD2. (D) Bath application of RPCH (10^{-6} M) activates the cardiac sac pattern generator, resulting in rhythmic activity (*ivn*, CD2). (E) Rhythmic activity ceases when RPCH is washed out. (F) When proctolin is applied after the wash, 10–15 minutes after the end of the last cardiac sac burst, rhythmic activity is once again induced.

modulators, many of which are located in the neuropile, where they are in an appropriate position to modulate the activity of the system (Marder, 1987). One question that arises is how these transmitters interact with one another to influence the output of the pattern generators. It is becoming clear that some modulatory neurons contain more than one transmitter; the Gastro-Pyloric Receptor (GPR), for example, appears to use both serotonin and acetylcholine (Katz *et al.*, 1989; Katz and Harris-Warrick, 1989). In addition, the same modulator can have different effects when applied alone than when applied with or after another modulator. For example, when the peptide proctolin is applied to the isolated STG, it has no effect on the cardiac sac pattern, as recorded in CD2 (Fig. 5A, B). However, if the ganglion has been previously bathed with RPCH, which induces cardiac sac activity, application of proctolin likewise induces rhythmic activity in the cardiac sac network (Fig. 5C–F). The mechanisms underlying this interaction are not yet understood, but it is clear that not only the presence of different neurotransmitters, but also the previous history of the system, is important in determining the response of the pattern generators to modulators, as well as in generating behavioral flexibility.

Changes in membrane properties cause changes in spike frequency

In considering how modulation of the stomatogastric pattern generators translates into behavioral changes, we must consider not only the changes in the patterns themselves, but also the ways they are likely to affect muscle contractions. One important consideration is that the properties of the muscle membrane are not necessarily constant, as we shall see later. In addition, the muscles of the crustacean foregut do not generate all-or-none twitches, but instead show graded contraction. The degree and rate of contraction is determined largely by summation and facilitation, which is in turn dependent on spike frequency. Thus, any

factors that profoundly alter spike frequency will also affect behavior.

A major mechanism by which modulations in the stomatogastric system are effected is changes in the plateau properties of the stomatogastric neurons. In the presence of an appropriate neuromodulator, they have two stable membrane potentials, and synaptic inputs can trigger the transitions between these levels. Spike frequency is high during plateaus, and is thus highly dependent on the plateau properties of individual neurons (Russell and Hartline, 1978, 1981, 1984). Consequently, modulatory inputs that induce or suppress plateau properties simultaneously change not only the neuron's role in generating the pattern, but also its spike frequency and hence the contraction of the muscles it controls. For example, the firing of the LG neuron, shown in Fig. 2, has two components, the first a plateau, the second due to synaptic input. When APM fires, the synaptic component is enhanced; but plateaus are suppressed, and so the firing frequency drops considerably, presumably leading to changes in muscle contraction as well.

MODULATION OF MUSCLE MEMBRANE PROPERTIES

At the level of the muscle itself, Meyrand and Moulins (1986) and Meyrand and Marder (1991) showed that one of the pyloric muscles in the shrimp can be converted into an endogenous oscillator by either dopamine or FMRFamide-like peptides. When either modulator is bath applied, the muscle membrane potential oscillates spontaneously (Fig. 6). This oscillation is accompanied by rhythmic contractions of the muscle. Early in a bath application of YGGFMRFamide, the membrane oscillates only when depolarized; in this state, the muscle displays regenerative properties, but does not oscillate spontaneously. Later it oscillates spontaneously, with the oscillation frequency increasing when depolarizing current is injected, as would be expected from an endogenous oscillator (Fig. 6).

Behaviorally, the consequences of this modulation are a function of the level of activation of both the muscle and the pyloric dilator (PD) motor neuron that innervates it. Meyrand and Marder (1991) divide the activation of the muscle into 3 possible states (Fig. 7): quiescent (no regenerative properties), oscillator, and "amplifier" (regenerative properties, no spontaneous oscillations). When the muscle is in the control or quiescent state, it does not move unless the motor neuron fires, and then it follows passively with small amplitude oscillations. When the muscle is in the oscillator state, it oscillates spontaneously, with large amplitude oscillations occurring regularly in the absence of any motor neuronal input. When PD does fire, it entrains the myogenic rhythm if the motor neuronal cycle frequency is close to the spontaneous muscle frequency; otherwise, it disrupts the myogenic rhythm. When the muscle is in the "amplifier" mode, it does not move unless the motor neuron fires. However, when PD is active, the muscle follows, over

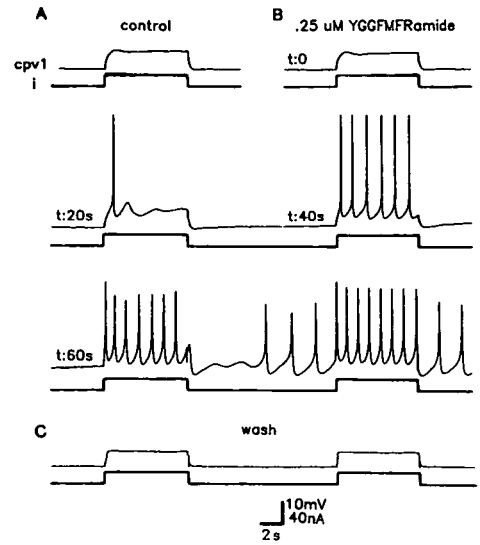

FIG. 6. Bath application of the peptide YGGFMRFamide (0.25 μM) alters membrane properties of the cpv1 muscle in the shrimp, so that it becomes an endogenous oscillator. Identical pulses of current (i) were injected into a cpv1 muscle fiber while the membrane potential was recorded. (A) In the control, the current caused a passive response. (B) During bath application of YGGFMRFamide, the membrane initially oscillated only during current injection (t = 20 sec, t = 40 sec, t = 60 sec). After 1 min in the peptide, the muscle membrane potential oscillated spontaneously; at this time, current injection increased the frequency of oscillations. (C) Spontaneous activity and responses to current injection returned to control levels in the wash. From Meyrand and Marder (1991).

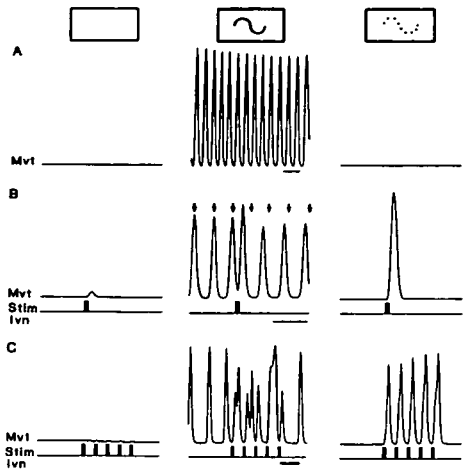

FIG. 7. The shrimp muscle shows three distinct states of activity: quiescent (left column), oscillator (middle column), and "amplifier" (right column). (A) In the absence of motor neuronal input (stimulations of *lvn*), no movement occurs when the muscle is in the control or the amplifier state. However, when it is in the oscillator state, large amplitude oscillations occur rhythmically. (B) A single burst of activity in the motor neuron provokes a single small amplitude movement in the control state; it provokes a single large amplitude movement in the amplifier state. In the oscillator state, the same motor input provokes an additional large movement, and resets the rhythm. (C) A series of bursts in the motor neuron provokes a similar series of small movements when the muscle is in the control state. When the muscle is in the oscillator state, this same series of bursts, at a frequency that differs considerably from the muscle's oscillation frequency, disrupts the rhythmic movements. When the muscle is in the amplifier state, a series of large amplitude movements results. From Meyrand and Marder (1991).

its entire range of cycle frequency, with large amplitude oscillations. Thus, the induction of regenerative properties in the muscle can serve either to generate a rhythm or to enhance the magnitude of the muscle response to a constant motor neuronal input (compare Fig. 7A3, C3).

CONCLUSIONS

Modulation both at the motor neuronal level and at the level of the muscle itself makes important contributions to behavioral flexibility and control in the stomatogastric system. Modulators acting at these levels, as well as at the interneuronal and sensory levels, can alter individual motor patterns by reconfiguring networks. They can likewise alter or even erase the boundaries between networks so that neurons switch from one network to another or entire networks fuse. Finally, they can alter the movements that result from the same motor neuronal input. Thus far, in investigations of the mechanisms that are responsible for all of these modulations, only two major classes of changes have been seen: alteration of the membrane properties of individual neurons or muscles, and alteration of the synapses that connect specific neurons.

ACKNOWLEDGMENTS

I am grateful to Jane Hauptman for help in preparing the figures. Supported in part by NSF BNS9008816 and BNS 931003.

REFERENCES

Dickinson, P. S., C. Mecsas, J. Hetling, and K. Terio. 1993. The neuropeptide red pigment concentrating hormone affects rhythmic pattern generation at multiple sites. J. Neurophysiol. 69:1475–1483.

Dickinson, P. S., C. Mecsas, and E. Marder. 1990. Neuropeptide fusion of two motor-pattern generator circuits. Nature 344:155–158.

Flamm, R. E. and R. M. Harris-Warrick. 1986*a*. Aminergic modulation in lobster stomatogastric ganglion. I. Effects on motor pattern and activity of neurons within the pyloric circuit. J. Neurophysiol. 55:847–865.

Flamm, R. E. and R. M. Harris-Warrick. 1986*b*. Aminergic modulation in lobster stomatogastric ganglion. II. Target neurons of dopamine, octopamine, and serotonin within the pyloric circuit. J. Neurophysiol. 55:866–881.

Getting, P. A. and M. S. Dekin. 1985. *Tritonia* swimming: A model system for integration within rhythmic motor systems. *In* A. I. Selverston (ed.), *Model neural networks and behavior,* pp. 3–20.

Harris-Warrick, R. M., F. Nagy, and M. P. Nusbaum. 1992. Neuromodulation of stomatogastric networks by identified neurons and transmitters. *In* R. M. Harris-Warrick, E. Marder, A. I. Selverston, and M. Moulins (eds.), *Dynamic biological networks: The stomatogastric nervous system,* pp. 87–138. MIT Press, Cambridge, Massachusetts.

Hooper, S. L. and E. Marder. 1987. Modulation of the lobster pyloric rhythm by the peptide proctolin. J. Neurosci. 7:2097–2112.

Hooper, S. L. and M. Moulins. 1989. Switching of a neuron from one network to another by sensory-induced changes in membrane properties. Science 244:1587–1589.

Katz, P. S., M. H. Eigg, and R. M. Harris-Warrick. 1989. Serotonergic/cholinergic muscle receptor cells in the crab stomatogastric nervous system: I. Identification and characterization of the gastro-

pyloric receptor cells. J. Neurophysiol. 62:558–570.

Katz, P. S. and R. M. Harris-Warrick. 1989. Serotonergic/cholinergic muscle receptor cells in the crab stomatogastric nervous system: II. Rapid nicotinic and prolonged modulatory effects on neurons in the stomatogastric ganglion. J. Neurophysiol. 62:570–581.

Kepler, T. B., E. Marder, and L. F. Abbott. 1990. The effects of electrical coupling on the frequency of model neuronal oscillators. Science 248:83–85.

Marder, E. 1987. Neurotransmitters and neuromodulators. *In* A. I. Selverston and M. Moulins (eds.), *The crustacean stomatogastric system: A model for the study of central nervous systems,* pp. 263–306. Springer-Verlag, Berlin.

Meyrand, P. and E. Marder. 1991. Matching neural and muscle oscillators: Control by FMRFamide-like peptides. J. Neurosci. 11:1150–1161.

Meyrand, P. and M. Moulins. 1986. Myogenic oscillatory activity in the pyloric rhythmic motor system of crustacea. J. Comp. Physiol. 158:489–503.

Nagy, F. and P. S. Dickinson. 1983. Control of a central pattern generator by an identified modulatory interneurone in Crustacea. I. Modulation of the pyloric motor output. J. Exp. Biol. 105:33–58.

Nagy, F., P. S. Dickinson, and M. Moulins. 1988. Control by an identified modulatory neuron of the sequential expression of plateau properties of, and synaptic inputs to, a neuron in a central pattern generator. J. Neurosci. 8:2875–2886.

Russell, D. F. and D. K. Hartline. 1978. Bursting neural networks: A reexamination. Science 00:453–456.

Russell, D. F. and D. K. Hartline. 1981. A multiaction synapse evoking both EPSPs and enhancement of endogenous bursting. Brain Res. 223:19–38.

Russell, D. F. and D. K. Hartline. 1984. Synaptic regulation of cellular properties in burst oscillations of neurons in gastric mill system of spiny lobster *Panulirus interruptus.* J. Neurophysiol. 52: 54–73.