Central pattern generation underlying *Limulus* rhythmic behavior patterns

Gordon A. Wyse*

Department of Biology, University of Massachusetts, Amherst MA 01003, USA

**Abstract** Many behavioral activities of the horseshoe crab *Limulus* are rhythmic, and most of these are produced in large part by central pattern generators within the CNS. The chain of opisthosomal (‘abdominal’) ganglia controls gill movements of ventilation and gill cleaning, and the prosomal ring of fused ganglia (brain and segmental ‘thoracic’ ganglia) controls generation of feeding and locomotor movements of the legs. Both the opisthosomal CNS and the prosomal CNS can generate behaviorally appropriate patterns of motor output in isolation, without movements or sensory input. Preparations of the isolated opisthosomal CNS generate rhythmic output patterns of motor activity characterized as fictive ventilatory and gill cleaning rhythms. Moreover, CNS preparations also express longer-term patterns, such as intermittent ventilation or sequential bouts of ventilation and gill cleaning. Such longer-term patterns are commonly observed in intact animals. The isolated prosomal CNS does not spontaneously generate the activity patterns characteristic of walking, swimming, and feeding. However, perfusion of octopamine in the isolated prosomal CNS activates central pattern generators underlying rhythmic chewing movements, and injection of octopamine into intact *Limulus* promotes the chewing pattern of feeding, whether or not food is presented. Our understanding of the ability of neuromodulators such as octopamine to elicit or alter central motor programs may help to clarify the central neural circuits of pattern generation that produce and coordinate these rhythmic behaviors [Current Zoology 56 (5): 537–549, 2010].

**Key words** *Limulus*, Rhythmic behavior, Central nervous system, Central pattern generator, Feeding, Respiration

1 Introduction

In this review I ask the question: What do we know about the neural control of behavior patterns in *Limulus*? Many recent studies of motor control have focused on other animals that can be analyzed genetically or in terms of uniquely identifiable neurons. The recent broadening of interest in the behavior and natural history of *Limulus*, however, is renewing attention to its underlying physiology. Here I will review studies that demonstrate that central pattern generators (CPGs) underlie many rhythmic behavior patterns in *Limulus*, and that suggest how these CPGs are controlled and modulated. My aim is to stimulate new work in this area, since *Limulus* has biomedical importance, behavioral relevance, and the advantages of an accessible nervous system that withstands experimental manipulation well.

Invertebrate nervous systems have been much studied in the past 50 years, both to clarify the neural bases of behavior in the invertebrates themselves, and as methodologically advantageous model systems that permit analysis of general issues of neural control at a network level, analysis that is daunting to pursue in the nervous systems of vertebrates (Marder et al., 2005; Clarac and Pearlstein, 2007). Recent genetic studies in mammals are clarifying the network circuitry of central motor pattern generation (Grillner and Jessell, 2009; Garcia-Campmany et al., 2010), but at the level of populations of interneurons rather than of uniquely identifiable neurons as in some invertebrate examples.

A central pattern generator (CPG) is a circuit within the central nervous system (CNS) that can produce a pattern of motor output appropriate to generate a behavior pattern, without requiring sensory input for timing or sequencing the motor output. Many rhythmic behavior patterns of invertebrates and vertebrates have been shown to depend, in large part, on underlying central pattern generators, and the CPG concept remains critically important in understanding the neural basis of rhythmic behavior (e.g., Delcomyn, 1980; Hooper and DiCaprio, 2004; Kullander, 2005; Marder et al., 2005; Dickinson, 2006; Grillner, 2006; Goulding 2009; Guerlin, 2009). Investigators test for the existence of a CPG by removing sensory feedback (deafferentation), often by isolating the relevant part of the central nervous system from the animal. A pattern of rhythmic motor output
recorded from an isolated preparation clearly cannot depend on sensory feedback and must be centrally patterned. Because the patterned output in isolation no longer drives muscle activity to produce the behavior pattern, it is called a *fictive* behavior pattern (fictive chewing, fictive gill cleaning, etc.). It is important to compare carefully the spatial and temporal pattern of motor activity recorded in isolation or deafferentation to that pattern underlying the normal behavior, to insure that the fictive pattern truly represents the normal motor program in intact animals.

2 Anatomy

The body of *Limulus* is divided into an anterior portion termed the *prosoma*, and a posterior portion, the *opisthosoma* (Fig. 1). The prosoma may correspond to the head and thorax of other arthropods, and the opisthosoma to the abdomen. Although the terms *opisthosoma* and *abdomen* may be used interchangeably, it is difficult to homologize these regions with any confidence. Lankester et al. (1885) described and numbered *Limulus* skeletal muscles, and their numbering system is generally followed. The most comprehensive general anatomy of the *Limulus* nervous system comes from Patten’s studies at the beginning of the 20th century (Patten and Redenbaugh, 1900; Patten, 1912). Although Patten’s anatomical descriptions and figures are generally correct and complete, his terminology is archaic and reflects a goal of deriving vertebrates from a *Limulus*-like ancestor.

![Graphical representation](image)

**Fig. 1** The central nervous system (CNS) of *Limulus* consists of a prosomal part and an opisthosomal part, shown in an orientation diagram on the left and enlarged in dorsal view on the right

In the prosoma, the brain and circumesophageal ring of fused pedal ganglia encircle the esophageal canal. Each pedal ganglion innervates one leg, via a pedal nerve. The opisthosomal chain of ganglia also has partial fusion at its posterior end. Each of the first five opisthosomal ganglia innervates a pair of gill plates via paired branchial nerves.

The *Limulus* central nervous system consists of the brain and circumesophageal ring of fused pedal (thoracic) ganglia in the prosoma, and a ventral nerve cord of six or more partially-fused abdominal ganglia in the opisthosoma (Fig. 1). The fusion of ganglia occurs during development. Pedal ganglia, for example, arise separately in post-oral positions, and hemiganglia migrate anteriorally to fuse around the esophagus. Typically each segmental ganglion has two pairs of roots or peripheral nerves exiting from it. The anterior roots (dorsal nerves) innervate the dorsal side of the animal, including a few muscles, the carapace, heart, and gut. The posterior roots (ventral nerves) innervate the ventral region of the segment, typically an appendage. In the prosoma the ventral nerve to a leg is called a pedal nerve, whereas in the opisthosoma the ventral nerve to a gill plate is called a branchial nerve (Fig. 1).

Chamberlain and Wyse (1986) and Fahrenbach and Chamberlain (1987) describe *Limulus* brain anatomy, and recent studies clarify the relationship of brain segments to those of other arthropods (Mittmann and Scholtz, 2003; Harzsch, 2006; Scholtz and Edgecombe, 2006).

3 Neural Basis of Behavioral Patterns

3.1 Gill ventilation

Horseshoe crabs have book gills on the posterior sides of five pairs of opisthosomal gill plates. The rhythmic movements that employ the gills are gill ventilation, hyperventilation, swimming, and gill cleaning. Ventilation, hyperventilation and swimming involve rhythmic promotions (forward movements) and remotions (backward movements) of the gill plates in a back-to-front metachronal sequence (Knudsen, 1973). In ventilation, only the gill plates beat at a relatively slow rate, with typical intervals of 2–3 sec in adults. In hyperventilation the gill plates and the genital operculum beat more strongly and at a faster rate, with shorter intersegmental latencies. In swimming the legs are also recruited in rowing movements, promoting and remoting together after the gills and operculum. Swimming is considered further in section 3.4.

The ventilatory movements of the gill plates that bear the book gills are the most prevalent rhythmic activities of *Limulus*. In addition to aerating the book gills, ventilation also produces water currents that draw water across chemoreceptors for finding food (Quinn et al., 1998). Hyde (1893; 1906) described the ventilatory rhythm, which she considered to be reflexive. Each opisthosomal ganglion innervates the muscles that move a
gill plate, via a branchial nerve (Fig. 2A) with separate branches of the branchial nerve innervating different muscle groups. The external branchial nerve (EBN) innervates promotor muscles, the medial branchial nerve (MBN) innervates remoter muscles, and the internal branchial nerve (IBN) innervates adductor muscles. This branching pattern allows clear recording of the motor programs for gill movements (Fourtner et al., 1971; Wyse, 1972; Knudsen 1975; Watson 1980a). Rhythmic bursts of motor activity characteristic of the gill ventilatory rhythm persist after the chain of opisthosomal ganglia is isolated from the animal and from sensory feedback (Fig. 2B; Fourtner et al., 1971; Wyse, 1972). The persistence in isolation of the appropriate motor pattern for ventilation (fictive ventilatory rhythm) demonstrates that central pattern generators underlie the gill ventilatory rhythm in intact animals. Fourtner et al. found that stimulation of roots or connectives was required to trigger the fictive ventilatory rhythm, but Wyse recorded the rhythm in both non-stimulated and stimulated preparations, and also recorded long-term patterns of fictive intermittent ventilation (see section 3.6 below).

There are central pattern generators for the ventilatory rhythm in each abdominal hemiganglion, and these are coordinated to produce the multisegmental ventilation pattern. Lesion experiments in which ventral nerve cords were cut at various levels confirm that each opisthosomal hemiganglion is sufficient to generate a ventilatory rhythm for its gill plate (Hyde, 1893; Knudsen, 1973; 1975). Coordinating neurons must maintain the appropriate coupling between the hemiganglonic CPGs. Although normal coordination of the ventilatory rhythm is in metachronal posterior-to-anterior sequence, it is possible to reverse the sequence transiently by stimulating input to a more anterior ganglion (Knudsen, 1973). After the connectives between opisthosomal ganglia are sectioned, the more posterior segment usually has a higher frequency of ventilatory rhythm. Therefore, the normal posterior-to-anterior metachronal rhythm presumably is maintained by the faster CPGs of more posterior segments entraining the intrinsically slower oscillators of more anterior segments. Tactile or electrical stimulation of more anterior segments results in a transient reversal of the metachronal rhythm to an anterior-to-posterior sequence (Knudsen, 1973). These experiments show that the CPGs of more posterior ganglia act as pacemakers that entrain the CPGs of successively more anterior ganglia via coordinating neurons, but that coordinating neurons also connect from anterior to posterior, allowing reversal of the metachronal sequence when stimulated anterior ganglia serve as pacemakers.

The neural network that acts as a ventilatory CPG is not known. Wyse (1972) recorded intracellular activity of motor neurons that produce the ventilatory rhythm. The motor neurons showed no evidence of intrinsic burst generation; instead they appeared to be synaptically driven. Phased synaptic excitation and inhibition produced the oscillation in membrane potential underlying each burst of motor neuron action potentials. Hyperpolarization of motor neuron membrane potentials reversed the burst-terminating compound IPSPs (inhibitory postsynaptic potentials) to become depolarizations. Altering motor neuron activity (by depolarization, hyperpolarization and antidromic stimulation) had no discernable effect on the underlying rhythm. These results suggest that the CPG circuitry in opisthosomal ganglia depends on interneuronal properties and connections, with motor neurons synaptically driven by CPG interneurons but contributing little to the pattern generation.

Command neurons in the opisthosomal ventral nerve cord can stimulate ventilatory CPGs. Three command tracts in each of the paired connectives anterior to the first opisthosomal ganglion contain axons that drive the ventilatory motor pattern when electrically stimulated (Wyse, 1972; Knudsen, 1975; Wyse and Page, 1976).

Gill ventilation, although under control of central pattern generators, is also influenced by sensory input. The ventilatory rhythm is greatly affected by oxygen concentration in the seawater around the animal, slowing and ceasing as the oxygen content is lowered (Hyde, 1906; Waterman and Travis, 1953; Page, 1973; Watson and Wyse, 1978). Ventilation resumes rapidly when oxygen is reintroduced, indicating that external oxygen receptors stimulate the rhythm (Waterman and Travis, 1953). Page and coworkers have characterized such oxygen receptors on the intercoxal cuticle between walking legs, and on the gills (Page, 1973; Crabtree and Page, 1974; Thompson and Page, 1975; Wyse and Page, 1976).

Proprioceptive reflexes, although not necessary for the generation of the ventilatory rhythm, can partially entrain the rhythm. Such reflexes are weak, and forced movement of a single gill plate has little effect on ventilation (Wyse and Page, 1976). When all five pairs of gill plates are driven by a motor at a frequency similar to the ventilatory frequency, the imposed movements can en-
train the centrally-generated ventilatory rhythm over a narrow range of frequencies. This entrainment is usually incomplete, producing a “lock-and-drift” mode of coordination in which the central pattern (recorded from muscles) ‘locks’ onto the imposed movement for a few cycles and then ‘drifts’ out of phase to partially lock onto another cycle of imposed movement at a preferred phase relationship (Wyse and Markey, 1975; Wyse and Page 1976). Such experiments show that although proprioceptive sensory feedback is not important in the generation of the ventilatory rhythm, it can play a minor role in ventilatory timing.

3.2 Gill cleaning

Hyde (1893) and Patten (1912) first described gill cleaning behavior, and Watson (1980a) first characterized it rigorously. Gill cleaning is a complex fixed action pattern occurring in multipartite bouts in which the paired gill plates are adducted across the midline for interaction. In this interaction the inner lobe of one gill plate rhythmically flicks between the gill lamellae of the contralateral side. There are two mirror-image patterns of gill cleaning, termed right-leading and left-leading (Watson, 1980a). A bout of gill cleaning has roughly equal probability of being left-leading and right-leading, but successive bouts do not strictly alternate between the two patterns.

The motor programs for gill cleaning depend on CPGs in opisthosomal ganglia (Fig. 2C). The basic pattern for a gill plate that is actively cleaning is for muscle 22 to partially promote the gill plate, while muscles 113 and 114 flick the medial lobe. This pattern of muscle activity is mirrored in isolation (Fig. 2C), with periods of promotor motor neuron activity in the external branchial nerve (EBN) that are punctuated by rapid bursts in the internal branchial nerve (IBN). Thus although there are differences in detail, the isolated opisthosomal nerve cord expresses the patterns of fictive gill cleaning, often with an approximate alternation of bouts of left-leading and right-leading cleaning patterns similar to that observed in intact animals (Wyse et al., 1980). Because a single abdominal ganglion is sufficient to control the gill cleaning movements of a pair of gill plates (Watson, 1980a), the CPGs for the two mirror-image patterns of gill cleaning must reside in each of the five opisthosomal ganglia that innervate the gill plates.

Fig. 2 Gill ventilation and gill cleaning

A. Opisthosomal muscles and nerves that control ventilation and gill cleaning. In this ventral view, the first gill plates are held forward to show the muscles and gill lamellae of their posterior surface (see orientation diagram on left). Below, the second gill plates are in their normal position, showing the muscles of the anterior surface. Muscle 22 is the main promotor of the gill plate; muscle 20 is the main remotor. Muscles 48 and 115 adduct the gill plate across the midline to interact in gill cleaning, during which muscles 113 and 114 flick the inner lobe of a gill plate between the lamella of the opposite gill. Each branchial nerve (black) divides into three branches: the external branchial nerve (EBN) innervates muscle 22, the medial branchial nerve (MBN) innervates muscle 20, and the internal branchial nerve (IBN) innervates muscles 113 and 114. B. The ventilatory rhythm is centrally patterned. The lower record shows ventilation motor output in an intact but dissected animal, with gill plate movement below (remotion is up). The upper record shows similar motor output in an isolated opisthosomal nerve cord. C. Central pattern generation also underlies gill cleaning behavior. The upper record shows a bout of gill cleaning in an intact Limulus, recorded with muscle electrodes. Muscle 22 holds the gill plate in a relatively promoted position while muscle 114 flicks the inner lobe between lamellae of the contralateral gill. The lower record shows a similar bout of fictive gill cleaning recorded from an isolated opisthosomal nerve cord. (A from Watson, 1980a; B after Wyse, 1972; C after Wyse et al., 1980.)
3.3 Rhythmic chewing movements of feeding behavior

In *Limulus* the mouth is at the ventral surface of the prosoma, surrounded by the bases of the walking legs (Fig. 3). There are no jaws; instead, the ventral portion of each coxal leg base has a spiny endite termed a gnathobase. Chemoreceptive spines on the leg gnathobases contact food and stimulate feeding behavior. Rhythmic feeding movements (termed *chewing*, Manton, 1964, Wyse and Dwyer, 1973) consist of alternate transverse abduction and adduction of the coxal segments of the walking legs. The chelicerae and walking-leg claws may pick up pieces of food and transfer them to the gnathobases, but this aspect of feeding behavior has been little examined. Each coxa, which is elongated dorsoventrally, moves in an arc around a dorsolateral pivot (*P*; Fig. 3A). The inwardly-directed spines on the gnathobases shred particulate food and push it into the mouth.

In these chewing movements, opposite legs are adducted in phase with each other, and adjacent legs are adducted out of phase (Fig. 3B). Thus, both first and both third legs move inward while both second and both fourth legs move outward, and vice versa.

Wyse and Dwyer (1973) recorded activity of the muscles that move the leg coxae during chewing and locomotion. The transverse movements of chewing result from simultaneous contractions of muscles inserting on the anterior face and the posterior face of the coxa (Fig. 3A), adducting the leg base. None of these muscles acts as an abductor; instead, abduction of a leg coxa results in part from the adduction of adjacent legs into the confined peri-oral space, passively pushing out the non-adducted leg.

Distinct nerves innervate the muscles that attach to the two faces of a coxa: the anterior entocoxal nerve (AEN) innervates the muscles of the anterior face, while the posterior entocoxal nerve (PEN) innervates the muscles of the posterior face (Patten and Redenbaugh, 1900).

The pattern of activation of coxal muscles differs between the chewing movements of feeding and the coxal swing of locomotion (Wyse and Dwyer, 1973). In both walking and swimming, anterior and posterior coxal muscles act antagonistically to alternately promote and remote the leg coxa. In contrast, during feeding eight of the nine muscles of each coxa contract together to adduct the coxa medially. The phase relationships of muscle activity between legs are also very different in feeding from those in walking and swimming: During feeding the coxae of adjacent legs are adducted

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**Fig. 3** Muscular basis of chewing and locomotor movements in *Limulus*

A. Cross-section of the prosoma at the level of the third legs; dorsal is up. Tergocoxal muscles (attaching to the dorsal carapace) and pleurocoxal muscles (attaching to a cartilaginous endopodite, *en*) insert on the basal coxal segment (*cx*) of a leg. The leg coxa bears gnathobase spines (*gn*), which contain chemoreceptors and shred food during chewing. (The mouth, not shown, is dorsal to the gnathobase spines and ventral to the endopodite.) In this anterior view, the muscles inserting on the anterior face of the elongated coxa are shown on the right. On the left the anterior face of a coxa is cut away to reveal the muscles that insert on the posterior coxal face. In chewing, simultaneous contraction of anterior-face muscles and posterior-face muscles adducts the coxa around a dorsolateral pivot (*p*). In walking and swimming, sequential contraction of anterior-face muscles and posterior-face muscles swings the leg forward and back, with a point near the gnathobase of the leg acting as a secondary pivot. The inset below shows that in chewing the coxal adduction-abduction cycle is not a simple arc. Three cycles of movement of a gnathobase spine are traced during ingestive chewing (at a larger scale that the main figure), showing dorsal coxal displacement (by muscles 29 and 27) at the end of adduction, pushing food into the mouth. B. Ventral view (see orientation diagram) showing the sequential pattern of adduction of leg coxae in the chewing rhythm of feeding. Both first and both third legs are adducted together, in alternation with the adduction of both second and both fourth legs. The jaw-like fifth legs do not participate in the rhythm, but are recruited to crush hard food. (A after Wyse and Dwyer 1973.)
out-of-phase with each other while opposite legs adduct in-phase, so both 2nd and both 4th legs adduct together but alternate with the 1st and 3rd legs (Fig. 3B). In contrast, during walking and swimming adjacent legs move metachronally, with smaller delays between adjacent legs (Wyse and Dwyer, 1973; Knudsen, 1973). These patterns of muscle activity result from differing patterns of CNS motor output in entocoxal nerves to the coxal muscles.

Because the phase relationships of muscle activity within and between legs in the chewing movements of feeding are quite distinct from those in other patterns of leg movements, the chewing pattern of motor output can be readily identified in the isolated Limulus CNS: bursts of motor neuron activity in the AEN and PEN of a pedal ganglion are in-phase with each other, but are out-of-phase with the bursts of activity recorded in pedal ganglia of adjacent legs (Fig. 4). These phase relationships define the motor pattern of fictive chewing.

Fictive chewing can be recorded from the isolated Limulus prosomal CNS following perfusion of octopamine or octopaminergic agonists (Lee and Wyse, 1986; Lee, 1989). Because this work is central to the topic under review but is not widely available, I summarize it here.

Most isolated preparations of the prosomal CNS (brain and circumesophageal ring of fused pedal ganglia, Fig. 4) exhibit only unpatterned spontaneous activity in the entocoxal nerves when perfused with Limulus saline. Octopamine ($10^{-6}$ to $10^{-5}$ M, perfused into the circumesophageal ring through a cannulated artery dorsal to a first pedal ganglion) elicited rhythmically patterned motor activity in 71 of 87 preparations (82%). The rhythmic activity began within 30-60 sec of perfusion, and was identified as fictive chewing using the phase relationships of bursts within and between leg segments (see Fig. 4). For example, in pairwise comparisons of burst activity in two entocoxal nerves, 48 of 56 cases (86%) that were expected to burst in-phase did so (e.g. 3R AEN and 3R PEN, or 2R AEN and 4R AEN). In pairwise comparisons of entocoxal nerves that were expected to burst out-of-phase (e.g., 3R and 4R), 57 out of 63 (90%) did so. Burst frequencies were typically 18 to 36 bursts/minute and increased with increasing concentrations of octopamine.

The phase relationships and frequencies of octopamine-stimulated rhythmic CNS output in isolation are characteristic of chewing, but differ from patterns producing rhythmic locomotor movements of the legs, as noted above. The CNS output in isolation may be characterized as fictive chewing, but it may or may not correspond to the motor output producing actual chewing in intact animals. For example, chewing movements in Limulus can be either ingestive (taking food in) or egestive (rejecting), a difference produced by the change in phase of contraction of two coxal muscles (#29 and #27 in Fig. 3; Wyse and Dwyer, 1973). Entocoxal nerve recordings from isolated CNS preparations lack single-muscle resolution, and would not show the difference between ingestive and egestive chewing. Therefore, it is important to examine the effects of octopamine injection on intact Limulus and ask: does octopamine evoke bona-fide chewing movements?

**Fig. 4** Demonstration that the Limulus CNS contains central pattern generators for producing rhythmic chewing behavior

The prosomal CNS (brain and ring of fused pedal ganglia to the walking legs, dorsal view) can be removed from a horseshoe crab and its motor activity recorded in isolation. Electrodes record motor neuron activity in nerves to muscles that move the coxal segments of (in this case) the second right (2R) and third right (3R) legs: AEN = anterior entocoxal nerve, PEN = posterior entocoxal nerve. A cannula perfuses octopamine through the preparation via one of the paired descending arteries, evoking bursts of spatiotemporally-patterned action potentials (shown as black rectangles). This pattern of motor activity is characteristic of the motor output producing the chewing pattern of feeding behavior: Simultaneous bursts of motor neuron activity in AEN and PEN of the right second leg would adduct that leg to chew, while the adductor bursts of two adjacent legs are out of phase with each other (2R AEN vs 3R AEN, etc.) (After Lee, 1989).

Moner (1989) demonstrated that octopamine injection into the hemolymph circulation of intact Limulus stimulates chewing behavior in the absence of food, and increases the frequency and duration of chewing behavior in response to food stimulation of gnathobase chemoreceptors. Because her master’s thesis is not widely available, I summarize Moner’s study here. Fifty-eight adult male Limulus were divided into 5 groups, and their chewing behavior was quantified before and after a single bolus injection of octopamine into the cardiac sinus. The injected octopamine mixed...
with hemolymph, and the heart pumped the mixture to
the CNS. The major output of the heart is through paired
descending anterior arteries directly to the prosomal
CNS (Fig. 4), which is enclosed in an arterial sheath
(Patten and Redenbaugh, 1900; Dumont et al., 1965).
Following a feeding test (in air) and a control injection
with Limulus saline, each Limulus received one 0.1 ml
injection of octopamine (10^{-3}, 3.2 \times 10^{-3}, 10^{-2}, 3.2 \times 10^{-2}
M) or Ringer. The number of chewing cycles in a
10-minute test period was assessed by electromyog-
raphic recording from coxal muscles (typically #25; see
Fig. 3), as well as by visual observation.

Injected octopamine significantly increased chewing
frequency in the absence of food. This effect was
dose-dependent, ranging from 0 cycles/10 min with 0 or
10^{-3}M octopamine injection, to a mean of 24.5 cycles/10
min for animals receiving 3.2 \times 10^{-2} M. Octopamine
also significantly increased chewing when food (a small
piece of flounder) was presented 23 min after the injec-
tion. This response was also dose-dependent.

Moner’s finding that octopamine both elicits and poten-
tiates chewing in intact animals strengthens Lee’s
finding that octopamine induces a chewing rhythm in
the isolated prosomal CNS, and vice versa. Taken to-
together, Lee’s results with isolated CNSs (Lee and Wyse,
1986; Lee, 1989) and Moner’s (1989) results with intact
animals provide a strong demonstration that octopamine
stimulates CPGs to produce the chewing motor program
of Limulus feeding behavior.

Evidence suggests that the chewing rhythm depends
on multiple distributed CPGs, rather that a single CPG
(Fig. 5). Weak chemical stimulation of gnathobase
spines of a single leg can evoke chewing movements of
only that leg coxa. Chemical stimulation of gnathobase
spines of more than one leg recruits chewing move-
ments of additional legs, which are appropriately coor-
dinated (see Fig. 3B). Patten (1912) lesioned the circum-
esophageal ring of prosomal ganglia in intact Limulus
and studied effects of the lesions on chewing and other
responses. Patten’s lesion studies show that pedal gan-
glia disconnected from the brain can still produce a
chewing rhythm, but that the cheliceral ganglia help to
coordinate and prolong the chewing rhythm. The cheli-
ceral ganglia are now considered to correspond to the
deuterocerebrum of mandibulate arthropods (Mittmann
and Scholtz, 2003; Harzsch, 2006; Scholtz and Edge-
combe, 2006). They receive large tracts of fibers from
the gnathobase and other chemoreceptors (Patten, 1912;
Wyse, 1971) and appear to relay chemoreceptive infor-
mation to other brain centers (Fahrenbach, 1979). The
results of behavioral studies and Patten’s lesions suggest
that chewing is controlled by segmental CPGs in each
of the pedal ganglia that innervate the first four pairs of
walking legs (Fig. 5). The CPGs on one side are stimu-
lated, and to some extent coordinated, by the cheliceral
ganglion on that side. Coordination between sides is
primarily via post-oral commissures that connect the
paired pedal ganglia.

The dependence of chewing on multiple segmental
CPGs is in keeping with the distributed control of gill
ventilation and gill cleaning, for both of which there is
clear evidence for segmental CPGs that control the
rhythmic output of a single gill plate or pair of gill
plates.

![Fig. 5](image-url) Chewing CPGs are segmental and coordinated
A. In principle, rhythmic chewing in Limulus could be controlled by a
single CPG, shown schematically by an oscillator symbol. B. Alterna-
tively, the two sides could be controlled by two separate CPGs that
coordinate and reinforce each other. C. Each of the pedal ganglia for
the first four pairs of walking legs could contain a CPG. Because
chemical stimulation of gnathobase spines of one leg can elicit cyclic
chewing of only that leg, C is the favored model. Moreover, lesion
experiments (Patten, 1912) have shown that pedal ganglia separated
from the rest of the prosomal CNS can still initiate rhythmic chewing.
Coordinating connections (not shown) are inferred to maintain phase
relationships between the CPGs of contralateral sides and different
segments.
In stimulation of the chewing rhythm of feeding behavior, octopamine could be acting as a neurotransmitter, a neuromodulator, or a hormone. Lee perfused octopamine via a major artery from the heart to flow throughout the circumesophageal ring of prosomal ganglia. Moner injected a bolus of octopamine into the heart, which perfused it through the paired arteries to the ring and presumably throughout the animal. Nevertheless the (short-term) changes recorded were rather specific to chewing behavior. Although this apparent specificity is consistent with a hormonal action, octopamine is known to have many sites of action in *Limulus* (see section 4.2), many of which appear unrelated to feeding. Both this multiplicity of sites of action and other evidence suggest a neurotransmitter-like role for octopamine. First, the concentrations of octopamine required to stimulate feeding-related motor activity were rather high. In Moner’s experiments, the injected bolus would be diluted in the blood by at least 3 orders of magnitude (0.1 ml injection into >100 ml of hemolymph, although initial concentrations before full mixing would be higher). In both Lee’s and Moner’s experiments further dilution would occur along octopamine’s diffusion path within the (capillary-free) ganglia. Nevertheless, the calculated effective concentrations are in the micromolar range expected of a neurotransmitter rather than the nanomolar range characteristic of the action of many hormones.

The anatomical distribution of octopaminergic neurons in *Limulus* also suggests that these neurons act synaptically (or via local neuromodulators) rather than neurohormonally. Lee and Wyse (1991) mapped the distribution of octopamine-immunoreactive (Oct-IR) neurons in the prosomal CNS. Somata of the Oct-IR neurons occur in discrete clusters in the segmental pedal ganglia of the prosomal CNS. Most appear to be interneurons with processes that appear confined to neuropile in the interior of the ganglia. Some Oct-IR neurons have axons in segmental nerves and connectives, but no neurohemal release sites were seen. This distribution in the CNS is comparable to that of serotonin (Chamberlain et al., 1986) and other presumed neurotransmitters, but in contrast to neurons with bombesin-like immunoreactivity (Wyse, 1983) and FMRFamide-like immunoreactivity (Lewandowski et al., 1989), which have rich concentrations of endings at apparent neurohemal release sites at the ganglion surface, adjacent to the hemolymph bathing the surface within the arterial sheath (see Dumont et al., 1965).

The chewing rhythm in *Limulus*, in addition to being programmed by CPGs, is likely to be subject to sensory feedback. Chemical stimulation of gnathobase spines triggers chewing and also reinforces it during a feeding bout. Proprioceptive feedback, in contrast, appears to play a more restricted role. Markey and Wyse (unpublished) altered proprioceptive feedback during chewing in air, by opposing adduction of legs with weights, by altering hardness of food, and by immobilizing some or all legs in dental impression plaster. These attempts to increase the load upon chewing movements produced little evidence for load compensation, but tended to slow the frequency of chewing and tended to recruit the jaw-like fifth legs, which play little role in normal chewing but are used to crush large and hard pieces of food. The interaction of chewing CPGs with other aspects of feeding behavior should be further studied.

### 3.4 Locomotion: walking and swimming

The major patterns of locomotion in adult *Limulus* are walking and swimming. In both walking and swimming, the coxal segments of the legs are rhythmically promoted and remoted, each leg swinging like a door around the main dorsolateral pivot (*P* in Fig. 3A) and around a second indistinct stationary point near the ventral gnathobase (*gn* in Fig. 3A; Manton, 1964; Wyse and Dwyer, 1973). Coordination between legs is variable in walking, but is stereotyped in swimming.

In swimming the legs are promoted and remoted in synchrony, with coordinated rapid beating of the gill plates. Gill plates are promoted synchronously with the legs and remoted in a back-to-front metachronal sequence that slightly precedes the near-simultaneous reulation of the legs (Knudsen, 1973). *Limulus* may also use the swimming pattern of appendage coordination for rapid walking, producing a “bounding” progression along the bottom (Watson, personal communication).

Opisthosomal ganglia must be connected to the prosomal CNS for their gill plates to participate in the swimming rhythm. In swimming the two sides of a hemisected opisthosomal ganglion (that remains connected to the prosomal CNS but severed from posterior abdominal ganglia) remain synchronously rhythmic, unlike the situation in ventilation, in which both sides of a hemisected ganglion may continue to express the ventilatory rhythm but maintain no coordination with each other (Knudsen, 1975). Fictive swimming and walking have not been reported in isolated CNSs of *Limulus*. Taken together, these observations suggest that the pattern generators for swimming lie in the prosomal CNS, with bilateral coordinating neurons that extend posteriorly to opisthosomal ganglia, where they entrain circuits that may correspond to the ventilatory CPGs.
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(Knudsen, 1975). The swimming pattern-generating circuits are presumed to be CPGs, but there is no direct evidence for this.

### 3.5 Burrowing

Eldredge (1970) and Vosotka (1970) have described burrowing behavior to a limited extent, but its neuromuscular basis and neural control have not been investigated. During burrowing and foraging, the hyperventilation pattern of gill plate and opercular movements helps to clear sediment from under the animal (Watson, personal communication). Watson and Chabot (2010) find that burrowing for foraging constitutes much of the tidal locomotor activity of *Limulus* in mud flats, a conclusion that warrants increased study of burrowing activity. It should be straightforward to study burrowing with wire electromyographic electrodes in leg coxal muscles and gill muscles (see Wyse and Dwyer, 1973; Knudsen 1973; Watson, 1980a).

### 3.6 Long-term patterns of rhythmic activity

Rhythmic activities of *Limulus* appendages are typically organized into higher-order patterns that persist for hours, in which bouts of one activity alternate with bouts of another (Watson, 1980b). In aquaria the most common pattern is alternate ventilation and gill cleaning; other patterns are swimming and ventilation, ventilation and apnea (intermittent ventilation), swimming and apnea, and swimming and gill cleaning (Watson, 1980b). Just as the fictive motor programs of gill ventilation and gill cleaning persist in isolated abdominal nerve cords, so do some of the long-term patterns of alternation. Alternate bouts of fictive ventilation and apnea, and alternate bouts of fictive ventilation and gill cleaning are recorded from isolated abdominal nerve cords (Wyse, 1972; Wyse et al., 1980). Figure 6 shows long-term alternation of fictive ventilation and gill cleaning in isolation. Such patterns are among the more elaborate and long-lasting (non-circadian) rhythmic behavioral activities that have been shown to be produced in isolation by central pattern generators.

### 3.7 Circadian and circatidal rhythms

*Limulus* have circadian rhythms of visual sensitivity (Barlow, 1983), mediated primarily by octopaminergic efferent neurons to the eyes (Battelle, 2002). Rhythms of locomotor activity are primarily circatidal (Chabot et al., 2008; Watson et al., 2008). Papers by Dalal and Battelle (2010) and by Chabot and Watson (2010) in this issue discuss these circadian and circatidal rhythms, and Watson and Chabot (2010) discuss evidence for tidal effects on locomotion in natural settings. It will be interesting to explore the relationship of circadian and circatidal rhythms to the long-term activity patterns (intermittent ventilation, ventilation and gill cleaning).

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**Fig. 6** Long-term pattern of sequential alternation between gill ventilation and gill cleaning rhythms in an isolated opisthosomal nerve cord

*Below* is a record of ca. 140 sec of nerve activity in branches of a first left branchial nerve: EBN = external branchial nerve to promotor muscles; MBN = medial branchial nerve to remotor muscles; IBN = internal branchial nerve to intrinsic gill-plate muscles. In the ventilatory rhythm short remotor bursts in MBN alternate with longer promotor bursts in EBN and IBN. During the period shown, three bouts of ventilatory rhythm alternate with two bouts of gill cleaning. *Above*: An 80 min period of recorded activity in the same isolated nerve cord, during which time ventilation bouts (white) and gill cleaning bouts (black) alternated fairly regularly. Such regular alternation of patterns is common in intact animals. (From Wyse, Sanes, and Watson, 1980.)
4 Neuromodulation of CPGs

4.1 Cardiac ganglion/heart rhythm

The neurogenic heartbeat of Limulus is controlled by a cardiac ganglion consisting of 231 pigmented neurons and a larger number of smaller unpigmented neurons (Bursey and Pax, 1970). The neural control of heartbeat, its cardioregulation by the CNS, and its neuromodulation by various amines and peptides have been much studied, but they are largely beyond the scope of this review’s focus on rhythmic behavior. Watson and Groome (1989) provide a good review of much of the literature on heartbeat generation and its modulation by various amines and peptides.

Although heartbeat is not a behavior, its rhythm is coordinated with rhythmic behavioral activities. Heart rate increases during any period of gill ventilatory activity and decreases whenever ventilation is inhibited. In intermittent ventilation the heart rate may decrease to a third during apnea, and with longer suppression of ventilation by hypoxia the heart rate changes may be still greater (Watson and Wyse, 1978). Bradycardia is also associated with gill cleaning (Watson and Wyse, 1978, Watson, 1980b).

In addition to the rate covariation of ventilation and heartbeat, there can also be a variable degree of phase coupling between the two rhythms. Heartbeat is usually slower, but can be entrained to a multiple of the ventilatory rhythm such as alternate ventilations. Although the degree of this phase coupling is variable, the preferred phase of heartbeat within a ventilation cycle is more consistent, characteristically entraining heartbeats that are out of phase with gill remotion (Watson and Wyse, 1978).

The main mechanism of coordination of heart and ventilatory rhythms is via segmental cardioregulatory nerves that extend dorsally from the CNS to the heart (reviewed in Watson and Groome, 1989). If the cardioregulatory nerves are sectioned, the heart does not slow in response to hypoxia, although ventilation is suppressed as usual (Watson and Wyse, 1978). Further studies are needed to clarify the mechanisms of covariation and phase coupling of heartbeat with ventilation, swimming, and other activities.

The likely function of coordination of heart and ventilatory rhythms involves the functional role of gill ven-

4.2 Central and peripheral sites of neuromodulator action

It is clear that the patterns of motor output that constitute the neural control of rhythmic behavior are influenced by neuromodulation, as has been widely shown in other invertebrates (see e.g. Marder et al., 2005, Dickinson, 2006). In the isolated preparations reviewed here, the site of action of the neuromodulator must be the CNS, but modulators in intact animals could act more broadly (hormonal action at peripheral sites as well as the CNS) or more narrowly (synaptic action at discrete postsynaptic neurons rather than general CNS perfusion).

In addition to their actions on the CNS, cardiac ganglia, and eyes, several modulators studied also act on other peripheral sites. These sites include skeletal neuromuscular junctions (Rane et al., 1984), the gut (Groome et al., 1992; Groome and Lent, 1992) and joint proprioceptors of the tibio-tarsal organ of the claw joint. In the latter (unpublished) study, Pasztor and Wyse recorded proprioceptive responses to a standard claw-opening stimulus, and found that octopamine (10^-6 M perfused through isolated claws) increased the num-
ber of spikes per stimulus of tonic proprioceptors by 139%, while serotonin (10^{-6} M) decreased the number of tonic spikes per stimulus by 42%. Thus, as in crustaceans (Pasztor and Bush, 1989; Pasztor and McMillan, 1990) and insects (Farooqui, 2007), primary sensory functions might be as subject to neuromodulation as other parts of neural circuits. Further work is needed to clarify the physiological roles, the sites of action, and the modes of action of neuromodulators that affect behavior.

5 Future Directions

The nervous system of *Limulus* continues to be an excellent model system for developing an understanding of the complex orchestration of neural generation and modulation of behavior. Much behavior of *Limulus* is rhythmic and is organized into temporal sequences and hierarchies. The neural bases of these patterns are experimentally tractable. There are several future directions of work that could profitably build on the studies described here:

1. **Analysis of the neural circuits underlying the behavioral rhythms.** This “circuit busting” may be more difficult in *Limulus* than in some other arthropods, because the *Limulus* nervous system is less tractable to analysis in terms of uniquely identifiable neurons. However, a combination of intracellular recording/labeling and modern imaging methods could greatly clarify these neural circuits.

2. **Activation and interaction of CPGs within a rhythmic activity.** Gill ventilation, gill cleaning, and chewing rhythms all result from the coordinated action of distributed, segmental CPGs. Command neurons are present at least for the ventilatory rhythm. The distributed nature of the CPGs and the ability of the *Limulus* CNS to tolerate lesions and other experimental manipulations make it an excellent preparation for studying the activation and coordination of CPGs. Does swimming behavior, for example, involve orchestrated interaction of CPGs in each pedal ganglion and each of the opisthosomal ganglia that innervates a gill plate, as well as modulation of the heart? We do not know, but we have good ideas about how to find out.

3. **Roles of neuromodulators in activating and orchestrating rhythmic behavior.** Numerous amine and peptide modulators affect the heart and cardiac ganglion, and some of these also affect behavior. The clearest examples so far are octopamine’s activation of chewing (described here) and its circadian modulation of the visual system (Dalal and Battelle, 2010). The sites of action and modes of delivery of octopamine need to be clarified for all roles except visual sensitivity. Moreover, octopamine and other modulators need to be tested for effects on other behavior patterns. Watson (personal communication) reports that octopamine can stimulate the ventilatory rhythm, suggesting that it may also play a general arousal role. Renewed immunohistochemical mapping of modulatory neurons and local application of modulators by pressure injection or iontophoresis will be valuable to assess sites and modes of action.

4. **Behavioral significance and interaction of rhythmic activities.** The behavioral activities of *Limulus* are strongly temporally patterned and arranged in hierarchies. The recent evidence that *Limulus* locomotor activity is under control of a circatidal rhythm (reviewed by Chabot and Watson, 2010) may be a third-level rhythm that organizes the long-term patterns discussed here (intermittent ventilation, ventilation and gill cleaning, swimming and ventilation, etc.), which in turn are composed of shorter-term rhythmic elements. It will be important to characterize the relationship between circatidal rhythms, circadian rhythms, and the bouts of sequential rhythmic activity that often last for hours. What is the adaptive significance of these sequential bouts? Does the tidal organization of overall locomotor activity suggest that the sequential activities, made up of elements of swimming, gill cleaning and ventilation, are also adaptations to a tidal environment? We do not know, but many interesting and tractable questions remain to be answered.

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