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Neuromodulation of rhythmic motor patterns in the blue crab *Callinectes sapidus* by amines and the peptide proctolin

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**Abstract** The blue crab *Callinectes sapidus*, provides an opportunity to study neuromodulation of three variations of rhythmic behavior produced by the same appendages. These behaviors are sideways swimming, backward swimming, and courtship display (CD). Each behavior has a different context, and despite similarities among them, each is quantifiably distinguishable. CD behavior occurs in males, is stimulated naturally by pheromone, and elements of the behavior are evoked by proctolin and dopamine. Sideways and backward swimming do not share these characteristics. Bath-applied proctolin, combined with either electrical or pheromonal stimulation, was used to search for interneurons influencing motor outflows from the fifth legs. Interneurons were found which, when stimulated electrically or pheromonally, initiated rhythmic behavior. At least one of these neurons responded to pheromonal stimulation. Application of proctolin combined with stimulation of descending 'trigger' cells resulted in changes from a backward swimming motor pattern toward a CD pattern. Dopamine applied with proctolin lowered the concentration threshold for proctolin-evoked changes in motor outflows. Octopamine co-applied with proctolin extinguished the proctolin effect unless dopamine was co-applied. Combinations of modulators appear to play critical roles in shaping patterns of rhythmic motor activity of the fifth legs.

**Key words** Neuromodulation · Proctolin · Dopamine · Octopamine · Invertebrate

**Abbreviations:** CD courtship display · DA dopamine · OA octopamine · PROC proctolin · EMG electromyogram · OC oesophageal connective · VNC ventral nerve cord · PIR proctolin-like immunoreactivity · SNK Student-Neuman-Keuls · PO pericardial organ

**Introduction**

Neuromodulation is an important mechanism for providing both neural flexibility and adaptiveness of behavior. Neuromodulators have been shown to function at multiple levels of the nervous system to coordinate behavior. Studies on the action of modulators within the central nervous systems of invertebrates imply that neuromodulators can play a permissive role in resetting the threshold for initiation of stereotyped behaviors (Kravitz 1988). Substances such as biogenic amines and peptides have been shown to mimic the action of interneurons that 'trigger' or 'command' behaviors (Harris-Warrick and Johnson 1989; Kravitz 1988). Neuromodulators also have been shown to play an instructive role in altering oscillating networks of neurons by functionally 'rewiring' the network so that unique patterns of motor outflow are correlated with neuromodulatory influences (Harris-Warrick and Johnson, 1989; Marder and Nusbaum, 1989; Getting 1988). Unfortunately, in some of the model systems where details of neuromodulatory mechanisms are known, the behavioral significance of modulator-induced neural flexibility remains unknown. Further, there are a number of examples of systems where multiple modulators are present, but again, the significance of this for behavior is not well understood. The blue crab *Callinectes sapidus* offers a model system in which the mechanisms of neuromodulation may be studied in the context of known behaviors.
This study concerns the role of neuromodulation in the production of three rhythmic behaviors in the blue crab: courtship display behavior (CD) by the male, sideways swimming, and backward swimming. These three behaviors have both postural and rhythmic components, and are related in that they use the same appendages to perform these distinct but similar patterns of rhythmic motor output. These three stereotyped action patterns are released in different behavioral contexts (Wood and Derby 1995). CD behavior occurs only in the male and is evoked by a pheromone released by female crabs prior to their pubertal molt (Gleeson 1980). CD behavior has been shown to be regulated by an unidentified humoral eyestalk factor and possibly by an androgenic hormone (Gleeson et al., 1987). Aspects of CD behavior are influenced by application of the neuromodulators proctolin (PROC) and dopamine (DA) (Wood et al., submitted). Both types of swimming are seen in escape or other locomotory contexts. Swimming did not appear to be initiated by either DA or PROC, but could be modulated during behavior (Wood et al., 1995).

The CD behavior consists of a postural component-standing high on the walking legs with a wide chelae spread—and a distinctive rhythmic component of the fifth legs in an anterodorsal fashion above the carapace (Wood and Derby 1995; Wood et al. 1995; Teytaud 1971). The posture and rhythm of CD very often are accompanied by the extension of the third maxillipeds and rapid beating of the flagella of the maxillae (and probably the scaphognathites); this results in the production of water currents around the animal (Wood unpublished data; Wood et al. 1995). Behavioral assays have shown that PROC injected into the animal can initiate both the rhythmic component and occasionally the postural component of CD (Wood et al. 1995). Injection of DA results in posture closely resembling that seen during CD as well as several additional behaviors such as grooming, tonic lifting or rhythmic lifting of the third walking leg (Wood 1993; Wood et al. 1995). In addition, octopamine (OA) initiates a submissive posture that is antagonistic to the posture evoked by DA and is seen in females during courtship (Wood et al. 1995).

Previous results using video analysis and electromyography have revealed that the rhythm of the fifth leg basal muscles during each of the three behaviors shows that the rhythmic portions of both CD and backward swimming have an important distinction from sideways swimming in that the sequence of muscular activity is asymmetrical between the two legs during sideways swimming (Wood and Derby 1995).

Given the similarity of the three rhythmic behaviors under examination, it is likely that neural pathways producing each behavior share common neural elements at several levels of the nervous system. In this study, I examined mechanisms of control of these three rhythmic behaviors and the interaction of neuromodulators to produce variations in rhythmic motor outputs. Interneurons in crustaceans that serve functions related to initiation of behavior have been identified (Harris-Warrick 1985; Ma et al. 1991), and many of these neurons have been shown to have axonal projections in the oesophageal connectives (OCs) that descend into the thoracic and abdominal ganglia (Wiersma and Ikeda, 1964; Bowerman and Larimer, 1976). The OCs are a logical starting point to search for interneurons that initiate rhythmic behaviors using the fifth legs. Since injection of either PROC or DA into a freely-moving animal results in the production of components of CD behavior and because injection of OA results in an antagonistic posture (Wood et al. 1995), this system also offers an opportunity to examine the effect of individual modulators and combinations of modulators on neurons that participate in the initiation of the behaviors of interest.

Materials and methods

Animals

Adult male crabs were obtained from commercial sources or collected at the Whitney Laboratory, St. Augustine, Florida. The animals were maintained in artificial sea water at 19 to 21°C. The reproductive maturity of males was judged by whether the abdomen is 'sealed' (i.e., adults but not juveniles have the ability to extend their abdomen due to a loss of fusion of the abdomen with the ventral thorax) (Pyle and Cronin 1950; Haefner 1988). After chilling the animal on ice for anesthesia, the ventral surface of the animal was thoroughly dried using 95% ethanol and glued dorsal side up to a rubber platform. The dish was filled to a level that submerged the animal with decapod crustacean saline at 4°C (Mulloney and Selverston, 1974, modified as in Hamilton and Ache, 1983). The claws were autotomized under cold saline at the thoraco-coxopodite joint. This saline was gradually exchanged for saline of a temperature that was 19°C to 21°C.

Dissection

A section of the carapace was removed using a dental drill and scissors. The heart of the animal was immediately cannulated and perfused with saline in a pressurized, oxygenated circulation system.