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REVIEW

Crustacean neuroendocrine systems and their signaling agents

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Abstract Decapod crustaceans have long served as important models for the study of neuroendocrine signaling. For example, the process of neurosecretion was first formally demonstrated by using a member of this order. In this review, the major decapod neuroendocrine organs are described, as are their phylogenetic conservation and neurochemistry. In addition, recent advances in crustacean neurohormone discovery and tissue mapping are discussed, as are several recent advances in our understanding of hormonal control in this group of animals.

Keywords Neuroendocrine organs · Neurotransmitters · Neurochemistry · Gut · Neurohormones · Genome mining · Mass spectral imaging · Decapod crustaceans

Introduction

For over half a century, crustaceans have provided important insights into our understanding of neuroendocrine control. For example, the process of neurosecretion was first formally demonstrated by using the brachyuran crab X-organ-sinus gland (XO-SG) system (Bliss 1951; Passano 1951) and the first invertebrate neuropeptide to be fully characterized, the red pigment concentrating hormone (RPCH), was from a caridean shrimp (Fernlund and Josefsson 1972). Moreover, many of the basic principles underlying the generation, maintenance and modulation of rhythmically active behaviors have been derived from studies of the crustacean cardiac and stomatogastric neuromuscular systems (Cook 2002; Hooper and DiCaprio 2004; Marder and Bucher 2007). Since the last comprehensive reviews of crustacean neuroendocrine signaling (e.g., Cooke and Sullivan 1982; Fingerman 1992), much in this field has changed. In the sections that follow, recent advances in our understanding of crustacean neuroendocrine control are summarized. Descriptions of several newly identified neuroendocrine organs, new groups of signaling molecules present in these structures, new methodologies for identifying and mapping neurohormones and new functional roles attributed to circulating hormones in these animals are discussed.

Crustacean neuroendocrine organs

Like all arthropods, crustaceans possess open circulatory systems. In these animals, the heart, which is typically neurogenic (Cook 2002), pumps hemolymph through a series of arteries into the hemocoel, where it bathes all internal organ systems (Brusca and Brusca 2003). The return of the hemolymph to the heart is accomplished via a noncoelomic pericardial sinus and ostia in the heart wall; contraction of the heart generates a pressure gradient that draws the hemolymph into the pericardial sinus and ultimately into the heart itself (Brusca and Brusca 2003). Because of the organization of their circulatory systems, crustacean neuroendocrine structures are defined as any

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region of the nervous system in which secretory nerve terminals are in direct contact with the hemolymph.

Although all crustaceans are assumed to possess neuroendocrine release sites, the vast majority of work directed at their identification and characterization has been performed on members of the Decapoda. In decapods, neuroendocrine sites vary from loosely associated clusters of release terminals located along the ventral nerve cord or in peripheral nerves, to well-organized neuroendocrine organs. Two neuroendocrine organs, the XO-SG complex and the pericardial organ (PO), appear to be ubiquitously conserved in members of this order, whereas others, including the post-commissural organ (PCO) and the anterior ramification (AR), as well as the newly identified anterior cardiac plexus (ACP) and anterior commissural organ (ACO), might be more limited in their phylogenetic conservation. A representation of the nervous system of the brachyuran crab *Cancer productus*, including its known neuroendocrine organs, is shown in Fig. 1. The general organizations, conservations and functional roles of the major decapod neuroendocrine organs are briefly summarized in the sections that follow; the chemical signaling agents present in these structures are discussed later in this review.

The XO-SG complex

The XO-SG complex is arguably the major neuroendocrine organ in decapod crustaceans. As stated earlier, this system appears ubiquitously conserved in members of this taxon. The XO-SG has a long history in the field of neuroendocrinology. In fact, this system was the subject of the first formal demonstration of neurosecretion in any animal (Bliss 1951; Passano 1951). In most decapod species, the XO-SG is located within the eyestalk (Fig. 1), with the collection of release terminals that form the SG being superficially located on the dorsal or dorso-lateral side of the optic ganglia, generally at the level of the medulla interna and/or medulla externa. The majority of somata innervating the SG are located in the XO, a tightly associated soma cluster situated at the ventral proximal margin of the medulla terminalis (e.g., Cooke and Sullivan 1982; Fingerman 1992). The XO is connected to the SG via the sinus gland nerve, alternatively termed the sinus gland tract, which typically runs deep within the neuropil of the medulla terminalis, emerging from this neuropil on the dorsal or dorso-lateral side of the ganglion (e.g., Cooke and Sullivan 1982; Fingerman 1992). In addition to the XO somata, a few cell bodies located in other regions of the eyestalk, in the supraesophageal ganglion (commonly referred to as the brain) and in the thoracic ganglia, are thought to project to and innervate the SG (e.g., Cooke and Sullivan 1982; Fingerman 1992).

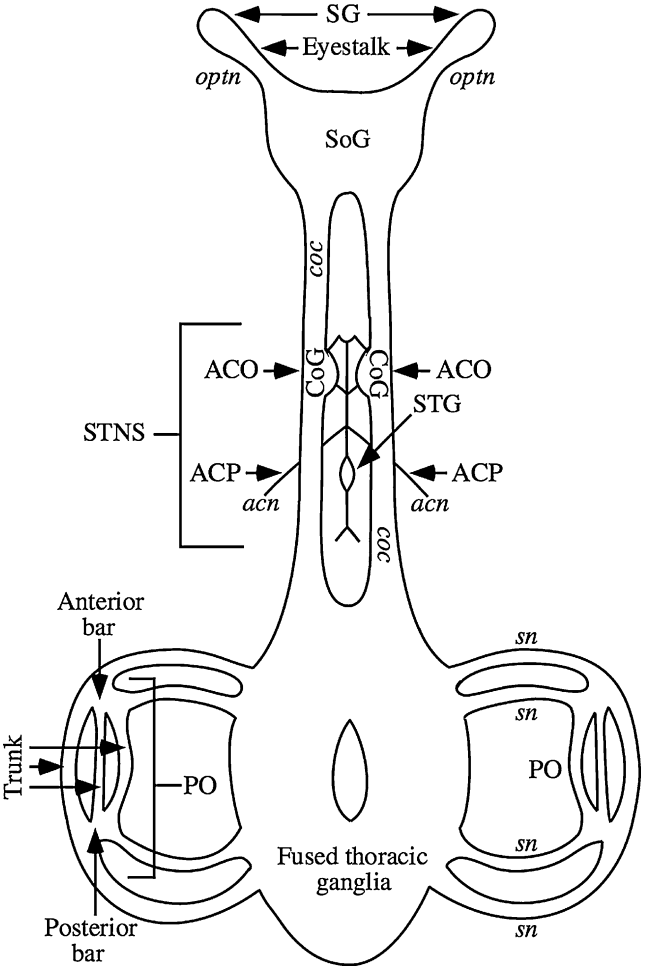

Fig. 1 Representation of the nervous system of the crab *Cancer productus* illustrating the relative locations of the known neuroendocrine organs of this species. The central nervous system (CNS) consists in the supraesophageal (*SoG*) and fused thoracic ganglia, which are connected via the circumesophageal connectives (*coc*). The optic nerves (*optn*) link the SoG with the ganglia of the eyestalks, the location of the neuroendocrine sinus gland (*SG*). Another well-known neuroendocrine site is the pericardial organ (*PO*), which is located in the pericardial chamber surrounding the heart. The PO consists in elaborations of the segmental nerves (*sn*), which project from the fused thoracic ganglia. Two additional neuroendocrine sites, the anterior cardiac plexus (*ACP*) and the anterior commissural organ (*ACO*), are contained within the stomatogastric nervous system (*STNS*), an offshoot from the CNS that overlies the foregut. The ACPs are located on the anterior cardiac nerves (*acn*) and the ACOs are located within the commissural ganglia (*CoG*). The location of the stomatogastric ganglion (*STG*) is also shown. Figure and legend used with permission from Hsu et al. (2006)

Ultrastructurally, the decapod SG is composed of a dense collection of neurosecretory terminals that are interspersed with, and in many regions enwrapped by, glial processes (e.g., Cooke and Sullivan 1982; Fingerman 1992). Several classes of neurosecretory terminals have been reported in decapod SGs, each distinguished by its vesicle complement (which can include clear synaptic-type and/or dense-core vesicles);

depending on the species, the mode of fixation and other factors, the number of classes of secretory terminals described in decapod SGs is variable (e.g., Cooke and Sullivan 1982; Fingerman 1992).

Being superficially located on the eyestalk, the outer surface of the SG is accessible to the circulatory system via a hemolymph space present between it and its overlying connective tissue. Likewise, its surface facing the optic ganglia is accessible to the circulatory system via a hemolymph space located between the gland and the outer surface(s) of the medulla interna and/or medulla externa. Invaginating hemolymph lacunae provide circulatory access to the interior of the SG (e.g., Cooke and Sullivan 1982; Fingerman 1992). Omega-figures, i.e., morphological correlates of hormone release (Fig. 2), have typically been reported only in SG terminals that appear to be in direct contact with the hemolymph, e.g., ones located directly under its neurolemma (e.g., Cooke and Sullivan 1982; Fingerman 1992).

Hormones, principally peptides, released from the XO-SG system (Table 1) are known to control many physiological processes, including molting, somatic growth and sexual maturation, as well as metabolic adaptation to changing environmental conditions (e.g., Chung et al. 2010). The XO-SG also plays prominent roles in the control of pigment migration, both in the retina and in chromatophores (e.g., Rao and Riehm 1993; De Kleijn and Van Herp 1995). SG-derived hormones also function as modulators of the motor programs that drive a variety of rhythmic behaviors, including those responsible for the chewing and filtering of food within the stomatogastric neuromuscular system (e.g., Christie et al. 1995a; Skiebe 2001, 2003).

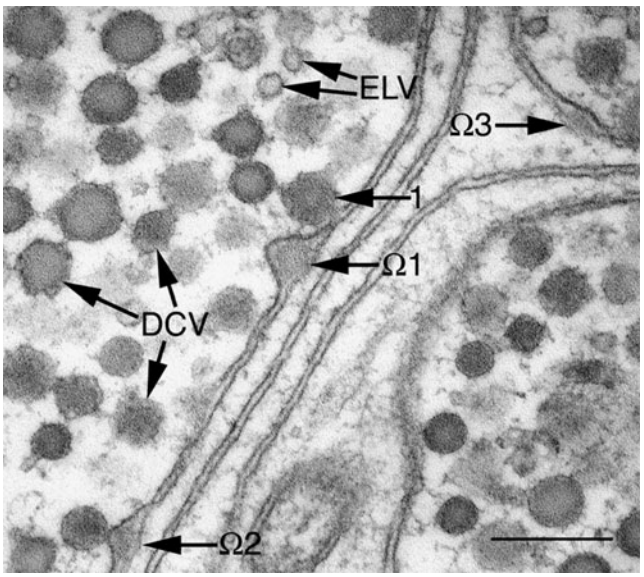

Fig. 2 Transmission electron micrograph illustrating morphological correlates of hormone secretion in the anterior cardiac plexus of *Cancer productus*. Both dense-core vesicles (*DCV*), which are probably peptidergic and electron-lucent vesicles (*ELV*) are present in these terminals. In this image, one DCV (*1*) has docked at the plasma membrane, whereas several others have fused with the membrane, exocytosing their contents and creating characteristic ultrastuctural features, i.e., omega (Ω) figures, on the plasma membrane (Ω*2*, Ω*3*). The docked DCV and the three Ω-figures visible in this micrograph create a pseudo-time course of peptide hormone secretion. Specifically, a DCV docks at the plasma membrane (*1*), then fuses with it releasing its dense-core and forming an Ω-figure (Ω*1*). The membrane of the DCV is rapidly incorporated into the plasma membrane of the terminal and the Ω-figure subsides (Ω*2*, Ω*3*). *Bar* 200 nm. Figure and legend used with permission from Christie et al. (2004a)

The pericardial organ

A second major neuroendocine organ present in decapod crustaceans is the PO (Alexandrowicz and Carlisle 1953), a structure typically located along the lateral wall of the pericardial chamber that surrounds the heart (e.g., Cooke and Sullivan 1982; Fingerman 1992). Ramifications of the segmental nerves that exit the thoracic nervous system are the primary contributors to the decapod PO; the organization of these ramifications varies considerably between members of the various infra-orders of the Decapoda (e.g., Cooke and Sullivan 1982; Fingerman 1992). For example, in brachyuran crabs, the segmental nerves give rise to a well-defined organ consisting in two or more longitudinal nerve trunks interconnected by two vertical nerve bars (Fig. 1). In contrast, the POs in members of the Astacidea (clawed lobster and freshwater crayfish) typically appear as a system of nerve trunks that ramify as diffuse plexuses along the lateral walls of the pericardial chamber and on the ligaments of the heart; the surfaces of the nerve trunks themselves are reported to contain dense collections of release terminals. Regardless of organization, the somata that innervate decapod POs include both intrinsic and extrinsic somata, the latter distributed throughout the central nervous system (CNS), although most are thought to reside within the thoracic nervous system (e.g., Cooke and Sullivan 1982; Fingerman 1992).

Ultrastructural analyses of decapod POs show that this neuroendocrine organ consists in a dense collection of neurosecretory terminals located just under its ensheathing epineurium; the axons giving rise to the release terminals project within the core of the nerves that form the PO, where they are enwrapped by glial processes (e.g., Cooke and Sullivan 1982; Fingerman 1992). As in the SG, a number of classes of release terminals are present in the PO, each defined by its vesicle complement, which, like those of the SG, can include clear synaptic-type and/or dense-core vesicles (e.g., Cooke and Sullivan 1982; Fingerman 1992).

Similar to the hormones secreted from the SG, the amines and peptides released from the PO (Table 1) have been implicated in the control of a broad array of

Table 1 Chemical complement of crustacean neuroendocrine organs. The data presented in this table are not species-specific but, rather, represent data pooled from a number of species. The presence/absence of a substance at a site can vary between species, including closely related species. Thus, care should be taken before assuming that the detection of any substance in the neuroendocrine organ of one species implies extensive conservation across species. The majority of data on peptides presented in this table are summarized from a recent review by Christie et al. (2010b). Data on amines are summarized primarily from Beltz 1999, whereas data on small molecule transmitters and gas transmitters are summarized primarily from Christie et al. (1995a), (2003) and Lee et al. (2000). Some data are unpublished (A.E. Christie)

Signaling agent		Neuroendocrine organ					
		Sinus gland	Pericardial organ	Post-commissural organ	Anterior cardiac plexus	Anterior commissural organ	Other
Peptides							
A-type allatostatin			+	+			
B-type allatostatin			+				
C-type allatostatin			+				
Allatotropin[a]		+					
Bursicon			+				
Corazonin			+	+			
Crustacean cardioactive peptide			+				
Crustacean hyperglycemic hormone[b]	CHH	+	+				
	MIH	+					
	MOIH	+	+		+		
Crustacean hyperglycemic hormone precursor-related peptide		+					
Calcitonin-like diuretic hormone		+	+				
FMRFamide-like peptide	Myosuppressin		+				
	Neuropeptide F[c]		+		+		
	Short neuropeptide F	+	+				
	Sulfakinin[d]		+				
	Extended -FLRFamide	+	+		+		
	Extended –YLRFamide		+				
Insect kinin			+				
Orcokinin		+	+				
Orcomyotropin		+	+				
Pigment dispersing hormone		+			+		
Proctolin		+	+				
Pyrokinin							
Red pigment concentrating hormone		+	+	+			
RYamide			+				
SIFamide		+					
Tachykinin-related peptide		+				+	
Amines							
Dopamine			+				
Octopamine			+				
Serotonin			+				
Small molecule transmitters							
Gamma-aminobutyric acid			+				
Gases							
Carbon monoxide							+
Nitric oxide		+					

[a] Based on the detection of a mass corresponding to that of *Manduca sexta* allatotropin (Fu et al. 2005b)

[b] Subfamilies: *CHH* crustacean hyperglycemic hormone, *MIH* molt-inhibiting hormone, *MOIH* mandibular organ-inhibiting hormone

[c] Based on neuropeptide Y-like immunolabeling (A.E. Christie, unpublished)

[d] Based on cholecystokinin-like immunolabeling (Turrigiano and Selverston 1991; Christie et al. 1995b)

physiological processes in decapods. The proximity of the PO to the heart makes the cardiac neuromuscular system a major target for its hemolymph-borne modulators (Cook 2002). Likewise, hormones derived from the PO have been postulated to modulate the output of the stomatogastric neuromuscular system (e.g., Christie et al. 1995a; Skiebe 2001, 2003) and to play roles in osmoregulatory control (e.g., Morris 2001).

The post-commissural organ

PCOs were first described by Knowles (1953) in several shrimp species, specifically, the penaeid *Farfantepenaeus brasiliensis* and carideans *Palaemon serratus* and *Palaemon affinis*. In *Farfantepenaeus brasiliensis*, the PCO is derived from the post-commissural nerves, which are offshoots of the tritocerebral commissure. Each post-commissural nerve projects from the triocerbral commissure dorso-posteriorly, terminating on and innervating an endophragmal muscle. At this muscular termination, the two post-commissural nerves flatten, producing a plate-like structure, which has been named the PCO. In terms of its organization, the PCO is similar to the PO in that it consists in neurosecretory terminals located directly under an epineurium, with a central core consisting in the axons that produce the terminals and motor neuron fibers-of-passage. The organization of the PCO in caridean shrimp is similar to that of *Farfantepenaeus brasiliensis*, except that the post-commissural nerves form no plate; instead, the nerves flatten along their proximal half to produce the organ. A structure apparently homologous to the PCO has also been identified in the brachyuran crabs *Pachygrapsus crassipes*, *Uca pugilator* and *Uca pugnax* (Maynard 1961a, b; Fingerman 1966). Here, the post-commissural nerves ramify over the *ligamentum ventrale capitis* to produce the PCO. Unlike the PCOs of shrimp, the brachyuran organ does not appear to be ensheathed by an epineurium but rather, its secretory terminals are freely exposed to the circulatory system (Maynard 1961a, b; Fingerman 1966). In at least the carideans, the somata that give rise to the PCO include somata located in the tritocerebral commissure and brain (Knowles 1953; Carlisle and Knowles 1959), and at least two cell bodies in the eyestalk ganglia (Dircksen et al. 2005). The functional roles played by the PCO remain unknown; however, peptides present in it (Table 1) suggest an involvement in the control of chromatophore pigment migration (e.g., Knowles 1953; Fingerman 1966; Dircksen et al. 2005).

The anterior ramification

The AR appears to be a brachyuran-specific neuroendocrine organ (for a review, see Fingerman 1992). In members of this infra-order, the AR is located below a sinus membrane separating the ventral respiratory muscles from the dorsal thoracic cavity. In terms of its structural organization, considerable species-specific variation has been noted. For example, in *Callinectes ornatus*, the AR consists in several well-defined stereotyped branches, whereas in *Plagusia depressa*, it is organized as a diffuse and irregular net-like structure (Maynard 1961a). The first segmental nerve projecting from the fused thoracic ring appears to be the primary contributor to the AR; this nerve also contributes to the brachyuran PO (e.g., Cooke and Sullivan 1982; Fingerman 1992). Little is known about the location of the somata that innervate the AR, although innervation by intrinsic and extrinsic cell bodies has been reported (e.g., Maynard 1961b). The ultrastructure of the AR is essentially identical to that of the PO (reviewed above), at least in *Callinectes sapidus* and *Uca pugilator* (Andrews 1973). Essentially, nothing is known about the neurochemistry of the AR, although, based on its secretory vesicle complement (Andrews 1973), it probably contains a variety of hormones, including both peptides and amines (e.g., Fingerman 1992). The functional roles served by the AR remain unknown.

The anterior cardiac plexus

The ACP is a newly described neuroendocrine organ from the brachyuran crab *Cancer productus* (Christie et al. 2004a). In this species, the ACP is located within the stomatogastric nervous system (STNS), which is an offshoot of the central nervous system and which controls the rhythmic movements of the foregut. In *Cancer productus*, the ACP consists in a tightly associated cluster of secretory nerve terminals located just beneath the sheath of the anterior cardiac nerve (*acn*; Christie et al. 2004a; Figs. 1, 3); the location of the ACP on the *acn* places it near the musculature of the foregut's cardiac sac and gastric mill regions. Four neurons, the anterior cardiac neurons or ACNs, one pair in each commissural ganglion (CoG), are the sole source of innervation to the *Cancer productus* ACP (Christie and Messinger 2005; Savage et al. 2006). Each ACN soma projects an axon via the superior esophageal (*son*) and stomatogastric (*stn*) nerves to the junction of the *acn*s, where the axon bifurcates, sending one process into the left *acn* and one into the right *acn* (Christie et al. 2004a; Christie and Messinger 2005; Savage et al. 2006). These left and right projecting branches terminate in the left and right ACP, respectively (Christie et al. 2004a; Christie and Messinger 2005; Savage et al. 2006). In addition to producing the ACPs, each ACN also arborizes within its CoG of origin (Christie and Messinger 2005; Savage et al. 2006).

At present, the extent of the conservation of the ACP system in decapods is unclear. Work carried out on other

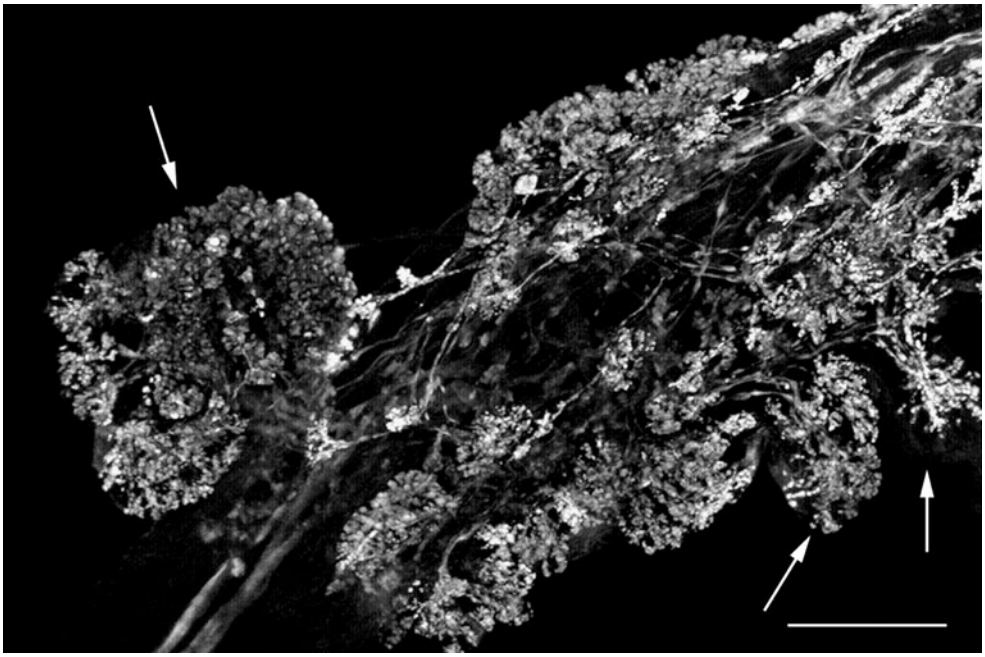

Fig. 3 General organization of the anterior cardiac plexus (ACP) of *Cancer productus*. In this and other *Cancer* species, the ACP is composed of nerve terminals contained within blister-like protuberances of the anterior cardiac nerve (*acn*) sheath (*arrows* several prominent protuberances). The location of the ACP on the *acn* places it in direct contact with the circulatory system. In the confocal micrograph shown, the ACP is visualized via FMRFamide-like immunoreactivity, one of three peptide immunoreactivities thus far identified in the ACP of this species. *Bar* 100 μm. Figure and legend used with permission from Christie et al. (2004a)

Cancer species shows that this system is structurally conserved in at least members of this genus (Savage et al. 2006). Interestingly, the neurochemistry of the ACP differs between *Cancer* species, e.g., the structure exhibits FMRFamide-like peptide (FLP) labeling in *Cancer productus* (Christie et al. 2004a) but not in either *Cancer borealis* or *Cancer magister* (Savage et al. 2006). A structure similar in organization to the *Cancer* ACP is present in *Callinectes sapidus*, another brachyuran, although in this species it is located at the junction of the dorsal posterior esophageal nerve (*dpon*) and the *acn*, projecting to this location via the *dpon* rather than the *acn* (A.E. Christie, unpublished). The presence of a putative ACP in *Callinectes sapidus* suggests this neuroendocrine organ may be conserved more broadly, at least within the Brachyura. No information is currently available regarding the presence/absence of the ACP system in non-brachyuran decapods.

The functional role(s) served by the hormones released from the ACP system (Table 1) remain(s) largely unknown. Its location within the STNS suggests that it might participate in local neuroendocrine control of the stomatogastric neuromuscular system and/or in the signaling of the feeding status of the animal to other tissues/organ systems. In *Cancer productus*, the former hypothesis is strengthened by the finding of a spatial pattern of FLP sensitivity in the foregut muscles, correlating with their proximity to the ACP (Verley et al. 2008). Specifically, the contractile forces produced by muscles located in close proximity to the ACP increase notably in the presence of 10^{-7} M TNRNFLRFamide (a relatively high but still hormonally relevant, concentration of peptide), whereas muscles more distal to this neuroendocrine organ show little or no response to the same concentration of TNRNFLRFamide (Verley et al. 2008). None of the muscles examined have any direct innervation by FLP-producing neurons in *Cancer productus* (Cruz-Bermúdez et al. 2006). In a different *Cancer* species, *Cancer borealis*, hormonally delivered FLPs are postulated to be crucial for maintaining appreciable muscle contractions in response to the low-frequency, low-intensity motor discharges that drive many foregut muscles (Jorge-Rivera and Marder 1996). It seems logical that this situation also holds true for *Cancer productus* and, at least in this species, one local source of hormonal FLP is the ACP.

The anterior commissural organ

Like the ACP, the recently identified ACO was first formally characterized in the crab *Cancer productus* (Messinger et al. 2005). This neuroendocrine organ is also contained within the STNS, where it is located within the paired CoGs (Fig. 1). Unlike the neuroendocrine organs described earlier, the ACO is not superficially located but is, instead, located deep within the synaptic neuropil of the CoG (Fig. 4), with access to the circulatory system provided by hemolymph channels fenestrating the ganglion (Messinger et al. 2005). Each ACO consists in a dense collection of release terminals surrounding these hemolymph lacunae (Messinger et al. 2005; Fig. 5). In addition to being in direct contact with the circulatory system, the ACO is directly apposed to regions of synaptic neuropil, suggesting that it functions as both an endocrine and paracrine organ (Messinger et al. 2005). Approximately 50–100 small-diameter (<1 μm) axons

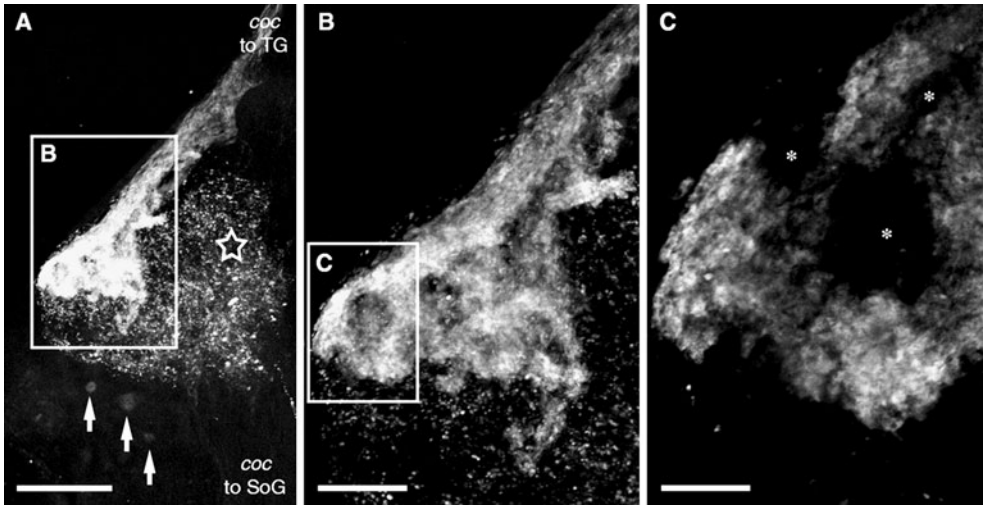

Fig. 4 Distribution of substance P-like labeling in the commissural ganglion (CoG) of the crab *Cancer productus*, including in the anterior commissural organ (ACO). **a** Confocal micrograph showing substance P-like labeling in the CoG. Within each ganglion, substance P-like immunoreactivity is present in approximately seven neuronal somata (three denoted by *arrows*) and in neuropilar processes (*star*) and a large club-shaped plexus, the ACO (*boxed*). The ACO is located in the anterior medial quadrant of the CoG and originates from a fascicle of small-diameter axons that project from the circumesophageal connective (*coc*), which connects the supraesophageal (*SoG*) and thoracic ganglia (*TG*) to the CoG. **b** Higher magnification view of the ACO *boxed* in **a**. As can be seen from this micrograph, the ACO is composed of tightly aggregated, flocculent varicosities. The aggregated varicosities cluster around unlabeled tubular structures (revealed in Fig. 4 to be hemolymph lacunae), particularly in the posterior portion of the ACO. **c** Projection of three optical sections taken at 1.0-mm intervals from the *boxed* region in **b**, showing aggregated substance-P-immunopositive terminals enveloping several unlabeled tubular structures (*asterisks*). *Bars* 200 μm (**a**), 75 μm (**b**), 25 μm (**c**). Figure and legend used with permission from Messinger et al. (2005)

projecting to the CoG via the circumesophageal connective arborize to produce each ACO (Blitz et al. 2008). The location of the somata that give rise to these fibers remains unknown, although they are thought to reside somewhere within the thoracic nervous system (Blitz et al. 2008).

In terms of its phylogenetic conservation, the ACO appears broadly, although perhaps not ubiquitously, conserved within the Decapoda. By using an antibody directed against substance P (a member of the tachykinin superfamily and the original marker used to visualize the ACO; Goldberg et al. 1988; Messinger et al. 2005), structures similar in organization to the *Cancer productus* ACO have been revealed in the CoGs of numerous other decapods, including members of the infra-orders Achelata, Anomura, Astacidea, Brachyura, Caridea and Thalassinidea (Goldberg et al. 1988; Messinger et al. 2004; A.E. Christie, unpublished). In the penaid shrimp, no ACO-like structure has been identified, although this finding is based only on tachykinin immunolabeling in *Litopenaeus vannamei* (A.E. Christie, unpublished). Whether this lack of immunoreactivity represents a true lack of the ACO in penaeids, or whether the structure is present but does not contain tachykinin remains unclear.

With respect to function, studies conducted on *Cancer* crabs suggest that the ACO plays both endocrine and paracrine roles, at least in the modulatory control of the stomatogastric neuromuscular system. In *Cancer productus*, the sole neuronal source of circulating tachykinin-related peptide (TRP) appears to be the ACO (Messinger et al. 2005); TRP is the only hormone thus far identified in the ACO of any species (Goldberg et al. 1988; Messinger et al. 2004, 2005; A.E. Christie, unpublished; Table 1). In this species, a 10^{-7} M concentration of APSGFLGMRamide (a native TRP) increases excitatory junctional potentials in several gastric mill and pyloric muscles, with concentrations as low as 10^{-8} M consistently increasing contraction amplitude in one of the gastric mill group. Given the lack of direct TRP innervation of these muscles and the finding that the ACO is the sole neurohormonal source of APSGFLGMRamide in the animal, this enhancement is attributed to circulating peptide released from the ACO (Messinger et al. 2005). If this hypothesis is true, then ACO-derived TRP, like the FLP derived from the ACP, might play a critical role in maintaining appreciable contractions in the musculature of the stomatogastric system.

Paracrine actions of the ACO have thus far been assessed only in the crab *Cancer borealis* (Blitz et al. 2008). In this species, the ACO appears to be responsible for the generation of a novel long-lasting gastric mill (chewing) motor pattern. This gastric mill rhythm includes a unique protractor phase activity pattern, in which the lateral gastric (LG) neuron (which innervates the muscles mediating protraction of the lateral teeth within the gastric mill) and modulatory commissural neuron 1 (MCN1) and commissural projection neuron 2 (CPN2), two projection

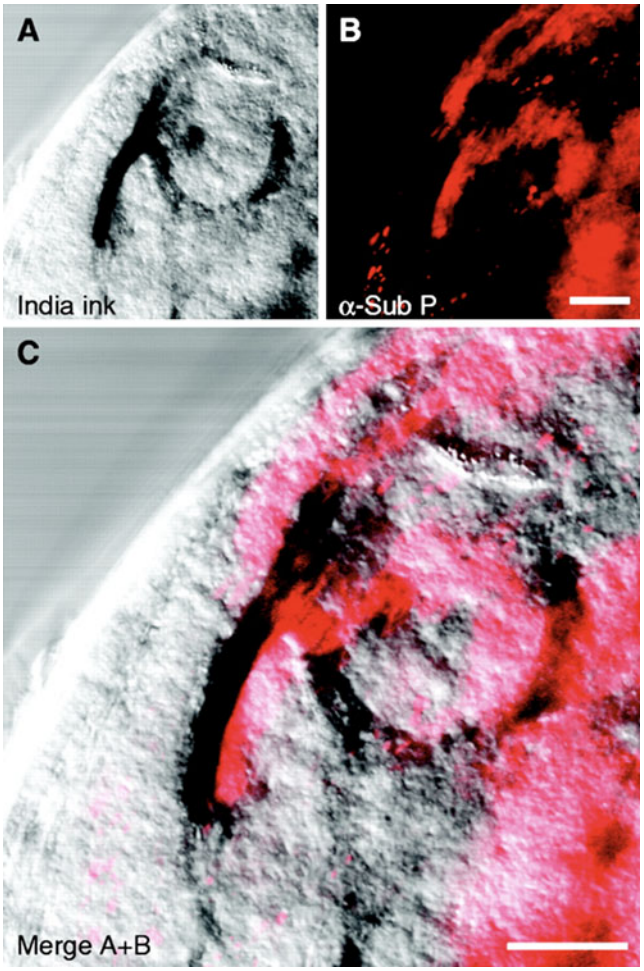

Fig. 5 Hemolymph lacunae are coincident with areas of immunolabel avoidance in the anterior commissural organ (ACO) of *Cancer productus*. In the commissural ganglion (CoG), the substance-P-immunopositive plexus is fenestrated by what appears to be a network of branched tubes. To determine whether these tubular structures were hemolymph lacunae, several India-ink-filled ganglia were immunoprocessed with the substance P antibody (*α-Sub P*) and the ink and immunolabel were simultaneously imaged via confocal microscopy; the filling of CoGs was accomplished by injection of ink into the circulatory system. As can be seen in this set of micrographs, ink-filling (**a**; same scale as **b**) was evident in numerous lacunae in the portion of the ganglion containing the substance-P-immunopositive ACO (**b**). As a merging of the micrographs shows (**c**), the substance-P-immunopositive nerve terminals that form the ACO envelop the hemolymph lacunae. This organization, with nerve terminals in direct apposition to the hemolymph space, is considered the defining characteristic of a crustacean neuroendocrine site. *Bars* 20 μm (**b**, **c**). Figure and legend used with permission from Messinger et al. (2005)

neurons whose somata are located in the CoG, fire in a pyloric rhythm-timed activity pattern, instead of the tonic firing pattern exhibited by these neurons during other gastric mill rhythms (Blitz et al. 2008). Activation of this motor program is hypothesized to result, at least in part, from the paracrine actions of TRP released from the ACO, targeting and generating a long-lasting activation of MCN1 and CPN2 within the CoG (Blitz et al. 2008). Functionally, the distinct pattern of LG neuron activity triggered by TRP release from the ACO is predicted to result in a unique mode of chewing, which includes pyloric-timed movements of the lateral teeth (Blitz et al. 2008), a motor pattern that has been documented in another *Cancer* species, *Cancer pagurus*, via in vivo endoscopic recordings of the gastric mill (Heinzel et al. 1993).

Chemistry of crustacean neuroendocrine sites

Crustaceans, like all multicellular animals, employ a variety of molecules as neurohormonal signaling agents. Peptides are by far the largest and most diverse class of crustacean neurohormones; biogenic amines, small molecule transmitters and recently, diffusible gases have also been identified in the neuroendocrine organs of these animals. In the sections that follow, the members of each neurochemical class that have been identified as contributing to crustacean neurohormonal signaling are described. Table 1 provides a summary of these data.

Peptides

Over the past decade, mass spectrometry (e.g., Huybrechts et al. 2003; Li et al. 2003; Fu et al. 2005a, b; Stemmler et al. 2007a; Ma et al. 2008, 2009a, b, c, 2010), in combination with transcriptomics (e.g., Christie et al. 2008, 2010a; Gard et al. 2009; Ma et al. 2009a, 2010) and, recently, genome mining (Christie et al. 2011a; see below), has lead to an explosion in crustacean peptide discovery (for a review, see Christie et al. 2010b). Via these and other methods, numerous peptide families have been identified in crustaceans; these include but are not limited to, the A-type allatostatin (AST-A), B-type allatostatin (AST-B), C-type allatostatin (AST-C), allatotropin (ATR), bursicon (both α and β subunit peptides), corazonin, crustacean cardioactive peptide (CCAP), crustacean hyperglycemic hormone (CHH; both CHH and molt-inhibiting hormone [MIH] subfamilies), crustacean hyperglycemic hormone precursor-related peptide (CPRP), calcitonin-like diuretic hormone (CLDH), ecdysis-triggering hormone (ETH), eclosion hormone (EH), enkephalin, FLP (myosuppressin, neuropeptide F [NPF], short neuropeptide F [sNPF], sulfakinin, extended –FLRFamide, extended –YLRFamide and extended –FVRFamide subfamilies), insect kinin, neuroparsin, orcokinin, orcomyotropin, pigment-dispersing hormone (PDH), proctolin, pyrokinin, RPCH, RYamide, SIFamide and TRP families (e.g., Christie et al. 2010b). For many of these peptide groups, multiple isoforms are known. These range from peptides

that are essentially ubiquitously conserved (e.g., SYWKQCAFNAVSCFamide, an isoform of AST-C; Gard et al. 2009; Dickinson et al. 2009) to ones that appear to be population-specific (e.g., the CPRPs of *Cancer productus*; Stemmler et al. 2007b) or individual-specific (e.g., the *Pagurus pollicaris* SIFamide variant GYRKPPFNGPIFamide, thus far identified from only a single individual; Cashman et al. 2007). Recent investigations have shown that, for many peptide families, large numbers of isoforms can be present in a single species, e.g., over 30 AST-A variants have been identified in the crab *Carcinus maenas* (Duve et al. 1997; Ma et al. 2009a). However, given the possibility of population-specific and individual-specific isoforms, care must be taken before assuming that all isoforms of any peptide family are present in all individuals of a given species.

Most but not all, of the peptide families/subfamilies identified in crustaceans probably serve as circulating neurohormones (Christie et al. 2010b). Interestingly, most of these peptide groups also appear to function as locally released neuromodulators (Christie et al. 2010b). For example, in *Cancer borealis*, proctolin is present in the neuroendocrine SG and PO (e.g., Christie et al. 1995a; Li et al. 2003) and in the synaptic neuropil of the stomatogastric ganglion (STG; e.g., Christie et al. 1997). As discussed at length in Christie et al. (1995a), this dual function is probably significant physiologically, as the effects of many modulators are highly concentration-dependent and the concentration of locally released versus hormonally released peptide is almost certainly different at any given target. For example, in *Cancer borealis*, the effects of proctolin on the stomatogastric neural circuits are concentration-dependent. Here, a low concentration of proctolin (i.e., 10^{-7} M) elicits a qualitatively distinct effect (activation/enhancement of the pyloric rhythm) from that seen at a high concentration (i.e., 10^{-5} M) of peptide (a rhythm that is a fusion of the pyloric and gastric mill motor patterns). Thus, in *Cancer borealis*, proctolin released from the SG or PO probably produces a qualitatively different effect on the STG circuits that are produced by release of the peptide from sites within the ganglion.

Table 1 provides a summary of the presence of the various neurochemicals thus far identified in the major crustacean neuroendocrine tissues. All of the neuroendocrine organs described earlier are peptidergic. For the ACO, the only hormone thus far identified in it is a peptide, specifically TRP (Goldberg et al. 1988; Messinger et al. 2004; A.E. Christie, unpublished). FLP, PDH and mandibular organ-inhibiting hormone (MOIH), a member of the CHH superfamily, have all been identified via immunohistochemistry in the ACP (Christie et al. 2004a; Hsu et al. 2006, 2008). Members of many peptide families have been described in both the SG and PO (e.g., Christie et al. 1995a; Pulver and Marder 2002; Li et al. 2003; Fu et al. 2005a, b; Ma et al. 2008, 2009a, 2010). It is important to note that Table 1 is not species-specific but, rather, represents data pooled from a number of species; the presence/absence of a substance in a site can vary between species, including those that are closely related. This is particularly true for peptides. For example, immunohistochemistry has shown that TRP is present in the SG of *Cancer borealis* (Christie et al. 1995a) but not in the SG of *Cancer productus* (Fu et al. 2005b). Similarly, the ACP of *Cancer productus* appears to contain at least one FLP (Christie et al. 2004a), which is absent in the ACPs of both *Cancer borealis* and *Cancer magister* (Savage et al. 2006). Thus, care should be taken before assuming that the detection of any substance in the neuroendocrine organ of one species implies extensive conservation across species.

Peptides released from neuroendocrine sites have been shown to play broad roles in the control of nearly all aspects of crustacean physiology and behavior. A recent comprehensive review of crustacean neuropeptides (Christie et al. 2010b) includes discussions of peptide hormone function and thus, in the interest of space, readers are referred to this article for summaries of the roles played by the individual peptide hormone families/subfamilies listed earlier. This said, several recent findings concerning peptide hormone signaling are highlighted in later sections of this review, specifically, a role for SIFamide in modulating aggression and social dominance (Vázquez-Acevedo et al. 2009) and a role for NPF in modulating food intake and growth (Christie et al. 2011b).

Biogenic amines and their derivatives

Four amines, dopamine, histamine, octopamine and serotonin, are commonly recognized as being produced by crustacean neural tissues (e.g., Beltz 1999). As for many neuropeptides, dopamine, octopamine and serotonin probably serve both as locally released neuromodulators and as circulating neurohormones in crustaceans (e.g., Beltz 1999), with the PO (the subesophageal ganglion dorsal nerves/thoracic ganglia segmental nerves included) appearing to be the primary neurohormonal source of these amines in at least the decapods (e.g., Evans et al. 1976; Beltz and Kravitz 1983; Siwicki et al. 1987; Schneider et al. 1993; Christie et al. 1995a; Pulver and Marder 2002; Fu et al. 2005b; Dickinson et al. 2008; Table 1). Although a number of studies have implicated histamine as a locally released neurotransmitter/modulator (e.g., Mulloney and Hall 1991; Callaway and Stuart 1999; Harzsch and Glötzner 2002; Pulver et al. 2003; Christie et al. 2004b; Rieger and Harzsch 2008; Hartline and Christie 2010; McCoole et al. 2011), little evidence exists for its presence in any crustacean neuroendocrine tissue (e.g., Fu et al.

2005b). Thus, histamine might well not serve as a crustacean neuroendocrine signaling agent.

Circulating serotonin, octopamine and dopamine have been shown to play many functional roles in crustaceans. For example, all three amines modulate neuromuscular transmission (e.g., Battelle and Kravitz 1978; Kravitz et al. 1980; Glusman and Kravitz 1982; Jorge-Rivera et al. 1998; Djokaj et al. 2001), serve modulatory roles in the control of neural circuit activity including those in the present in the STNS (e.g., Flamm and Harris-Warrick 1986a, b; Johnson and Harris-Warrick 1990, 1997; Johnson et al. 1993, 1994, 1995; Peck et al. 2006), are cardio/vasoactive (Battelle and Kravitz 1978; Florey and Rathmayer 1978; Saver et al. 1999; Wilkens and Taylor 2003; Fort et al. 2004; Cruz-Bermúdez and Marder 2007) and play roles in the control of osmoregulation (e.g. Lohrmann and Kamemoto 1987; Morris et al. 2000; Morris and Ahern 2003). In addition, both serotonin and octopamine are well-known players in the control of aggression (e.g., Edwards and Kravitz 1997; Kravitz 2000; Huber et al. 2001; Sosa and Baro 2002; Panksepp et al. 2003; Huber 2005; Pedetta et al. 2010), whereas dopamine has been implicated in the control of immune function (e.g., Cheng et al. 2005; Li et al. 2005; Chang et al. 2007). As discussed later in this review, hormonally delivered serotonin has recently been implicated in the control of adult neurogenesis within several regions of the crustacean brain (e.g. Benton et al. 2008).

Small molecule transmitters

Transmission electron microscopy carried out on a number of crustacean neuroendocrine sites has demonstrated the presence of electron-lucent vesicles in the release terminals of these tissues (e.g., Fu et al. 2005a, b; Christie et al. 2003, 2004a, b). As a number of small molecule transmitters are packaged into this vesicle class (e.g., acetylcholine and glutamate), a variety of small molecules might be utilized as signaling agents by crustacean neuroendocrine organs. Interestingly, only one small molecule transmitter has been formally detected in a crustacean neuroendocrine organ, specifically γ-aminobutyric acid (GABA) identified via immunoreactivity in the PO of the crabs *Cancer borealis* and *Cancer productus* (Christie et al. 1995a; Fu et al. 2005b;Table 1). The function that GABA might serve in the PO of *Cancer* crabs is unclear, as this molecule is not generally thought of as a circulating hormone. One possibility is that its role in the PO is to control the release of other hormones from this neuroendocrine site (Christie et al. 1995a) and previous work has shown that at least some crustacean neurosecretory somata (García et al. 1994; Rogers et al. 1997; Duan and Cooke 2000) and their secretory terminals (Rogers et al. 1997) exhibit an increase in Cl^- conductance in response to GABA. Alternatively, the GABAergic axons in the PO might simply be fibers-of-passage, whose ultimate functional destination is outside this neuroendocrine organ, e.g., projecting to the cardiac ganglion and contributing to the local control of the cardiac neuromuscular system (Delgado et al. 2000; Cruz-Bermúdez and Marder 2007).

Diffusible gases

Recently, evidence has been presented for the existence of at least two gas transmitters, namely nitric oxide (NO) and carbon monoxide (CO), in crustacean neuroendocrine tissues (Table 1). For NO, immunohistochemisty using an antibody generated against nitric oxide synthase (NOS), the rate-limiting biosynthetic enzyme of NO, suggests that this gas transmitter is produced in the SG of the crayfish *Procambarus clarkii* (Lee et al. 2000). Likewise, in the lobster *Homarus americanus*, transcripts encoding several NOS isoforms have been identified from XO mRNA (M.C. Chapline and A.E. Christie, unpublished), suggesting that NO is also produced by the XO-SG system of this species. Although currently only conjecture, NO has been hypothesized to regulate peptide release from the XO-SG system (Lee et al. 2000), a role played by this gas in vertebrate species (e.g., Mancuso et al. 2010); this gas has been shown to play additional roles in other regions of the crustacean nervous systems, including modulation of central pattern generators in the stomatogastric and cardiac neuromuscular systems and the control of neurogenesis/morphogenesis in the brain (Scholz et al. 2001, 2002; Mahadevan et al. 2004; Stein et al. 2005; Benton et al. 2007).

At present, the identification of CO as a putative crustacean neuroendocrine signaling agent is limited to a single study conducted on the crayfish *Cherax quadricarinatus* (Christie et al. 2003). In this report, an antibody generated against heme oxygenase 2 (HO-2), the rate-limiting biosynthetic enzyme for CO, was found to label an extensive neuroendocrine plexus in the anterior portion of the STNS. A group of approximately 12 HO-2-immunopositive somata in each CoG is postulated to be the source of this HO-2-like labeling. As for NO, CO is hypothesized to play a role in controlling hormone secretion from this neuroendocrine site, a role ascribed to it in vertebrates (e.g., Mancuso et al. 2010).

A recent advance in the identification of crustacean hormones: genome mining

For many years, the common strategy employed for the identification of crustacean neuropeptides was the chromatographic or biochemical purification of a single peptide from a large pool of starting material, following it through

the purification process via bio- and/or immunoassay. Once isolated to purity, the structure of the peptide was determined by using a combination of proteolytic cleavage, Edman analysis and/or mass spectrometry. For example, this strategy was used for the isolation and structural identification of RPCH from the caridean shrimp *Pandalus borealis*, the first neuropeptide fully characterized from any invertebrate (Fernlund and Josefsson 1968, 1972). Today, a shift in focus has occurred, moving from the targeted identification and characterization of single peptides to identifying peptidomes, the full complement of peptides present in a tissue or species. Over the last few years, mass spectrometry and transcriptome mining have been the primary methods used for characterizing crustacean peptidomes (e.g., Huybrechts et al. 2003; Li et al. 2003; Fu et al. 2005a, b; Christie et al. 2008; Ma et al. 2008, 2009a, b, c, 2010; Gard et al. 2009; Christie et al. 2010a). The use of these methods for crustacean peptide discovery is one of the subjects of a recent comprehensive review of crustacean neuropeptides (Christie et al. 2010b) and readers are directed to that article for detailed descriptions of the methods and results that they have generated.

As in crustaceans, mass spectrometry and transcriptome mining have been major contributors to peptide discovery in members of other arthropod subphyla (e.g., Huybrechts et al. 2005; Audsley and Weaver 2006; Clynen et al. 2006; Christie 2008a, b; Weaver and Audsley 2008; Neupert et al. 2009; Ons et al. 2009; Christie et al. 2011c). However, the sequencing of several insect genomes has provided an additional resource for peptide discovery in members of this taxon (e.g., Hummon et al. 2006; Liu et al. 2006; Amare and Sweedler 2007; Li et al. 2007; Roller et al. 2008; Wegener and Gorbashov 2008; Hauser et al. 2010; Huybrechts et al. 2010; Predel et al. 2010).

Recently, the genome of *Daphnia pulex*, a cladoceran crustacean, was sequenced; this is the first and currently only, crustacean genome available for public use (e.g., Colbourne et al. 2005; Bauer 2007; Stollewerk 2010). Utilizing this resource, Christie and colleagues have just published the first description of a crustacean peptidome based solely on the analyses of genomic data (Christie et al. 2011a). Here, a strategy similar to that employed for crustacean transcriptome mining was used for gene identification and subsequent peptide prediction (e.g., Christie et al. 2008, 2010a; Gard et al. 2009; Ma et al. 2009a, 2010). Specifically, these authors used known pre/prepro-hormone sequences to query the *Daphnia pulex* genome for genes encoding putatively orthologous proteins; standard BLAST-based searching was used for these analyses. All genes identified were translated and checked for homology with the target query and if the hit seemed likely to represent a viable gene, post-translational processing of the deduced protein was predicted by using a combination of online peptide prediction programs and homology to known peptide processing schemes (Fig. 6). In this study, Christie and colleagues (Christie et al. 2011a) used 34 known arthropod peptide precursors to query the *Daphnia pulex* genome for genes encoding putative orthologs, with 23 of the searches resulting in gene identifications. From these genes, the authors were able to predict the putative mature structures of nearly 100 peptides (Table 2), including members of the AST-A, AST-B, AST-C, ATR, bursicon α, bursicon β, CLDH, corazonin, CCAP, CHH, ETH, EH, insulin-like peptide (ILP), MIH, NPF, orcokinin, PDH, proctolin, RPCH, sNPF, SIFamide, sulfakinin and TRP families/subfamilies; the prediction of Dappu-ATR (i.e., GFKTVGLATARGFamide) is the first ATR described in any crustacean species. This complement of peptides identified via genome mining represents the most diverse peptidome thus far described in any crustacean and is also among the largest thus far annotated, demonstrating the power that this approach has for peptide discovery in Crustacea. As the genomes of other crustaceans are sequenced and released (e.g., the *Daphnia magna* genome), this study will provide a model for deducing other peptidomes via genomic analyses.

A recent advance in mapping the distributions of crustacean hormones: mass spectral imaging

For over 40 years, immunohistochemisty has been the primary method used for mapping neurochemical distributions in crustaceans (e.g., Jaros and Keller 1979; Van Herp and Van Buggenum 1979; Hartline and Christie 2010; Sharp et al. 2010; Wilson and Christie 2010). Via this method, numerous molecules, including peptides, amines, small molecule transmitters and diffusible gases (e.g., Christie et al. 1995a, 2003, 2004a, b; Pulver and Marder 2002; Fu et al. 2005b), have been identified and/or mapped in crustacean neuroendocrine organs. This technique has also proven useful in determining co-transmitter complements in many identified neurons, including a number of neurosecretory cells, e.g., both dopamine and the peptide proctolin in the lobster *Homarus americanus* L-cell (Siwicki et al. 1987), a known contributor to the innervation of the PO.

Although a powerful tool, immunohistochemical mapping does have its limitations. For example, whereas antibodies can be paired to determine the co-localization patterns of neurochemicals, the antibodies used typically need to be generated in different host species and/or be of distinct immunoglobulin type/subtypes. One must also possess the capability of visualizing each antibody distinctly. Thus, the number of molecules that can be mapped simultaneously is often small. In addition, the distributions of neurochemicals that differ from one

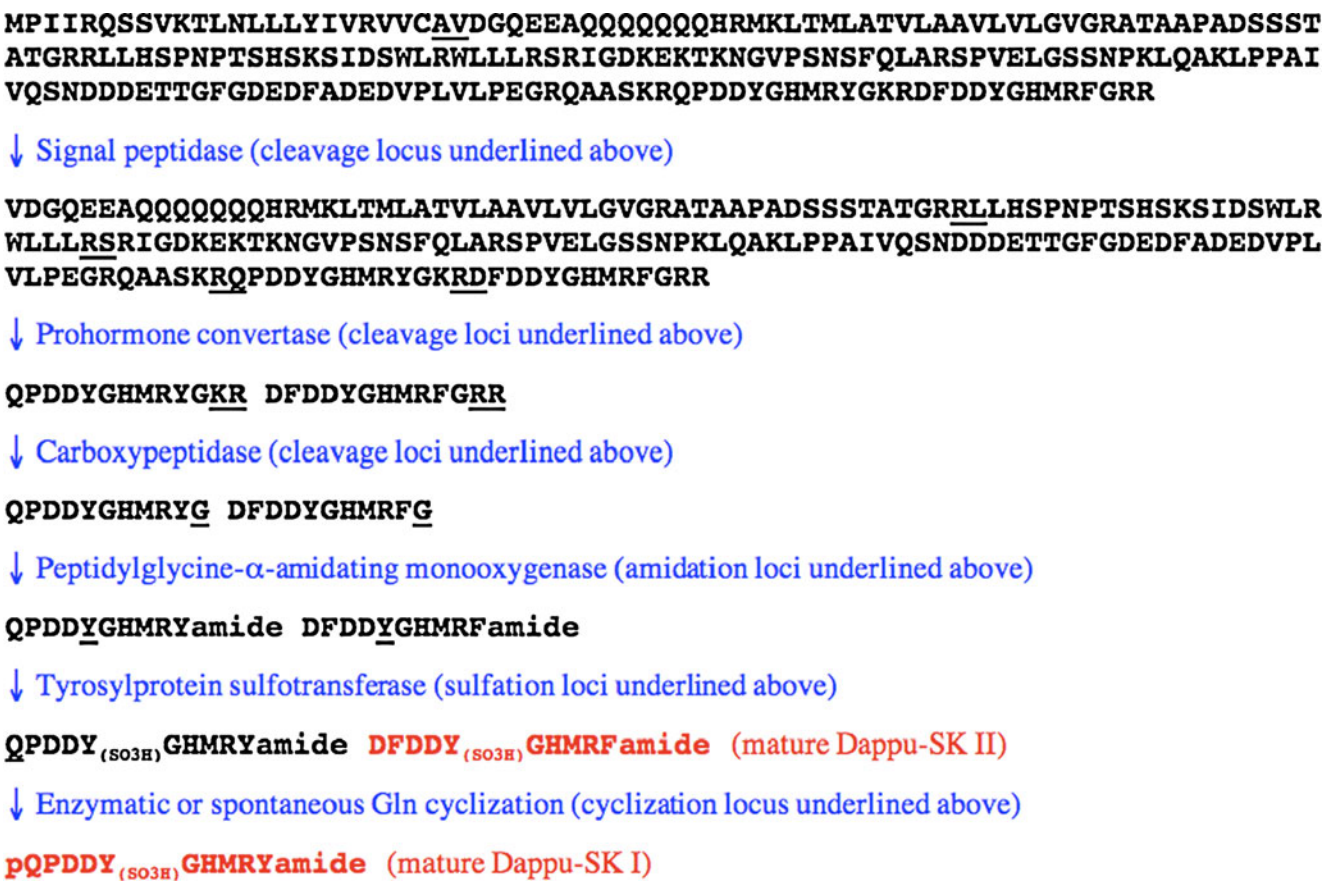

Fig. 6 Putative processing scheme resulting in the production of the two isoforms of Dappu-sulfakinin from its precursor protein (see Table 2). The mature conformations of the two sulfakinin isoforms (Dappu-SK I and II) are colored *red*. Prediction of the signal peptide cleavage locus was accomplished by using the online program SignalP 3.0 (http://www.cbs.dtu.dk/services/SignalP/; Bendtsen et al. 2004). Prohormone convertase cleavage loci were predicted based on information provided in Veenstra (2000). Prediction of the sulfation state of Tyr residues was accomplished by using the online program Sulfinator (http://www.expasy.org/tools/sulfinator/; Monigatti et al. 2002). Prediction of all other post-translational processing was based on homology to known sulfakinin processing schemes. Data modified with permission from Christie et al. (2011a)

another by subtle structural changes, e.g., small variations in sequence, post-translational modifications, etc., are often difficult to separate. This is particularly true for the mapping of peptides, although exceptions can be found to this rule, e.g., the immunohistochemical mapping of D- and L-epimers of members of the CHH superfamily in the XO-SG system of the lobster *Homarus americanus* (Ollivaux et al. 2009). Moreover, the production of the antibodies used in immunohistochemistry is often costly and time consuming and the labeling protocols themselves can be labor- and time-intensive.

Mass spectrometric imaging (MSI) has recently emerged as an alternative to immunohistochemisty for determining the distributions of molecules of interest, particularly peptides, in neural tissues (for reviews, see Rubakhin et al. 2007; Rubakhin and Sweedler 2010). This approach overcomes several of the challenges faced when mapping substances via immunolabeling; specifically, large numbers of molecules can be mapped simultaneously and subtle modifications in structure can often be resolved. However, the spatial resolution provided by MSI is still somewhat limited relative to that of the optical imaging employed with most immunohistochemical mapping. While a number of mass spectrometric platforms have been used for MSI, the ultra-high resolution and mass accuracy of matrix-assisted laser desorption/ionization (MALDI) mass spectrometry makes it one of the most commonly employed with respect to peptide analyses.

In several recent publications, Li and colleagues have demonstrated the power of MSI for mapping crustacean neuropeptides (DeKeyser et al. 2007; Chen et al. 2009, 2010). Specifically, the authors used MALDI tandem time-of-flight (TOF/TOF) mass spectrometry to produce two- and three-dimensional maps of neuropeptides in the neural tissues of the crab *Cancer borealis*. In these studies, Li and co-workers employed peptide profiling to generate detailed information about peptide localization in the investigated tissues, with high-energy tandem mass spectrometric experi-

Table 2 *Daphnia pulex* peptides predicted via genome mining (*AST-A* A-type allatostatin, *AST-B* B-type allatostatin, *AST-C* C-type allatostatin, *ATR* allatotropin, *CCAP* crustacean cardioactive peptide, *CHH* crustacean hyperglycemic hormone, *MIH* molt-inhibiting hormone, *CLDH* calcitonin-like diuretic hormone, *ETH* ecdysis-triggering hormone, *EH* eclosion hormone, *FLP* FMRFamide-like peptide, *NPF* neuropeptide F, *sNPF* short neuropeptide F, *ILP* insulin-like peptide, *PDH* pigment-dispersing hormone, *RPCH* red pigment concentrating hormone, *TRP* tachykinin-related peptide, *PRP* precursor-related peptide, *a* carboxy-terminal amide group, *pQ* amino-terminal pyroglutamic acid). Peptide structures shown in *standard font* are identical to those previously predicted *Daphnia pulex* peptides (Gard et al. 2009). Peptide structures shown in *italics* are similar in structure to those previously predicted *Daphnia pulex* peptides (Gard et al. 2009). Peptide structures shown in *bold* are novel *Daphnia pulex* peptides. Table modified from Christie et al. (2011a) and used with permission

Family (subfamily)	Predicted peptide structure
AST-A	*TDVNNDSSVIEAVGAGGGDSQAKAESLASAEDKLSALTADGMATRARYFKSFGGNPTGDPNLNIYSFGLa*
	TSRSYSINPYSFGLa
	GGNAKSYPQQIPYSFGLa
	NPTKYNFGLa
	PDRFGFGLa
	LPVYNFGLa
AST-A PRP	*NLKDDDLDQWLNDEEYSQFDDTEEEIEGREDDSQEIMDVN*
	SQMAAGQQQQQQQQAFFPSHLQSAFYGGPFMSNLARANTHALGKSRTFNQQAATHDLPNLa
AST-B	**NNWNRMQGMWa**
	AWSDLSQQGWa
	SWTQLHGVWa
	RWDQLHGAWa
	SGWNKMQGVWa
	GWNQLQGVWa
	NWNNLRGAWa
	ESGWNNLKGLWa
AST-B PRP	**INTQQTPSQRLEPNQVAGLVELHQQLEQPREHQQQQQHHQQQPEQPAQADNKDYAPSPAVLLQLTPSDWKNTQD**
	QQTPSQRLEPNQVAGLVELHQQLEQPREHQQQQQHHQQQPEQPAQADNKDYAPSPAVLLQLTPSDWKNTQD
	SQQQSDESALSEMTPPRQMV
	TPDQLEDDSKAEQPENSQEDEDQQSSSEVEREEANDSDDTVENS
	SSSSSKTNSGPAIEGMGNNDLLLLISGTGDQLYQQREDDQAKANVEDAGNEESVD
	ALSAMAAGY
	EIPAAIAKGMEWSR
AST-C	SYWKQCAFNAVSCFa
AST-C PRP	*KSTDREETESTDFGQDIEVVKFRHFSTDKDELICFILKENIHLGAVPDDGSVETALLNYLFAKQIVARLRTNANPQDLMR*
ATR	**GFKTVGLATARGFa**
ATR PRP	**APHPADYTSSSVNNQRDF**
	APSLSNFNSFQDAAEQMMQQQEENPNSDPDVFPVDWLVNYLQNKPDVIRYMVEHLLDHNGDGQVTSQEMMTSLQQQRED
Bursicon α	*DECQLTPVIHVLQYPGCIPKPIPSFACTGKCTSYVQVSGSKLWQTERSCMCCQESGERETVSLLCPKAAPGEPKLRRVVTRAPVDCMCRPC TALEESAVMPQEIARFLDDGSFPFKL*
Bursicon β	*SKTNLMSGTCETLPSTIHITKEEYTDGGILSRTCEGDIGVAKCEGSCSSQVQPSVVHPSGFLKECMCCRESFLRERVVTLTHCYDANGNRLT GKSSSLDVKMREPADCKCFRCGDSAE*
Corazonin	**pQTFQYSRGWTNa**
Corazonin PRP	**RSDPSFVQQQQWIQRNGHPMVIPAEFRSNSFEDWSRYRINGEKVNEDGDSWLVHVSHCAKLATSLGSVLKNKDAKSDDNP LIDVIH**
CCAP	PFCNAFAGCa
CCAP PRP	QPLKNNQNDSDSAEEIEQWSFKE
	RSMIKDTKHPPYEKAASNHPRLPNADTKLLDKLFAKIQHQRANFVQLDDPEY(SO3H)Y
CHH (CHH)	*SFFDINCKGLYDKSIFARLDRICQDCYSLYREPELHTLCRKNCFTTNYFKGCLDALLINDEKDIQRVMKDISIIHQIPI*
CHH PRP	MSALSSGHHSLS
	ALSSGHHSLS
CHH (MIH)	*MSALSSDHHSSCKGLYDKSIFYRLNRICHDCFSLYRSPELHTLCRSECFTTPFFKACLKVLLMGDQDPDSSEMIDKIa*
	ALSSDHHSSCKGLYDKSIFYRLNRICHDCFSLYRSPELHTLCRSECFTTPFFKACLKVLLMGDQDPDSSEMIDKIa
CLDH	GVDFGLGRGYSGSQAAKHLMGLAAANYAIGPa
CLDH PRP	APP
	PMLVDLDDPDSVMEVITRLERSLL
	NSDY(SO3H)EHQ
	DTTESTPEDVKTGAIN
ETH	DPSPEPFNPNYNRFRQKIPRIa

Table 2 (continued)

Family (subfamily)	Predicted peptide structure
	GEGIIAEY(SO3H)MNSESFPHEGSLSNFFLKASKAVPRLa
ETH PRP	KDISTESGRAAMVGEEPFGRISNEIPIMNQKQDLWPNMNINELTGALNKELNYPGPRIPKDLQDNYIQDLIHSWINQYENLNEN
EH	**PMLGNIHLCMMNCGQCKEMYGEYFEGQRCAEFCLASYKPSQAGSVSGGGGGSGWAPMPDCNEPETVDQFLKLSLMPQS DGPSVQQLIDDSDSDGGGGYMSPISQALMGSAYSGRYAGR-**
	KPSASAQQNTLYSSRTGAEFKGHNQLLKNRKW
EH PRP	**QGSDGS**
	RINGSSKLLGGSPSMPRWYFV
FLP (NPF)	VCVTTTKADGGDVMSGGEGGEMTAMADAIKYLQGLDKVYGQAARPRFa
NPF PRP	GRLPYFDMEDLPLHPQHAY
FLP (sNPF)	SDRSPSLRLRFa
sNPF PRP	QNIASASPTPLLSGFEDYSEDRLNGEQPSLYELLLQREMLADKLDSEGRGHLIV
	SPTPLLSGFEDYSEDRLNGEQPSLYELLLQREMLADKLDSEGRGHLIV
	ADPDVPRVSAASNQHD
FLP (sulfakinin)	**pQPDDY(SO3H)GHMRYa**
	DFDDY(SO3H)GHMRFa
Sulfakinin PRP	**APADSSSTATa**
	LLHSPNPTSHSKSIDSWLRWLLL
	SRIGDKEKTKNGVPSNSFQLARSPVELGSSNPKLQAKLPPAIVQSNDDDETTGFGDEDFADEDVPLVLPEGRQAAS
ILP	**NARYCGSYLADALRMACSKSSYLPLFa**
	GVHDECCVKGCTFKELTSYCTRPN
ILP PRP	**HTLQNPVNLGLLKDEETQARNYFADFLRVLYAPQAPKDDVSLSAGSSNDMPNEIFPMISSDEDDHEEENY(SO3H)DKI**
	KEFPVDGVLLPILQ
	STLPGKSLGLTTTAATPNAELGSWPFINDDKAHSILSNHHLFHRYT
Orcokinin	NLDEIDRSNFGTFA
	NLDEIDRSDFGRFV
	NLDEIDRSDFSRFV
Orcokinin PRP	LNYQSEEAVGVEHERDRDSLGGGHILRGLDSIGESNLLRAIYREKPRDFLRIN
	GLDSLSGASFGIE
	LDSLTGLGFGSQ
	RETMEAESSQQQH
PDH	NSELINSLLGLPRFMKVVa
PDH PRP	*APPSISSNNRPEAQMSIQEMEKFLEGLTRYLHRQHLDLPKVHQQSQEEQPGSYEADAIDRSGDMSAPTETERSSSSSSSELANHSLLSHPRP PMANKWPWSLSHLERIEDDPDFKERQQPYA*
Proctolin	**SSWGVDARYLPT**
	RYLPT
Proctolin PRP	**SDPLSPIGPPRGEDP**
	FDRLYDIIIRELLRNGGGDMLDEAKYPIKNQFSSGAY
RPCH	**pQVNFSTSWa**
RPCH PRP	**SPSTSTKAAEPPSAPSYRQNFHSKKVEPGTLETLPNNQHLPESFDTVSSTIYDDAEEQRISISLPSPCLSILKSLLLVNQIVE FKNSPLDGRMH**
	FKIENLFPLPNRTC
	LYI
SIFamide	TRKLPFNGSIFa
SIFamide PRP	SNQGTDKLESPSNLQLLCDAAMNACSDWLPIGS
TRP	**TPNSRAFLGMRa**
	KMHGEKFLGMRa
	RAPSSNSFMGMRa
TRP PRP	**AATAVTDDDELMARQT**
	GLVLRSW
	NAQQQTDHSADKTPSAKVAEPILPSQKEAMVFNGLPISMRLVLLQHLAGYD
	SSPPGADALTMEDNQLDDASGWPQGDILPDTYYFGPAPQ
	MMNGLADGTAFIPNWRERYIYQEPFE
	SESTTPTPNDYQFFNDDIIVDEELPDVDSKVSP
	SQERTPI

ments being undertaken to confirm the identity of the neuropeptides in question. Moreover, the authors used intensity maps of mass-to-charge ratios (*m/z*) to provide information about the three-dimensional distributions of a number of selected neuropeptides within tissues. Figure 7 shows an example of the three-dimensional distributions of six peptides (the orcokinins NFDEIDRSGFGFA and NFDEIDRTGFGFH, the FLPs NRNFLRFamide and SMPLRLRFamide, the SIFamide GYRKPPFNGSIFamide and the TRP APSGFLGMRamide) in the brain of *Cancer borealis* (Chen et al. 2009). As this methodology evolves, it has the potential to improve greatly our ability to map neurochemicals, particularly peptides, within crustacean neuroendocrine organs and other neural tissues, theoretically allowing the production of the first distribution maps of full peptidomes in these structures.

Recent advances in the understanding of hormonal control of crustacean physiology and behavior

In crustaceans, circulating hormones are critical components of all physiological control systems. A recent review by Christie and colleagues (2010b) describes many recent advances in our understanding of hormonal control in crustaceans, particularly those involving the peptidergic control of the cardiac and stomatogastric neuromuscular systems, including cotransmission, metamodulation and developmental/evolutionary aspects of hormonal signaling. Other recent reviews have described recent advances in our understanding of the physiological roles served by specific peptide families, e.g., members of the CHH and allatostatin families (Fanjul-Moles 2006; Stay and Tobe 2007; Nakatsuji et al. 2009; Chung et al. 2010), or for particular target tissues, e.g., the stomatogastric neuromuscular system (Stein 2009). In addition, several recent reviews have focused on endocrine disruption and other aspects of endocrine toxicology, including the roles of neuroendocrine signaling relevant to this topic (LeBlanc 2007; Rodríguez et al. 2007). Thus, rather than repeat discussions of these topics, the sections that follow discuss three recent advances in our understanding of hormonal control in crustaceans not recently reviewed, i.e., peptidergic control of aggression and social dominance, peptidergic control of food intake and growth and aminergic control of adult neurogenesis.

Modulation of aggression and social dominance by SIFamide

Decapods, particularly members of the Astacidea, have long been used in studies of the neurochemical basis of

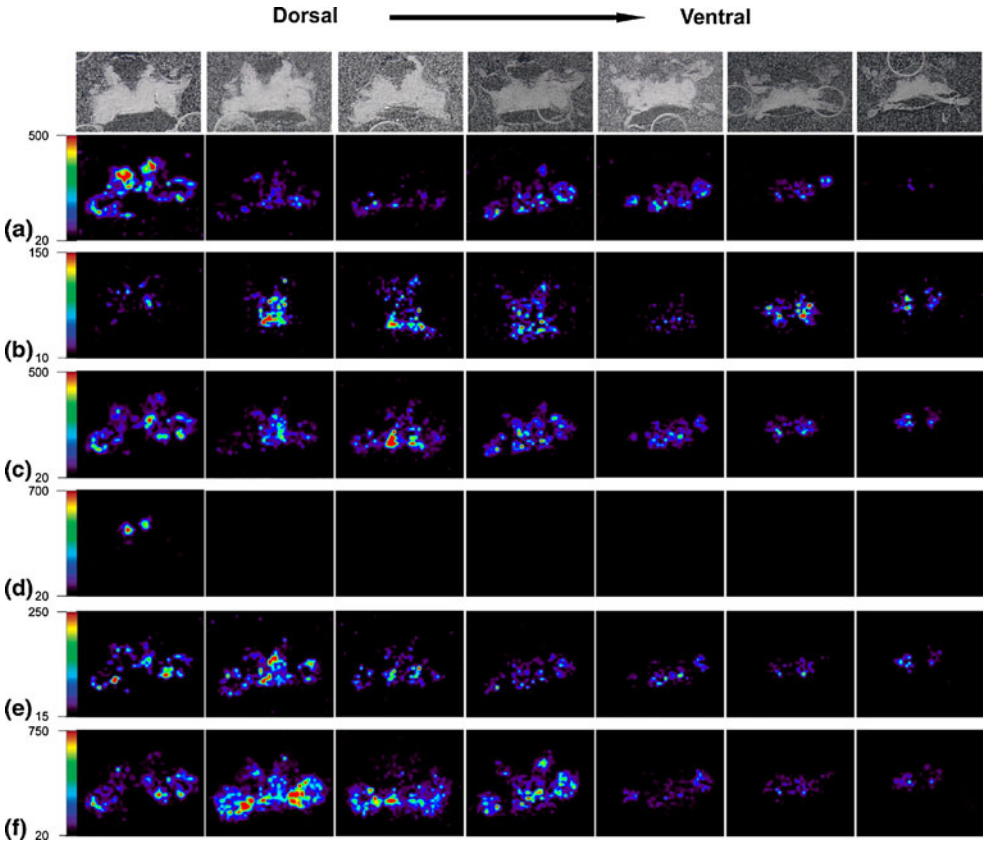

Fig. 7 Three-dimensional distributions of neuropeptides in the brain of the crab *Cancer borealis* as revealed by mass spectrometric imaging. From *left* to *right*, the brain slices are arranged from *dorsal* to *ventral*. The individual optical images of each layer of section are shown *top*. Tissue sections were prepared by using regular matrix spraying methods for neuropeptide detection. Six neuropeptides are shown: two orcokinins, (**a**) NFDEIDRSGFGFA (*m/z* 1474.7), (**b**) NFDEIDRTGFGFH (*m/z* 1554.7); two FMRFamide-like peptides, (**c**) NRNFLRFamide (*m/z* 965.5), (**d**) SMPSLRLRFa (*m/z* 1105.6); a SIFamide, (**e**) GYRKPPFNGSIFamide (*m/z* 1381.7); and a tachykinin-related peptide (**f**) APSGFLGMRamide (*m/z* 934.5). Various distribution patterns are evident between the different families and between the different isoforms of individual families. Figure used with permission from Chen et al. (2009)

aggression and social dominance (for reviews, see Edwards and Kravitz 1997; Kravitz 2000; Huber et al. 2001; Panksepp et al. 2003; Huber 2005). As stated earlier, the amines serotonin and octopamine appear to play key, and opposing, roles in mediating/modulating the physiological control systems that underlie these behaviors, with increased levels of the former correlating with increasing aggression and increasing levels of the latter correlating with decreases in this behavior. Although they are clearly key players in the control of aggression, the actions of these amines alone cannot account for all aspects of aggressive behavior/social dominance, particularly its adjustment to varying conditions (e.g., Vázquez-Acevedo et al. 2009). Thus, a search has begun for other players in the control of aggression and given their diversity and pleiotropy, peptides represent likely candidate molecules.

Sexual behavior is often intimately associated with social dominance and aggression. Recently, the peptide AYRKPPFNGSIFamide, an insect-specific member of the SIFamide family, has been implicated in the control of sexual behavior in the fruit fly *Drosophila melanogaster* (Terhzaz et al. 2007). In this species, removal of the SIFamide system either via targeted cell ablation or RNA interference radically modifies courtship behavior, inducing promiscuity in both sexes, with males indiscriminately courting members of either sex and females exhibiting sexual hyper-receptivity.

Given the link of SIFamide to male courtship in *Drosophila*, Sosa and colleagues recently investigated the possibility that a member of the SIFamide family plays a role in modulating aggression/social dominance in the caridean shrimp *Macrobrachium rosenbergii* (Vázquez-Acevedo et al. 2009). Specifically, they assessed the levels of aggression/submission between dominant (blue-clawed morphotype)/subordinate (yellow-clawed morphotype) male pairs before and after the injection of SIFamide into the circulatory system. In both morphotypes, injection of GYRKPPFNGSIFamide, the native caridean SIFamide (Stemmler et al. 2007a), into the hemolymph increased their dominance indexes. Interestingly, when the subordinate animal was administered SIFamide, not only was its dominance index modulated (increased aggression) but so was that of its formerly dominant pair-mate (which exhibited decreased aggression; Fig. 8). This reversal of dominance status is, thus far, unique to SIFamide injection in *Macrobrachium rosenbergii*; no shift has been noted for serotonin injection in Astacidean species. At present, the hormonal source of SIFamide in *Macrobrachium rosenbergii* is unclear, as no systematic study has been made of its neurochemistry to date. Likewise, the molecular/cellular mechanisms underlying SIFamide's modulation of aggression/social dominance in this species remain to be determined. However, the data from the Sosa laboratory show, for the first time, that peptides contribute to the control of these behaviors in crustaceans, opening the door for a variety of future studies into the peptidergic control of aggression in this and other crustacean species.

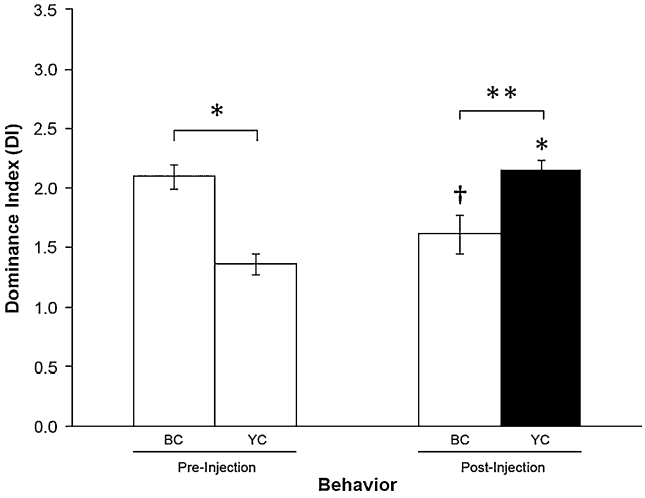

Fig. 8 Gly[1]-SIFamide injection into yellow clawed (*YC*) morph of *Macrobrachium rosenbergii*. Effect on mean dominance index (*DI*) before (*Pre*) and after (*Post*) injection of the YCs with 1×10^{-3} M Gly[1]-SIFamide (n=5). Before the injection, blue clawed (*BC*) morph individuals established themselves as the dominant animals and YCs as the subordinates (one-way analysis of variance, Tukey's test, P=0.001). When the YCs were injected with Gly[1]-SIFamide, they behaved much as did BCs under control conditions. The behavior of BCs confronted with Gly[1]-SIFamide-injected YCs was also modified, showing a significant decrease in the levels of aggression compared with control conditions. The *asterisk* over the YC post-injection group represents a significant difference from the YCs under control conditions (before the injection). The *cross* over the BC post-injection group represents a significant difference from the BCs under control conditions. *Filled bar* indicates the mean DI of the injected (YC) prawns; *P=0.001, **P=0.025, P=0.050. Figure used with permission from Vázquez-Acevedo et al. (2009)

Modulation of food intake and growth by NPF

Aquaculture is an increasingly important source of marine and freshwater species for human consumption. Large-scale farming of crustaceans is currently limited to the penaeid shrimp. In the past, the generation of marketable-sized individuals involved rearing the animals through multiple developmental stages under controlled environmental conditions. However, in recent years, the production per unit area has leveled off, largely because of the exhaustion of new options in environmental control. To reverse this trend, a shift has occurred toward developing strategies that involve modifying the physiology of the animals themselves, with a focus on strategies that control important components of aquaculture, e.g., increasing food intake and, hence, growth rates.

Circulating hormones modulate feeding-related behavior in most animals. In vertebrates, one group of peptides that plays a key role in the control of food intake, among other functions, is the neuropeptide Y (NPY) superfamily (e.g., Pedrazzini et al. 2003; Emeson and Morabito 2005; Karl and Herzog 2007; Chee and Colmers 2008; Thorsell 2008; Benarroch 2009). Whereas no authentic NPY has yet been identified in any invertebrate, a broadly conserved subgroup of the FLP superfamily, commonly referred to as the NPFs, has been proposed as their homolog (Walker et al. 2010). Members of the NPF subfamily are typified by –RPRFamide C-termini, Tyr residues located 10 and 17 amino acids from their carboxyl ends and an overall length of approximately 36 amino acids (Christie et al. 2010b). Since the discovery of the first NPF from the platyhelminth *Moniezia expansa* (Maule et al. 1991), isoforms have been identified in many other groups of invertebrates (e.g., Rajpara et al. 1992; Brown et al. 1999; Dougan et al. 2002; Stanek et al. 2002; Humphries et al. 2004; Roller et al. 2008; Nuss et al. 2010), including a number of insects, where the functions thus far ascribed to them mirror those played by NPY in vertebrates, including their participation in the control of feeding-related behavior (e.g., Wu et al. 2003, 2005; Gonzalez and Orchard 2009; Krashes et al. 2009).

Recently, the first crustacean isoforms of NPF were identified via transcriptome/genome mining (Christie et al. 2008, 2011a; Gard et al. 2009), including one, KPDPSQLANMAEALKYLQELDKYYSQVSRPRFamide, from the penaeid shrimp *Marsupenaeus japonicus* (Christie et al. 2008). Molecular cloning of NPF precursors from two additional penaeid species, *Litopenaeus vannamei* and *Melicertus marginatus*, has followed, showing that these animals also possess the same NPF as *Marsupenaeus japonicus* (Christie et al. 2011b). Collectively, these findings strongly suggest the presence of a single, highly conserved isoform of NPF in penaeids.

Given the role of NPF in the control of feeding in insects, Christie and colleagues have recently investigated the effects of NPF on food intake and growth in *Litopenaeus vannamei* (Christie et al. 2011b), the most commonly farmed penaeid. In this study, a total daily dose of 2 μl 10^{-3} M KPDPSQLANMAEALKYLQELDKYYSQVSRPRFamide (1 μl 10^{-3} M peptide administered twice daily), delivered orally, increased food consumption by approximately 30% in the treated animals relative to control individuals (Fig. 9). This increase in food intake was observed only during the 5-day trial period (which was bracketed by 5-day pre- and post-trials); thus, a direct and short-lived effect of the peptide on appetite was proposed. In addition, the final weights of the NPF-supplemented shrimp were approximately 10% higher than those in the control group, a difference that was statistically significant, despite the short treatment period (Fig. 10). Collectively,

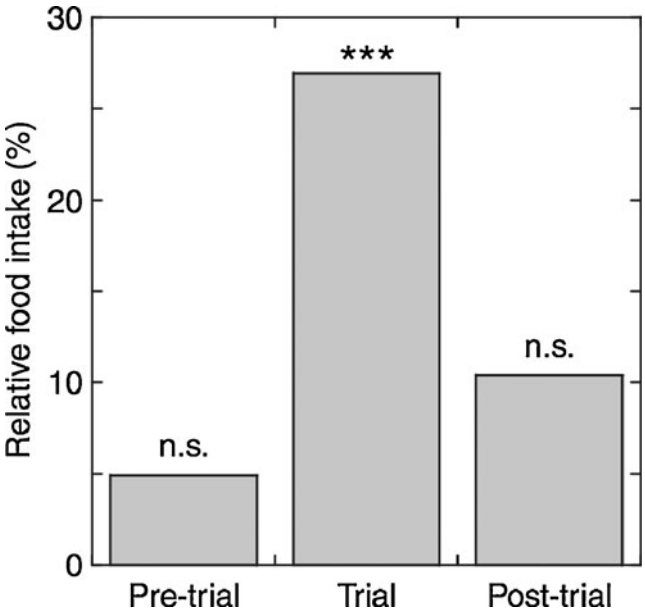

Fig. 9 Relative food intake computed as a percent difference between neuropeptide F (NPF)-treated and control *Litopenaeus vannamei* during the pre-trial, trial and post-trial periods of an experiment designed to test the effects of orally administered NPF on food intake and growth. NPF-treated individuals had their diet enhanced with a daily addition of 2 μl 10^{-3} M synthetic KPDPSQLANMAEALKYLQELDKYYSQVSRPRFamide during the trial period only. Significance: ***P<0.001 (*n.s* not significant). Figure used with permission from Christie et al. (2011b)

these findings suggest that NPF is a potent orexigenic agent in penaeid shrimp and that oral administration of NPF and potentially other peptide hormones, might provide a novel avenue for increasing growth rates in this and other aquacultured crustaceans.

At present, the neuronal source(s) of NPF in *Litopenaeus vannamei* is/are unclear and thus the origin of the native peptide influencing food intake is unknown. However, reverse transcriptase-polymerase chain reaction (RT-PCR) tissue profiling suggests that NPF is broadly distributed within the nervous system of penaeid species (Christie et al. 2011b), including the eyestalk ganglia (the location of the XO-SG system; Fig. 11) and the thoracic nervous system (the location of many somata that contribute innervation to the PO; Fig. 11). Thus, both the XO-SG and PO are candidates for the neuronal source of a circulating NPF in *Litopenaeus vannamei* (Christie et al. 2011b).

The pathway through which NPF exerts its orexigenic influence in *Litopenaeus vannamei* remains to be determined. In terms of sites of action, since the NPF was orally administered, the digestive tract is a likely target, as apparently is true in insects (e.g., Brown et al. 1999; Nuss et al. 2008; Gonzalez and Orchard 2009). If this is the case, NPF might modulate nutrient/ion/water absorption through

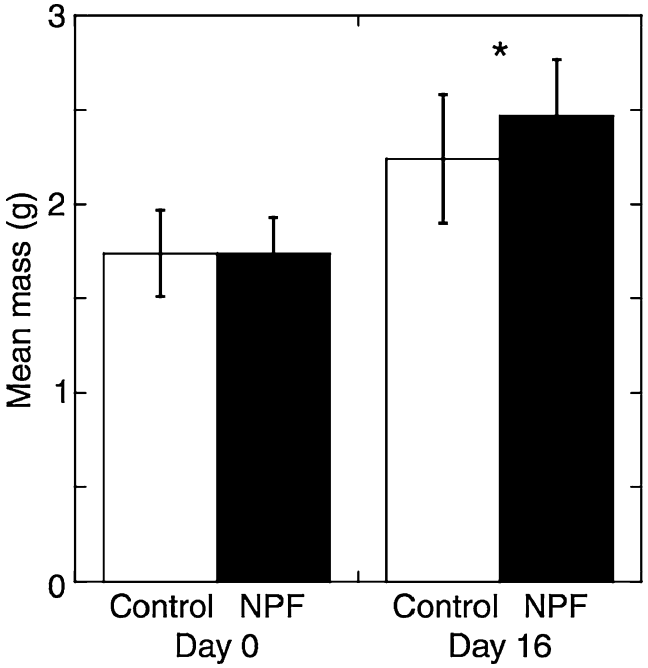

Fig. 10 Mean weights of control and neuropeptide F (NPF)-treated shrimp at the beginning (day 0) and at the end (day 16) of an experiment designed to test the effects of orally administered NPF on food intake and growth. NPF-treated individuals had their diet enhanced with a daily addition of 2 μl 10^{-3} M synthetic KPDPSQLANMAEALKYLQELDKYYSQVSRPRFamide during the 5-day trial period (but not during the pre- and post-trial periods). Initial mean weights of control and NPF-treated shrimp were the same. An approximately 10% difference in mean weights was seen between the NPF-treated animals and the control shrimp at day 16 (*error bars* standard deviation). Significance: *$P \leq 0.05$. Figure and legend used with permission from Christie et al. (2011b)

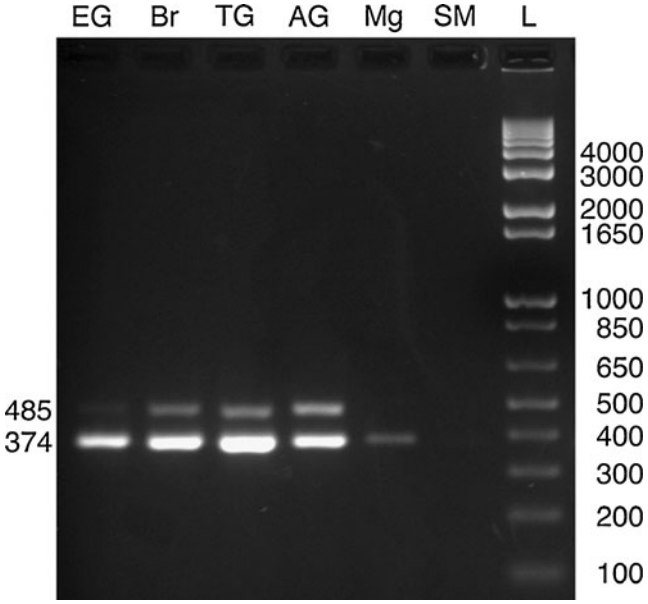

Fig. 11 Profiling by reverse transcriptase followed by the polymerase chain reaction (RT-PCR) in *Litopenaeus vannamei* (*Litva*) *prepro-NPFs* nervous system, midgut and skeletal muscle. Note that *Litva-prepro-NPF I* encodes the peptide KPDPSQLANMAEALKYLQELDKYYSQVSRPRFamide, whereas *Litva-prepro-NPF II* encodes an internally extended isoform of this peptide, possibly a splice variant (Christie et al. 2011b). By means of a gene-specific primer that amplifies products from *Litva-prepro-NPF I* and *II*, RT-PCR tissue profiling was conducted to assess the distributions of the two transcripts. A robust band of the predicted *Litva-prepro-NPF I* product (374 bp in length) was consistently detected in eyestalk ganglia (*EG*, *lane 1*), brain (*Br*, *lane 2*), thoracic ganglia (*TG*, *lane 3*) and abdominal ganglia (*AG*, *lane 4*), which are all neural tissues. Weaker bands corresponding to the predicted *Litva-prepro-NPF II* product (485 bp in length) were also consistently detected in these tissues. A weak band corresponding to the *Litva-prepro-NPF I* product was detected in some but not all, midgut samples (*Mg*, *lane 5*); no detection of the *Litva-prepro-NPF II* product was seen in this tissue. Neither *Litva-prepro-NPF I* nor *Litva-prepro-NPF II* was detected in samples of skeletal muscle (*SM*, *lane 6*). The base-pair ladder is shown in *lane 7*. Figure and legend used with permission from Christie et al. (2011b)

the gut directly and/or modulate the release of other paracrines/hormones from endocrine cells located within fore-, mid-, or hindgut epithelia (discussed later in this review). In addition, NPF receptors in the gut might act to modulate the frequency and amplitude of spontaneous contractions of the digestive tract, changing the rate of food passage or processing through it. Clearly, future experiments directed at assessing the influence of NPF on a variety of target tissues need to be conducted, as do studies exploring the possible influences of other crustacean hormones on food intake and growth.

Modulatory control of adult neurogenesis by serotonin

In both invertebrates and vertebrates, new neurons are added to the nervous system over the course of development, as well as during adulthood (e.g., Beltz and Sandeman 2003). In crustaceans, adult neurogenesis has been documented in regions of the brain and the peripheral nervous system associated with olfaction (e.g., Beltz and Sandeman 2003) and in regions of the optic system (Sullivan and Beltz 2005). Neurogenesis within the olfactory system has been extensively investigated in members of the Decapoda, particularly in asticidean species and has been shown to have a number of features in common with vertebrate adult neurogenesis (e.g., Beltz and Sandeman 2003). In astacidean crustaceans (freshwater crayfish and clawed lobsters), glial cells residing in a specialized niche on the ventral surface of the accessory lobe of the brain divide to produce daughter cells that travel in migratory streams along processes derived from niche cells to two proliferation zones: the lateral (LPZ) and medial (MPZ) proliferation zones. Here, the daughter cells divide at least once more before becoming neurons,

with those derived from the LPZ becoming new olfactory projection neurons whose somata reside in cell cluster 10 of the brain and those derived from the MPZ generating new local olfactory interneurons with somata located in cell cluster 9 (for detailed descriptions of the process of adult neurogenesis in astacideans see: Zhang et al. 2009, 2011)

Although many factors are likely to influence adult neurogenesis in crustaceans, several lines of evidence from Beltz, Sandeman and colleagues suggest a prominent role for hormonally delivered serotonin, a player in the control of neurogenesis in vertebrates (e.g., Gould 1999; Brezun and Daszuta 2000). Initially, Beltz and co-workers observed that the rate of neurogenesis in the lobster *Homarus americanus* brain decreased when serotonin was pharmacologically depleted using 5,7-dihydroxytryptamine (Benton and Beltz 2001; Beltz et al. 2001). Later, they demonstrated that the rate of neurogenesis in *Homarus americanus* was highly dependent on the serotonin concentration, with in vitro application of a low dose of serotonin (10^{-10} M) to the brain eliciting a pronounced increase in neurogenesis and application of higher concentrations of serotonin resulting in a dose-dependent decrease in neurogenesis (Benton et al. 2008; Fig. 12). More recently, the Beltz/Sandeman team have demonstrated that the paired serotonergic dorsal giant neurons (DGNs) of the crayfish *Cherax destructor* are a potential source of serotonin for affecting neurogenesis (Sandeman et al. 2009); the DGNs are located in cell cluster 11, with one DGN soma on each side of the midbrain, innervating the ipsilateral olfactory and accessory lobes. Specifically, these authors have shown that stimulation of one of the DGN pair results in a 10-fold increase in serotonin concentration in the ipsilateral cluster 10 of the brain relative to that of the contralateral cluster (Fig. 13). Moreover, they have found a significant elevation in the rate of neurogenesis within cluster 10 on the stimulated side of the brain (Fig. 14). In a just-published study (Zhang et al. 2011), Beltz and colleagues have demonstrated that, in the crayfish *Procambarus clarkii*, the actions of serotonin on neurogenesis occur not at the level of the precursors within the niche but, rather, are limited to late second-generation daughter cells and their progeny. They have likewise demonstrated that the action of serotonin on these cells is mediated through at least two distinct serotonin receptors subtypes.

Clearly, many factors are likely to influence adult neurogenesis in crustaceans (e.g., Beltz and Sandeman 2003). Whether serotonin is the sole hormonal player in the control of this process in crustacean remains an open question. As additional studies are conducted, the possible influence of other hormones on this control system will be of interest, as will whether such hormones are among those involved in the control of vertebrate neurogenesis.

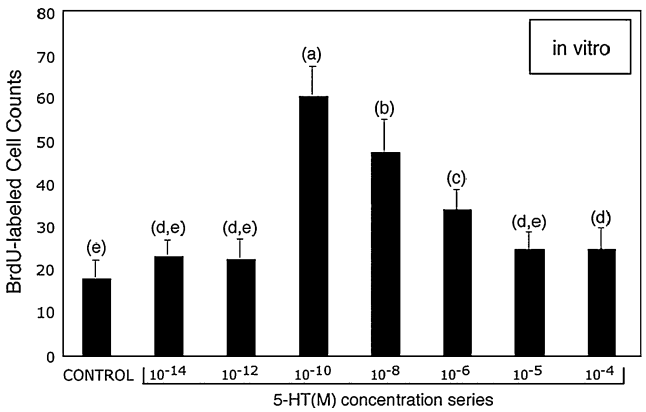

Fig. 12 Dose-response curve for serotonin (*5-HT*) and levels of adult neurogenesis in cluster 10 of *Homarus americanus* brains in vitro. The graph of 5′-bromodeoxyuridine (*BrdU*)-labeled cell counts versus serotonin levels (10^{-14} M–10^{-4} M) demonstrates that the rate of cell proliferation in cluster 10 is most sensitive to neurohormonal levels (10^{-8} M–10^{-10} M) of this amine. Analysis of variance: F=78, P<0.0001; subsequent Tukey–Kramer HSD: P<0.05). Note significant differences at serotonin levels between 10^{-10} M and 10^{-6} M relative to control brains. Statistical similarity is indicated by the *same letters* (n=10 per group for each serotonin concentration and control). Figure and legend used with permission from Benton et al. (2008)

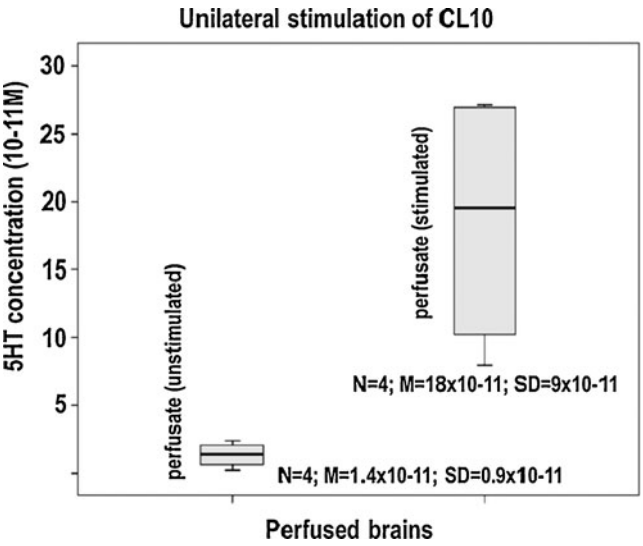

Fig. 13 Comparison between the levels of serotonin (*5-HT*) collected in the perfusate over 4 cycles from the brain of the crayfish *Cherax destructor* during periods of no stimulation (*first column*) and stimulation (*second column*) of the cluster 10 cell bodies, as measured by high pressure liquid chromatography. Serotonin levels in samples taken over 10-min periods preceding the stimulation of cell cluster 10 are about 10 times lower than levels in samples taken over 10-min periods during stimulation (independent samples t-test: P<0.013). Figure and legend used with permission from Sandeman et al. (2009)

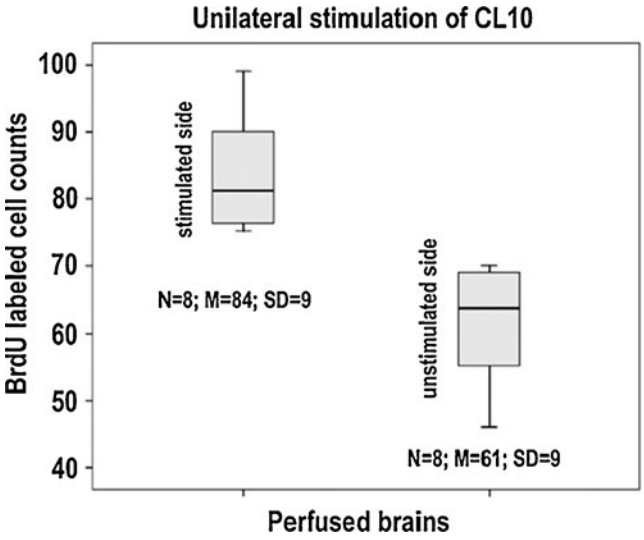

Fig. 14 Effects of stimulation of the serotonergic dorsal giant neurons (DGNs) of the crayfish *Cherax destructor* on neurogenesis in cell cluster 10 of the brain. This graph compares the counts of BrdU-labeled cells in cell cluster 10 from the stimulated and unstimulated sides of brains in which stimulation was applied unilaterally to various areas of the brain. In cell cluster 10, more cells were labeled on the stimulated ipsilateral side of the brain than in the unstimulated contralateral side (paired samples *t*-test: *P* 1⁄4 0.008). Figure and legend used with permission from Sandeman et al. (2009)

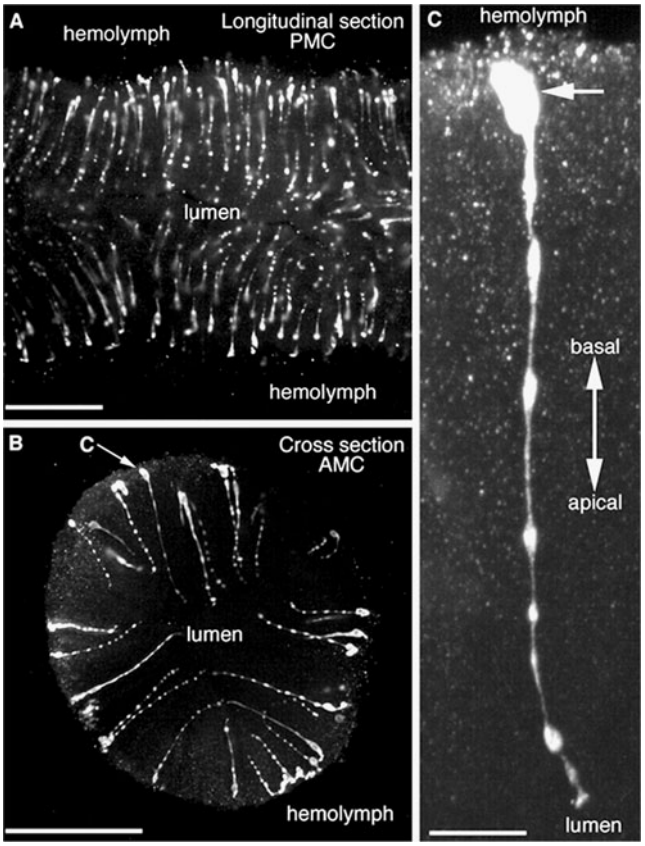

Fig. 15 General organization and morphology of peptidergic endocrine cells in the midgut of *Cancer productus*. **a** Posterior midgut caecum (*PMC*); SIFamide immunolabeling. **b** Anterior midgut caecum (*AMC*); substance P immunolabeling. Regardless of location within the midgut or of peptide content, the gross organization and morphology of the intrinsic peptidergic endocrine cells appear similar. Specifically, all cells possess an enlarged basal region and extended a thin beaded projection apically toward the midgut lumen. This organization is shown in a longitudinal section in **a** and in a cross section in **b**. The morphology of one peptidergic cell from **b** (*arrow*) is shown at higher magnification in **c**. *Bars* 200 μm (**a**, **b**), 25 μm (**c**). Figure and legend used with permission from Christie et al. (2007)

New non-neuronal sources of peptide hormones

Although the focus of this review has been neuroendocrine signaling in crustaceans, it should be noted that the nervous system is not the only endocrine source of "neurohormones" in crustaceans, particularly peptide hormones. Recent studies from several laboratories have identified epithelial endocrine cells in the crustacean fore-, mid- and hindguts as potential sources of circulating peptides originally identified from the nervous system (Chung et al. 1999; Webster et al. 2000; Christie et al. 2007; Figs. 15, 16). Likewise, recent transcriptome mining suggests that the lymphoid organ too might produce at least one "neuropeptide" (Ma et al. 2010).

With respect to the fore- and hindgut, epithelial endocrine cells containing CHH have been identified, with peptide expression and release from these cells being correlated with ecdysis (Chung et al. 1999; Webster et al. 2000). The ecdysis-timed release of CHH from these cells is hypothesized to function to stimulate the water uptake needed for recently molted animals to swell to their final postmolt dimensions (Chung et al. 1999); binding studies suggest that CHH receptors are present in the mid- and hindgut and in the gill, all of which are involved in osmoregulatory control (Chung and Webster 2006). Whether or not other peptide hormones are produced by and released from these or other fore- and hindgut epithelial endocrine cells is currently unknown.

The midgut, like the fore- and hindgut, is known to possess epithelial endocrine cells (Christie et al. 2007). In *Cancer* crabs, large numbers of these cells have been reported, with immunohistochemistry suggesting the region-specific expression of distinct complements of peptides in this tissue, i.e., SIFamide is present in epithelial endocrine cells of the anterior midgut and its associated caeca and TRP is present in those of the posterior midgut and the posterior midgut caecum (Christie et al. 2007; Figs. 15, 16). Unpublished mass

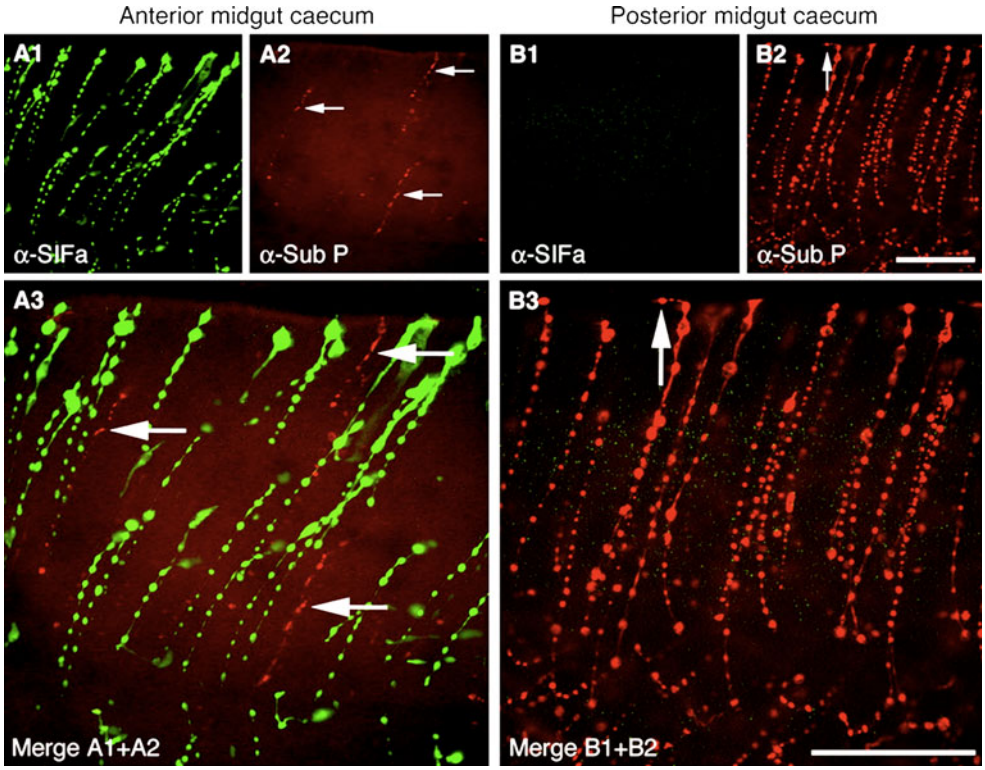

Fig. 16 SIFamide-like and substance P-like immunopositive endocrine cells are differentially distributed within the *Cancer borealis* midgut epithelium. In this and other *Cancer* species, the cells labeled by the SIFamide and substance P antibodies were differentially distributed; the substance P antibody detects a member of the tachykinin-related peptide (TRP) family in this tissue. Specifically, the SIFamide (*SIFa*)-labeled cells (pseudocolored *green*) are restricted to the epithelium of the anterior portion of the midgut proper and the anterior midgut caeca (**a**), whereas substance P (*Sub P*) immunopositive cells (pseudocolored *red*) are concentrated in the posterior portion of the midgut proper and the posterior midgut caecum (**b**). As the few substance-P-like immunopositive cells seen in the anterior midgut (*arrows*) are not among those labeled by the SIFamide antibody and *vice versa* (e.g., the *red* and *green* but not *yellow*, cells present in **a**), no colocalization of the two peptides is present in the midgut. Notably, some epithelial endocrine cells possess a short thin basal process that projects along the outer surface of the midgut (*arrows* in **b**). This type of projection is also seen in a subset of both the SIFamide- and TRP-like immunopositive cells (not shown). *Bars* 200 μm. Figure and legend used with permission from Christie et al. 2007

spectrometric studies conducted on species from a diverse set of crustacean infra-orders suggest that the complement of peptides present in the midgut is large, including not only SIFamide and TRP but also members of the FLP superfamily, orcokinins and pyrokinins (E.A. Stemmler and A.E. Christie, unpublished). Physiologically, midgut-derived peptides have been implicated in the control of feeding-related behavior/digestion in *Cancer* crabs, but are likely to be highly pleiotropic in the roles that they serve in crustaceans.

Finally, a recent investigation of the neuropeptidome of the penaid shrimp *Litopenaeus vannamei* (Ma et al. 2010) has identified transcripts encoding putative corazonin precursors from the lymphoid organ, a hemolymph filtering structure that is purported to play a role in innate immune control (van de Braak et al. 2002; Pongsomboon et al. 2008). Clearly, the interrelationship of the lymphoid organ and the hemolymph makes it a logical candidate for hormone production and release, warranting additional study as a potential endocrine organ.

Concluding remarks

Since the publication of the last comprehensive reviews on neuroendocrine signaling systems, much has changed in the field. New neuroendocrine organs have been identified and new functional roles have been ascribed to those that were known. Methodologies, including genome and transcriptome mining and mass spectrometry, have led to an explosion in peptide hormone identification. In addition, mass spectral imaging is beginning to provide a platform for mapping the three-dimensional distributions of hormones within neuroendocrine organs and other tissues. Concurrently, our understanding of the functional roles served by circulating hormones in crustaceans is expanding,

providing tools for research in new fields, including translational studies for use in aquaculture.

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