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OBSERVATIONS OF SEROTONIN AND FMRFAMIDE-LIKE IMMUNOREACTIVITY IN PALP SENSORY STRUCTURES AND THE ANTERIOR NERVOUS SYSTEM OF SPIONID POLYCHAETES

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ABSTRACT Evidence suggests that ciliated sensory structures on the feeding palps of spionid polychaetes may function as chemoreceptors to modulate deposit-feeding activity. To investigate the probable sensory nature of these ciliated cells, we used immunohistochemistry, epi-fluorescence, and confocal laser scanning microscopy to label and image sensory cells, nerves, and their organization relative to the anterior central nervous system in several spionid polychaete species. Antibodies directed against acetylated alpha tubulin were used to label the nervous system and detail the innervation of palp sensory cells in all species. In addition, the distribution of serotonin (5-HT) and FMRFamide-like immunoreactivity was compared in the spionid polychaetes *Dipolydora quadrilobata* and *Pygospio elegans*. The distribution of serotonin immunoreactivity was also examined in the palps of *Polydora cornuta* and *Streblospio benedicti*. Serotonin immunoreactivity was concentrated in cells underlying the food groove of the palps, in the palp nerves, and in the cerebral ganglion. FMRFamide-like immunoreactivity was associated with the cerebral ganglia, nuchal organs and palp nerves, and also with the perikarya of ciliated sensory cells on the palps.

Key Words: Annelida, Spionidae, neurotransmitter, chemosensory, CLSM, SEM

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Polychaete annelids commonly possess sensory cells with microtubule-containing cilia that pass through the cuticle into the environment. Ciliated sensory cells occur alone or grouped into sense organs (*e.g.*, nuchal organs) and may have one or more cilia (Mill, 1978; Storch and Schlötzer-Schrehardt, 1988). Ciliated cells underlie several annelid sensory modalities (including vision, mechanoreception and chemoreception) that are processed by the central nervous system (Mill, 1978). Among these senses, chemoreception modulates a variety of ecologically important behaviors including spawning (Hardege et al., 1996; Hardege and Bentley, 1997; Hardege, 1999), possibly spermataphore transfer in spionids (Rice, 1978, 1991), larval settlement (reviewed by Qian, 1999), juvenile post-settlement movement (Woodin et al., 1995), and feeding (Copeland and Weiman, 1924; Rullier, 1950; Ferner and Jumars, 1999; Kihslinger and Woodin 2000; Riordan and Lindsay, 2002; Mahon and Dauer, 2005)

Among the polychaetes, nuchal organs are typically considered to function in chemoreception based on histological, ultrastructural and positional criteria (Storch and Schlötzer-Schrehardt, 1988; Rhode, 1990; Purschke, 1997, 2005, but see Fewou and Dhainaut-Courtois, 1995 for a potential osmoregulatory function). Although physiological evidence for chemoreception is limited, several researchers have linked nuchal organs to chemoreceptive behaviors. For example, Rullier (1950) demonstrated that nereidid polychaetes without nuchal organs failed to feed. Nuchal organs are composed of ciliated supporting cells, bipolar primary sensory cells with cilia, unmodified epidermal cells and retractor muscle cells (in those species where nuchal organs can be retracted) (Storch and Schlötzer-Schrehardt, 1988; Purschke, 2005). Other presumed chemosensory structures have been described, including epidermal papillae of the deposit-feeding lugworm *Arenicola marina* (Jouin et al., 1985), compound sensory organs on the prostomial cirri and palps of *Nereis diversicolor* (Dorsett and Hyde, 1969), and the parapodial cirri of nereidid polychaetes (Boilly-Marer, 1972). In

Platynereis dumerlii, the receptors of the parapodial cirri function in perception of sexual pheromones (Boilly-Marer, 1968, 1974). In spionid polychaetes, females of some species use feeding palps to manipulate spermatophores transferred from males (Rice, 1978), and some females can discriminate between spermatophores produced by conspecifics *versus* closely related species (Rice 1978, 1991).

Presumed sensory structures have been observed on the feeding palps of several species of spionid polychaetes (Dauer, 1984, 1997; Worsaae, 2001; Riordan and Lindsay, 2002). Whether such cells are mechanosensory or chemosensory has largely been argued based on ultrastructural and positional criteria. Very few functional studies exist, but recent activity-dependent cell labeling studies by Lindsay et al. (2004) suggest that the ciliated cells found on spionid feeding palps function as chemoreceptors and possibly also as mechanoreceptors. The same chemical cues that elicited feeding responses from the spionid *Dipolydora quadrilobata* (Riordan and Lindsay, 2002) also activated the sensory cells on the palps (Lindsay et al., 2004).

A number of functional ciliary groups on the palps of spionids have been described (Dauer, 1985; Qian and Chia, 1997; Worsaae, 2001). The ciliary groups can generally be classified as 1) *frontal*: cilia of the medial food groove, 2) *latero-frontal*: a single row at the lateral edge of the food groove, 3) *lateral*: (including papillae) a row of cilia beyond the latero-frontals, and 4) various motile and non-motile groups scattered on the medial and dorsal surfaces of the palp, including the dorsally located *abfrontal* cilia. The lateral and abfrontal cilia are presumed to be chemoreceptors, while the latero-frontal cilia are considered good candidates for mechanoreceptors. The relationship of these ciliated cells to the central nervous system has not been well described. The goal of this study was to describe the relationship between these cells and the nervous system, and to compare the composition of the anterior nervous system in spionid polychaetes by examining the occurrence and distribution of two common neuropeptides, serotonin and FMRFamide.

Serotonin (5-hydroxytryptamine, 5-HT) is a widely distributed biogenic indolamine (Aghajanian, 1987) created by enzymatic modification of the essential amino acid tryptophan. Serotonin occurs in most invertebrate and vertebrate nervous systems studied (Goldberg et al., 1994). Invertebrates (particularly leeches and molluscs) have been model systems for serotonin neurobiology since the 1950's (Osborne, 1980). Serotonin appears to be the most widespread amine in the polychaete nervous system (Golding, 1992). Serotonin may be found in the cerebral and segmental ganglion, and in several species serotonergic fibers innervate the somatic muscles, pharynx and gut. As a neurotransmitter and modulator, 5-HT has a wide range of physiological effects and in polychaetes it may be a major transmitter in both muscular and sensory systems (Golding, 1992). When examined using confocal laser scanning microscopy, immunoreactivity to 5-HT typically results in detailed reconstructions of the polychaete nervous system (e.g., Müller and Westheide, 2002; Orrhage and Müller, 2005).

The neural tetrapeptide FMRFamide (Phe-Met-Arg-Phe-NH_2), and related compounds: FaRPs (FMRFamide-related peptides, Price and Greenberg, 1989) or FLPs (FMRFamide-like peptides, Mercier et al., 2003) have a range of physiological actions in various organisms, including the modulation of muscle contraction potential, digestive enzyme secretion, synaptic transmission to the central nervous system, and cardiac output (Price and Greenberg, 1977; Favrel et al., 1998; Baux et al., 1992; Mercier et al., 2003). Widespread throughout the annelid CNS, FMRFamide probably functions as a neurotransmitter and modulator. Immunoreactivity to the neuropeptide (or related compounds) is particularly strong in the perikarya of mono- and bi-polar neurons (Golding, 1992). As a neurotransmitter in the polychaete *Nereis virens*, FMRFamide appears to act (perhaps with serotonin) on the muscles of the esophagus and gut to control intestinal motility (Krajniak and Greenberg, 1992). FMRFamide-like peptides also increase muscular contractions in the pharynx of the leech *Hirudo medicinalis* (O'Gara et al., 1999). Interestingly, immunoreactivity to FMRFamide antibodies revealed portions of the stomatogastric nervous system in dinophilid polychaetes that were not labeled by 5-HT antibodies (Müller and Westheide, 2002).

The neuroanatomy and sensory systems of spionids have been studied since at least the early part of the twentieth century, and probably much earlier. Researchers were debating the evolution and sensory nature of spionid nuchal organs since the 1920's (Schlötzer-Schrehardt, 1987 citing Söderstrom, 1920, 1930 and Jeener, 1927). Much of the anterior spionid CNS morphology (specifically of the cephalic region, including the

circumesophageal connectives and cerebral ganglion) was meticulously examined by Lars Orrhage in the early 1960's. According to Orrhage, spionids contain seven to eight palp nerve roots (which branch into several more palp nerves), more than any other 'sedentary' polychaete family (Orrhage, 1965, 1990, 1995). Consequently, spionids have a relatively well developed and complex cerebral ganglion innervated by palp nerves that presumably transmit sensory information from ciliated receptors on the palp. Previous studies of the spionid polychaete nervous system have focused mainly on the anterior CNS and dorsal ciliated organ morphology and ultrastructure (Orrhage, 1965; Schlötzer-Schrehardt, 1987; Jelsing 2003). In this study, we focus primarily on the sensory cells of the feeding palps and their relationship to the central nervous system.

MATERIALS AND METHODS

Animal collection and maintenance The spionid polychaetes used in these experiments were maintained in laboratory colonies at the University of Maine in Orono. Sediment cores (120 mL) were taken at low tide from mudflats at Lowes Cove, Walpole, ME (Darling Marine Center) and transported to the laboratory in insulated coolers containing seawater. Worms were later collected with a 0.5 mm sieve, removed from their tubes, and identified to the species level (*Dipolydora quadrilobata* (Jacobi), *Streblospio benedicti* Webster, *Pygospio elegans* Claparède, and *Polydora cornuta* Bosc). Worms of each species were housed in separate aquaria containing aerated seawater and 0.5 mm sieved sediment. Aquaria were maintained in an environmental chamber (14°C; 12 H light:dark cycle). Colonies were fed sieved, defaunated sediment mixed with algae paste (Innovative Aquaculture Products). Water was changed monthly and excess algal growth on the walls of the aquaria was removed. A surface layer of diatoms was usually present on the sediment surface.

Scanning Electron Microscopy For scanning electron microscopy, worms were relaxed in chilled isotonic magnesium chloride in seawater, fixed for 1 h on ice in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) with 10% sucrose, then post-fixed on ice in 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7.4) for 1 h, and dehydrated in an ethanol series. Samples were then critical point dried with liquid CO_2, mounted on stubs, and coated with gold palladium in a Conductavac I (SeeVac, Inc.) sputter coater. Samples were viewed with an AMRay AMR1000A scanning electron microscope operating at 5 kV. Negatives were scanned at 600 dpi with an Epson V700 Photo flatbed scanner and the resulting images were saved as TIFF files.

Immunohistochemistry Indirect immunohistochemistry methods were used to label ciliated sensory cells and nerves. Worms were removed from their tubes and put into the wells of a porcelain spot plate (CoorsTek) in a humid chamber; each plate well holds 0.5 mL of solution. Worms were relaxed (~5 min.) with isotonic magnesium chloride ($MgCl_2$) then fixed at 4°C overnight with 4% paraformaldehyde in artificial seawater (ASW, Forty Fathoms ®, 'Marinemix'; adjusted to 32‰ and pH 7.4). After three, 10 min ASW rinses, specimens were incubated in blocking solution (0.5% bovine serum albumin, BSA, and 0.5% Triton X-100 in ASW) for 5 h at room temperature. After rinsing, primary antibodies were applied overnight at 4°C in 0.5% Triton X-100 in ASW (ASW/T). The primary antibodies used were: 1) monoclonal mouse anti-acetylated α-tubulin (Sigma; clone 6-11-B1, dilution 1:100); 2) polyclonal rabbit anti-serotonin (Sigma; 1:100); and 3) polyclonal rabbit anti-FMRFamide (ImmunoStar; 1:1000). After three rinses, fluorophore-conjugated secondary antibodies were applied (~ 5 h, 1:100 in ASW/T at room temperature in a dark box). Secondary antibodies used were: 1) FITC-goat anti-mouse (Sigma), and 2) TRITC-goat anti-rabbit (Sigma) or 3) Alexa Fluor 647-goat anti-rabbit (Molecular Probes). After three final ASW rinses, the specimens were mounted in Fluoromount-G ® (Southern Biotechnology Associates) on glass slides, coverslipped and stored in slide folders at 4°C. For negative controls the specimens were treated as described only omitting the primary antibody. Reproducibility was checked with repeated preparations of multiple worms. Acetylated αtubulin antibodies have been used to label and image the nervous systems and ciliated sensory organs of several families of polychaetes (e.g., Muller and Westheide, 2000, 2002; Purschke and Hessling, 2002). Because the antibody to FMRFamide may recognize only the RFamide-motif, we follow the practice of describing positive antibody staining as "FMRF-amide-like immunoreactivity" (e.g., Orrhage and Müller, 2005). FMRFamide-like IR was examined only in *Dipolydora quadrilobata* and *Pygospio elegans*, while serotonin IR was examined in all four species.

Digital Imaging Conventional epifluorescence and confocal microscopy were used to visualize labeled cells. Digital images were captured with a SPOT RT CCD camera mounted to an Olympus BX-60 (epifluorescence) microscope, or a Leica TCS SP2 confocal laser scanning microscope system. Digital image processing (contrast, level and/or gamma adjustments, image overlays, measurement, and depth coding) was done with Image-J (http://rsb.info.nih.gov/ij/), Adobe Photoshop, SPOT Advanced (Diagnostic Instruments) and Leica software.

RESULTS

In *Dipolydora quadrilobata*, the ciliated nuchal organs lie on either side of the dorsal caruncle, and

extend from the prostomium through the first three setigers (Fig. 1A). An additional dorsal ciliated organ was observed lateral to the nuchal organs in some specimens (Fig. 1B). FMRFamide-like immunoreactivity was observed in the sensory cells underlying the cilia of the nuchal organs, and associated with perikarya adjacent to the dorsal ciliated organs (Fig. 1B), as well as with the several (but not all) palp nerves (Fig. 1C, F), the stomatogastric nerves (Fig. 1C), and sensory cells on the mouth and prostomium (Fig. 1C). FMRFamide-like immunoreactivity was also observed in the supraesophageal ganglion and dorsal and ventral roots of the circumesophageal connectives (data not shown). Serotonin immunoreactivity was particularly strong in cells of the buccal lips of the mouth, and in cells underlying the ciliated food groove of the palp (Fig. 1D). On either side of the ciliated food groove of the palp was a row of laterofrontal cilia, and the lateral and abfrontal palp surfaces were covered with scattered ciliated structures presumed to be sensory. Perikarya of the laterofrontal, lateral and abfrontal bipolar sensory cells showed FMRFamide-like immunoreactivity (Fig. 1F) but not serotonin IR.

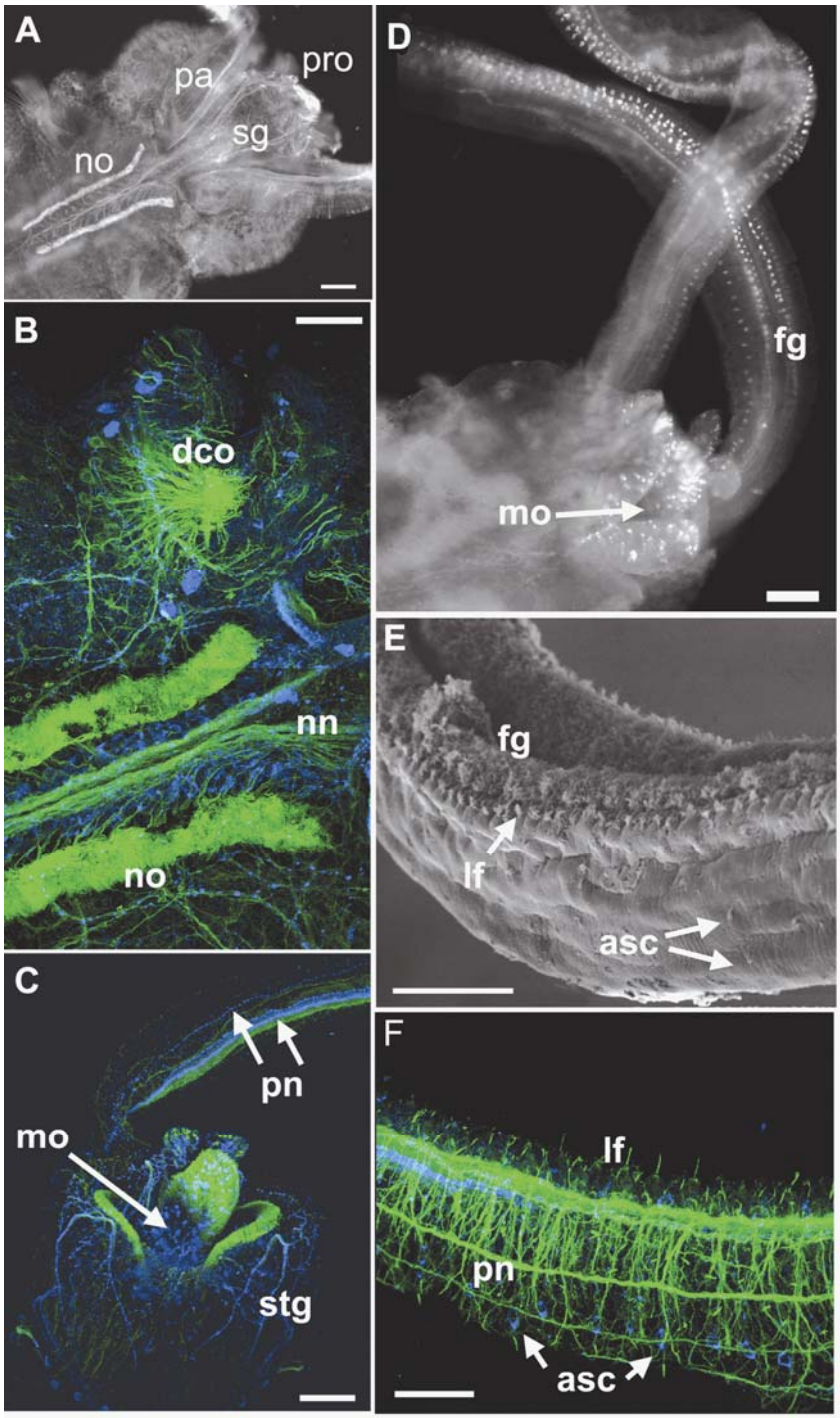

Fig. 1. *Dipolydora quadrilobata* anterior and palp sensory structures showing immunoreactivity to acetylated αtubulin (light gray or green, A-C, F), FMRFamide (blue, B,C,F), or serotonin (light gray , D). In A-D, worms are oriented with anterior to the right or up. A: Epifluorescence, dorsal view, scale 25 μm. B: Confocal maximum intensity projection (MIP), composite image, dorsal view, scale 40 μm. C: Confocal MIP, ventral view of prostomium, scale 80 μm. D: Epifluorescence, ventral view, scale 100 μm. E: Palp, middle portion, scale 25 μm, SEM. F: Confocal MIP, middle portion of palp, food groove is up, scale 40 μm. asc, abfrontal sensory cells; dco, dorsal ciliated organ; fg, food goove of palp; lf, laterofrontal cilia; mo, mouth; no, nuchal organ; nn, nuchal nerve; pa, palp; pn, palp nerve; pro, prostomiuim; sg, supraesophageal ganglion; stg, stomatogastric nerves.

In *Pygospio elegans*, the nuchal organs are bands of cilia on the dorsal surface of the first setiger, behind the palps (Fig. 2A-C). Acetylated αtubulin immunoreactivity showed processes from the sensory cells in the nuchal organs bundling into the nuchal nerves, which join the dorsal root of the circumesophageal connective at the dorsal ganglion in the ventral portion of the brain (Fig. 2 B,C), along with one of the main palp nerve roots. Serotonin immunoreactivity was observed in the supraesophageal ganglion and several palp nerves (Fig. 2B, F). Serotonergic cells were observed underlying the ciliated food groove of the palps (Fig. 2B, red cell bodies), but these cells were less abundant and more widely spaced than those observed for *Dipolydora quadrilobata*.

Palps of *P. elegans* have a central ciliated food groove, with two distinct rows of laterofrontal cilia, a row of lateral cilia on either side of the palp, and scattered abfrontal ciliated structures (Fig. 2D). Acetylated αtubulin immunoreactivity labeled the cilia of the food groove, other ciliated cells and palp nerves (Fig. 2E). The perikarya of the abfrontal cells and the palp nerves under the food groove also showed FMRFamide-like immunoreactivity.

Streblospio benedicti have a single pair of branchiae located immediately posterior to the feeding palps (Fig. 3A); we did not examine nuchal organs in this species. Cilia of the branchiae and palp food groove, main palp nerves, and anterior

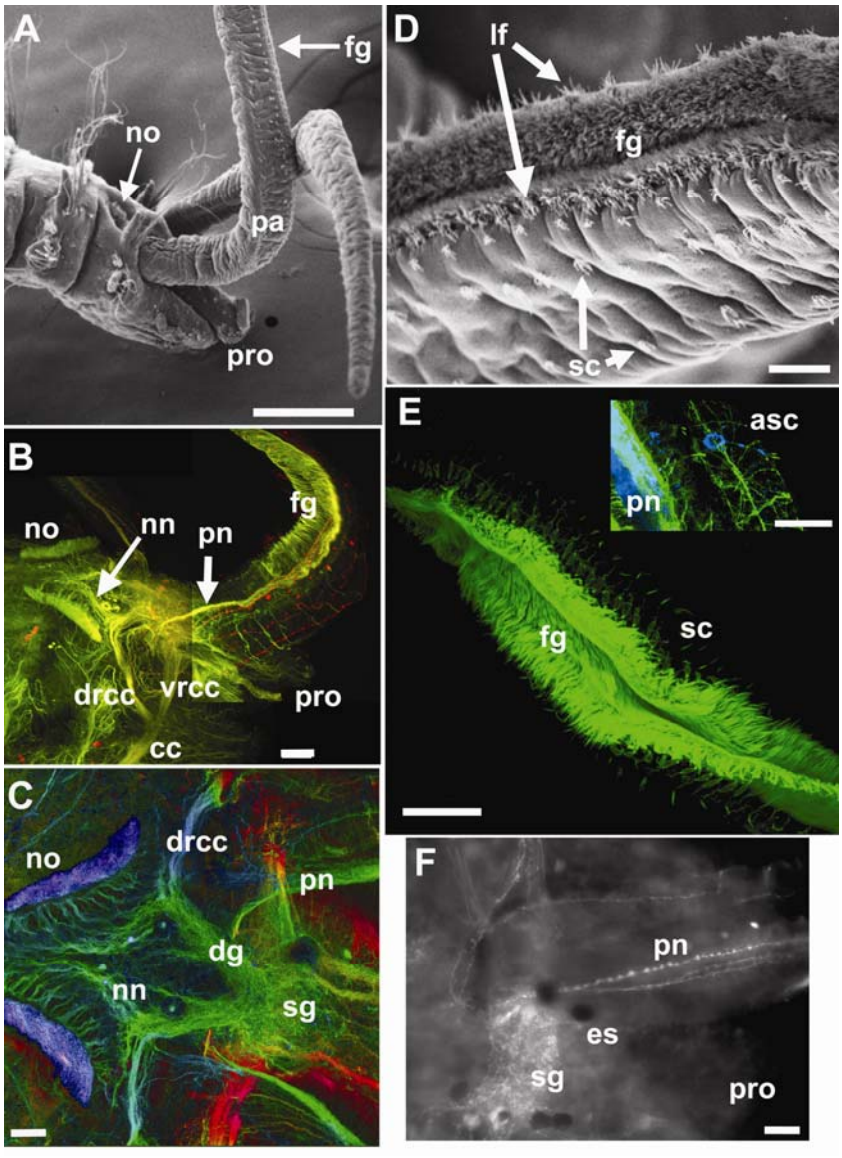

Fig. 2. *Pygospio elegans* anterior and palp sensory structures showing immunoreactivity to acetylated αtubulin (yellow or green, B, E), FMRFamide (blue, E inset), or serotonin (red or light gray, B, F). In A-C and F, worms are oriented with anterior to the right. A: Head, scale 100 μm, SEM. B: Confocal maximum intensity projection (MIP) of the head, composite image, acetylated αtubulin and serotonin IR, lateral view, scale 40 μm. C: Confocal depth-coded MIP showing acetylated αtubulin IR in the nuchal organs and central nervous system; dorsal view, scale 20 μm. D: Palp, middle region, lateral view, scale 10 μm, SEM. E: Confocal MIP of the cilia on the palp, acetylated αtubulin IR, scale 40 μm with FMRFamide-like IR in the cell body of an abfrontal sensory cell on the palp (inset, scale 20 μm). F: Epifluorescence, serotonin-IR in the palp and central nervous system, dorsal view, scale 25 μm. asc, abfrontal sensory cell; cc, circumesophageal connective; drcc, dorsal root circumesophageal connective; dg, dorsal ganglion; es, eyespot; fg, food groove; lf, laterofrontal cilia; no, nuchal organ; nn, nuchal nerve; pa, palp; pn, palp nerve; pro, prostomium; sc, sensory cells; sg, supraesophageal ganglion; vrcc, ventral root circumesophageal connective.

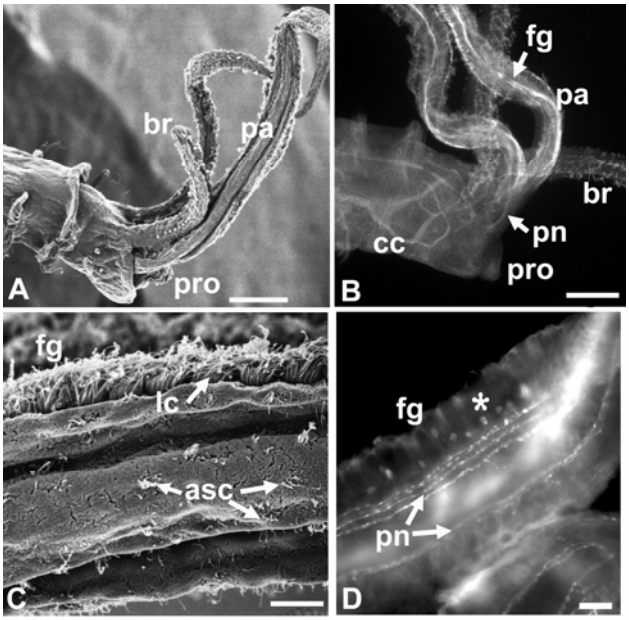

Fig. 3. *Streblsopio benedicti* anterior and palp sensory structures. A: head, showing feeding palps and the single pair of branchiae, scale 100 μm, SEM. B: Acetylated αtubulin IR reveals the central nervous system, palp nerves, and cilia of the feeding palps and branchiae, epifluorescence, lateral view, scale 200 μm. C: Palp, middle portion, lateral view, scale 10 μm, SEM. D: Serotonin IR in the palp labels the palp nerves and cells underlying the food groove (asterisk), epifluorescence, scale 25 μm. br, branchiae; cc, circumesophageal connective; fg, food groove; lc, lateral cilia; pa, palp; pn, palp nerve; pro, prostomium; sc, sensory cell.

central nervous system showed strong acetylated αtubulin immunoreactivity (Fig. 3B). Palp ciliation was similar to *Dipolydora quadrilobata* and *Pygospio elegans*, with a row of laterofrontal cilia on either side of the food groove. Next to the laterofrontal cilia was a row of lateral cilia (images not shown, see also Dauer 1984). Presumed sensory cilia were on the lateral and abfrontal surfaces of the palp (Fig. 3C). Similarly, several palp nerves showed strong serotonin immunoreactivity, as did cells under the ciliated food groove (Fig. 3D).

Acetylated αtubulin labeled the nuchal organs, nuchal nerves, food groove cilia and palp nerves of *Polydora cornuta* (Fig. 4A). The palps had two rows of laterofrontal cilia on papillae in addition to the lateral and abfrontal sensory cilia (Fig. 4B) that were observed in other species. As in the other species, serotonergic cells were observed under the ciliated food groove, occurring in clusters at

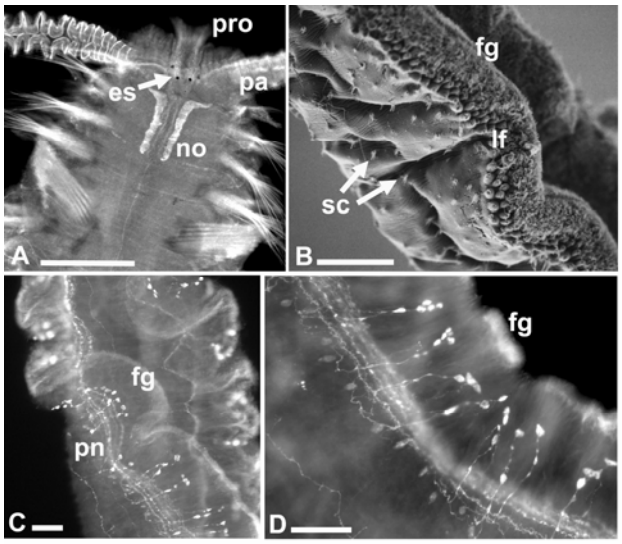

Fig. 4. *Polydora cornuta* anterior and palp sensory structures. A: Acetylated αtubulin IR in the palps and nuchal organs shown in light gray, epifluorescence, dorsal view, scale 500 μm. B: Palp, middle portion, scale 50 μm, SEM. C, D: Serotonin IR in the palp nerves and cells underlying the food groove of the palp (light gray), epifluorescence, scales 50 μm. es, eyespot; fg, food groove; lf, laterofrontal cilia; no, nuchal organ;pa, palp; pn, palp nerve; pro prostomium; sc, sensory cells.

approximately 25 μm intervals, and projecting to the palp nerves (Fig. 4C,D).

DISCUSSION

The organization of the central nervous system in the spionid polychaetes examined in this study conforms to the general polychaete ground plan (Orrhage and Müller , 2005). As in previous studies with annelids (Hessling and Purschke, 2000; Müller and Westheide, 2000), the acetylated αtubulin antibody labeled the anterior central nervous system as well as the nuchal organs, dorsal ciliated organs, the ciliated food groove, and lateral and abfrontal ciliated cells of the palps. The results for *Pygospio elegans* generally agree with previous ultrastructural studies: the nuchal nerves are composed of axon bundles (fascicles of fibers) originating from the posterior circumesophageal connectives, and an efferent nerve plexus at the base of the ciliated cells which emanates from a dorsal nerve cord (Schlötzer-Schrehardt, 1987). In *Dipolydora quadrilobata* and *Polydora cornuta*, the nuchal nerves are longer, but the nerve plexus at the base of the ciliated cells is similar to that described for *P. elegans* (Schlötzer-Schrehardt, 1987).

The palp nerves are probably composed of afferent sensory and efferent motor fibers that are related to feeding behavior. As has been described for other polychaetes (Orrhage, 1995; Orrhage and Müller, 2005) the palp nerves innervate various regions of the mid- and posterior cerebral ganglion and circumesophageal connectives. Latero-frontal and abfrontal palp cells are linked to the CNS *via* different palp nerves; this finding is illustrated most clearly for *Dipolydora quadrilobata* (Fig. 1) but was consistent in all four species.

Distribution of FMRFamide and Serotonin

In previous studies of spionid feeding palp functional morphology, ciliated cells on the lateral and abfrontal surfaces of the palps have been presumed to be sensory based on morphological and positional characteristics (i.e., *Streblospio benedicti*, Dauer ,1984; *Dipolydora quadrilobata* and *Polydora cornuta*, Worsaae, 2005). Such characterizations are supported by results of activity dependent cell labeling studies in *D. quadrilobata* palps showing an amino acid-induced increase in (abfrontal) ciliated palp cell activity (Lindsay et al., 2004). In the current study, FMRFamide-like immunoreactivity was specifically localized in several palp nerves, perikarya and neural processes of abfrontal ciliated cells in the palps of *D. quadrilobata* and *Pygospio elegans*. These results lend additional support to the hypothesis that such cells are primary olfactory neurons, but electrophysiological studies will be required to definitively characterize their sensory modalities. Moreover, a chemosensory function need not be limited to feeding as the palps are also involved in spermataphore transfer and discrimination in several spionids (Rice, 1978, 1991).

FMRFamide-like immunoreactivity was also observed in the stomatogastric nerves and and in perikarya in the pharynx of *Dipolydora quadrilobata* (Fig. 1). This finding is interesting given that FMRFamide in the pharynx of the leech has been associated with a protein kinase C second messenger system that controls motility of the pharyngeal muscles (O'Gara et al., 1999). O'Gara et al. (1999) also provide evidence of an RFamide-IP_3 (inositol phosphate) second messenger system. Müller and Westheide (2002) also observed complex FMRFamide-like immunoreactivity in the stomatogastric nervous system of four dinophilid polychaetes. In *D. quadrilobata*, FMRFamide- like immunoreactive perikarya of the nuchal nerves probably correspond to the "bipolar primary sensory cells" described by Jelsing (2003). It is possible that FMRFamide in spionids has several functions and may be acting as a neuromuscular transmitter in the CNS and pharynx, and as a sensory neurotransmitter (*via* second messengers) in the abfrontal cells of spionid palps.

Perhaps the most significant discovery of this study is the presence of 'serotonergic palp cells' (SPCs) underlying the food groove in all spionids studied. These cells do not correspond to any known polychaete serotonergic cell type. The SPCs show considerable morphological variation between species. *Polydora cornuta* has the most intricate structure, with clusters of cell bodies occurring at regular intervals along the palp. In contrast, the distribution of SPCs was somewhat sparser *Pygospio elegans* and S*treblospio benedicti*.

The SPCs are found along the length of the food groove, particularly in the lateral margins (e.g., Figs. 1,4). The SPCs are innervated by serotonergic palp nerves that extend toward the posterior region or the cerebral ganglion or circumesophageal connectives (Figs. 2 - 4). The function of the spionid SPCs is unknown but they may have a role in feeding behavior, perhaps either by modulating the beating frequency of palp cilia, or else by controlling mucus secretion. In phoronid actinotroch larvae, for example, 5-HT-immunoreactive fibers are found under the ciliated bands of the feeding tentacles (Hay-Schmidt, 1990), and 5-HT was shown to increase the ciliary beat frequency (CBF) of *Mytilus edulus* gill cilia (Paparo and Aiello, 1970).

In summary, serotonin immunoreactivity was concentrated in cells underlying the food groove of the palps, in the palp nerves, and in the cerebral ganglion of all four spionid species examined. FMRFamide-like immunoreactivity was associated with the cerebral ganglia, nuchal organs and palp nerves, and also with the perikarya of ciliated sensory cells on the palps in *Dipolydora quadrilobata* and *Pygospio elegans*. The FMRFamide-like immunoreactive palp cells were not the same as the serotonergic palp cells distributed under the lateral margins of the ciliated food groove. Although additional functional assays will be required to definitively assign a specific sensory modality (i.e., chemoreception vs. mechanoreception) to the ciliated cells of the feeding palps, our results clearly confirm the sensory nature of these cells.

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