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REGULAR ARTICLE

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Histochemical survey of transmitters in the central ganglia of the gastropod mollusc *Phestilla sibogae*

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Abstract The aeolid nudibranch *Phestilla sibogae* is well studied in terms of its larval nervous system and neuronal involvement in metamorphosis. Central neurones in the adult have also been identified anatomically and electrophysiologically. We describe the neurotransmitter contents of these neurones and provide details of neuritic projections and developmental changes during growth (3 to 18 mm body length). Central ganglia from specimens of all sizes contained 100–115 serotonin-immunoreactive neurones, some of which appeared to be homologues of cells identified in other gastropods. Tyrosine hydroxylase immunoreactivity and aldehyde-induced fluorescence marked a common set of 28–30 catecholaminergic neurones located anteriorly in the cerebropleural ganglia and laterally in the pedal ganglia. Ganglionic neuropile and nerve trunks also contained many catecholaminergic fibres. About 65–100 intensely labelled FMRFamide-immunoreactive neurones were located symmetrically throughout the central ganglia, although one population was located only in the right pedal ganglion. Another 40–45 FMRFamide-immunoreactive neurones were weakly or variably stained. Central ganglia also contained 27–29 intensely labelled pedal-peptide-immunoreactive neurones, including those that were apparently homologues of cells previously described in *Tritonia diomedea*, and 16–19 weakly labelled pedal-peptide-immunoreactive neurones, including giant cerebropleural neurones coexhibiting FMRFamide immunoreactivity. Little cell addition involving any transmitter phenotype occurred as animals grew in body length, body growth being accommodated by growth in the size of individual cells, consistent with an approximate doubling in the size of the ganglia themselves.

Keywords Serotonin · Catecholamine · Dopamine · FMRFamide · Pedal peptide · Central ganglia · Gastropod · *Phestilla sibogae* (Mollusca)

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Introduction

The nudibranch gastropod *Phestilla sibogae* has been well studied as a model for understanding several aspects of the neuronal bases of behaviour. The larvae of this tropical species also serve as convenient models of early development (Bonar and Hadfield 1974; Kempf et al. 1992, 1997) since they lay eggs all the year round and the larvae hatch within a week of oviposition. After a few days, the larvae can be induced to metamorphose into juvenile sea slugs by means of extracts of the sole prey of adult *P. sibogae*, viz., a species of coral, *Porites compressa* (Hadfield 1978; Hadfield and Pennington 1990). *P. sibogae* is therefore highly amenable to studies of neural involvement in triggering molluscan metamorphosis (Hadfield et al. 2000; Pires et al. 1997, 2000; Pires and Hadfield 1991). In the adult, individual cells have been characterized morphologically and electrophysiologically, and their electrical activities have been correlated with specific behaviour patterns and movements (Willows 1985). The anterior sensory organs (rhinophores, tentacles and oral and cephalic shields) of *P. sibogae* have been studied intensively, because these structures are highly sensitive to specific cues emanating from their exclusive prey (Boudko et al. 1998, 1999). In addition, the time from hatching to egg-laying in *P. sibogae* has previously been reported to be as short as 27 days (Miller and Hadfield 1990) but recent studies suggest that the interval until maturation of the testes

may even be more abbreviated (Todd et al. 1997). Such a short generation time provides a unique opportunity for genetic studies of neurodevelopment and behaviour.

Although extensive research has been conducted into the physiology and development of *P. sibogae*, nothing is known about the distribution of various neurotransmitters within adults of this species. In the present study, we have begun to address this deficiency by examining the central distributions of four types of neurotransmitters common in the gastropod nervous system. Previous work has established that serotonin (5-HT), various catecholamines and the tetrapeptide, Phe-Met-Arg-Phe-NH_2 (FMRFamide), and related peptides occur abundantly in the nervous systems of other gastropods (Greenberg and Price 1992; Walker 1986) and/or within the larvae of *P. sibogae* (Kempf et al. 1992, 1997; Pires et al. 1997, 2000). In addition, a family of pedal-peptides (e.g. TPep; first characterized in *Aplysia californica* (Hall and Lloyd 1990; Pearson and Lloyd 1990), has more recently also been found in other gastropods (Lloyd et al. 1996; Willows et al. 1997). All four of these transmitter types can be reliably detected by using standard histochemical and immunohistochemical procedures. These procedures have therefore been applied to whole-mounted ganglia of *P. sibogae* to offer the best correspondence between our findings and previous electrophysiological work. Finally, because whole-mount immunocytochemistry facilitates comparisons between individuals of different sizes, we have also exploited this opportunity to provide a general description of post-larval development of the central nervous system while *P. sibogae* grows six-fold in body length.

Materials and methods

Animals

Specimens of *Phestilla sibogae* were collected from the wild and maintained in flow-through sea-water tables under ambient light and temperature at the Kewalo Marine Laboratories, Honolulu, Hawaii. *Porites compressa* was fed to the slugs *ad libitum*. Although *P. sibogae* routinely attains lengths of 30–40 mm (Todd et al. 1997), whole-mount staining worked best in smaller specimens. We therefore examined specimens 3–18 mm in length. Over 150 specimens were examined with a minimum of ten animals being examined for each transmitter-specific procedure described below. Extended body lengths of animals were measured by using a dissecting microscope and ocular micrometre while specimens crawled about in Petri dishes containing natural sea water.

Immunocytochemistry

Procedures modified from Croll and Chiasson (1989) were used for whole-mount immunohistochemistry. Briefly, slugs of various sizes were first decapitated by using a razor. The entire head region was then fixed for 4–12 h either in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) at 4°C for the subsequent detection of 5-HT or neuropeptides or in methanol at –18°C for the detection of tyrosine hydroxylase (TH), which catalyzes the initial step in the conversion of tyrosine to the various catecholamines (Cooper et al. 1996; Pani and Croll 1998; Pires et al. 2000). After fixation, the tissues were washed several times in phosphate-buffered saline (PBS; 50 mM Na_2HPO_4, 140 mM NaCl, pH 7.2) and bathed overnight in a blocking solution of 1% Triton X-100 and 1% bovine serum albumen (BSA) in PBS. The tissues were then incubated at 4°C for 2–3 days in one of the primary antibodies. The anti-5-HT and anti-FMRFamide IgGs were raised in rabbits and obtained from IncStar (DiaSorin, Stillwater, Minn.). Antibodies to *Tritonia* pedal-peptide (TPep) were also raised in rabbits and donated by A. O. D. Willows (University of Washington). These polyclonal antibodies were used at a 1:500 dilution. The monoclonal anti-TH antibody was developed in mouse and obtained from IncStar. This latter antibody was diluted 1:50.

Following incubation in primary antibodies, the tissues were given an additional three or four 1-h washes in PBS and then incubated for 12–24 h in goat anti-rabbit or sheep anti-mouse antibodies (Jackson Labs, West Grove, Pa.) labelled with either fluorescein isothiocyanate (FITC) or Texas Red. After several further washes in PBS, the central ganglia were dissected from surrounding tissues and mounted between glass coverslips in a 3:1 solution of glycerol to 0.1 M TRIS buffer (pH 8.0) with the addition of 2% n-propyl gallate (Giloh and Sedat 1982). Preparations were viewed and photographed on a Zeiss Axiophot microscope equipped with filter blocks with a 510–560 nm excitation and 590 nm longpass barrier filter for viewing Texas Red and a 450–490 nm excitation and 515–565 nm bandpass barrier filter for viewing FITC.

Use of FITC- and Texas Red-labelled secondary antibodies yielded identical results. Additional control experiments involved the use of similar procedures as described above except for the replacement of the primary antisera with either 1% normal rabbit or mouse serum or with the serum diluant alone. No staining was observed in any of these control preparations.

Aldehyde-induced histofluorescence

Head regions from other specimens were processed to yield aldehyde-induced fluorescence of catecholamines (Croll and Chiasson 1990; Furness et al. 1977). The freshly dissected tissues were placed directly into a solution of 4% paraformaldehyde and 0.5% glutaraldehyde (FaGlu) in 0.1 M phosphate buffer with 15% sucrose. After 1–12 h, the central ganglia were dissected from the surrounding tissues and placed directly on a glass coverslip. The ganglia were air-dried for several hours and then mounted in paraffin oil before the placement of a top coverslip. These preparations were viewed and photographed on a Zeiss Axiophot microscope equipped with a filter block with a 365 nm excitation and 420 nm longpass barrier filter.

Photography and measurements of neural development

Histological preparations were photographed by using Kodak TMAX 100 film. Either the developed negatives or printed photographs were digitally scanned and the images were then labelled and assembled into plates by using Photoshop 5.0 (Adobe Systems, San Jose, Calif.). The contrast and brightness of the images were adjusted to provide consistency within plates.

Several whole-mounted ganglia were also used to provide measures of neuronal development. Ganglia from animals of known sizes were digitally photographed by using a Nikon TE 300 microscope equipped with a Croma 31004, Texas Red /Cy3.5 filter set and an Optronics Engineering DEI-470 charge-coupled device video camera. Images were passed to a Windows NT 4.0-operated computer by using a Genesis video processor board and Millight software (Matrox Electronic Systems, Dorval, PQ, Canada). These images were then analysed with Scion Image 3b software (Scion, Frederick, Md., USA). Measurements were made of interocular distance and the lengths of the cerebropleural and the pedal ganglia from anterior to posterior margins (see Fig. 1). Similarly, immunoreactive neurones were photographed digitally to measure soma diameters. When a population of cells was being examined, photographs were made at different focal planes and all

visible cells were measured to obtain a mean size for the population. Counts of labelled cells in specific neuronal populations were obtained directly from observations of ganglia through the microscope. Data were then collected into SigmaPlot 5 (SPSS, Richmond, Calif.) spreadsheets for statistical analyses and graphical presentations. Linear regression lines were fitted to several data sets (see below) where appropriate. Where linear regressions appeared inadequate, data sets were fitted with three-parameter sigmoidal functions to estimate growth trends (see below).

Results

Morphology and growth of central ganglia

Central ganglia (Fig. 1) grew in size as the animals increased in body length (Fig. 2). The interocular distance and the lengths of the cerebropleural and pedal ganglia roughly doubled in size as the animals increased by about six-fold in body length. Thus, the size of the central nervous system decreased relative to the size of the body over this time. In the smallest animals (range: 3.6–4.8 mm, n=10), the mean interocular-distance to body-length ratio was 9.02% (±0.34%, SEM) of the body length. In the largest animals (range: 12.8–18.0 mm, n=10), the mean interocular-distance to body-length ratio was 4.55% (±0.17%, SEM) of the body length. These values were significantly different (t-test; P<0.01).

Within this context of growing animals and ganglia, we report the distribution of cells exhibiting immunoreactivity to 5-HT, TH, FMRFamide and TPep. Summary diagram showing the distributions of the various transmitter phenotypes are provided in Fig. 3.

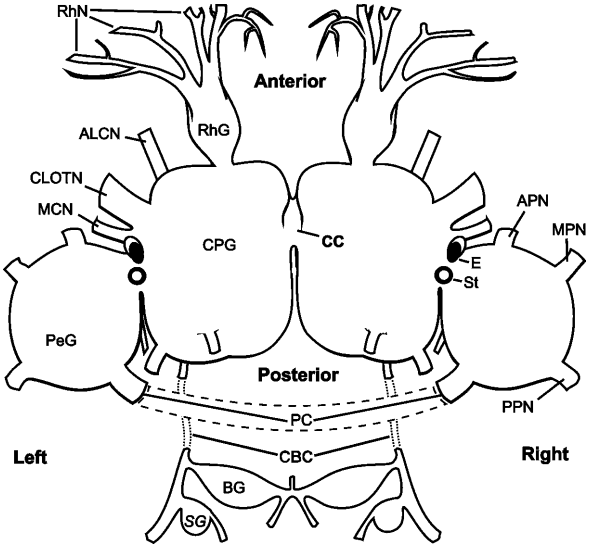
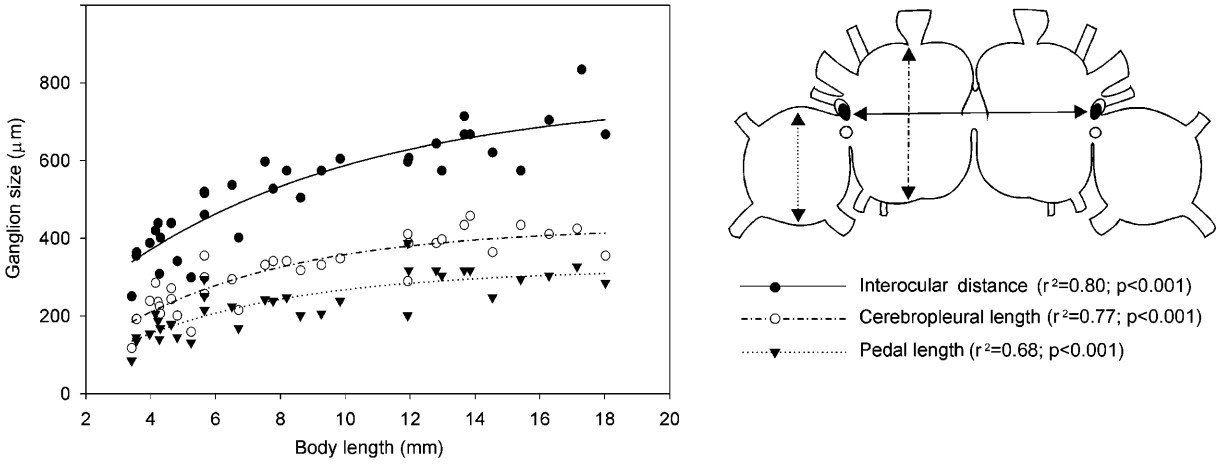


Fig. 1 Schematic diagram of a dorsal view of the central ganglia of *Phestilla sibogae* after the cutting of the pedal commissure and both cerebrobuccal connectives (*APN* anterior pedal nerve, *BG* buccal ganglion, *CLOTN* common labial and oral tentacle nerve, *CBC* cerebrobuccal connective, *CPG* cerebropleural ganglion, *CSN* cephalic shield nerve, *E* eye, *MPN* middle pedal nerve, *PC* pedal commissure, *PeG* pedal ganglion, *PON* posterior oral nerve, *PPN* posterior pedal nerve, *RhG* rhinophoral ganglion, *RhN* rhinophoral nerve, *SG* stomatogastric ganglion, *St* statocyst)

5-HT immunoreactivity

The central ganglia from specimens of all sizes contained approximately 100–115 5-HT-immunoreactive neurones. The dorsal surface of each cerebropleural ganglion contained five medial somata near the cerebral commissure. Two of these somata (small arrows in Fig. 4A) were small, with one cell being located immediately posterior to the commissure and the other being generally located just over the base of the commissure. The other three somata (large arrows in Fig. 4A) were larger and formed a tighter cluster in a slightly more lateral position. Several additional somata were located along the anterior margin of the cerebropleural ganglia. Occasionally, one of these neurones was found on the dorsal surface (arrowhead in Fig. 4A) but, more frequently, the cells were located on the ventral surface (arrowheads in Fig. 4B). Each cerebropleural ganglion consistently contained two moderately sized somata

Fig. 2 Ganglionic growth. Ganglionic dimensions (interocular distance, length of cerebral ganglion, length of pedal ganglion) plotted against body length of animals. Nonlinear regression lines (sigmoidal three-parameter function), correlation coefficients and P values are shown



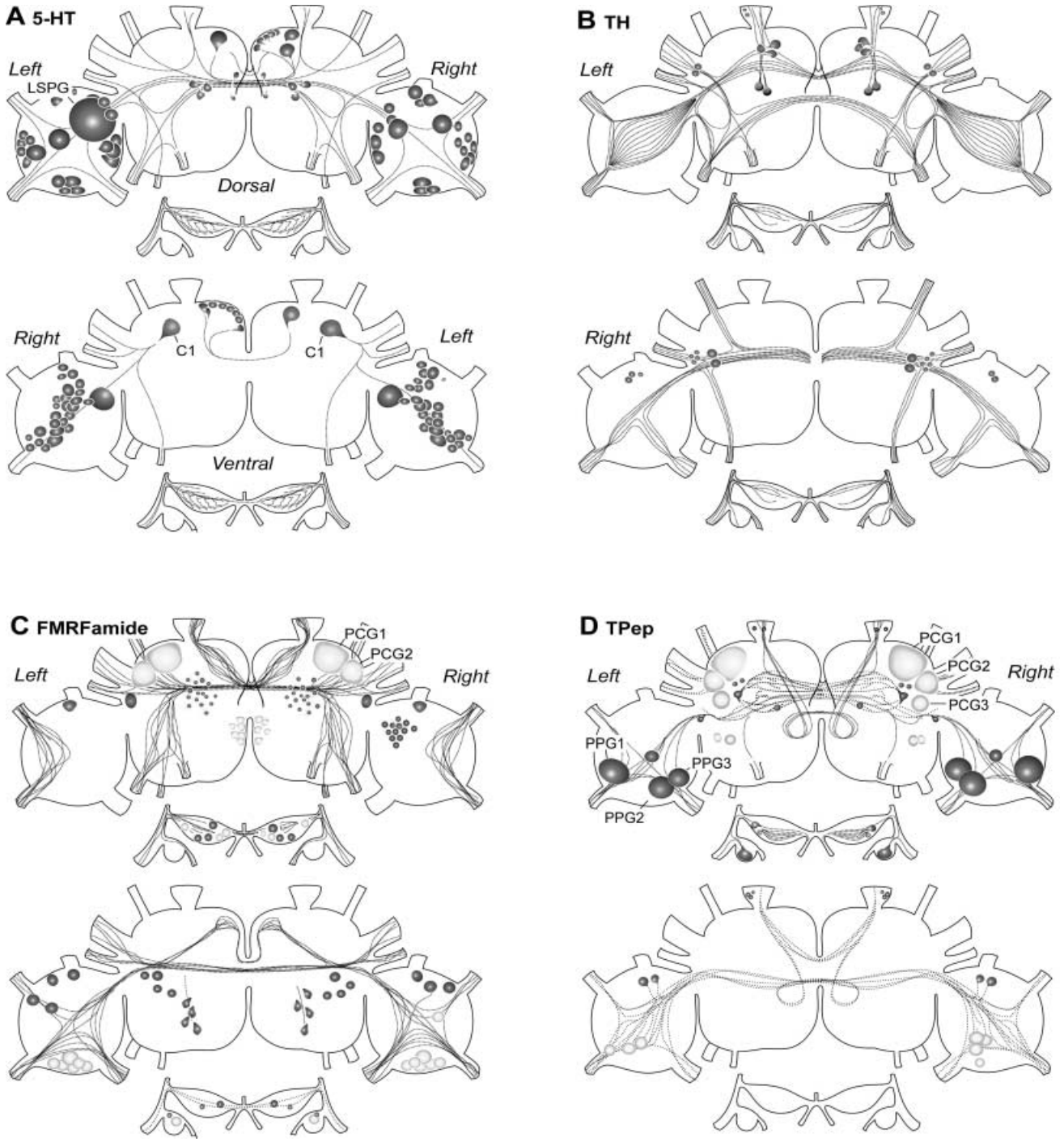


Fig. 3 Summary of the dorsal (*top*) and ventral (*bottom*) distributions of serotonin (*5-HT*) immunoreactivity (**A**), tyrosine hydroxylase (*TH*) immunoreactivity (**B**), *FMRFamide* immunoreactivity (**C**) and *Tritonia* pedal-peptide (*TPep*) immunoreactivity (**D**) in the central ganglia of *Phestilla sibogae*. Darkly coloured cells represent intensely immunoreactive somata, whereas faint cells represent weakly immunoreactive somata. Patterns of major immunoreactive fibre pathways are also indicated. The locations of various identified cells are marked as follows: left serotonergic pedal giant cell (*LSPG*), the C1 cells (*C1*), the peptidergic cerebral giant cells 1, 2 and 3 (*PCG1*, *PCG2*, *PCG3*) and the peptidergic pedal giant cells 1, 2 and 3 (*PPG1*, *PPG2*, *PPG3*)

along the anterior margin but only the right ganglion possessed additional smaller somata in this region. The number of these smaller immunoreactive somata was variable, ranging from 6 to 16, but the population size appeared to be independent of the animal body size. Finally, each cerebropleural ganglion also possessed a single moderately sized soma on the ventral surface roughly posterior to the base of the rhinophoral ganglion and lateral to the base of the cerebral commissure (Fig. 4B). A single axon (small arrow in Fig. 4B) could be traced from each of these somata into the ipsilateral cerebral-

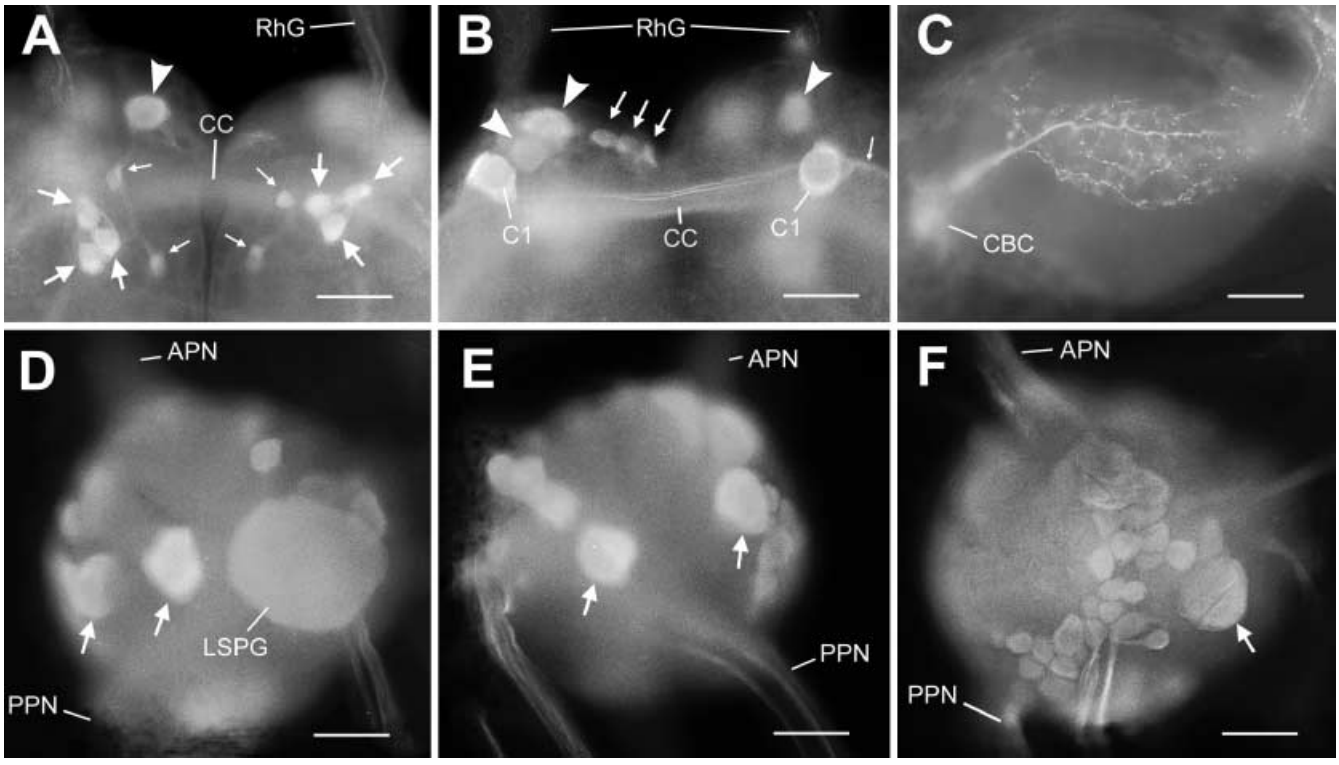


Fig. 4A–F Serotonin immunoreactivity in the central ganglia of *Phestilla sibogae*. **A** Dorsal surface of cerebropleural ganglia (*RhG* rhinophoral ganglion, *CC* cerebral commissure, *large arrowhead* large cell along the anterior margin of the left ganglion, *medium-sized arrows* clusters of three cells, *small arrows* two distinctly smaller and more medial cells in each ganglion). **B** Ventral surface of cerebropleural ganglia, with the locations of the two C1 somata and the axon of the left cell (*small arrow*) indicated (*right* in this ventral view; *large arrowheads* large anteromedial cells found in all specimens, *medium-sized arrows* examples of cells found only in the right ganglion). **C** Neuropile in the buccal ganglion. Immunoreactive terminals appear to originate from a single fibre that enters the ganglion via the cerebrobuccal connective (*CBC*). **D** Dorsal surface of the left pedal ganglion with medial margin *right* (*APN* anterior pedal nerve, *PPN* posterior pedal nerve, *LSPG* left serotonergic pedal giant cell, *arrows* a pair of moderately large cells found in all preparations). **E** Dorsal surface of the right pedal ganglion with the medial margin *left* (*arrows* a pair of moderately large cells found in all preparations). **F** Ventral surface of right pedal ganglion with the medial margin *right* (*arrow* a single large cell consistently found in all preparations). *Bars* 45 μm for **A**, **B**, **D–F**, 25 μm for **C**

buccal connective. The terminals of these axons provided the only source of 5-HT to the buccal ganglia (Fig. 4C). Based upon these unique characteristics, we identified these cells as the C1 neurones, homologous to C1 (also known as the metacerebral giant) neurones found in many other gastropods (Croll 1987b; Pentreath et al. 1982; Sakharov 1976; Weiss and Kupfermann 1976).

The pedal ganglia possessed large numbers of 5-HT-immunoreactive neurones. The left and right PG each contained about 15 moderately sized cells scattered over central regions and along the posterior, lateral and medial margins of the dorsal surface (Fig. 4D, E). Whereas these cells were generally bilaterally symmetrical in their distributions, the dorsal surface of the left pedal ganglion contained a giant 5-HT-immunoreacative neurone (the left serotonergic pedal giant, LSPG), which had a diameter at least two to three times larger than any other pedal cell and was located along the medial margin. The left and right pedal ganglia each contained an additional band of approximately 23–27 5-HT-immunoreactive neurones, which extended across the ventral surface (Fig. 4F). The most medial cell in this population was consistently larger than its ventral neighbours.

Aldehyde-induced histofluorescence and TH immunoreactivity

The same set of neurones and fibres appeared to be labelled by aldehyde-induced fluorescence and exhibited TH immunoreactivity. For instance, both labels were found in two somata (small arrows in Fig. 5A, B) along the lateral margin of the rhinophoral ganglion and another three somata on in the nearby anterior region of the cerebral ganglion (large arrows in Fig. 5A and the anterior-most region of the ganglion shown in Fig. 5D). The locations of labelled somata and the distribution of neuropilar staining were also similar in the pedal ganglia with the two techniques (compare Fig. 5B, E). However, the locations of the various cells were more variable in ganglia prepared for histofluorescence, presumably because of tissue distortion caused by the dessication required for this technique, and the clarity and resolution afforded by immunocytochemistry were superior. We therefore employed TH-immunoreactivity to

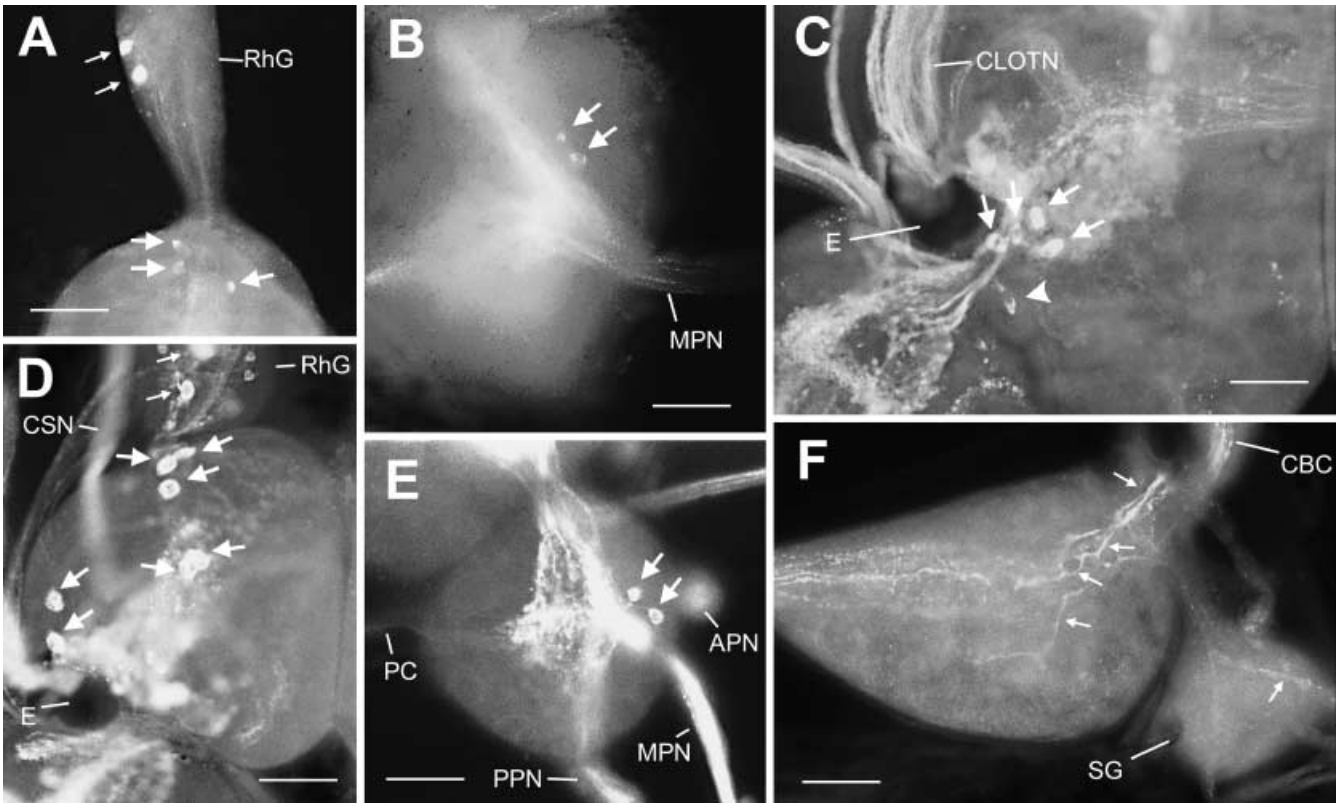


Fig. 5A–F Catecholamine-containing cells in the central ganglia of *Phestilla sibogae*. **A** Aldehyde-induced fluorescence of two cells *(small arrows*) in the rhinophoral ganglion (*RhG)* and three cells (*large arrows*) in the anterior-most portion of cerebropleural ganglion. **B** Lateral margin of the pedal ganglion stained with FaGlu (*arrows* fluorescent somata, *MPN* middle pedal nerve). **C** TH immunoreactivity on the ventral surface of the right cerebropedal ganglion near the eye (*E* eye, *arrows* labelled somata in the cerebropleural ganglia, *arrowhead* an additional cell that might be located in the optic ganglion). Abundant immunoreactive fibres are found in the common labial and oral tentacle nerve (*CLOTN*). **D** TH immunoreactivity in cells in the rhinophoral ganglion (*small arrows*) and on the dorsal surface of the left cerebropleural ganglion (*large arrows*). The cephalic shield nerve (*CSN*) is indicated. **E** Abundant TH-immunoreactive fibres are located in the neuropile of the pedal ganglia (*arrows* a pair of immunoreactive somata near the lateral margin, *APN* anterior pedal nerve, *PPN* posterior pedal nerve, *PC* pedal commissure). **F** TH-immunoreactive fibres (*arrows*) in the neuropile of buccal ganglion and the attached stomatogastric ganglion (*SG*). The cerebrobuccal connective (*CBC*) is also indicated. *Bars* 50 µm for **A** and **D**, 40 µm for **B**, 30 µm for **C** and **F**, 60 µm for **E**

provide further details of catecholaminergic neurones in *P sibogae*.

TH immunoreactivity was exhibited by relatively few central somata (approximately 28–30) and these cells were consistently observed in specimens of all sizes. In addition to the three TH-immunoreactive somata located near the base of the rhinophoral nerve (Fig. 5D), four more somata were also located on the dorsal surface of each cerebropleural ganglion: two cells were located laterally near the base of the common labial and oral tentacle nerve (CLOTN) and another two cells were located directly over the centre of the ganglion. On the ventral surface, five cells were located near the base of the eye (Fig. 5C). One of these cells (arrowhead in Fig. 5C) may have been located in the optic ganglion. In addition, brightly labelled TH-immunoreactive fibres were located in all nerves originating from the cerebropleural ganglia but were particularly plentiful in the CLOTN and cephalic shield nerve (CSN). TH-immunoreactive fibres were also abundant in each of the pedal nerves and in the neuropile in the centre of the pedal ganglia (Fig. 5E). Two to three TH-immunoreactive somata were observed in lateral regions of each pedal ganglion (arrows in Fig. 5E). No TH-immunoreactive somata were observed in the buccal ganglia or the attached stomatogastric ganglia (Fig. 5F). TH-immunoreactive fibres were observed in the buccal ganglia and appeared to enter primarily from the cerebrobuccal connective.

FMRFamide immunoreactivity

The central ganglia of specimens 5–7 mm in length contained 65–75 brightly labelled FMRFamide-immunoreactive neurones and another 40–45 FMRFamide-immunoreactive neurones that were either weakly labelled or were variable in staining intensity. The most prominent FMRFamide-immunoreactive neurones, the peptidergic cerebral giant cells (PCG1 and PCG2), resided along the anterolateral margin of the dorsal surface of each cerebropleural ganglion (Fig. 6A, C). Another more moderately sized cell was located slightly more posterior in a position just medial to the eye on the dorsal surface of each cereropleural ganglion (Fig. 6A, C). All three of

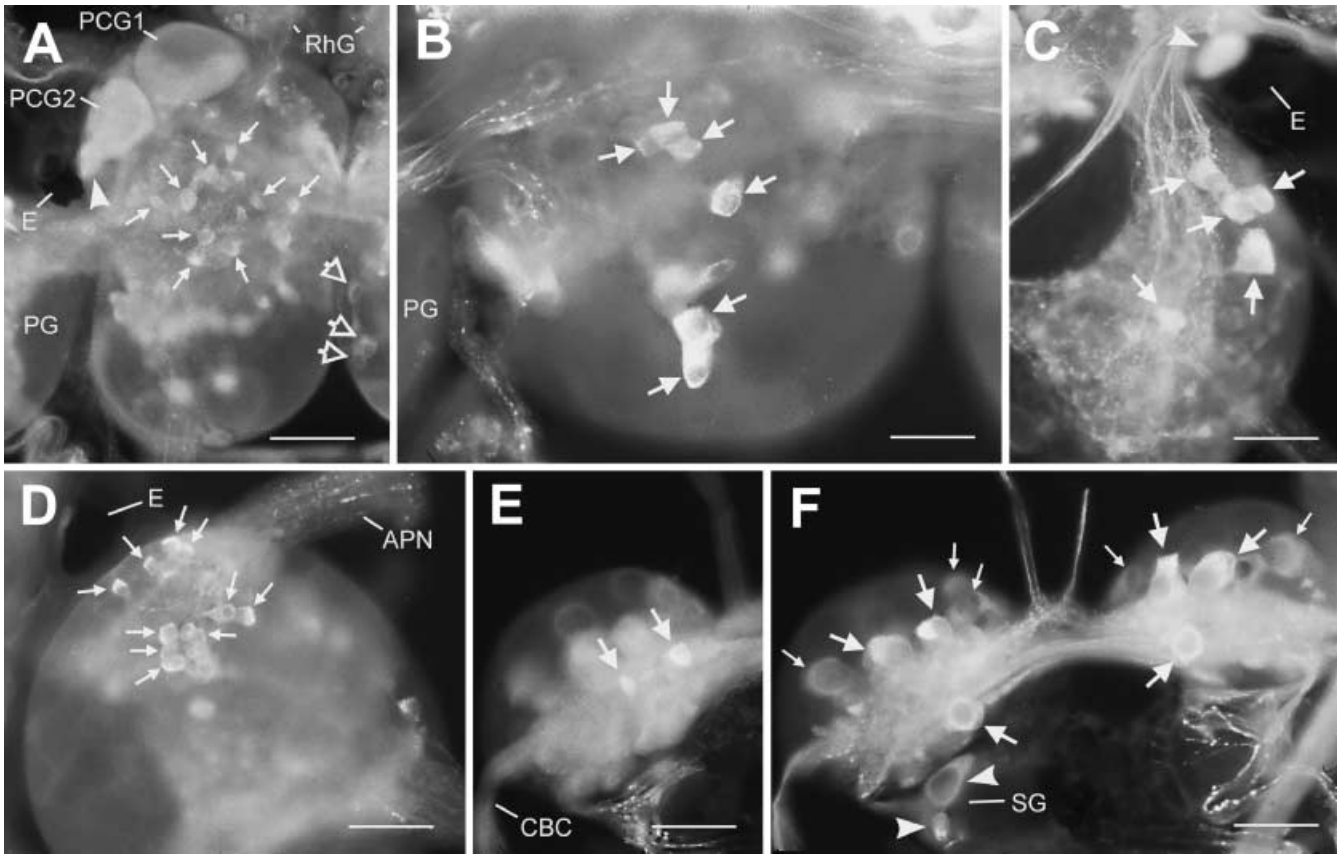


Fig. 6A–F FMRFamide immunoreactivity in the central ganglia of *Phestilla sibogae*. **A** Dorsal surface of left cerebropleural ganglion (*E* eye, *RhG* portions of the rhinophoral ganglia, *PG* pedal ganglia, *PCG1*, *PCG2* immunoreactive peptidergic cerebral giant cells 1 and 2, *solid arrowhead* another soma located on the dorsal surface of the cerebropleural ganglion near the eye, *open arrowheads* weakly fluorescent somata along the medial margin of the right cerebropleural ganglion, *arrows* immunoreactive cells loosely scattered over central regions of dorsal surface of the ganglion). **B** Higher magnification view of posterior regions of the ventral surface of the right cerebropleural ganglion (*arrows* intensely FMRFamide-immunoreactive somata). **C** Ventral surface of the left pedal ganglion (*arrows* intensely immunoreactive somata, *arrowhead* a fluorescent soma located out of the focal plane and on the dorsal surface of the cerebropleural ganglion near the eye). **D** Dorsal surface of right pedal ganglion (*APN* anterior pedal nerve, *arrows* examples of immunoreactive somata in anteromedial regions). **E** Ventral surface of buccal ganglion (*CBC* cerebrobuccal connective, *arrows* immunoreactive cells). **F** Deeper focus of same preparation shown in **E** showing the dorsal surfaces of both buccal ganglia (*small arrows* examples of weakly immunoreactive somata, *large arrows* intensely immunoreactive somata, *arrowheads* two cells in the left stomatogastric ganglion, *SG* stomatogastric ganglion). *Bars* 50 μm for **A**, 30 μm for **B** and **C**, 35 μm for **D** and 40 μm for **E** and **F**

these cells were variable in their intensity of labelling. In particular, they were often undetectable in the smallest (<8 mm body length) and largest (>14 mm body length) specimens. The cerebropleural ganglia in specimens of all sizes also contained a population of 13–17 smaller and more intensely labelled cells scattered over central regions of the dorsal surface (solid arrows in Fig. 6A). Each cerebropleural ganglion also contained 7–9 intensely labelled cells scattered over the posterior regions of the ventral surface (Fig. 6B). Finally, another cluster of 3–7 dimly labelled cells was found posteriorly along the medial margin of the dorsal surface of each cerebropleural ganglion (Fig. 6A).

The ventral surface of each pedal ganglion generally contained 3–5 intensely labelled FMRFamide-immunoreactive neurones near its anterior margin (Fig. 6C) and 6–8 weakly labelled neurones along its posterior margin. The dorsal surface of each pedal ganglion also contained a single neurone located along the anterior margin. In addition, the dorsal surface of the right pedal ganglion of specimens 5–7 mm in length contained a population of 15–18 intensely labelled FMRFamide-immunoreactive neurones (Fig. 6D). Smaller specimens generally contained slightly fewer cells, whereas larger specimens contained up to 30 neurones within this population (Fig. 7). The dorsal surface of the left pedal ganglion contained no comparable cluster of FMRFamide-immunoreactive neurones, regardless of the size of the specimen from which they were dissected.

The buccal ganglia of all specimens each contained a total of five intensely labelled FMRFamide-immunoreactive neurones: two on the dorsal surface (Fig. 6E) and three on the ventral surface (large arrows in Fig. 6F). The dorsal surface of each buccal ganglion also possessed three weakly labelled FMRFamide-immunoreactive neurones, with two lying along the medial margin and one along the lateral margin (small arrows in Fig. 6F). In addition, each stomatogastric ganglion con-

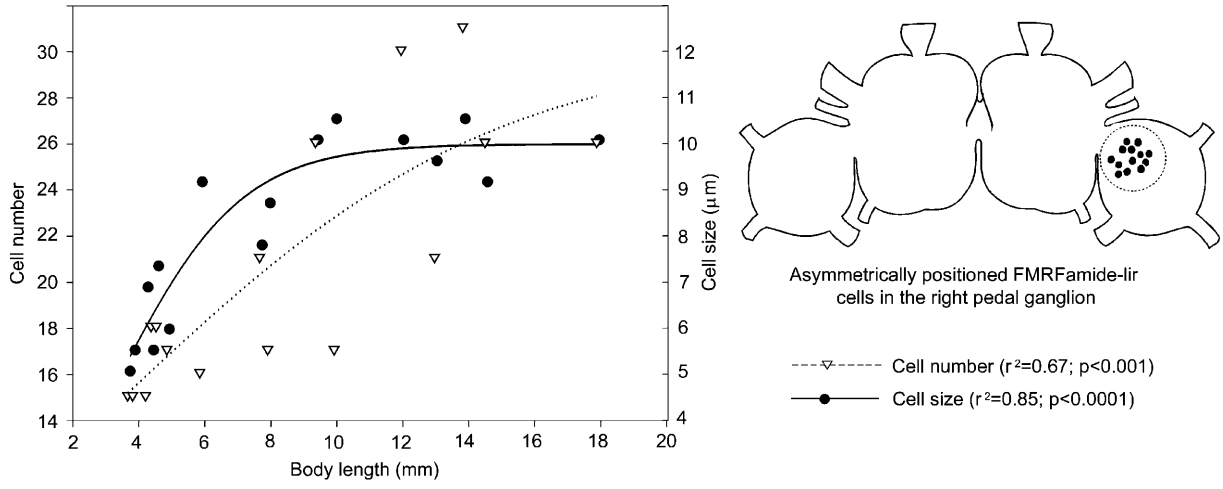


Fig. 7 Growth in size and number of asymmetrically positioned FMRFamide-immunoreactive cells on the dorsal surface of the right pedal ganglion. Cell number (*left axis*) and cell size (*right axis*) are plotted against body length of animals. Nonlinear regression lines (sigmoid three-parameter functions), correlation coefficients and *P* values are shown

tained one small but intensely labelled FMRFamide-immunoreactive neurone and one larger but more dimly labelled FMRFamide-immunoreactive neurone (Fig. 6F).

TPep immunoreactivity

The central ganglia of specimens of all sizes contained a total of approximately 27–29 intensely labelled TPep-immunoreactive neurones and 16–19 dimly labelled TPep-immunoreactive neurones. The most prominent TPep-immunoreactive neurones of each cerebropleural ganglion were three giant neurones located laterally along the anterior margin (Fig. 8A–C). The two most anterior neurones, PCG1 and PCG2, had previously been shown to be FMRFamide-immunoreactive (see above). The most posterolateral cell of the trio, PCG3, was also the smallest. The staining intensity of all three of these giant cells was variable and we therefore categorized them as weakly TPep-immunoreactive. No attempt was made to correlate their staining intensities with animal size. Two much smaller, weakly labelled TPep-immunoreactive neurones were located posterolaterally within each cerebropleural ganglion (small arrows in Fig. 8A) and a final small, dimly labelled TPep-immunoreactive neurone was located adjacent to the eye, perhaps in the optic ganglion (arrow in Fig. 8C).

Medial to the three peptidergic cerebral giant cells was a group of four intensely labelled cells, the fibers from which appeared to form a basket-like collection of neurites in the adjacent neuropile (small arrows in Fig. 8B).

Each pedal ganglion contained three large, intensely labelled neurones (Fig. 8D). All three peptidergic pedal giants (PPG1, PPG2, PPG3) were located along the posterior margin of the dorsal surface, whereas a single smaller TPep-immunoreactive neurone was located towards the centre of the dorsal surface. The anterior margin of each pedal ganglion contained one or two smaller, intensely labelled TPep-immunoreactive neurones (Fig. 8D, E) and 3–4 larger but more weakly labelled TPep-immunoreactive neurones along the posterior margin (Fig. 8A).

The buccal ganglia each contained two intensely labelled TPep-immunoreactive neurones in their lateral regions (arrows in Fig. 8F). These cells appeared to produce fibres that contributed to the buccal neuropile. A single, larger, intensely labelled TPep-immunoreavtive neurone was located in the stomatogastric ganglion (arrowhead in Fig. 8F.

Changes in cell sizes during postembryonic development

Cell numbers appeared to change very little over the range of postembryonic development examined in this study (see above) but various cells did grow significantly in size. Figure 9 shows the growth rates of four different 5-HT-immunoreactive cells or members of cell populations. The smallest somata (represented by the pair of small medial cells) roughly doubled in size as the animals grew from 3–18 mm in body length. The slightly larger and more lateral three somata on the dorsal surface of the cerebropleural ganglia grew more slowly, increasing by only about 50% in diameter. The moderately large 5-HT-immunoreactive soma immediately lateral to the LSPG also roughly doubled in size, whereas the LSPG itself grew at the fastest rate and roughly tripled in size as the animal grew from 3–18 mm in body length.

A similar range of growth rates was observed in selected FMRFamide-immunoreactive cells. Both the posterior somata on the ventral surface of the cerebropleural ganglion (Fig. 10) and the somata found only on the dorsal surface of the right pedal ganglion (Fig. 7) grew relatively slowly, roughly doubling or less in size as animal body length increased from 3–18 mm. Although the PCG1 neurone was not detectable in either the smallest

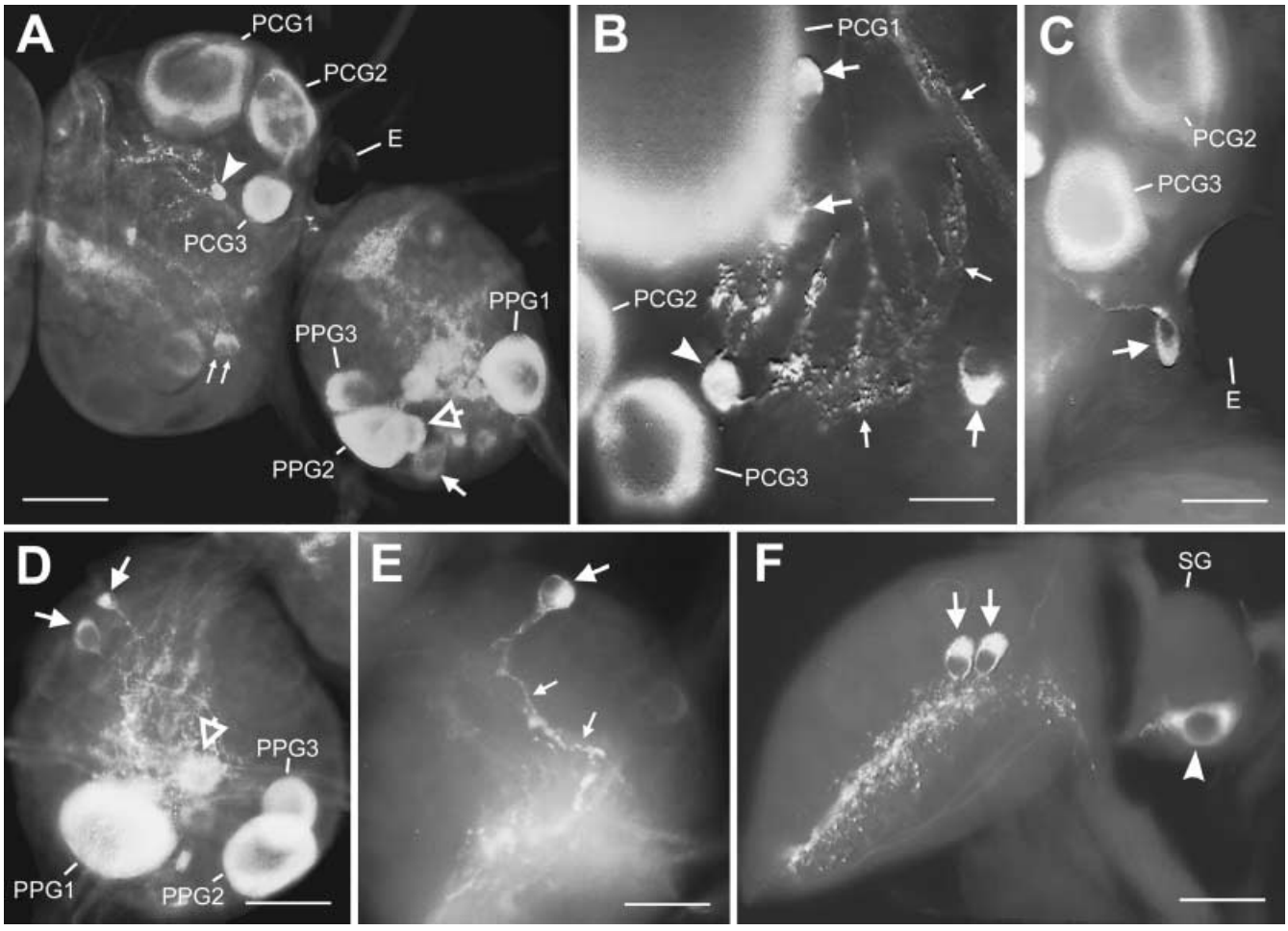


Fig. 8A–F TPep immunoreactivity in the central ganglia of *Phestilla sibogae*. **A** Dorsal surface of right cerebropleural and pedal ganglia (*E* location of the eye, *PCG1*, *PCG2*, *PCG3* peptidergic cerebral giant cells 1, 2 and 3, *PPG1*, *PPG2*, *PPG3* peptidergic pedal giant cells 1, 2 and 3, *large solid arrowhead* and *open arrowhead* other intensely TPep-immunoreactive somata, *small arrows* weakly fluorescent cells in the posterior regions of the cerebropleural ganglion, *middle-sized arrow* example of a weakly fluorescent cell in the pedal ganglion). **B** Higher magnification view near the anterior margin of the left cerebropleural ganglion showing intensely TPep-immunoreactive neurones (*large arrows*) and associated network of fibres (*small arrows*). One of these cells (*large arrowhead*), which is consistently found in all preparations, is distinct because of its most lateral position and bipolar morphology. **C** Immunoreactive cell (*arrow*) next to the eye. **D** Dorsal surface of left pedal ganglion showing the three peptidergic pedal cells, another unnamed but intensely fluorescent cell (*open arrowhead*) and two weakly fluorescent somata (*arrows*) along the anterolateral margin. **E** Ventral surface of left pedal ganglion showing one of the weakly TPep-immunoreactive somata (*large arrow*) and its processes (*small arrows*) as they enter the neuropile. **F** Dorsal surface of the buccal ganglia (*arrows* two immunoreactive somata, *large arrowhead* immunoreactive soma located in the stomatogastric ganglion, *SG* stomatogastric ganglion). *Bars* 50 µm for **A**, 25 µm for **B** and **C**, 45 µm for **D**, 40 µm for **E** and 30 µm for **F**

or largest specimens (see above), the cell appeared to grow much more rapidly over the limited body lengths sampled (Fig. 10).

Discussion

Approximately 265–325 neurones have been described in the present study. Based upon the sizes and positions of the various labelled cells, there appears to be only minor overlap in the populations of cells expressing immunoreactivities against 5-HT, TH, FMRFamide and TPep (Fig. 3A–D). However, whereas colocalisation of neurotransmitters and/or neuromodulators appears to have little impact upon the estimated number of cells sampled in this study, previous research suggests that it is likely to be more widespread than that reported here. For instance, Beck et al (2000) have recently demonstrated that numerous TPep-immunoreactive cells in *T. diomedea* also exhibit immunoreactivity for another neuropeptide, SCP_b. Other studies have suggested that FMRFamide immunoreactivity colocalises with immunoreactivities for APGWamide, myomodulin and enkephalins within certain gastropod neurones (Dyakonova et al. 1995; Li and Chase 1995; Santama et al. 1994). With the physiological importance of multiple transmitters in neurones

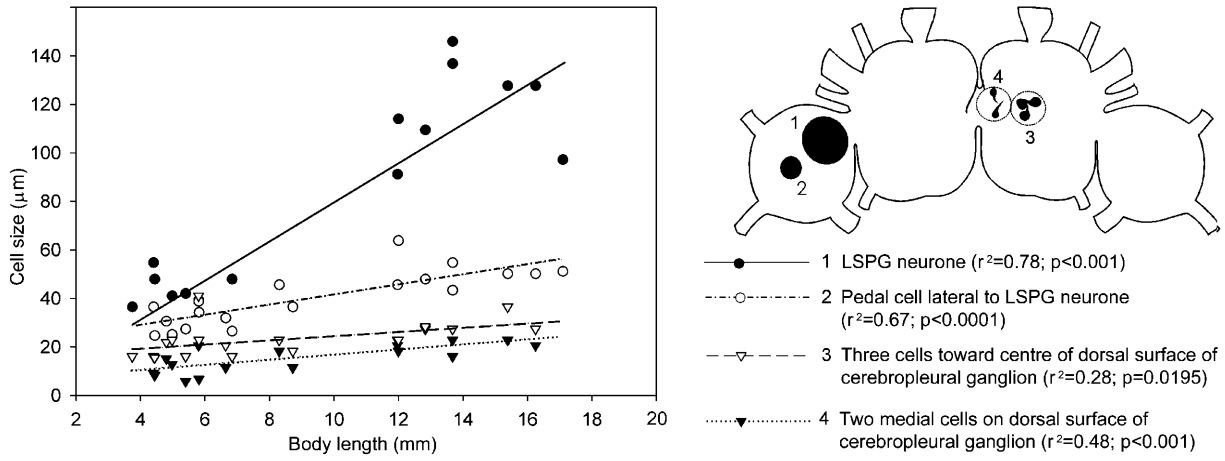


Fig. 9 Growth in sizes of 5-HT-immunoreactive cells. Diameters of two identifiable individual cells in the left pedal ganglion and representatives of two populations of cerebral cells are plotted against body length of animals. Linear regression lines, correlation coefficients and *P* values are shown

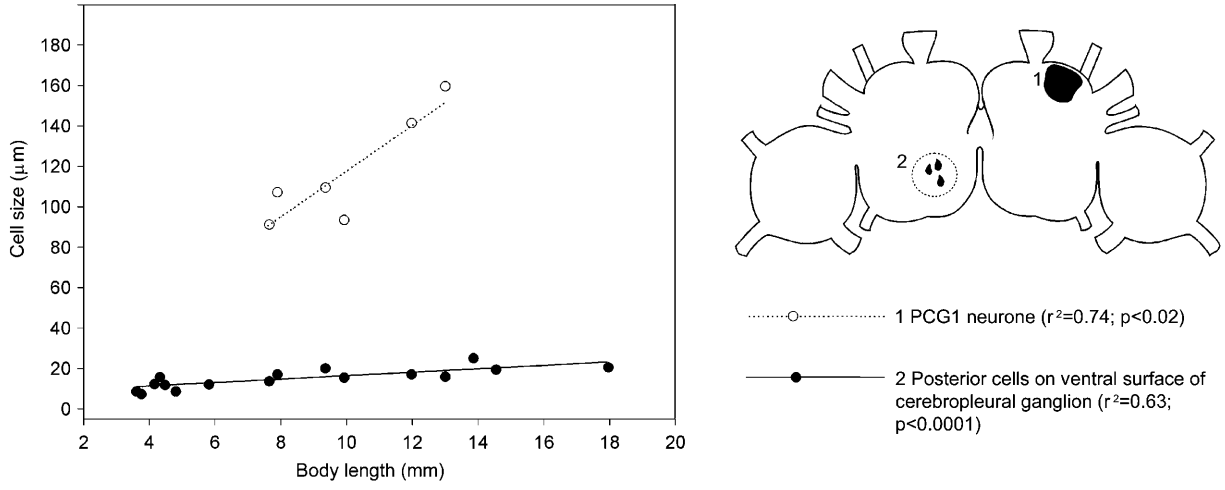


Fig. 10 Growth in sizes of FMRFamide-immunoreactive cells. Diameters of a giant cell in the cerebral ganglion and representatives of another population of cells on the ventral surface of the cerebral ganglion are plotted against body length of animals. Linear regression lines, correlation coefficients and *P* values are shown

becoming more apparent (De Lange et al. 1998; Weiss et al. 1992; Whim and Lloyd 1990) in other gastropods, future work is needed to ascertain both the degree of colocalisation of transmitters within the various identified cells of *P. sibogae* and the significance of co-transmission within this species.

5-HT immunoreactivity

5-HT-immunoreactive neurones constitute the largest population of cells revealed in our studies. This finding is consistent with the results of numerous other studies showing large numbers of such neurones in gastropods, including other opisthobranchs (Croll 1987a; Land and Crow 1985; Longley and Longley 1986; Satterlie et al. 1995; Sudlow et al. 1998), pulmonates (Croll 1988; Croll and Chiasson 1989) and prosobranchs (Barlow and Truman 1992; Croll and Lo 1986) and even Polyplacophora (Moroz et al. 1994) and Bivalvia (Croll et al. 1995). In all gastropod species examined, including *P. sibogae*, the majority of 5-HT-immunoreactive neurones are located in the pedal ganglia. Several of these neurones appear to play important roles in the control of ciliated cells in the sole of the foot (Audesirk et al. 1979; Syed and Winlow 1989) but other 5-HT-immunoreactive neurones are likely to have a variety of roles. For example, Willows (1985) has reported that electrical stimulation of cells in *P. sibogae* with locations consistent with the 5-HT-immunoreactive neurones described here causes movements of the cerata. In addition, a large asymmetric serotonergic cell, similar to the LSPG neurone, has previously been described in the closely related nudibranch *Hermissenda crassicornis* (Croll 1987a; Land and Crow 1985) and stimulation of this cell also elicits movements of the cerata (Jerussi and Alkon 1981). A similar cell has also been reported in the left

pedal ganglion within a wide variety of gastropods (Croll 1987a, 1988; Croll and Chiasson 1989; Sakharov 1976; Satterlie et al. 1995) but has not been found in all species examined, e.g. *Pleurobranchaea californica* (Sudlow et al. 1998) or *A. californica* (Longley and Longley 1986). The roles of this cell in most species are unknown but, in *Lymnaea stagnalis*, the cell innervates the mantle (R. P. Croll and B. J. Chiasson, unpublished) and receives synaptic input that may co-ordinate its activity with the respiratory cycle (Benjamin and Winlow 1981; Syed and Winlow 1991).

Within the cerebropleural ganglia, the most prominent 5-HT-immunoreactive cells are the neurones that we refer to as the C1 cells. Similar cells that appear to provide the sole source of 5-HT to the buccal ganglia have been reported in all opisthobranch and pulmonate species examined to date (Croll 1987b; Granzow and Rowell 1981; Pentreath et al. 1982; Sakharov 1976; Weiss and Kupfermann 1976). Based only on the size and position of the soma, Willows (1985) appears to have mis-identified the PCG1 cells as C1 homologues in *P. sibogae*. He has noted, however, that these cells are distinct from the C1 cells in other species. Whereas the C1 cells identified in the present report have more significant features in common with the C1 cells in other species, they are smaller and positioned more posteriorly than the C1 cells of other nudibranchs (Croll 1987a; Land and Crow 1985; Sudlow et al. 1998), thus explaining their being overlooked by Willows (1985).

Another notable population of 5-HT-immunoreactive neurones consists of five pairs of neurones located on the dorsal surface of the cerebral ganglia lateral to the cerebral commissure. Similar neurones have been described in other nudibranchs (Croll 1987a; Land and Crow 1985; Sudlow et al. 1998), other opisthobranch orders (Longley and Longley 1986; Sudlow et al. 1998), pulmonates (Croll 1988; Croll and Chiasson 1989; Marois and Croll 1992) and prosobranchs (Croll and Lo 1986). These cells play several of roles in the various species (Katz et al. 2000). For instance in *A. californica*, they appear to modulate the gill withdrawal reflex circuitry (Mackey et al. 1989). In *Clione limacina*, *Tritonia diomedia* and *P. californica*, they initiate or modulate swimming responses (Jing and Gillette 1999; Katz and Frost 1995a, 1995b; Satterlie and Norekian 1995). In both *L. stagnalis* and *P. californica*, at least one of these cells also projects to the tentacles and/or rhinophores, which are anterior sensory structures found widely on gastropods (Croll 1983). 5-HT-immunoreactive fibres have also been reported to innervate these structures in *P. sibogae*, although the origin of these fibres has not been determined (Boudko et al. 1998).

The final populations of 5-HT-immunoreactive cells are located in the anteromedial region of the cerebropleural ganglia near the C1 cells. 5-HT-immunoreactive cells have been reported in corresponding regions of the central ganglia of many other gastropods (Croll 1987a, 1988; Croll and Chiasson 1989; Croll and Lo 1986; Longley and Longley 1986; Satterlie et al. 1995). Sudlow et al. (1998) have reported that these neurones have pleural projections in *P. californica*, whereas Satterlie and Norekian (1995) have previously demonstrated pedal projections from such cells in *C. limacina*. Although other species have anteriomedial 5-HT-immunoreactive cells, *P. sibogae* is the first species reported to have asymmetrically positioned 5-HT-immunoreactive cells on the right side. Bilaterally asymmetric populations of cells containing other transmitters have been reported in other gastropods and such cells have been shown or assumed to be involved in the control of male reproductive organs found on the right side only (Croll and Van Minnen 1992; Fan et al. 1997; Li and Chase 1995; Smith and Croll 1998). Consistent with these findings, 5-HT-immunoreactive fibres have also been found in the penial complex of *P. sibogae* (D. M. Boudko and R. P. Croll, unpublished observations).

TH immunoreactivity

Because of the need for different fixatives, it was not possible to double-label individual cells by using both FaGlu and immunocytochemical techniques for detecting TH. Both techniques, however, revealed similar patterns with abundant labelling of neuropilar regions and fibres in peripheral nerves and a relative paucity of central neurones. In addition, the sizes and positions of the central neurones exhibiting aldehyde-induced fluorescence were consistent with those exhibiting TH immunoreactivity. We are confident therefore that the TH immunoreactivity reported here provides an accurate representation of catecholamine-containing elements within the central ganglia of *P. sibogae*.

Previous studies have employed high performance liquid chromatography or radioimmunoassays to measure abundant levels of catecholamines within the central ganglia of gastropods (Carpenter et al. 1971; McCaman 1984; McCaman et al. 1973; Walker 1986) and other molluscs (Pani and Croll 1995; Stefano and Catapane 1977; Stefano et al. 1978). Such studies have consistently shown that dopamine constitutes the majority of catecholamine found in molluscs, although lesser amounts of noradrenaline are also often found. Accordingly, we assume that the majority of cells and fibres labelled in the present report contain dopamine and presumably use this monoamine as a neurotransmitter. The presence of noradrenergic cells, however, cannot be discounted. Given the relative abundance of catecholamines measured in the central nervous systems of gastropods and other molluscs by biochemical methods (McCaman 1984; McCaman et al. 1973; Pani and Croll 1995), a greater number of TH-immunoreactive somata might have been expected. However, the central ganglia of *P. sibogae* contain only about 30 neurones, none of which attains a large size. This finding is consistent with other reports of the distribution of catecholamine-containing cells in gastropods (Croll 1987a, 1988; Croll et al. 1999), although a single giant soma is located in the right pedal ganglion in

some species (e.g. *L. stagnalis*; Cottrell et al. 1979) or in the left pedal ganglion of sinistral species (e.g. *Planorbis corneus*; Osborne et al. 1975). Unfortunately, the small sizes of TH-immunoreactive neurones and our inability to follow their axons among the numerous other labelled axons in the neuropile hinders comparisons with specific cells in other species. However, as in *P. sibogae*, a single catecholamine-containing cell has been reported in the optic ganglion of *H. crassicornis* (Heldman and Alkon 1977). Moreover, catecholamine-containing cerebrobuccal cells have been reported near the cerebrobuccal connective in other species (Croll 1988; Croll et al. 1999; Rosen et al. 1991); homologues may account for the lateral cells in the cerebropleural ganglia and for fibres in the cerebrobuccal connective in *P. sibogae*.

Although catecholamine-containing cells in *P. sibogae* share similarities with catecholamine-containing cells in other gastropods, significant differences can also be noted. For instance, *P. sibogae* appears to lack the catecholaminergic buccal cells found in many other species (Croll 1988; Croll et al. 1999; Kabotyanski et al. 1998; Teyke et al. 1993; Trimble et al. 1984). Also noteworthy is the finding that *H. crassicornis* possesses a giant catecholamine-containing cell in the right pedal ganglion (Croll 1987a), whereas such a cell is lacking in *P. sibogae*, a similar aeolid species.

Despite difficulties in identifying specific homologies of catecholamine-containing cells in various gastropods, all species examined to date possess abundant catecholamine-containing fibres in various nerves and neuropilar regions of the central ganglia (Croll 1987a, 1988; Croll et al. 1999; Kabotyanskii and Sakharov 1990; Salimova et al. 1987b). Several reports also suggest that gastropods and bivalves possess numerous catecholaminergic peripheral neurones, at least some of which possess axons that project to and terminate within the central ganglia (Croll et al. 1999; Kabotyanskii and Sakharov 1990; Salimova et al. 1987a; Smith et al. 1998). It is thus appears that these putative sensory cells are both a general feature of molluscs and the source of much of the catecholamine found within the central nervous system.

FMRFamide immunoreactivity

A notable feature of the FMRFamide immunoreactivity in *P. sibogae* is the existence of what appear to be two populations of cells distinguished by their relative staining intensities. The intensely FMRFamide-immunoreactive cells were consistently found in all preparations, whereas the neurones that we designated as weakly FMRFamide-immunoreactive cells were variable in the intensity of their staining. Occasionally, these latter cells were relatively brightly labelled but often their immunofluorescence only barely exceeded background levels or they exhibited no detectable immunoreactivity whatsoever. The basis for differences in staining intensities is unclear but appears to be age-related; cells were most consistently labelled in specimens ranging from 8–14 mm in body length and were generally unlabelled in larger and smaller specimens. Different staining intensities may have been caused by differences in the concentrations of immunoreactive peptides within the cells or by the presence of different FMRFamide-related peptides (Gaus et al. 1993; Greenberg and Price 1992; Santama et al. 1995) with different antibody-binding affinities.

A relatively large number of intensely labelled FMRFamide-immunoreactive cells was located in the cerebropleural ganglia. Each cerebropleural ganglia possessed a population of several immunoreactive cells scattered over central regions of the dorsal surface and another population of several more cells scattered over posterior regions of the ventral surface. Other intensely labelled immunoreactive cells were found on the ventral surface of the pedal ganglia and a few such cells were located in the buccal ganglia. This pattern of large numbers of FMRFamide-immunoreactive cells throughout the nervous system is generally consistent with staining in other gastropods (Cooke and Gelperin 1988; Schot and Boer 1982) but specific homologies have neither been suggested in the literature nor are they apparent from the present study. Generally, FMRFamide immunoreactivity is expressed in small to moderately sized neurones, which are difficult to recognize as individuals within species or as homologues between species. However, the FMRFamide-immunoreactive cells in the right pedal ganglion of *P. sibogae* are distinct in this regard. Like the anteromedial 5-HT-immunoreactive cells of the right cerebropleural ganglion, these pedal cells are possibly involved in the control of the penial complex. Both FMRFamide-immunoreactive cells and various cells in the right pedal ganglia have been found to innervate the male copulatory organs in other gastropods (De Lange et al. 1998; Li and Chase 1995; Smith and Croll 1998).

Among the various weakly labelled FMRFamide neurones found throughout the nervous system, the giant cells, PCG1 and PCG2, along the anterior margin of the cerebropleural ganglia are the most prominent. As mentioned previously, the PCG1 neurones, the largest and most anterior of these cells in *P. sibogae*, were originally thought to be the C1 cells by Willows (1985) who noted that their electrical stimulation caused pursing and protraction of the lips and mouth.

TPep immunoreactivity

The three giant pedal cells are the most prominent intensely labelled TPep-immunoreactive neurones in the central ganglia of *P. sibogae*. These cells are similar in their relative sizes and positions to the Pd5, Pd6 and Pd7 neurones that are TPep-immunoreactive in the nudibranch *T. diomedia* (Willows et al. 1997) and can therefore be considered as their homologues. Indeed, TPeps were originally isolated and characterized from

Pd5 and Pd6 of *T. diomedia* (Lloyd et al. 1996). TPep-immunoreactive neurones have also been located in *L. stagnalis* (Willows et al. 1997) and related peptides have been found in abundance in the pedal ganglia of *A. californica* (Hall and Lloyd 1990; Pearson and Lloyd 1990). In both *T. diomedia* and *A. californica*, the various related peptides have been implicated in the control of locomotion. The peptides contained in the Pd5 and Pd6 neurones of *T. diomedi* have been demonstrated to affect the activity of ciliated cells that propel the animal as it glides over a thin layer of secreted mucus (Willows et al. 1997) and, more recently, Popescu and Willows (1999) have correlated the electrical activity of Pd5 with the crawling rate of the animals. In *A. californica*, a related peptide has been shown to increase the amplitude of muscular contractions that underlie the galloping locomotory programme characteristic of this species (Hall and Lloyd 1990). Given the very different modes of locomotion regulated by the peptides in the two species, it is perhaps not surprising that individual cells containing the peptides may be different in *T. diomedia* and *A. californica*. In *P. sibogae*, however, which is more closely related to *T. diomedia* and is similarly propelled by ciliated pedal cells during locomotion, one could have reasonably predicted major similarities in the neuronal circuitry responsible for this behaviour. It should be noted, however, that the distribution of TPep-related peptides differs significantly in the cerebropleural and buccal ganglia of *P. sibogae* and *T. dimomeda* (Beck et al. 2000), thus suggesting that the involvement of these peptides in other behaviours might show less evolutionary consevation. The functions of the various other intensely labelled TPep-immunoreactive neurones in *P. sibogae* are unknown but related peptides in *T. dimomeda* have been shown to be active in the salivary ducts (Gaston 1998), whereas related peptides in *A. californica* have been found in a wide variety of peripheral tissues, such as the reproductive organs and gut, thereby indicating an involvement in a multitude of functions in addition to locomotion (Pearson and Lloyd 1990).

As with FMRFamide immunoreactivity, the reasons for differences in the intensity of TPep-immunoreactive staining are unknown. Again, it may be that some neurones simply contain lower concentrations of the same peptides than the intensely labelled neurones. Alternatively, they may contain other peptides that cross-react with the antibodies. Pearson and Lloyd (1990) have previously demonstrated the presence of additional immunoreactive but otherwise uncharacterized peptides in *A. californica*.

Development

Although total cell counts have not been conducted in *P. sibogae*, the number of central neurones in other opisthobranchs is estimated as 10,000 to 20,000 (Kandel 1979). Thus, we have examined about 1%–3% of the neurones of the central nervous system of *P. sibogae* in this study. Whereas this is a relatively small percentage, the cells, in addition to containing different transmitters, also cover a wide range of sizes and are located in all regions of the central ganglia. We therefore suggest that developmental changes in these cells probably represent general changes during the growth of the nervous system in postembryonic life.

We report here that the growth of the central nervous system in *P. sibogae* is attributable almost exclusively to growth in the sizes of individual cells, at least over the range of body sizes examined. This growth ranges from approximately the doubling in the sizes of many cells to much greater increases in the sizes of a limited number of other cells. For example, by the time that the animal reaches 14 mm or more in body length, the LSPG neurone consistently measures over 100 µm in diameter and covers approximately 25%–30% of the entire diameter of the pedal ganglion. The PCG1 neurone attains even larger sizes. The growth rates of the various cells reported here are generally consistent with changes in cell sizes reported in other gastropods (Arvanitaki and Tchou 1942; Coggeshall 1967; Croll and Chiasson 1989). However, other species examined in this regard also add new cells during postembryonic and/or postlarval development (Cash and Carew 1989; Coggeshall 1967; Croll and Chiasson 1989; Zakharov et al. 1998). Much of the cell addition in other species occurs within tight clusters of generally smaller cells (Croll and Chiasson 1989). In contrast, *P. sibogae* appears to possess few tightly clustered populations of cells. The one population that does appear to increase in number, however, is the group of FMRFamide-immunoreactive cells on the dorsal surface of the right pedal ganglion. As stated above, these cells may innervate the male copulatory organs, which, as Todd et al. (1997) have demonstrated, are functional even in animals at the smallest sizes examined in this study.

Postembryonic and postlarval development of the nervous system have not been studied frequently in gastropods and it is therefore unclear why cell addition appears to be rarer in *P. sibogae* than in other species studied. Three hypotheses might be proposed. First, this developmental strategy might be characteristic of certain opisthobranch gastropods such as nudibranchs but not anaspidean opisthobranchs (e.g. *Aplysia*) or basommatophoran pulmonates (e.g. *Lymnaea)*. Although postlarval development of the nervous system has not been studied in other nudibranchs, the adult nervous systems of species such as *P. sibogae*, *H. crassicornis* and *T. diomedia* share many seemingly homologous cells (Croll 1987a; Land and Crow 1985; Sudlow et al. 1998; Watson and Willows 1992; Willows et al. 1997). It is possible therefore that nudibranchs have generally opted for a similar developmental strategy whereby a limited number of neurones is generated early in ontogeny and subsequent body growth is accommodated largely by the growth of neuronal somata, which can, in turn, support increasingly larger neuritic arborizations. This developmental strate-

gy, however, may stand in contrast to a more general trend in gastropods favouring increasing numbers of smaller cells, as illustrated by the evolution of the nervous systems of many neogastropod prosobranchs and stylommatophoran pulmonates (Bulloch and Horridge 1965). Second, the small amount of cell addition observed in the present study may, at least in part, reflect the fact that *Phestilla* grows to a smaller final body size than *A. californica* (Kandel 1979) or even *L. stagnalis* (Croll and Chiasson 1989). Larger animals may depend more heavily upon cell addition than smaller animals do. Finally, although our animals ranged over a six-fold increase in size, we nonetheless examined only a restricted range of development within this study. Specifically, we did not examine animals before the onset of reproductive competence (Todd et al. 1997). In comparison, *L. stagnalis* adds new serotonergic cells to the PeIb cluster, which innervates the penis (De Lange et al. 1998), coincident with male maturation (Croll and Chiasson 1989). Similarly, *A. californica* adds the neuroendocrine bag cells at the time of female maturation (Coggeshall 1967; McAllister et al. 1983). Examination of *P. sibogae* at earlier ages or examination of different transmitter phenotypes might therefore reveal a larger amount of cell addition than is apparent in this study.

Conclusions

The present study provides an initial view of the organization of the central ganglia of the well-studied species *P. sibogae*. Whereas previous research has identified a few central neurones on the bases of cell size and position and electrophysiological characteristics, this study provides the first details of neuritic morphology and transmitter phenotypes. Furthermore, 200–300 additional cells have been described, many of which appear to be homologous with previously identified neurones in other species. However, several unique characteristics of the nervous system of *P. sibogae* suggest intriguing possibilities for the study of the evolution of brain and behaviour in nudibranchs in terms of changes in single identifiable neurones. As a pioneering study of postlarval development of the nudibranch nervous system, the present study also indicates that this taxon can provide an important counterpoint to themes issuing from studies of other gastropod species, such as *L. stagnalis* and *A. californica*. Finally, the details of the morphology and developmental trends noted here set the stage for research into the way in which the molluscan nervous system changes with metamorphosis, a process that mediates the abrupt transition from a suspension-feeding swimming veliger to a rasping crawling sea slug. The veliger of *P. sibogae* is well studied in terms of its neuronal organization. Questions regarding the way in which the nervous system of *P. sibogae* changes from its larval form to that of the sexually mature sea slug described here promise rich grounds for future exploration.

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