

# Regeneration and Molting Effects on a Proprioceptor Organ in the Dungeness Crab, *Cancer magister*

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## SUMMARY

Decapoda Crustacea molt in order to grow; some species, such as the Dungeness crab *Cancer magister*, achieve a very large size. Does sensory neuron hyperplasia in internal proprioceptors accompany this growth? To determine this, neurons in propodite-dactylopodite (PD) chordotonal organs were counted in first walking legs of juvenile (5th through 9th instar) and adult (10th through 13th instar) *C. magister*. We found that the PD organs of J5 crabs have about 56 neurons; the number increases to about 61 neurons in J6 crabs. Significant hyperplasia now occurs because an average of 79 neurons are found in the PD organs of J7 crabs. Little to no hyperplasia accompanies the several succeeding juvenile and adult molts (ca. 82–86 neurons are present). Because autotomized limbs are regenerated upon molting, we also examined how the number of PD organ neurons in regenerated legs compares with those of pristine legs. Newly regener-

ated legs (termed 1st stage regenerates) have fewer sensory neurons than do their contralateral pristine partners (65 vs 81); larger regenerated legs which have attained nearly normal size as a result of additional molts (2nd stage regenerates) still have fewer neurons than their pristine partners (69 vs 81). Additionally, in contrast to those of pristine walking legs, the elastic strand of PD organs from 1st stage regenerates is a misshapen sheet containing a cluster of small neurons with no obvious functional organization. Nonetheless, neurophysiological recordings indicate that all the receptor types typical for pristine legs (movement and position cells) are represented. The PD organs of 2nd stage regenerates differentiate to the shape and neuronal organization of pristine legs. © 1994 John Wiley & Sons, Inc.

**Keywords:** hyperplasia, regeneration, proprioceptor, chordotonal organ.

## INTRODUCTION

Decapoda Crustacea such as crabs, lobsters, and crayfish have a life cycle that includes zoeal, megalopal, juvenile, and adult stages. During the course of this development, animals grow via molting from less than 1 mm to a very large size. For example, the Dungeness crab, *Cancer magister*, grows from 0.7 cm (across the carapace) and 0.15 g in

body mass as a 1st instar juvenile crab to at least 21 cm carapace width and 1 kg by the 13th instar (3–4 years old). Is this increase in size and mass accompanied by neuronal hyperplasia?

The number of motor neurons that innervate individual leg muscles of adult crabs is very small, ranging from as few as 3 in the dactyl opener (Wiersma and Ripley, 1952) to about 10 in the levator and promotor muscles (Hoyle and Burrows, 1973; Moffet, 1975). Therefore, it seems unlikely that an increase in body size is accompanied by an increase in motor neuron number. Are mechanosensory neurons likewise constant in number during this increase in body mass and size? Certain mechanoreceptor neurons at the base of limbs, whose cell bodies lie within the CNS, have

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large axon diameters, produce graded potentials rather than actively propagating action potentials, and are few in number. For example, those of the walking legs, called the *thoracico-coxal muscle receptor organs* (TCMRO), have three or four neurons (Alexandrowicz and Whitear, 1957; Bush and Laverack, 1982). Similarly, there are only three neurons in the oval organs of the maxilla (Pasztor, 1969; Pasztor and Bush, 1983), two such neurons in the swimmerets (Miyay and Neil, 1986), and four mechanosensory neurons in the uropods (Paul, 1976). Thus, because there are so few neurons, it would seem that these particular mechanoreceptors are present in final number early in the life of the animal. In contrast, numerous external sensilla called *cuticular articulated pegs*, or CAP organs, are positioned at the articulations of maxillipeds, chelipeds, and walking legs (Barth, 1935; Wales et al., 1970). Because of their location, CAP organs have been proposed to be responsive to joint movements (Alexandrowicz, 1972). Laverack (1976) has shown that CAP organs at the leg joints of the lobster, *Homarus gammarus*, increase at an approximate rate of 10% at each molt. He has recently reviewed the topic of numbers of neurons in Decapoda Crustacea (Laverack, 1988).

Chordotonal organs that are analogous to vertebrate joint receptors are located at the various joints within crustacean limbs (see Mill, 1976 for a review). These proprioceptors consist of an elastic strand or sheet into which are inserted the dendrites of numerous bipolar sensory neurons (Burke, 1954; Whitear, 1962). Movement of the respective joints elongates and relaxes the elastic strand, which, in turn, excites specific sensory neurons (Hartman and Boettiger, 1967; Boettiger and Hartman, 1968). Is molting accompanied by an increase in the number of sensory neurons of these internal chordotonal organs; if so, then does the increase continue throughout life? As they progress through their life cycle, crabs may suffer loss of limbs. These limbs are replaced by regenerated ones as early as the next molt. How does the anatomy, neuron number, and function of proprioceptors from regenerated legs compare with those of pristine legs?

We report in this article that the number of sensory neurons in chordotonal organs of the crab *Cancer magister* increases at one juvenile molt; the number of neurons then remains nearly constant in succeeding juvenile molts and throughout adulthood. Furthermore, initially regenerated legs have significantly fewer neurons in their chordotonal organs than do their pristine contralateral controls.

Although this number may increase at the next molt, it does not attain the amount found in pristine legs.

## METHODS

Because of their great range in size as well as their ready availability, the Dungeness crab *Cancer magister* was used in this research. Juvenile crabs were netted or seined from the shallow water adjacent to the Oregon Institute of Marine Biology or at Sunset Beach, Oregon; adult animals were obtained by trapping from either Charleston Harbor or Coos Bay, Oregon.

The propus-dactylus (PD) organs of first-walking legs from 82 male crabs ranging from 2.8 to 21 cm in carapace width (CW), representing 5th through 13th instar animals, were chosen for sensory neuron counts. We were unable to dissect and expose the proprioceptors of smaller crabs. Every effort was made to select animals of known instar for neuron counts, but it is difficult to know with absolute certainty the instar of field-caught animals, particularly early juveniles, by size. For our experiments, we grouped field-caught crabs in juvenile and adult instars according to the carapace width data of Orsensanz and Gallucci (1988) and our own experience with stock from the Coos Bay, Oregon region. As may be noted in Table 1, there was an excellent likelihood that we assigned animals to correct instars. The mean carapace width values in all instars (except between the fifth juvenile (J5) and sixth juvenile (J6) instars) were significantly different from one another (ANOVA and Scheffe test,  $p < 0.05$ ). Because we wished to determine the number of sensory neurons in PD organs of pristine first-walking legs, it was necessary to differentiate them from regenerated legs. Pristine legs are longer than newly regenerated ones. Rather than measure the entire length of limbs, the dorso-lateral edge dimension of early propus was more conveniently measured and found to have a very narrow range in pristine legs at each instar (Table 1). We also selected crabs with a regenerated first-walking leg and a pristine contralateral partner for comparison of PD organ sensory neuron counts, noting both the carapace width and propus lengths. Crabs were induced to autotomize the first-walking leg by cutting slowly with scissors across the distal region of the meropodite. The isolated limb was bathed in cold *Cancer magister* saline (Macmillan and Dando, 1972) at pH 8.1, the pH of freshly drawn crab blood. The composition of the saline (salt/g/l) is as follows: NaCl/27.29, KCl/0.81, MgSO<sub>4</sub>·7H<sub>2</sub>O/4.81, CaCl<sub>2</sub>·2H<sub>2</sub>O/1.85, Na<sub>2</sub>SO<sub>4</sub>·10H<sub>2</sub>O/0.97, HEPES acid/0.476, HEPES salt/2.08. The PD organ was exposed by cutting a large window on the anterior side and a smaller window on the posterior side of the propus, leaving intact a cuticular bridge at the condyle of the latter side. The tendon of the opener muscle was severed at the dactyl attachment, and the entire muscle was removed and discarded. The muscle fibers of

**Table 1** Measurements of Carapace Width and Propus Length of the Pristine First-Walking Legs of Juvenile (J5–J9) and Adult (A1–A4) Instars of *Cancer magister* Used for Neuron Counts

Instar	Carapace Width (cm)	F Statistic	Propus Length (cm)	N
J5	3.08 ± 0.22		0.52 ± 0.04	5
J6	3.98 ± 0.40 N.S.	0.709	0.65 ± 0.10	12
J7	5.54 ± 0.24	3.084	0.89 ± 0.06	9
J8	6.90 ± 0.35	2.432	1.12 ± 0.07	13
J9	8.51 ± 0.47	2.352	1.35 ± 0.10	6
10 (A1)	10.74 ± 0.80	4.910	1.66 ± 0.14	10
11 (A2)	13.73 ± 0.61	11.504	2.17 ± 0.17	11
12 (A3)	16.40 ± 1.42	11.157	2.66 ± 0.31	15
13 (A4)	21		3.6	1

Note: Values are means ± S.D. The carapace widths are significantly different from each other (ANOVA and Scheffe test,  $p < 0.05$ ) except where noted. *F* value for 95% level is 2.068.

the closer were carefully dissected so as to completely expose the PD organ and its nerve bundle (Hartman and Boettiger, 1967). The PD organ bipolar sensory neurons stain deeply when bathed *in situ* for several hours, or overnight in a 0.05% solution of methylene blue in non-buffered saline (pH 6.6). Somata were then easily observed using transmission light microscopy, traced with the aid of a camera lucida attachment on an Olympus SZH stereomicroscope, and counted. The number of PD organ sensory neurons from 82 pristine walking legs of crabs ranging from the 5th through 13th instars was determined. Likewise, PD organ neuron counts of 23 regenerated first-walking legs were made and compared with those of their pristine contralateral partners.

Measurements of carapace width, propus length, and PD organ neuron numbers (total, movement, and position cells) at the various instars were analyzed using the one-way analysis of variance. Comparison between means were made with the Scheffe test. The Wilcoxon Signed-Rank test was used to compare the cell number counts of PD organs from regenerated legs with their contralateral pristine partners. To determine if the PD organ cell number means of 1st and 2nd stage regenerates differed from one another, the *t* test was employed (Sokal and Rohlf, 1981). Significance levels were established *a priori* at  $p < 0.05$ .

PD organs were prepared for transmission electron microscopy using the method of Gnatzy et al. (1984). Receptors were prefixed for 4 h at room temperature in 0.1 M phosphate buffer containing 5% glutaraldehyde and 15% sucrose at pH 7.4. After a wash with four changes of phosphate buffer/sucrose solution, the preparations were postfixed in 2% OsO<sub>4</sub> in phosphate buffer/sucrose for 2 h, washed in four changes of phosphate buffer/sucrose solution, and dehydrated in an ascending ethanol series. Upon imbedding in Epon 812, ultrathin sections of the PD organ nerve were cut with a diamond knife. Sections were double stained using lead citrate and uranyl acetate and examined with a JOEL electron microscope.

To compare the anatomical arrangement of neurons

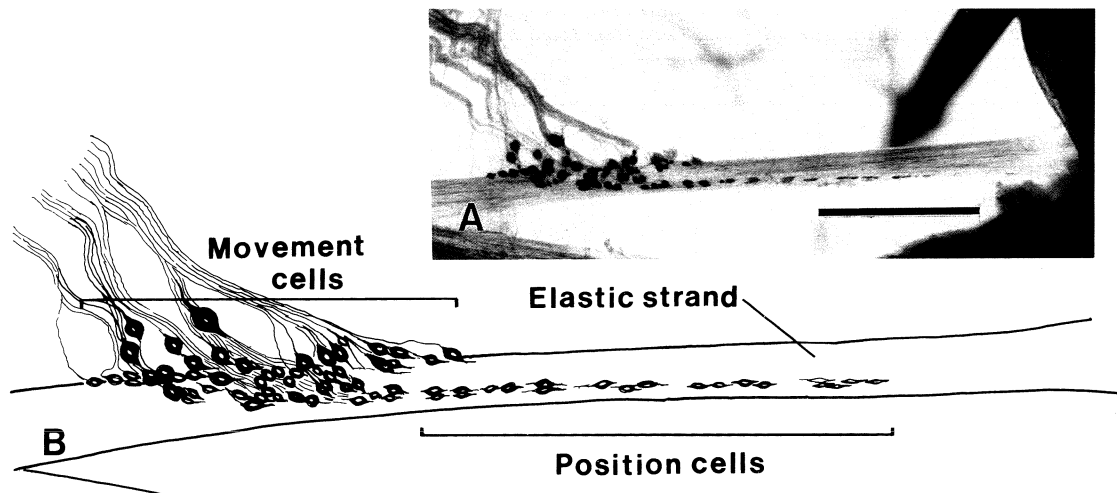
in the PD organ of regenerated and pristine walking legs, we used cobalt lysine to backfill the PD organ nerves (Lazar et al., 1983). The cobalt lysine was applied to the cut end of nerves placed in a petroleum jelly well. The preparation, immersed in filtered *C. magister* serum, was then incubated for 72 h at 4°C. Several washes in saline were followed by conventional ammonium sulfide precipitation, Carnoy's fixation, alcohol dehydration, and methyl salicylate clearing. While we did intensify some of the backfills using Delaney and Gelperin's (1990) modification of Lazar et al. (1983), most preparations stained so completely that intensification was unnecessary. *In situ* and isolated PD organs were photographed or traced with the aid of the camera lucida.

In order to determine if the sensory neuron responses typical of PD organs from pristine legs are to be found in those of regenerated legs, we recorded action potentials from nerve bundles of the latter using glass suction electrodes (Hartman and Boettiger, 1967). The elastic strand was moved over its physiological range by an electromechanical servomechanism (Hartman, 1985) that was controlled by an Apple Macintosh SE/30 computer, using MacLab (Mark II version) hardware. Neuron responses were displayed, stored, and printed using the system. To achieve maximum viability of the PD organ neurons in these experiments, we bathed the preparation in filtered *C. magister* serum at 15°C during the dissection and the recording sessions. The servomechanism, preparation, and manipulators were mounted on a TMC Vibration Isolation Systems table.

## RESULTS

### Neuron Count in PD Organs of Pristine Legs

The neurons of chordotonal organs are organized on the elastic strand according to their functions (Wiersma and Boettiger, 1959; Hartman and Boet-

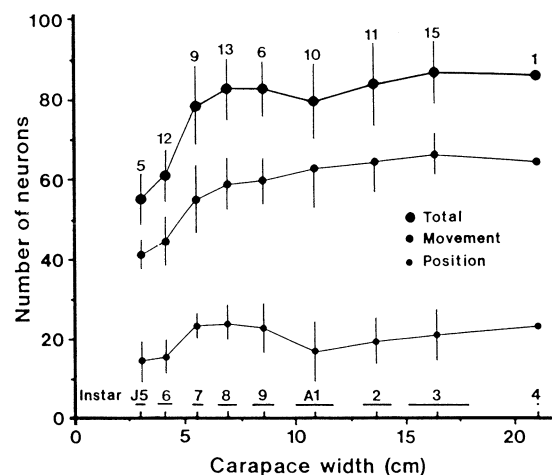


**Figure 1** Photograph (A) and camera lucida drawing (B) of a methylene blue-stained propus-dactylus (PD) organ from the pristine first-walking leg of a 10th instar (A1) *Cancer magister*. Twenty small position cells are arrayed along the elastic strand distally; 55 medium and larger movement cells are located along and atop the strand proximally. Scale bar = 1 mm.

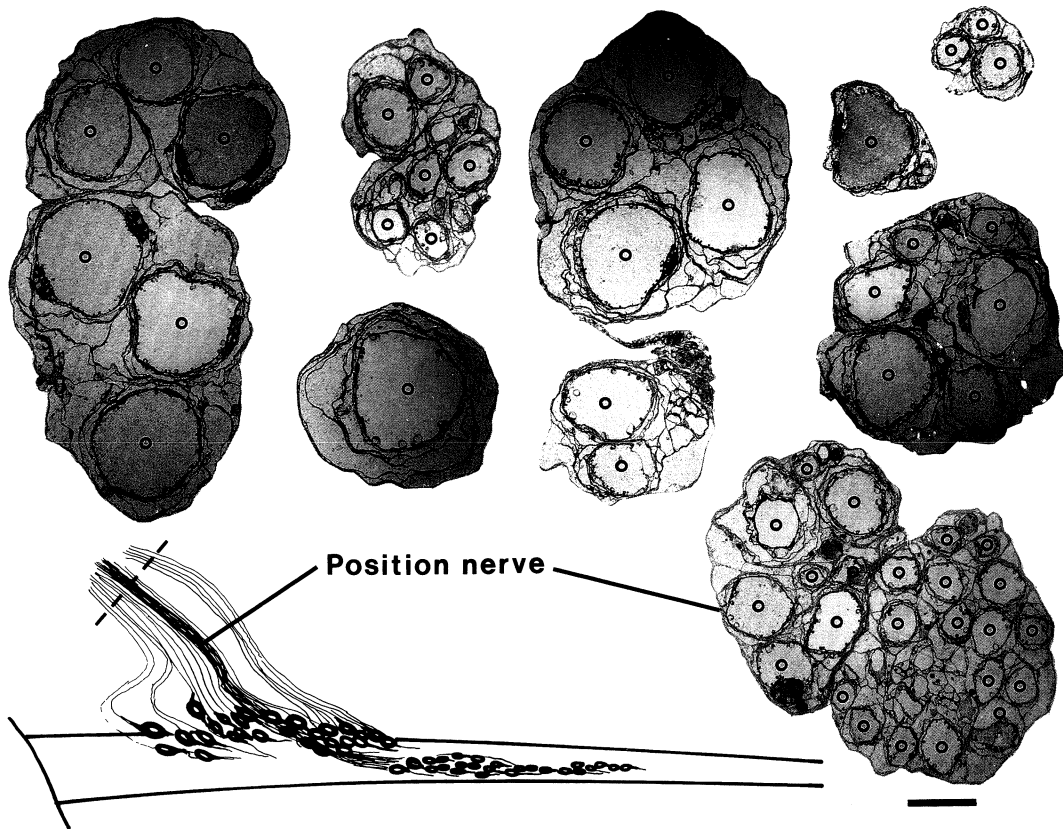
tiger, 1967). Figure 1(A) is a photograph of a typical PD organ stained with methylene blue which illustrates the general organization. Distally, small "position cells," which fire tonically to maintained static positions of the dactyl, are arranged in near single file. Phasic "movement-sensitive cells" with medium to large somata are located at the proximal end of the strand (Hartman and Boettiger, 1967). Observations and the camera lucida drawing made from this preparation reveals the somata of 20 position and 55 movement neurons [Fig. 1(B)].

The results of PD organ cell counts from pristine legs of several juvenile (5th through 9th instar) and all adult (10th through 13th instar) stages are shown in Figure 2. Crabs at the 5th instar (ca. 3 cm CW) have  $56.2 \pm 5.8$  (mean  $\pm$  S.D.) neurons; 6th instar animals have  $60.8 \pm 6.8$  neurons, although the difference in neuron number count for 5th and 6th instar PD organs is not statistically significant. Seventh instar crabs (ca. 6 cm CW) clearly show the results of hyperplasia; there are  $79.6 \pm 9.9$  neurons present, about 23 position cells and 55 movement cells. The total neuron counts (movement plus position cells) of 6th and 7th instar crabs are statistically different (ANOVA and Scheffe test,  $p < 0.05$ ). The cell count rises to  $83.1 \pm 7.2$  neurons at the 8th instar and remains there at the 9th instar ( $82.1 \pm 6.5$ ). The count falls slightly to  $79.8 \pm 9.8$  neurons at the adult molt (A1) or 10th instar, apparently due to a decline in the position cell number. In the molts that follow, the number of posi-

tion and movement neurons fluctuates slightly to produce total counts of between 84 and 86 neurons (Fig. 2). The total neuron counts from the 7th instar through the 13th instar (A4) are not statistically different.



**Figure 2** Graph summarizing the number of neurons (movement, position, total) in PD organs from pristine first-walking legs of juvenile and adult instars of *Cancer magister*. Cell counts are indicated as means ( $\pm$ S.D.) for the number of preparations indicated above the total bar. Animals are grouped into instars by carapace width according to the method of Orensanz and Gallucci (1988). Note that there is little to no increase in neuron number after the 7th instar even though there is significant growth of the animals.



**Figure 3** Cross-section montage of PD organ nerves from the preparation figured in the camera lucida drawing (inset). Dashed lines indicate the region of the section. Fifty-six somata were counted in the methylene-blue stained organ; a total of 53 axon profiles (indicated by small circles), 24 position cells, and 29 movement cells were found in the nine bundles. Scale bar = 10  $\mu\text{m}$ .

Although we were confident that our staining and microscopy methodology yielded accurate neuron count data, we examined cross-sections of axons of the PD organ nerve using electron microscopy to verify our somata counts. The adult (A1) preparation shown in the inset of Figure 3 revealed 56 neurons with methylene blue staining. The axons of 26 small somata extending in a line from the distal part of the strand were observed to form a small subnerve and judged to be position cells. Thirty other somata which were located elsewhere on the elastic strand, most of which were larger than the former, were evaluated to be movement neurons. This same preparation was then fixed *in situ*, embedded in Epon, and the PD organ nerve bundles were cross-sectioned at a point before the merger with tension nerve (closer apodeme tension nerve). Using low-magnification transmission electron microscopy, the axon cross-sections were assembled into a montage for counting purposes. As seen in Figure 3, there is a distinct bundle (the position nerve) containing 24 small (3–5  $\mu\text{m}$ )

and medium size (6–9  $\mu\text{m}$ ) axons. Twenty-nine axons, several very large (15  $\mu\text{m}$ ), are arranged in the eight remaining bundles. Thus, the axon count (53 cells) from TEM cross-sections of nerve bundles from this preparation is in very close agreement with the cell count (56 cells) data obtained by staining.

### PD Organs in Regenerated Legs

Regenerated legs in *C. magister* fall into two recognizable groups. Newly regenerated legs which we have chosen to designate 1st stage regenerates are easily identified because they are considerably shorter, more rotund, and much less pigmented than their pristine contralateral partners. The dorso-lateral edge dimension of the propus of these legs is considerably less than that of their pristine partner. Specifically, it ranged from 58% to 73% (64.1%  $\pm$  5.5%) of the partner's length for the 10 animals examined (Table 2).

Each time the crab molts, the regenerating leg

**Table 2 Propus Length and PD Organ Neuron Number of Pristine and 1st Stage Regenerate Walking Legs in the Dungeness crab, *Cancer magister***

Case	Instar Number	Propus Length (cm)		Neuron Number	
		Pristine	Regenerate	Pristine	Regenerate
1	6	0.6	0.4	70	59
2	8	1.1	0.65	76	57
3	10	1.7	1.1	86	72
4	10	1.8	1.2	73	52
5	10	2	1.15	85	81
6	11	2	1.4	79	57
7	11	2	1.3	79	57
8	11	2.2	1.6	80	70
9	11	2.1	1.5	86	63
10	12	2.5	1.5	82	75

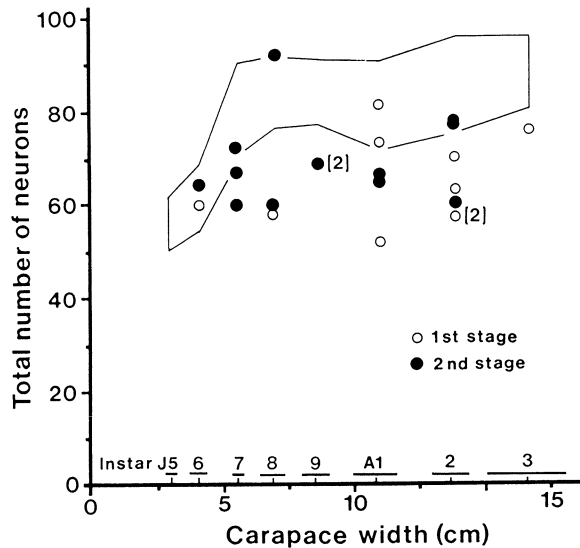
increases in size until its dimensions approximate those of its intact contralateral partner. The propus length for 13 multiply-molted animals that we examined ranged from 80% to 97% ( $86.3\% \pm 5.0\%$ ) of their partner's (Table 3). Such regenerated legs are as flattened and pigmented as pristine ones are. Because we have no way to determine how many times animals in this group may have molted since the loss of the limb, we have collectively grouped these legs as 2nd stage regenerates.

The number of neurons found in the PD organs of regenerated first-walking legs was less than that found in pristine contralateral legs (Fig. 4). Only three cell counts from 10 preparations of 1st stage regenerates fell within a standard deviation of the mean for pristine legs, while five cell counts of 13 preparations for 2nd stage regenerates fell within

that range. The two regenerated J6 preparations, while having cell counts within a standard deviation of the mean, had fewer cells than did their pristine partners. Because J6 walking legs ordinarily have significantly fewer neurons in the PD organs (Fig. 2) than later instars, their counts were omitted from the following cell count data summary. First stage regenerate neuron counts of 7th through 12th (A3) instar animals were  $64.9 \pm 9.9$ , while the number of cells in the PD organs of their pristine partners numbered  $80.7 \pm 4.5$  (Table 2). The neuron count of 2nd stage regenerates was higher,  $68.8 \pm 9.2$ , but still far less than their partners of  $80.7 \pm 9.1$  (Table 3). However, the number of neurons found in PD organs of 1st stage regenerates was not significantly different from those of 2nd stage regenerates ( $t$  test,  $p > 0.05$ ). In

**Table 3 Propus Length and PD Organ Neuron Number of Pristine and 2nd Stage Regenerate Walking Legs in the Dungeness Crab, *Cancer magister***

Case	Instar Number	Propus Length (cm)		Neuron Number	
		Pristine	Regenerate	Pristine	Regenerate
1	6	0.8	0.7	71	63
2	7	1	0.9	75	72
3	7	1	0.8	76	66
4	7	1	0.8	93	59
5	8	1.1	0.9	92	91
6	8	1.15	1	76	59
7	9	1.2	1.1	69	68
8	9	1.45	1.4	75	68
9	10	1.6	1.45	69	64
10	10	1.8	1.45	77	65
11	11	2	1.7	84	60
12	11	2.1	1.8	93	77
13	11	2.1	1.8	89	76



**Figure 4** Graph summarizing the total number of neurons (movement and position) in PD organs from 1st and 2nd stage regenerate first-walking legs of juvenile and adult instars of *Cancer magister*. In each case the number of neurons in the intact contralateral walking leg was determined. The boxed area is the mean total cell number  $\pm$  one standard deviation for pristine legs from Figure 2. All cell counts for regenerated legs were less than that for their intact partners and most fell below the range seen for pristine legs.

every case, the number of neurons on the PD organ of a regenerated leg was less than that found on its pristine contralateral partner (Tables 2, 3); the Wilcoxon Signed-Rank test comparing pristine and regenerate cell numbers showed the differences to be highly significant ( $p = 0.0051$  for 1st stage regenerate,  $p = 0.0022$  for 2nd stage regenerate). In two cases, the difference in cell number was small (68 vs 69, 91 vs 92) for 2nd stage regenerates, but more typically the difference was substantially greater (Tables 2, 3).

The appearance of the elastic strand and neuronal organization of the PD organ of 1st stage regenerated walking legs is very different from that of pristine legs. The elastic strand and neurons of a newly regenerated PD organ are tightly woven with an envelope of connective tissue, and there is a branch of a blood vessel that directs circulation to the region. Removal of the envelope reveals that, rather than a long tapered strand with the neurons arrayed according to function, the strand of 1st stage regenerates is a flattened sheet with small neurons tightly clustered at the proximal end. Typically, no position cells were seen aligned along the distal length of the strand in 1st stage regenerates,

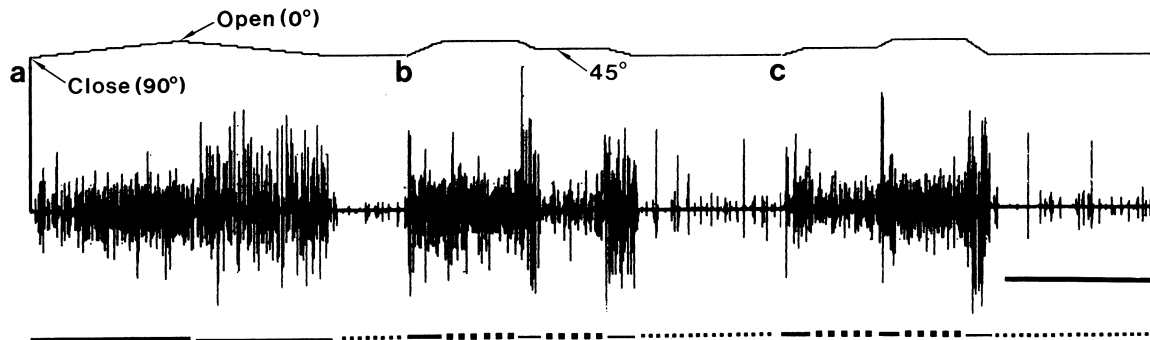
suggesting that position cells may not be represented in these PD organs. This organization was observed in methylene blue preparations in which cell counts were made, and in three cobalt lysine backfilled specimens. It should also be noted that the somata of 1st stage regenerate neurons are uniformly smaller in size than those found in the PD organs of pristine contralateral legs. For instance, in one preparation, the somata measured  $37.8 \pm 12.7 \mu\text{m}$ ; somata of a pristine leg at this instar included large movement cells and small position cells. The latter diameters were thus  $53.4 \pm 23.6 \mu\text{m}$ .

Examination and methylene blue staining of 2nd stage regenerates revealed that the elastic strand in these legs is tapered as it is in pristine legs, and that the sensory neuron somata are larger and distributed along the strand in an organization which is indistinguishable from that of pristine legs (Fig. 1). Such legs, however, continue to have fewer neurons.

### Physiological Recordings from 1st Stage Regenerates

Do the PD organs of 1st stage regenerates with their lower than normal neuron number and abnormal anatomical arrangement have both movement and position cells represented? In particular, because there is no anatomically distinct row of small position cells along the distal margin of the strand, and no accompanying discrete position nerve as in pristine legs (Fig. 3), there appear to be no position cells present. Recordings of action potentials were made from small nerve bundles about 15 mm proximal to their exit from the PD organ while the strand was moved over its normal physiological excursion and held briefly at various positions. A record from one such bundle, which is typical for the three preparations examined, is shown in Figure 5. This particular bundle was selected for illustration because it contains not only position cells but representative movement neurons as well. For the most part, the other nerve bundles were composed of movement neurons.

When the strand is moved from the closed position ( $90^\circ$ ) so that it becomes increasingly relaxed to mimic opening the dactyl to  $0^\circ$ , relaxation-sensitive movement cells (RSMC) fire throughout the movement. Upon changing direction to cause the strand to be increasingly stretched and mimic closing the dactyl, larger elongation-sensitive movement cells (ESMC) respond during the movement.



**Figure 5** Extracellular recording from one bundle of the PD organ nerve of a 1st stage regenerated leg of *Cancer magister*. The distal end of the elastic strand was moved over its normal excursion to mimic opening (upwards toward  $0^\circ$ ) and closing (downwards toward  $90^\circ$ ), and static positions of the dactyl. The strand is relaxed by opening joint movements (upper record). The lower record shows action potentials from large phasic movement cells, either relaxation-sensitive movement cells (RSMC) or elongation-sensitive movement cells (ESMC), and smaller cells which fire tonically to maintained positions. The latter are classified as either relaxation-sensitive position cells (RSPC) or elongation-sensitive position cells (ESPC). RSMC and ESMC are seen to fire throughout the movement from  $90^\circ$  to  $0^\circ$  and return to  $90^\circ$  in record (a) and during short movements in records (b) and (c). ESPC activity is seen at  $90^\circ$  at the end of records (a), (b), and (c). RSPC fire at low frequency at  $45^\circ$  as seen in record (c), and at high frequency at  $0^\circ$  in records (b) and (c). Time base = 0.5 s.

Very small elongation-sensitive position cells (ESPC) fire when the dactyl is held closed at  $90^\circ$  [Fig. 5(a)]. With a rapid movement to  $0^\circ$ , the RSMC fire a high-frequency burst, but relaxation-sensitive position cells (RSPC) are seen to fire tonically with the strand maintained at  $0^\circ$ . The ESMC fire a high-frequency burst during a rapid movement to  $45^\circ$ , and firing by position cells is seen with the strand maintained at  $45^\circ$ . This firing is by the same position cells (RSPC) that are seen at  $0^\circ$ ; hysteresis is typical of relaxation-sensitive position cells (Hartman and Boettiger, 1967). The rapid movement from  $45^\circ$  to  $90^\circ$  is accompanied by a high-frequency burst by the ESMC. Again, small ESPC tonic activity follows the return to the  $90^\circ$  position [Fig. 5(b)]. When the strand is rapidly moved to  $45^\circ$ , there is a burst of firing by the RSMC. If the  $45^\circ$  position is maintained, then the small RSPC fire tonically at the low rate. A quick movement to  $0^\circ$  is again accompanied by a burst from the RSMC, and upon achieving the  $0^\circ$  position, the RSPC fire tonically at a still greater rate. There is a high-frequency burst by ESMC when the strand is rapidly moved to  $90^\circ$ , and tonic activity is again seen by ESPC upon reaching  $90^\circ$  [Fig. 5(c)]. The pattern of firing by movement and position cells in this 1st stage regenerated PD organ preparation is indistinguishable from pristine preparations (Hartman and Boettiger, 1967; Boettiger and Hartman, 1968).

## DISCUSSION

The initial objective of this research was to determine if there is continuous addition of sensory neurons to internal chordotonal organs of crab walking legs as the animals grow. To do this, we examined the PD chordotonal organs of many specimens of *C. magister* representing several juvenile and all adult instars. Our results clearly indicate that, over the range of instars that we observed, sensory neuron hyperplasia is confined to the J6–J7 molt. Later juvenile (J8, 9) and four adult (A1–4) molts are unaccompanied by neuron hyperplasia in spite of great increases in mass and size of the crabs (Fig. 2). Approximately 82–86 neurons are present from the 8th instar onward. Thus, in *C. magister* the final number of chordotonal organ neurons is achieved during a juvenile instar, with other juvenile molts to follow, and is not correlated with molting to the adult stage. The Dungeness crab metamorphoses from a megalops to a 1st instar juvenile in the springtime. It molts rapidly during the summer months and overwinters as a J7 crab. The cessation of PD organ neuron hyperplasia is thus correlated with the first overwintering period. Interestingly, this is also about the time the animals begin their shift from juvenile to adult hemocyanin (N. Terwilliger, personal communication).

In marked contrast to our findings, recent stud-



ies by Cooper and Govind (1991) investigating the PD organs of the asymmetrical claws of *Homarus americanus* show that in an age-graded series of lobsters (1, 2, 5, and 15 years), the number of PD organ neurons increases from 214 to 1855 in the cutter, and from 281 to 4677 in the crusher over that span of years. This result is similar to that obtained by Laverack (1976) for *H. gammarus* in which he noted that CAP organs are also added at each molt. But as far as the sensory neurons of the PD organs are concerned, those of crab walking legs reach a final number at a juvenile stage (J7), whereas those of the lobster claws would appear to continue to be added as the animal ages.

The results of these two studies are difficult to compare directly because the animals, even though they are both Decapoda Crustacea, are very different in many ways. The Dungeness crab *C. magister*, a member of the infraorder Brachyura, is a true crab with symmetrical claws. Growing to about 1 kg in its life span of 6 or more years, it has a terminal molt. By contrast, the American lobster *H. americanus* (with its first three pairs of legs being chelate, the first pair becoming asymmetrical and increasingly robust as the animal grows) is in the infraorder Astacidea (Williams, 1984). It reaches at least 20 kg in mass, may live beyond 50 years, and does not reach a final size because it has no terminal molt (Phillips et al., 1980). Thus, crabs and lobsters are in disparate taxonomic groups, have developmental differences, are grossly different in body structure, and in addition, use dissimilar walking patterns.

The number of neurons in the PD organ, and in all likelihood those of other internal chordotonal organs of *C. magister*, does not increase much once the animal has reached the J7 instar. It may be that the basic rhythm for walking which is generated in the central nervous system requires input from only a limited number of sensory neurons to signal joint movement and position, and that additional parallel channels of chordotonal input are unnecessary beyond those present at juvenile instars. Perhaps, instead of hyperplasia, these neurons now remodel their connections with central target cells by increased axonal branching as an adjustment to growth. Laverack (1987) has speculated that "centrally located receptors may well indicate a once-for-all origin, whilst chordotonal and setal receptors add to their numbers continuously throughout life." While this is not the case of chordotonal organs in crab walking legs, it may yet be true for those in lobster chelipeds and walking legs. It is obvious that the chelipeds of *C. magister* and the

subchelate and walking legs of *H. americanus* will have to be examined to resolve the issue. Our work in progress indicates that, in contrast to the great numbers found in the lobster, PD organs in the chelipeds of adult *C. magister* have 80 or fewer neurons.

The second objective of this research was to compare the PD organs of regenerated legs with pristine ones. Regenerated first walking legs of *C. magister*, particularly 1st stage regenerates, are easily distinguished from their contralateral pristine partners. They are distinctly shorter, the dactyl and propus are more rotund, and the cuticle is much less pigmented than pristine legs (Weis, 1976). Unlike pristine legs, the PD organ at this stage is clothed in an envelope of connective tissue at the terminus of a blood vessel. This arrangement is in all likelihood a remnant of the efficient folding and packaging necessary to the construction of the regenerating limb and its enclosed constituents. Rather than a tapered strand, the elastic strand of PD organs of 1st stage regenerates is frequently a misshapen sheet in some ways similar in appearance to that of normal CP1 and MC1 organs (Whitewar, 1962). Receptor cells of uniform small size are clumped at the proximal portion of the strand. Because of this arrangement, it is impossible to distinguish position from movement cells based on cell location. Our cell counts of such preparations reveal that 1st stage regenerates have about 16 fewer sensory neurons than do their pristine partners (Fig. 4, Table 2). The cell counts fall within the range expected for J5 and J6 animals. Nonetheless, in spite of the reduced number of sensory cells and clustered anatomical arrangement, our neurophysiological recordings (Fig. 5) indicate that all sensory cell types (RSMC, ESMC, RSPC, and ESPC), which are typical of PD organs of pristine legs, are represented in 1st stage regenerated legs. Although there is no soma size gradation, it is probable that the more distal of the neuron clusters are the position cells.

When the crab molts again, the resultant 2nd stage regenerate legs have normal pigmentation. However, they are still distinguishable from pristine legs on the basis of their smaller size. The appearance of the PD organ elastic strand and its neuronal organization becomes pristine-like, the position and movement cells in the 2nd stage regenerates being organized according to function along the elongate and tubular strand. However, there are about 12 fewer neurons in these organs than found on their pristine partners [Fig. 2(B)]. Mayes and Govind (1988) noted that when major

and minor claws of male fiddler crabs, *Uca pugnax*, are compared, the number of sensory axons in the nerve of the major is 3× greater than that on the minor side. However, newly regenerated major claws (equivalent to our 1st stage regenerated legs) have only 1.8× as many as the pristine minor (Mayes and Govind, 1988). Our findings suggest that some of that diminution (particularly in the medium to larger axon range) is due to the reduced number of chordotonal organ neurons.

Since PD organ neuron hyperplasia occurs during early juvenile instars (up to J7), legs regenerated during those instars may eventually attain the neuron number found in pristine legs. Such regenerated legs ultimately become indistinguishable from original limbs in size and appearance. Longitudinal studies beginning with early juvenile animals should reveal if neuron number of PD organs in these regenerated legs ever returns to the range typical of pristine legs.

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