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Tentacle morphogenesis in hydra

I. The role of head activator

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Summary

Stimulation of tentacle-specific cell differentiation by the neuropeptide head activator was investigated in *Hydra magnipapillata*. Tentacle-specific sensory nerve cells were identified by a monoclonal antibody, NV1. Treatment of hydra with 1 pM head activator for 18 h stimulated differentiation of NV1$^+$ nerve cells and tentacle epithelial cells in tissue from the distal gastric region. Head tissue and tissue from the proxmial gastric region did not respond to head activator treatment with increased NV1$^+$ differentiation. Hence the distal gastric region appears to be the site of tentacle formation in hydra. Tentacle precursors in head tissue seem to be committed since they fail to respond to head activator or to changes in tissue size with altered amounts of tentacle formation. We suggest that NV1 precursors form a complex with tentacle epithelial cell precursors, which then moves distally through the head region into the tentacles. The signal for NV1$^+$ differentiation appears to be formation of this complex.

Key words: hydra, head activator, nerve cell differentiation, tentacle morphogenesis.

Introduction

In many developing embryos and, in some cases, also in adult organisms, the differentiation pathway of a cell is determined by its position within the tissue. This is especially true for interstitial stem cells in hydra. Stem cells in adult polyps of hydra form a population of multipotent interstitial cells (David and Murphy, 1977; Bosch and David, 1987), which give rise continuously to differentiated products, such as nematocytes and nerve cells (David and Gierer, 1974). Whereas differentiation of nematocytes is restricted exclusively to the gastric region, nerve cell differentiation occurs primarily in the head and foot of hydra (for review see David *et al.* 1987).

In an attempt to identify signals controlling morphogenesis and patterns of cell differentiation in hydra, Schaller (1973, 1976) characterized factors in extracts from hydra tissue that enhanced head formation and nerve cell differentiation. The active component in these extracts was isolated and identified as the neuropeptide 'head activator' (Schaller and Bodenmüller, 1981). Synthetic head activator (0.1–10 pM) has recently been shown to stimulate formation of committed nerve cell precursors (Holstein *et al.* 1986). These cells then require a second signal, which can be released by injuring tissue, to develop into mature nerve cells; otherwise they accumulate in an arrested state before final differentiation (Holstein and David, 1986; Hoffmeister and Schaller, 1987).

In the present experiments, we have investigated the role of head activator in position-specific cell differentiation in hydra. We have used a monoclonal antibody, NV1, which specifically recognizes a subpopulation of tentacle-specific nerve cells. The results indicate that head activator stimulates formation of NV1$^+$ nerve cells and, in addition, formation of tentacle-specific battery cells. Since stimulation only occurs in the distal gastric region, we conclude that this region is the site of tentacle formation. Committed tentacle precursors, which are no longer able to respond to head activator, then move through the head region into tentacles. Nerve cell differentiation *per se*, however, is not inhibited in head tissue since differentiation of nerve cells recognized by a second monoclonal antibody, NV4, is stimulated by head activator.

Since differentiation of tentacle-specific NV1$^+$ nerve cells is closely correlated with tentacle formation both quantitatively and in terms of the kinetics of appearance, we suggest that NV1$^+$ differentiation is not directly stimulated by head activator but rather indirectly *via* formation of a tentacle-specific complex between a nerve cell precursor and a battery cell precursor. The requirements for complex formation are investigated in detail in the accompanying report (Hobmayer *et al.* 1990).

Materials and methods

Animals and culture conditions

Hydra magnipapillata strain 105 (Sugiyama and Fujisawa,

1977) were used in all experiments. Animals were cultured at 18°C in a modified Loomis and Lenhoff (1956) medium containing 1 mM $CaCl_2$, 0.1 mM $MgCl_2$, 0.1 mM KCl, and 1 mM $NaHCO_3$ in deionized water, adjusted to pH 7.5 with HCl. Hydra were fed daily with freshly hatched *Artemia* nauplii and washed 6–8 h later. Experimental animals bearing a young stage 1 to 3 bud (Otto and Campbell, 1977) were collected 24 h after feeding. All experiments were carried out in a constant temperature room maintained at 18±1°C.

Maceration of hydra tissue

Hydra tissue was macerated (David, 1973) in a modified maceration solution containing glycerin:acetic acid:water (1:1:7) at room temperature. Identification of cell types was also done according to the classification given by David (1973).

Production of monoclonal antibodies

Monoclonal antibodies were raised as described elsewhere by Schmidt and David (1986). Supernatants from hybridoma cultures were used directly for antibody staining or diluted appropriately in PBS (phosphate-buffered saline).

Visualization of antibody binding

Using indirect immunofluorescence, binding of monoclonal antibodies to specific cell types of hydra was visualized on whole mounts and macerated cells.

Whole-mount staining

Animals were anesthetized in 2 % urethane in hydra medium for 1 min. Then the relaxed and elongated hydra were fixed with Lavdowsky's fixative (ethanol:formalin:acetic acid:water – 50:10:4:40) for more than 12 h. Thereafter, they were washed 30 min in PBS with 0.1 % BSA (bovine serum albumin) and 0.02 % azide and transferred in wells of a 96-well microtitre plate (Costar). Next, the whole mounts were incubated for at least 2 h, normally overnight, in 50 µl of a specific monoclonal antibody solution and after this for 2–6 h in 50 µl of fluorescein isothyocyanate (FITC)-conjugated goat anti-mouse immunoglobulin IgG/IgM (TAGO, California) diluted 1:50 in PBS/BSA/azide. Finally, they were washed 30 min in PBS and mounted on microscope slides in PBS/glycerin (1:9). All steps were done at room temperature.

In a sequential double-staining procedure, binding of a monoclonal antibody was combined with nuclear DNA staining using the DNA-specific fluorochrome DAPI (Leeman and Ruch, 1982). Binding of the monoclonal antibody was visualized as described above using FITC-conjugated immunoglobulin. After washing the whole mounts briefly in PBS/BSA/azide, they were transferred to ethanol (96 %) for 15 min. Then they were washed again in PBS/BSA/azide (30 min) and incubated in DAPI (4′,6-diamidino-2-phenylindole-dihydrochloride, Serva) for 2 h. The staining procedure was again finished by washing the whole mounts in PBS for 30 min.

Staining of macerated cells

Fixed cell suspensions were spread on gelatin-coated slides with a small drop of detergent (Tween 80, Merck) and dried overnight. Then the macerates were washed in PBS/BSA/azide for 30 min and transferred to a humid chamber. The slides were stained with a specific monoclonal antibody for 2–6 h, rinsed briefly in PBS/BSA/azide, and subsequently stained for 2–6 h with FITC-labelled goat anti-mouse IgG/IgM diluted 1:50 in PBS/BSA/azide. After that, cells were washed in PBS for 30 min and, finally, cover slips together with a drop of PBS/glycerin (1:9) were placed on the slides. All steps were done at room temperature.

Whole mounts and macerated cells were observed with a Leitz Dialux 20 microscope equipped with epifluorescence attachment and filterblocks I2 (excitation wavelength 450–490 nm, barrier filter 515 nm) and A (excitation wavelength 340–380 nm, barrier filter 430 nm).

Determination of cell numbers

Numbers of $NV1^+$ and $NV4^+$ nerve cells were counted in antibody-stained maceration preparations. 500–1000 epithelial cells and the corresponding number of nerve cells were counted in a given sample.

In addition, the number of $NV1^+$ nerve cells was also counted in whole mounts. To determine the number of $NV1^+$ cells relative to the number of tentacle epithelial cells, we used the DNA-specific fluorochrome DAPI (Leeman and Ruch, 1982). Large epithelial cell nuclei became clearly visible after DAPI-staining and could be easily distinguished from nuclei of other cell types. Since FITC and DAPI have very different excitation and emission maxima, NV1- and DAPI-stained whole mounts could be analyzed without interference by switching from filterblock I2 to A.

Cell number/tentacle set refers to the total number of cells in all tentacles of a single animal.

Preparation of hydra head activator

The neuropeptide head activator [pGlu-Pro-Pro-Gly-Gly-Ser-Lys-Val-Ile-Leu-Phe (Schaller and Bodenmüller, 1981)] was synthesized by BACHEM (Switzerland). Head activator was lyophilized in 5 nM samples and stored at −20°C. To stabilize its biologically active monomeric form (Bodenmüller *et al.* 1986), 5 nM were solubilized in 250 µl 3 M $(NH_4)_2SO_4$ and 250 µl distilled water. Thereafter, the solution of 10 µM head activator was diluted with 1 % BSA in PBS to a concentration of 10 nM and diluted with hydra medium to an end-concentration of 1 pM. The dilution procedure was done on ice.

Results

NV1 staining pattern in adult polyps

The monoclonal antibody NV1 stains about 350 epidermal sensory nerve cells located in the tentacles of hydra (Fig. 1A). A few additional $NV1^+$ nerve cells are located near the basal disc (Fig. 1B); $NV1^+$ nerve cells do not occur in the rest of the body column.

Each tentacle-specific $NV1^+$ nerve cell is embedded within a battery cell; the cell body is polar and extends a cilium apically to the epidermal surface and two or more processes basally to the mesoglea (Fig. 2). Whole-mount preparations show that these sensory nerve cells innervate the nematocyst batteries of several neighboring battery cells, whereas there is no connection between individual $NV1^+$ cells (see Fig. 1 in accompanying paper).

A small number of $NV1^+$ ganglion cells (15–20 per animal) forms a narrow ring around the peduncle near the basal disc (Fig. 1B). These multipolar ganglion cells are characterized by a thick cell body and short processes. Like $NV1^+$ sensory cells in the tentacles, the $NV1^+$ ganglion cells in the peduncle ring do not contact neighbouring $NV1^+$ cells.

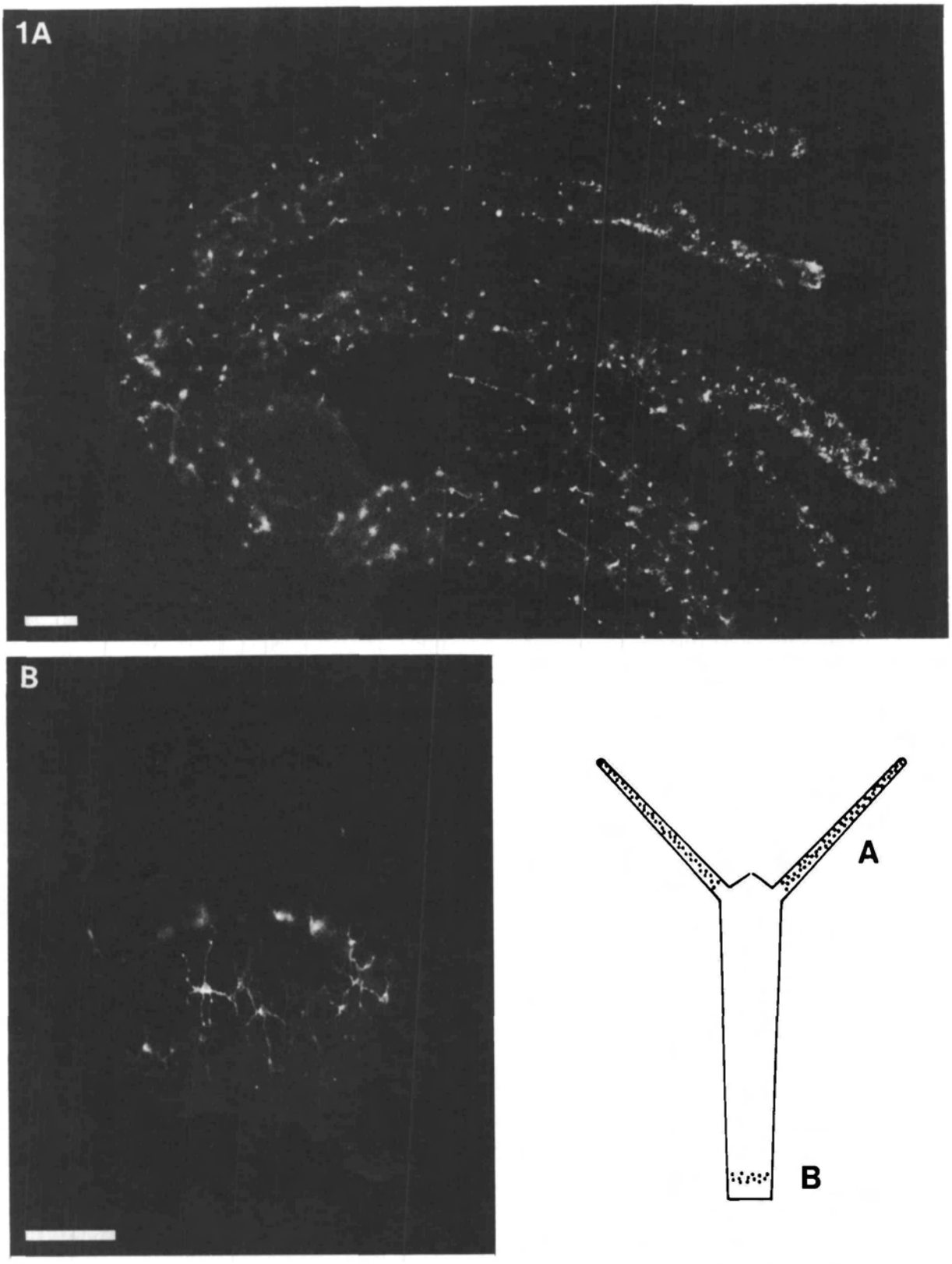

Fig. 1. The spatial distribution of $NV1^+$ nerve cells in *Hydra magnipapillata*, visualized *in situ* by indirect immunofluorescence. (A) Epidermal sensory nerve cells in the tentacles. (B) Ganglion cells which form a narrow ring around the lower peduncle. Bars: 50 μm.

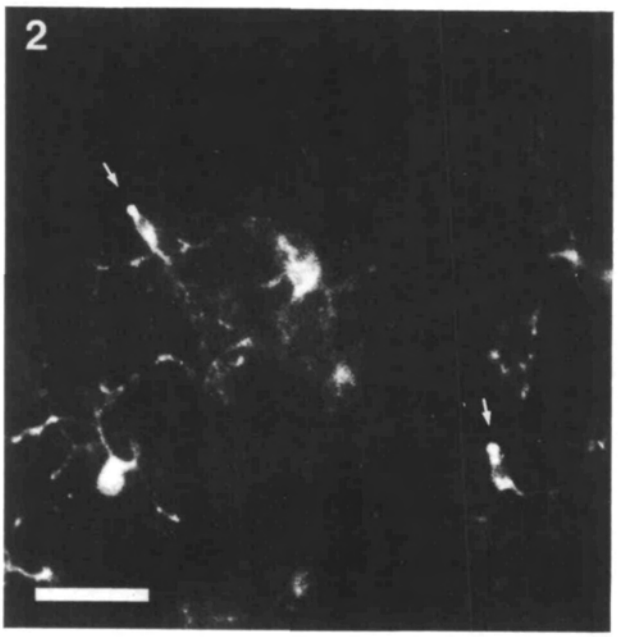

Fig. 2. $NV1^+$ epidermal sensory nerve cells in tentacles of *Hydra magnipapillata*. The intensely stained tips of the cell bodies (arrows) extend to the epidermal surface. Bar: 25 μm.

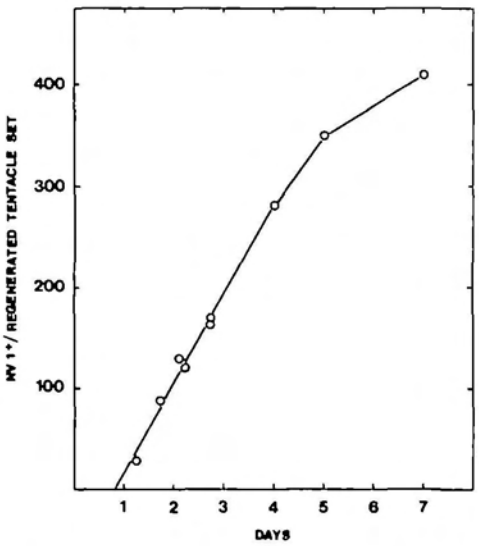

Fig. 3. Formation of $NV1^+$ nerve cells during tentacle regeneration in whole animals. Tentacles were removed at t_0. Each point represents analysis of 10 regenerates.

Head activator stimulates differentiation of tentacle-specific $NV1^+$ nerve cells

Schaller (1973) demonstrated that the neuropeptide head activator stimulates tentacle formation in head-regenerating hydra. To investigate the action of head activator on tentacle-specific cell differentiation, whole hydra were incubated in 1 pM head activator for 18 h. Then the tentacles were excised at their base and allowed to regenerate.

Removal of tentacles from hydra eliminates all $NV1^+$ nerve cells from the distal end of the animal. Fig. 3 shows that $NV1^+$ cells begin to reappear in such regenerates after 1 day and increase in number rapidly for the next 5–6 days, at which point tentacles are fully regenerated. When animals were treated with head activator, formation of tentacle-specific $NV1^+$ nerve cells was stimulated. The results in Table 1 demonstrate that head-activator-treated animals regenerated about 25 % more $NV1^+$ cells than untreated controls at day 2 after tentacle removal. Parallel to this increase in $NV1^+$ cells, there was an increase in tentacle size as demonstrated by the increased number of epithelial cells per tentacle set.

Tentacles are formed by evagination from the head region. However, tissue entering tentacles is ultimately derived from the distal body column (Campbell, 1967; Dübel *et al.* 1987). To localize the site of the head activator effect on tentacle formation and $NV1^+$ differentiation, we treated whole animals with head activator and then excised the head or head plus the distal quarter of the body column. Tentacles were removed from the excised pieces and they were allowed to regenerate.

The results in Table 1 indicate that pieces of head plus distal quarter of the body column differentiated the same number of $NV1^+$ nerve cells and tentacle epithelial cells as whole animals and also showed a head activator stimulation of roughly 25 %. In contrast, isolated head pieces from head-activator-treated animals differentiated only the same number of $NV1^+$ cells as untreated controls and also contained the same number of epithelial cells in their tentacles (Table 1).

Table 1. *Stimulation of $NV1^+$ and tentacle epithelial cell differentiation in head activator (HA)-treated hydra*

		Whole animal	Head plus distal gastric region	Head
$NV1^+$/tentacle set	HA-treated	161±23	160±28	111±45
	Control	130±29	133±29	113±44
Epi/tentacle set	HA-treated	1934±289	1878±570	1185±427
	Control	1527±339	1478±259	1178±338

Hydra were incubated in 1 pM head activator for 18 h. Tentacles were then removed and the animals and isolated head or head plus distal gastric region pieces (see text) were incubated in hydra medium for 2 days and scored for $NV1^+$ and epithelial cells in tentacles.

Each value represents mean (±one standard deviation) from ten polyps.

Whole animals and head plus distal gastric region pieces: Means of head-activator-treated and control animals are significantly different at a 95 % confidence limit (Student's *t*-test).

Head pieces: Null hypothesis accepted (Student's *t*-test); no significant difference.

Since head activator stimulates tentacle formation in tissue of head plus distal quarter of the body column but not in isolated head tissue, it appears that the distal gastric region is the site of tentacle formation.

Head tissue contains committed precursors of developing tentacle cells

The simplest explanation for the inability of head activator to stimulate $NV1^+$ differentiation in head tissue is that head tissue contains only committed tentacle precursors. Uncommitted cells, from which extra tentacle tissue could be recruited by head activator treatment, may not be present in heads.

To test this hypothesis, we have taken advantage of the striking ability of hydra tissue to proportion regulate. Over a wide size range, pieces of hydra tissue regenerate animals in which the size of the head is directly proportional to the size of the regenerating piece. Small pieces regenerate animals with small heads; large pieces regenerate animals with large heads (Bode and Bode, 1980).

The protocol used to test proportion regulation is shown in Fig. 4. Tentacles and varying amounts of proximal body column tissue were removed from hydra and the animals allowed to regenerate. Fig. 5 shows that the size of regenerating tentacles (expressed as number of tentacle-specific $NV1^+$ nerve cells) was the same in all pieces during the first 2 days of regeneration. In particular, head pieces developed huge tentacles compared to their very small size. After 2 days, however, no more tentacle tissue developed in the regenerating head pieces whereas tentacle tissue continued to differentiate in the larger pieces of tissue. Furthermore, the amount of additional tentacle tissue was roughly proportional to the size of the regenerating piece: isolated 1/4 animals differentiated 80, isolated 1/2 animals differentiated 160 and whole animals differentiated 280 additional $NV1^+$ cells. Thus, in agreement with previous observations (Bode and Bode, 1984), proportioning signals appear to control the size of tentacles relative to the size of the animal.

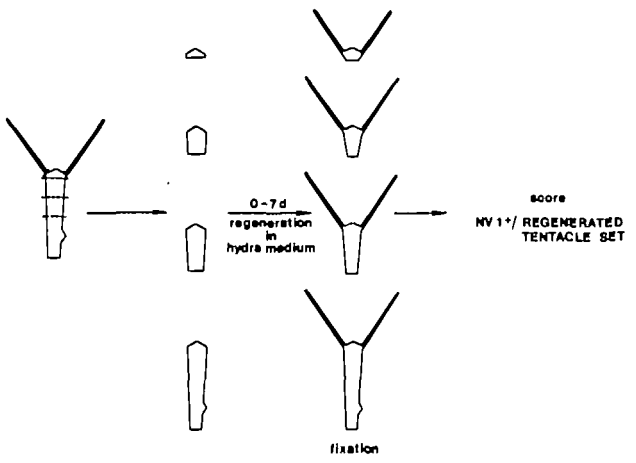

Fig. 4. Experimental protocol used to analyze tentacle regeneration in tissue pieces of different size.

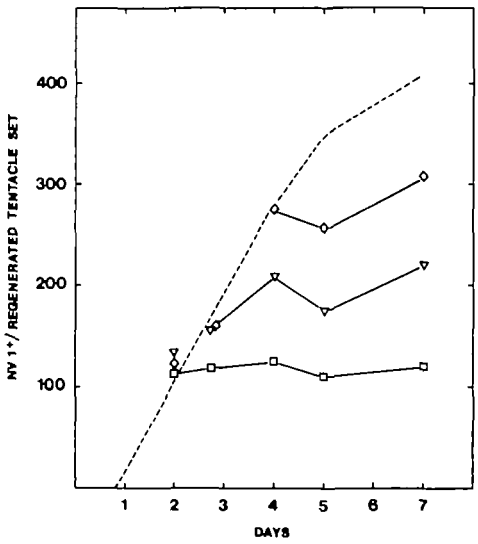

Fig. 5. Tentacle regeneration in tissue pieces of different size: head pieces (□), distal 1/4 body column pieces (▽), distal 1/2 body column pieces (◇). Tentacles were removed at t_0. Each symbol represents analysis of 10 regenerates. The dashed line indicates tentacle regeneration of whole animals (from Fig. 3).

The fact that tentacle size was independent of piece size during the early phase of regeneration indicates that the precursors to this tentacle tissue did not respond to proportioning signals. Since the amount of newly differentiated tentacle tissue only became proportional to tissue size after 2 days, at which time isolated head pieces had completed tentacle differentiation, we conclude that head tissue contains precursors that are already committed to develop tentacle cells.

Head tissue contains uncommitted nerve cell precursors

Previous observations have demonstrated that head activator stimulates nerve cell differentiation in head tissue (Holstein *et al.* 1986). Since head activator does not stimulate tentacle-specific $NV1^+$ differentiation in heads, this implies that differentiation of nerve cells other than $NV1^+$ cells must be stimulated. To test this hypothesis, we have used a second monoclonal antibody, NV4, that shows no positional specificity.

NV4 stains 60% of all nerve cells, both sensory and ganglionic, throughout hydra tissue. $NV4^+$ ganglion cells comprise a dense network which is spread homogeneously throughout the tentacles (Fig. 6B), gastric region (Fig. 6D) and peduncle (Fig. 6E). $NV4^+$ nerve cells with sensory cell morphology are present in both the hypostome (Fig. 6A,C) and basal disc (see Noda, 1969).

To investigate the effect of head activator on the formation of nerve cells in head tissue, whole hydra were incubated in 1 pM head activator for 18 h and then heads were explanted. At different times after explantation, the pieces were assayed for the presence of $NV1^+$ and $NV4^+$ nerve cells. Within the first day after explantation, there was a 30% increase in the $NV4^+$/

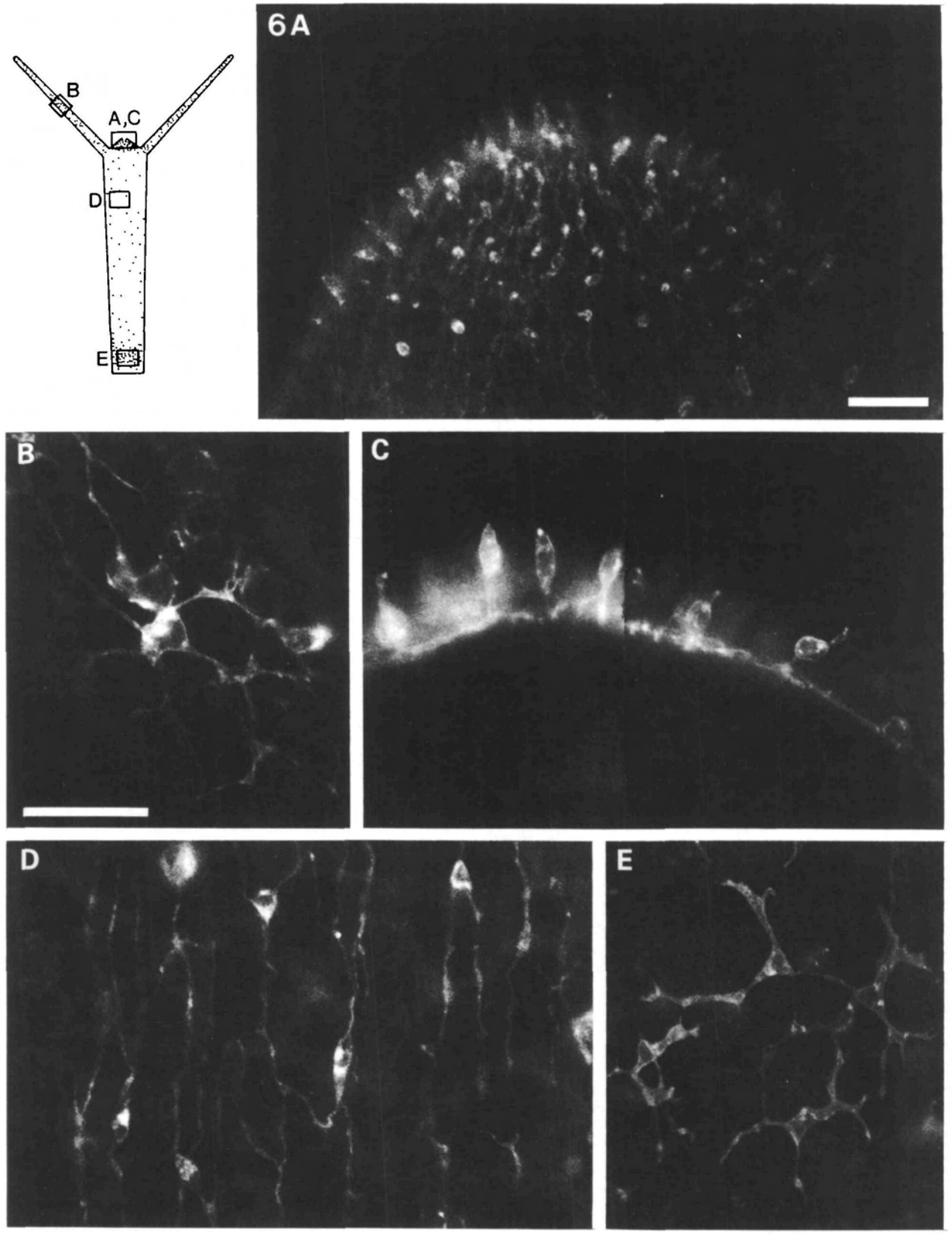

Fig. 6. Whole mount of *Hydra magnipapillata* stained with the monoclonal antibody NV4 and visualized by indirect immunofluorescence. (A,C) Side view of the hypostome showing epidermal sensory nerve cells. Ganglion cells in tentacles (B), gastric region (D), and lower peduncle (E). Bars: 25 μm.

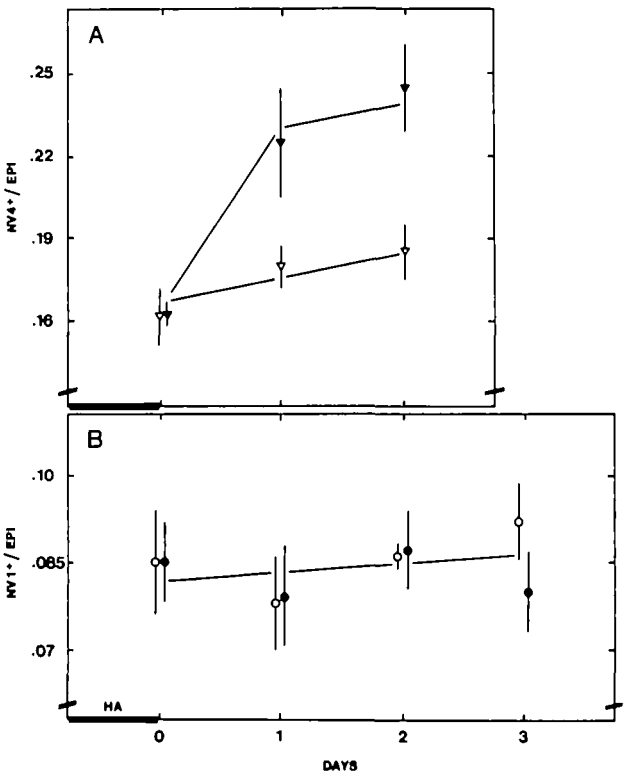

Fig. 7. Effect of head activator (HA) on differentiation of $NV4^+$ (A) and tentacle-specific $NV1^+$ (B) nerve cells in isolated head pieces. Open symbols: untreated control hydra; closed symbols: head-activator-treated hydra. At each time point five explants were macerated. Each point represents mean (±S.D.) of at least three independent experiments.

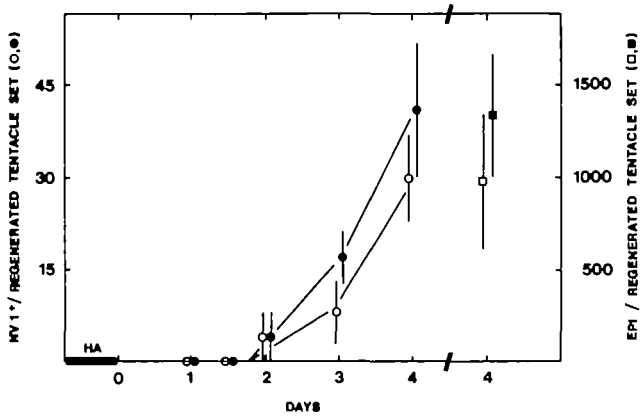

Fig. 8. Effect of head activator (HA) on differentiation of tentacle-specific $NV1^+$ nerve cells in explants of the distal gastric region. The number of epithelial cells in tentacles is also shown. Open symbols: untreated control hydra; closed symbols: head-activator-treated hydra. Each time point represents mean (±one standard deviation) of 10–30 explants. Means of head-activator-treated and control explants are significantly different at a 99% confidence limit (Student's *t*-test).

Epi ratio in head-activator-treated animals compared to untreated controls (Fig. 7A). Since the number of epithelial cells remains constant in such explants (Holstein *et al.* 1986), this represents a 30% increase in the number of $NV4^+$ cells as a result of head activator treatment. Thus, we conclude that head tissue contains uncommitted precursors capable of nerve cell differentiation.

By comparison, the ratio of $NV1^+$/Epi did not change following treatment with head activator (Fig. 7B). Thus, in agreement with the results in Table 1, $NV1^+$ differentiation in head tissue is not stimulated by head activator. Since head activator treatment alone is not sufficient to stimulate $NV1^+$ differentiation, some other event associated with tentacle formation must be the immediate signal for $NV1^+$ differentiation.

Differentiation kinetics of tentacle-specific $NV1^+$ nerve cells

If formation of $NV1^+$ nerve cells is controlled by tentacle formation and not by head activator treatment directly, then the kinetics of appearance of $NV1^+$ cells should be the same in treated and untreated tissue. To test this, we treated whole animals with head activator, then isolated the distal gastric region and followed the appearance of $NV1^+$ cells during head regeneration. The results in Fig. 8 demonstrate that the first $NV1^+$ cells appeared two days after isolation in both head-activator-treated and untreated explants. The appearance coincided closely with the evagination of tentacle tips in the regenerates. Thus, $NV1^+$ nerve cell formation is correlated with tentacle formation and not with head activator treatment.

In head-activator-treated explants, the number of $NV1^+$ nerve cells increased more rapidly such that on day 4 the treated explants had about 30% more $NV1^+$ nerve cells than untreated control explants. Such animals also exhibited an equivalent increase in the number of tentacle epithelial cells (Fig. 8). Both results agree well with the observations in Table 1.

Comparison of head activator stimulation of $NV1^+$ differentiation in distal and proximal gastric tissue

The results in Table 1 and Fig. 8 demonstrate that head activator treatment of distal gastric tissue stimulates tentacle cell differentiation. To investigate whether more-proximal gastric tissue also responds to head activator, we repeated the experiment in Fig. 8 but analyzed both distal and proximal pieces of gastric tissue. The results in Table 2 show that distal pieces from head-activator-treated animals differentiated about 30% more $NV1^+$ nerve cells than those from untreated animals. By comparison, proximal pieces did not differentiate significantly more $NV1^+$ cells than controls. Thus the stimulation of $NV1^+$ differentiation by head activator is restricted to tissue adjacent to tentacles. This localization of $NV1^+$ differentiation is particularly striking since differentiation of total nerve cells is stimulated by head activator in both distal and proximal gastric tissue (Holstein *et al.* 1986).

Table 2. *Effect of head activator (HA) on NV1$^+$ differentiation in distal and proximal gastric region*

		Distal gastric region*	Proximal gastric region†
NV1$^+$/tentacle set	HA-treated	46±16	38±17
	Control	31±11	34±16

Hydra were treated with 1 pM head activator for 18 h. Then pieces of 1/5 distal and 1/5 middle (=proximal) gastric region were explanted, incubated in hydra medium for 4 days and scored for NV1$^+$ nerve cells.

*Mean (±one standard deviation) from 21 polyps. Means of head-activator-treated and control pieces are significantly different at a 99 % confidence limit (Student's *t*-test).

†Mean (±one standard deviation) from 27 polyps. Null hypothesis accepted (Student's *t*-test); no significant difference.

Discussion

Tentacle morphogenesis in hydra

Tentacle tissue is replaced continuously in hydra. Tissue moves from the body column into the head region at the base of the tentacles and finally out into the tentacles themselves. Movement is rapid such that about 20 % of total tentacle length is added to the base of tentacles daily (Campbell, 1967; Dübel *et al.* 1987).

Both head activator treatment (Schaller, 1973) and changes in tissue size (Bode and Bode, 1984) have been shown to alter the amount of tentacle tissue differentiated. We have used both effects to localize the site of tentacle formation in hydra. The results in Table 1 and Fig. 8 indicate that tissue in the distal gastric region responds to head activator with increased tentacle formation. The same tissue responds to a decrease in animal size by regenerating less tentacle tissue (Fig. 5). Since the distal gastric region alters the amount of tentacle tissue that it differentiates under different conditions, we conclude that this region is the site of tentacle formation in hydra.

By comparison, the head region, from which tentacle tissue actually evaginates, does not respond to head activator or to proportioning signals by changing the amount of tentacle tissue differentiated. Fig. 5 shows that isolated heads regenerate tentacles which are far too large for the size of the piece, while Table 1 and Fig. 7B indicate that head tissue does not differentiate more tentacle tissue as a result of head activator treatment. Thus, we conclude that head tissue contains only committed tentacle precursors, which are insensitive to head activator treatment and proportioning signals. These tentacle precursors are committed in the distal gastric region and move into developing tentacles in the course of 2 days (Fig. 8).

The role of head activator in tentacle morphogenesis and nerve cell differentiation

The stimulatory activity of head activator on tentacle morphogenesis was first demonstrated by Schaller (1973). In her experiments, treatment of regenerating hydra with head activator caused a 15 % increase in the number of tentacles regenerated. In our experiments, treatment with head activator increased the number of tentacle-specific NV1$^+$ nerve cells and epithelial cells by roughly 25 % (Table 1 and Fig. 8).

The effect of head activator on tentacle morphogenesis was restricted in our experiments to the distal gastric region. Tissue in the proximal gastric region did not respond to head activator treatment with increased tentacle formation (Table 2). Thus, our results indicate that head activator can increase the size of an existing region of tentacle morphogenesis but that it cannot induce ectopic tentacle formation. We interpret this to mean that head activator is not the only signal involved in tentacle formation.

The absence of a head activator effect in proximal tissue in our experiments was surprising since Schaller (1973) could stimulate tentacle formation with head activator at proximal sites in hydra. Her experiments, however, were different from ours in that she treated regenerating tissue whereas we treated intact animals.

Comparison of the present experiments with the results of Holstein *et al.* (1986) suggests that head activator has two different effects on hydra tissue. The results presented here demonstrate a **local** effect of head activator on tentacle formation in the distal gastric region. By comparison, Holstein *et al.* (1986) demonstrated that head activator stimulates nerve cell formation in all parts of hydra tissue. Whether these two effects of head activator are related to each other is presently not clear.

Signal for tentacle-specific NV1$^+$ differentiation

By comparison with total nerve cell differentiation, which is stimulated by head activator in all parts of hydra, NV1$^+$ differentiation is only stimulated locally in the region of tentacle formation. Thus, head activator alone can not be the signal for NV1$^+$ differentiation. Rather, the signal appears to be tentacle formation itself. This conclusion is supported by the close quantitative (Table 1, Fig. 8) and temporal (Fig. 8) correlation between tentacle formation and NV1$^+$ differentiation. Together these facts suggest that tentacle formation, i.e. differentiation of battery cells, is a prerequisite for differentiation of NV1$^+$ cells. Additional experiments with a regeneration-deficient mutant (reg 16; Sugiyama and Fujisawa, 1977) also support this interpretation: inhibition of tentacle formation in the mutant completely blocks NV1$^+$ differentiation (Hobmayer and David, 1990).

Since NV1$^+$ cells are intimately associated with battery cells (see also Fig. 1 in the accompanying paper) and since tentacle formation is a prerequisite for NV1$^+$ differentiation (see above), it appears likely that the immediate signal for NV1$^+$ differentiation is battery cell formation itself. We interpret this to mean that battery cell precursors and NV1$^+$ precursors form a complex in tissue adjacent to the tentacle base which then differentiates when it moves into tentacles. The failure of head activator to induce NV1$^+$ differentiation in head tissue (Fig. 7B), despite the availability of nerve precursors (Fig. 7A), also suggests that all sites for NV1$^+$ differentiation are occupied, i.e. in complexes of

committed tentacle precursors. The role of specific cell–cell interactions in the formation of sensory cell complexes is discussed in more detail in the accompanying paper (Hobmayer *et al.* 1990).

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