Evolution of the First Nervous Systems

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Differentiation of a Nerve Cell-Battery Cell Complex in *Hydra*

ENGELBERT HOBMAYER and CHARLES N. DAVID

1. Introduction

Complex cell-cell interactions appeared early in the evolution of metazoans. One of the most interesting examples of such complexity is the battery cell in tentacles of cnidarians. This cell consists of a modified ectodermal epithelial cell which has nematocytes and sensory nerve cells embedded in it. To investigate the formation of this complex, we use the simple fresh water cnidarian *Hydra*. In this organism, epithelial cells of the gastric region are continuously displaced into tentacles (Campbell, 1967; Dübel et al., 1987), where they interact with sensory nerve cells and nematocytes to form battery cells.

Using the monoclonal antibody NV1 as a marker for tentacle-specific nerve cells (Hobmayer et al., in preparation) we have investigated formation of tentacle tissue on a cellular level. Formation of a NV1-battery cell complex occurs during head formation and is stimulated by treatment with the neuropeptide head activator (HA) (Schaller and Bodenmüller, 1981), which has been shown to stimulate tentacle (Schaller, 1973) and nerve cell formation (Holstein et al., 1986) in *Hydra*. Differentiation of NV1 immunoreactive (NV1+) nerve cells, however, does not appear to be stimulated directly by HA, but rather by cell-cell interactions with battery cell precursors during tentacle formation.

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2. Morphology of Battery Cells

Battery cells in the tentacles of *Hydra* constitute an association of different cell types (Hufnagel et al., 1985). As shown schematically in figure 1E, 15-20 nematocytes and one epidermal sensory nerve cell are embedded in an ectodermal epitheliomuscular cell, in a typical arrangement: one stenotele or one or two isorhizas lie in the center of a ring of desmonemes. The body of the sensory nerve cell is located to the side of the central nematocyte.

Using a monoclonal antibody, NV1, we were able to identify these tentacle-specific nerve cells in *H. magnipapillata* (Hobmayer et al., in preparation). With the exception of a few ganglion cells in the lower peduncle, no NV1+ cells occur in the rest of the body column. In *H. oligactis*, the same type of nerve cell is recognized by the monoclonal antibody JD1 (Dunne et al., 1985).

Based on *in situ* observations, using indirect immunofluorescence, on either NV1-stained whole mounts or maceration preparations NV1+ cells can be classified as bipolar and multipolar epidermal sensory nerve cells (Fig. 1A; Yu et al., 1986). They have an apical cilium which extends to the surface of the surrounding epithelial cell. Two or more processes extend laterally from the basal part of the cell body (Fig. 1C). They run along the base of the battery cell adjacent to the mesoglea and innervate several neighboring battery cells; short sidebranches make contact with the battery cell's nematocytes (Fig. 1A,B).

3. Development of NV1+ Nerve Cells During Head Formation

In both budding and head regeneration, the first NV1+ cells appear at the time of evagination of short tentacle tips (Fig. 2). Earlier stages of head formation, when the prospective head is only discernible as a rounded protrusion, contain no NV1+ cells and no battery cells. During outgrowth of tentacles, the density of newly formed NV1+ cells remains constant along the entire length of the tentacles. Thus, in general, differentiation of NV1+ cells shows a strong correlation with the formation of battery cells.

This dependence of NV1+ differentiation on battery cell formation is also clearly demonstrated in a regeneration deficient mutant, reg-16 (Sugiyama and Fujisawa, 1977). Animals of strain reg-16 are blocked at an early stage of head regeration, and do not form tentacles. To investigate whether such animals form NV1+ cells during head regeration, it was necessary to introduce interstitial cells of *H. magnipapillata* wild-type strain into reg-16, because reg-16 nerve cells do not express the NV1 antigen. Such reg-16/105 chimeras are defective in head regeration, like the reg-16 parent (Wanek et al., 1986), but can differentiate NV1+ nerve cells from wild-type strain 105

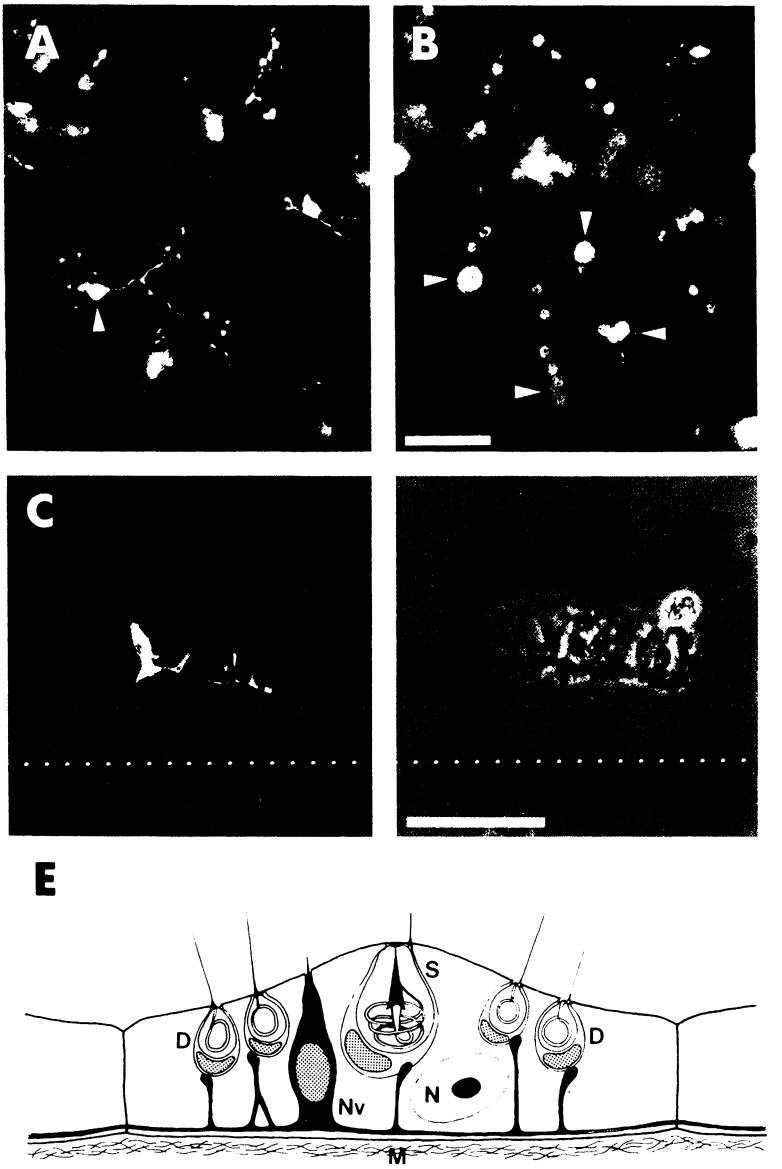


Figure 1. Tentacle-specific NV1+ nerve cells in *Hydra magnipapillata* visualized by indirect immunofluorescence. **(A).** NV1+ nerve cells in tentacles *in situ*. **(B).** Double staining with the nematocyte-specific monoclonal antibody H22 shows innervation of nematocytes of several battery cells by one NV1+ sensory cell (arrows indicate NV1+ cell body **(A)** and battery cell's stenoteles **(B)**). **(C).** Single NV1+ nerve cell in maceration preparation. D. Surrounding battery cell in phase-contrast. E: Schematic representation showing the location of a NV1+ nerve cell within the battery cell. Nv, NV1+ nerve cell; N, battery cell nucleus; S, stenotele; D, desmonemes; M, mesoglea. Bars: 25 μm.

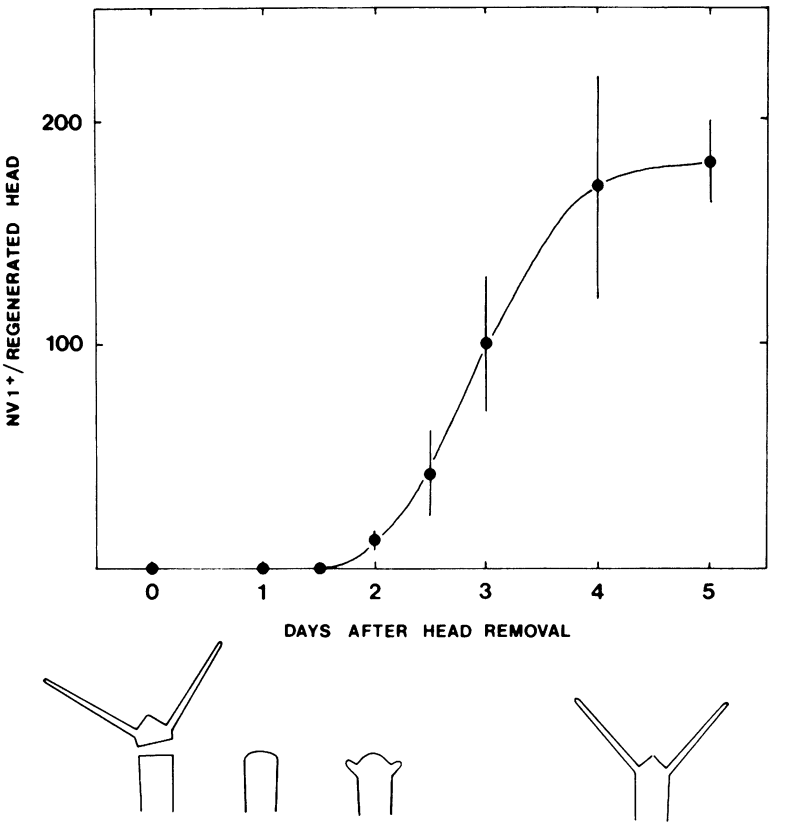


Figure 2. Reappearance of NV1+ nerve cells during head regeneration. Typical stages of head formation at the times indicated are given as schematic drawings.

interstitial cells. When chimeric animals were decapitated below the tentacle ring and allowed to regenerate, three types of regenerates were observed (Table 1): regenerates with completely normal heads (about 50%), incomplete regenerates having less than four tentacles per head (about 5%), and regenerates showing no regeneration of tentacle structures (about 45%). In the latter case, head formation was terminated by a rounded cap at the site of head removal.

In regenerates with normal heads, formation of NV1+ nerve cells was comparable to regeneration of the wild-type strain (Table 1). Tentacles contained normal numbers of NV1+ cells and the kinetics of appearance of these NV1+ cells was comparable to wild-type 105 (see Fig. 2). No NV1+ cells appeared in the regenerating tips of animals in which tentacle formation was inhibited (Table 1). NV1+ nerve cells formed, however, in partially inhibited animals with reduced numbers of tentacles (Fig. 3). There, NV1+ cells appeared only in tentacle tissue. Thus, formation of NV1+ nerve cells is tightly coupled with formation of tentacle morphology.

Table 1. Head Regeneration in Regeneration Deficient reg-16/105 Chimeras

regeneration of head structures	number of head regenerates	development of NV1+ nerve cells
complete	86	wild-type like reformation of NV1+ nerve cell pattern
incomplete	7	appearance of NV1+ nerve cells in tentacle structures
inhibited	66	no appearance of NV1+ nerve cells

Chimeras were decapitated below the tentacles, allowed to regenerate, and analyzed 7 days after head removal. Sample size: 159 head regenerates.

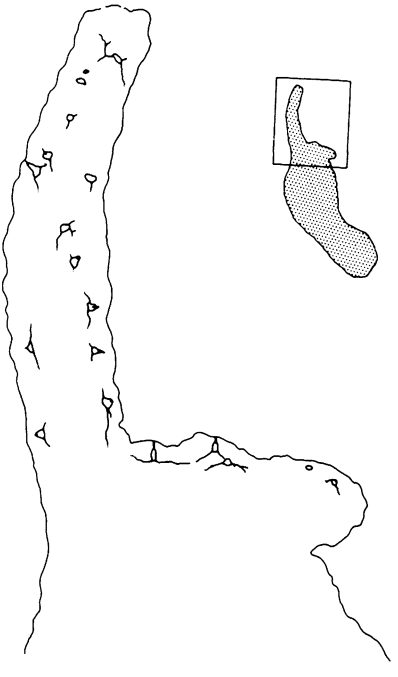


Figure 3. Camera lucida drawing showing NV1+ nerve cells in a partially inhibited reg-16/105 chimera 7 days after head removal. Inset indicates orientation of drawing.

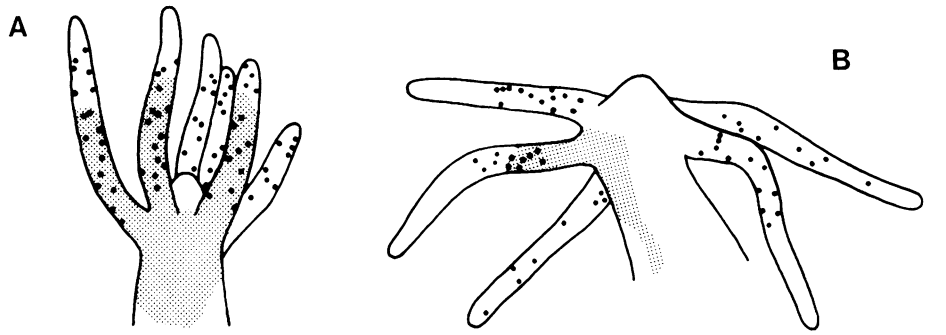


Figure 4. Camera lucida drawings showing a tentacle regenerating head (A) and an intact (B) NV1-free head, 4 days after transplantation. Black spots represent NV1+ cell bodies; stippled areas indicate the position of ink marked cells.

4. Requirements for Formation of a NV1-Battery Cell Complex

4.1. Formation of NV1+ Nerve Cells Requires Interstitial Cell Differentiation

Cell proliferation occurs continuously in the body column of *Hydra*. The new tissue is displaced into buds and into the head (tentacles) and foot, at either end of the body column (Campbell, 1967). During this displacement process, nerve cells from the body columnn become part of the head. Additional head-specific nerve cells also differentiate from interstitial cells at this time (Yaross et al., 1986). Thus, nerve cells in the head and tentacles are derived from two sources. These two sources can be distinguished by analyzing nerve cell formation in interstitial cell-free animals. Nerve cells, which appear in newly formed heads of such animals arise from nerve cells pre-existing in the body column; nerve cells which fail to form under these conditions must arise in normal animals by differentiation from interstitial cells.

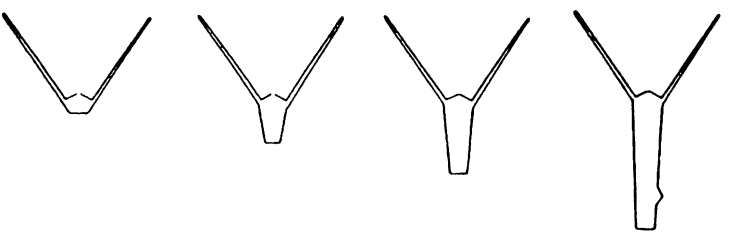
To differentiate the source of NV1+ cells in head tissue interstitial cell-free polyps (Diehl and Burnett, 1964) were allowed to regenerate heads. After six days of regeneration no NV1+ cells appeared in the regenerated tentacles; other types of nerve cells could be recognized in these tentacles using a different monoclonal antibody (NV4; Hobmayer et al., in preparation). Thus, tentacle-specific NV1+ nerve cells arise only by differentiation from interstitial precursor cells.

4.2. Formation of NV1+ Nerve Cells Requires Differentiation of New Battery Cells

Since differentiation of NV1+ nerve cells is closely correlated to differentiation of tentacle structures, it appeared possible that NV1 formation only occurs during differentiation of new battery cells. To investigate this, we grafted NV1-free heads onto the body columns of normal animals and followed the appearance of newly differentiated NV1+ nerve cells in the NV1-free tentacles. To permit tracking of epithelial cell movement from the body column into tentacles, ectodermal epithelial cells in the body column were labelled with India Ink at the site of transplantation (Campbell, 1973). Some experimental animals were left intact; in others, the tentacles were excised to follow formation of new tentacle cells.

The appearance of newly differentiated NV1+ cells was the same in both intact and tentacle regenerating transplants, and the amount of tentacle tissue containing

Table 2. Stimulation of NV1+ and Tentacle Epithelial Cell Differentiation in HA Treated Tentacle Regenerates



NV1+/ tentacle ring	HA treated	111 ± 45	160 ± 28	158 ± 28	161 ± 23
	control	113 ± 44	133 ± 27	127 ± 25	130 ± 29
Epi/ tentacle ring	HA treated	1185 ±427	1878 ±570	1805 ±238	1934 ± 289
	control	1178 ±338	1478 ±259	1510 ±252	1527 ± 339

Hydra were incubated in 1 pM HA for 18 hr. Pieces of different size (heads, distal 1/4, distal 1/2, and whole animals) were then cut, as shown above. Tentacles were excised from all pieces and the pieces were incubated in hydra medium for 2 days to permit tentacle regeneration. The regenerated tentacles were scored in whole mounts for NV1+ cells, by antibody staining, and for epithelial cells by staining their nuclei with the DNA-specific fluorochrome DAPI. The numbers in the Table refer to the total number of cells in all tentacles of a regenerate (the number of tentacles per regenerate varied from 4-7). The means of HA-treated and control animals differ (95% confidence limit, Students t-test) for distal 1/4, distal 1/2 and whole animal pieces.

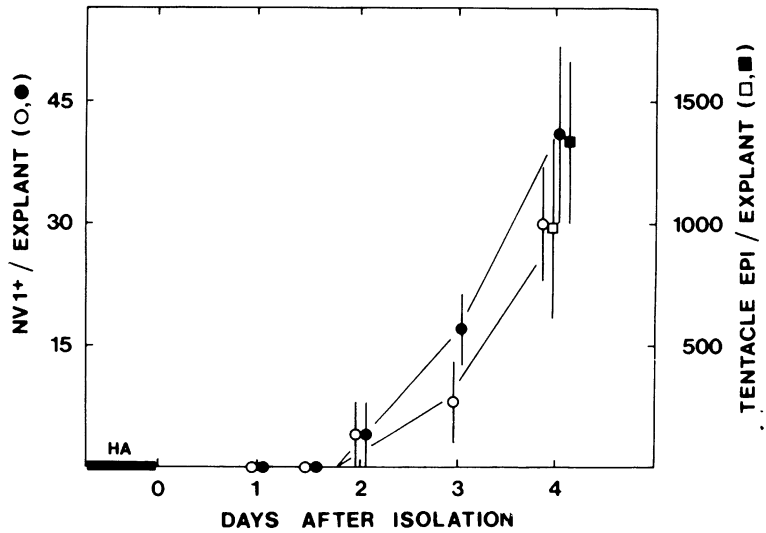


Figure 5. Kinetics of NV1+ and tentacle epithelial cell differentiation in distal gastric region explants of *Hydra* treated with 1 pM HA for 18 hr (closed symbols). Open symbols represent untreated control animals.

NV1+ cells was roughly the same. NV1+ cells filled almost the entire length of regenerated tentacles (Fig. 4A), while in intact animals there was a well defined distal boundary of NV1+ cells and an essentially empty area at the ends of the tentacles which corresponded to "old" tentacle tissue present at the time of grafting (Fig. 4B). Thus, NV1 precursors did not differentiate in association with already differentiated battery cells. Rather, it appears that the NV1-battery cell complex can only be formed by interaction between an interstitial cell precursor and a battery cell precursor.

5. Stimulation of NV1+ and Battery Cell Differentiation in Head Activator-treated Polyps

In order to characterize signals which control battery cell formation and to localize the site of their action in *Hydra* we have analyzed the effect of HA (Schaller, 1973) on differentiation of tentacle-specific NV1+ nerve cells and battery cells. Whole animals were incubated in 1 pM HA for 18 hr. Then tentacles were excised and after two days of regeneration in *Hydra* medium, the number of newly differentiated NV1+ nerve cells and tentacle epithelial cells was scored. In some animals, various amounts of proximal body column tissue were also removed. The results in Table 2 show that HA-treated animals contained about 25% more NV1+ nerve cells and tentacle epithelial cells than untreated control animals. Thus, HA stimulates formation of battery cells.

Truncated 1/4 and 1/2 animals differentiated the same number of NV1+ cells as intact animals and also showed the same HA effect (Table 2). In contrast, isolated head pieces regenerated reduced numbers of battery cells and showed no stimulation of NV1+ differentiation in HA treated animals (Table 2). Thus, head tissue itself is insensitive to HA.

Table 2 shows that pieces of *Hydra* which contained the distal gastric region responded to HA treatment with increased tentacle differentiation; head pieces which lacked this tissue did not respond. This suggests that tissue in the distal gastric region is the site of formation of the NV1-battery cell complex. To test this directly, we treated whole animals with 1 pM HA for 18 hr and then isolated the distal gastric region. Each isolated piece regenerated a small polyp with a head and tentacles. The first tentacle-specific NV1+ nerve cells appeared two days after isolation, coincident with the outgrowth of tentacle tips in both treated and untreated explants (Fig. 5). The number of NV1+ cells and the number of tentacle epithelial cells was about 30% higher in HA-treated animals than in control animals on day four.

In contrast, isolates from the proximal body column showed no stimulation of NV1+ differentiation by HA. From this we conclude that battery cell formation does not occur in this region. The distal gastric region seems to be the only site of battery cell formation in normal animals. In this region NV1 precursors and epithelial cell precursors interact to form a complex, which then differentiates to a battery cell during movement into the base of the tentacles.

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