

# Formation of a Primitive Nervous System: Nerve Cell Differentiation in the Polyp Hydra

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Nerve cell differentiation in the polyp *Hydra* is strikingly patterned: head and basal disk contain high densities of nerve cells. The pattern is formed by migration of interstitial cell precursors from the gastric region to sites of nerve cell differentiation in the head and basal disk. Migration occurs early in the final cell cycle of precursors and appears to be coincident with commitment to nerve cell differentiation. Following migration, each precursor divides and gives rise to a pair of nerve cells. Treatment of whole hydra with a neuropeptide stimulates formation of nerve cell precursors; differentiation of these precursors is regulated by a second signal, which is provided in vivo by budding or in vitro by injuring tissue.

**Key Words:** *hydra*, cell migration, nerve differentiation, head activator, stem cell, Dil labeling

Coelenterates are the phylogenetically simplest organisms with a clearly defined nervous system.<sup>1</sup> It has the form of a nerve net spread throughout the entire organism and has been best investigated in various hydrozoan polyps, primarily *Hydra* and *Hydractinia*.

The nerve net is formed from two morphologically distinct types of cells: ganglion and sensory (for review, see Bode et al<sup>2</sup>). These morphological classes include a large and expanding number of subclasses characterized by the presence of different neuropeptides or specific antigens recognized by specific monoclonal antibodies. The net is not a random mix of these cells but is clearly highly organized with specific classes of neurons in specific locations where they presumably fulfill specific functions.

The function of specific nerve cells and the nerve net as a whole is largely unknown in hydrozoans. It is clear, however, that various aspects of hydrozoan behavior, such as feeding, require an intact nervous system. Deletion of nerve cells by chemical treatment or mutation leads to so-called epithelial hydra, which exhibit no feeding behavior and are essentially motionless (for review, see Campbell<sup>3</sup>). Interestingly, they are also unable to attach to substrates and to open their mouths.

The most unusual feature of the polyp nerve net is its dynamic nature (for review, see Bode<sup>4</sup>). This is a consequence of the continuous growth of polyp tissue: the body column expands due to mitotic activity of epithelial cells while tentacle tissue at the distal end and the

basal disk at the proximal end are continuously renewed from epithelial cells of the body column. Since the distribution of nerve cells remains constant in polyps—in particular the high density of nerve cells in tentacles and basal disk—new nerve cell differentiation must occur continuously to maintain the status quo. Moreover, nerve cells displaced from one position to another as a result of growth must adapt their phenotype to the appropriate position (transdifferentiation).

What are the precursors to these differentiation events and what are the signals that generate the localized differentiation of nerve cells at the appropriate sites in tissue?

Although early work had tended to favor the idea of local commitment of nerve cell precursors (NVP) at the site of differentiation,<sup>5–7</sup> more recent work is providing support for an alternative idea: commitment of precursors dispersed throughout the body column followed by migration to the site of final differentiation.<sup>8–11</sup> The present review pulls together the existing evidence on nerve cell differentiation in hydra and attempts to provide a unified picture of the NVP and its behavior.

## NERVE CELL DIFFERENTIATION AND THE NERVE CELL PRECURSOR

Figure 1 outlines schematically the differentiation pathway of nerve cells in hydra. Nerve cells are

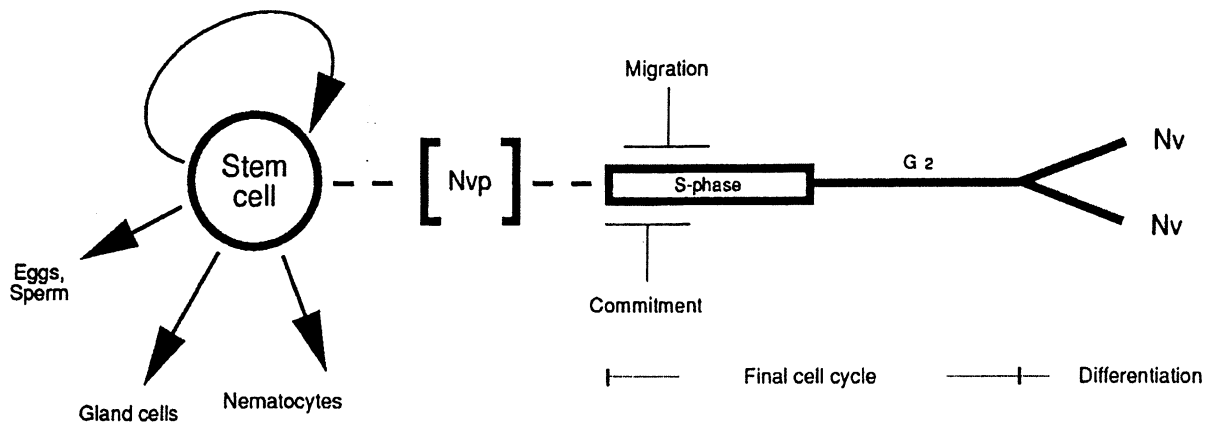


FIGURE 1 Schematic representation of the nerve cell differentiation pathway in Hydra. Nerve cell precursor (Nvp) properties are discussed in the text. The final cell cycle is about 18 hours in regenerating Hydra and 24 hours in intact Hydra; postmitotic differentiation requires about 6 hours.

derived from interstitial stem cells that are distributed uniformly throughout the body column.<sup>12,13</sup> At the other end of the pathway, the immediate precursor to nerve cells undergoes a final cell cycle and both daughter cells differentiate to nerve cells. Although the two ends of the pathway, stem cells and nerve cells, are well defined, the nature of the cell in the middle, the NVP, has been more difficult to define.<sup>14,15</sup> Recent experiments, to be reviewed later, are now helping to clarify the picture.

A series of cell cloning experiments has demonstrated that the interstitial cell population contains multipotent stem cells that are capable of giving rise to all differentiation products of the interstitial cell lineage, including nerve cells, nematocytes, gland cells, and gametes.<sup>12,16,17</sup> These stem cells constitute a large fraction of the interstitial cell population. Moreover, the cloning experiments provided no evidence for committed intermediates with extensive proliferation capacity, since no clones were observed with monospecific differentiation potential. In particular, no clones were observed that only gave rise to nerve cell differentiation. Hence, nerve cell differentiation in hydra appears to occur relatively directly from the stem cell pool (Fig. 1).

To identify the immediate precursors of newly differentiated nerve cells, several laboratories have investigated nerve cell differentiation during head regeneration.<sup>6,7,18,19</sup> Since head tissue normally contains a high level of nerve cells, this regeneration process includes formation of large numbers of new nerve cells. Labeling with [<sup>3</sup>H]thymidine has shown that the precursors to these new nerve cells are in S phase at the onset of head regeneration. They complete the cell cycle and differentiate as nerve cells beginning 12 hours after head removal (Fig. 1). Based on these ob-

servations, it has seemed likely that the signals stimulating nerve cell differentiation cause commitment of precursors in S phase. Similar results have also been obtained using feeding as a stimulus for nerve cell differentiation: precursor cells in S phase at the time of feeding are induced to differentiate as nerve cells within 18 hours.<sup>20</sup>

Although these experiments clearly defined the cell cycle position of the immediate precursor, they did not indicate whether the precursor arose directly from a multipotent stem cell or from an intermediate cell type capable of limited proliferation and poised to respond to signals inducing nerve cell differentiation. This uncertainty is indicated in Figure 1 by the dotted line.

## NERVE CELL PRECURSORS ARE MIGRATORY

A number of experiments have documented extensive migration of interstitial cells along the body column (see Bode and colleagues<sup>10,11,21</sup> and references therein). These experiments, however, did not reveal the function of the migrating cells. Thus, it has been uncertain whether cell migration played a role in the pattern of nerve cell differentiation.

The two experiments shown schematically in Figure 2 demonstrate that migrating cells play a central role in the pattern of nerve cell differentiation. Both experiments used axial grafting between labeled and unlabeled tissue and assayed quantitatively the formation of labeled nerve cells in the unlabeled tissue. In the case of head regeneration,<sup>7,8</sup> roughly half of the newly differentiated nerve cells in the head were labeled and hence derived from proximal tissue. Since the labeled tissue constituted half the interstitial cell population, the results indicated that essentially all newly differen-

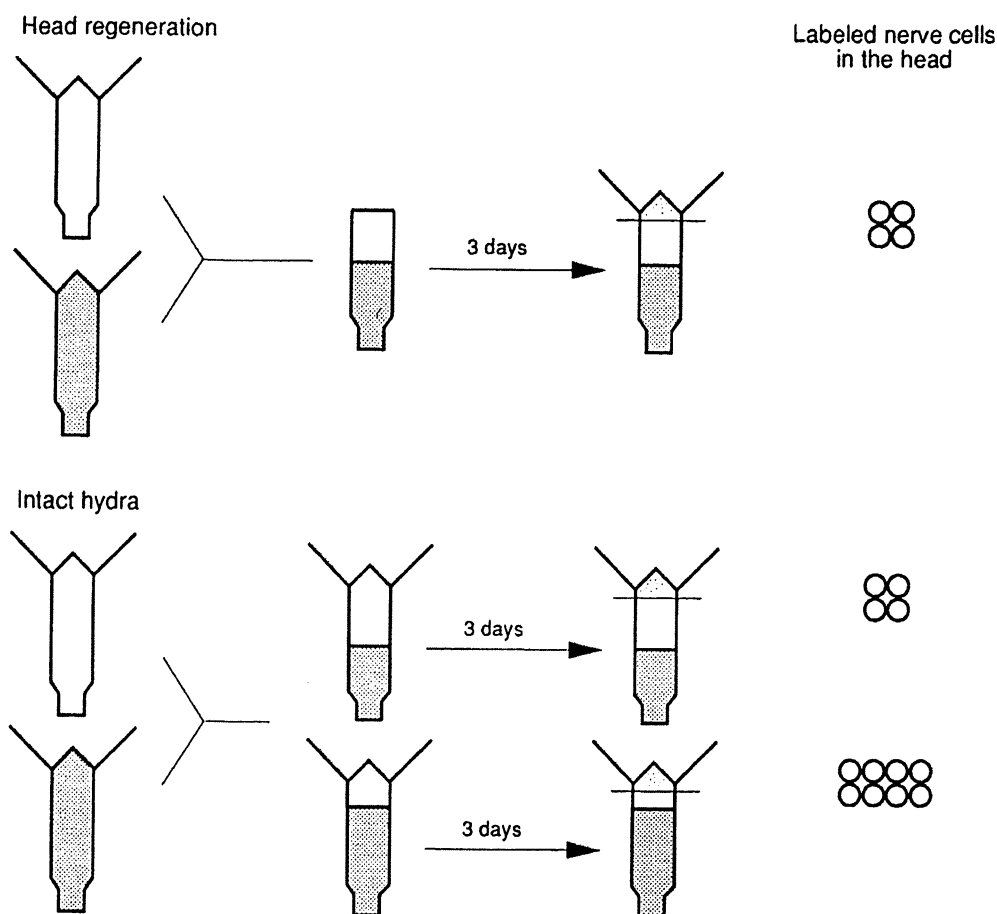


FIGURE 2 Nerve cell formation from migrating precursor cells. The figure shows schematically the results of axial grafting experiments on regenerating<sup>7,8</sup> and intact Hydra,<sup>12</sup> indicating that precursor cells from the labeled tissue (shaded) contribute to nerve cell formation in the unlabeled head. Labeling was done with [<sup>3</sup>H]thymidine. The number of labeled cells is indicated qualitatively, since the results are derived from experiments done in different laboratories under somewhat different conditions.

tiated nerve cells arose from precursors that migrated from the body column to the site of head regeneration.

In a similar experiment carried out on intact animals, the appearance of labeled nerve cells in the head was assayed in axial grafts containing different proportions of labeled tissue.<sup>12</sup> The results showed that, beginning 1 day after grafting, newly differentiated labeled nerve cells began to appear in the head. As in the regeneration experiment, the rate of labeled nerve cell differentiation was proportional to the fraction of the body column that was labeled. Thus, the precursors for nerve cell differentiation in the heads of intact animals were derived from the entire body column.

More detailed information about the properties of migrating cells has recently been obtained by labeling cells with the fluorescent vital dye DiI. Teragawa and Bode<sup>10</sup> showed that injection of DiI into the ectoderm of hydra caused vital staining of a discrete small patch of cells. Over the course of several days, a few labeled cells migrated out of such patches. Based on their behavior and morphology, they could be identified either

as single nematocytes migrating to the tentacles or as single interstitial cells.

The results of a similar DiI labeling experiment done in our laboratory are shown schematically in Figure 3. We have succeeded in following individual migrating cells over the course of several days and determining their fate. In addition to large numbers of migrating nematocytes, only single interstitial cells were observed to migrate. Pairs of interstitial cells and nests of nematoblasts did not migrate. Migrating interstitial cells divided once at their final destination, usually 1 day after ceasing to migrate, and gave rise to two nerve cells in close proximity to each other (Fig. 4). Thus, migrating cells are NVP. We have also occasionally observed cells that divided a second time, giving rise to a group of four nerve cells.

Almost all migrating interstitial cells gave rise to nerve cells. Although our data are still not extensive, we have not yet observed migrating cells that gave rise to nests of nematoblasts or to groups of interstitial cells that could be considered stem cell clones. Thus, we

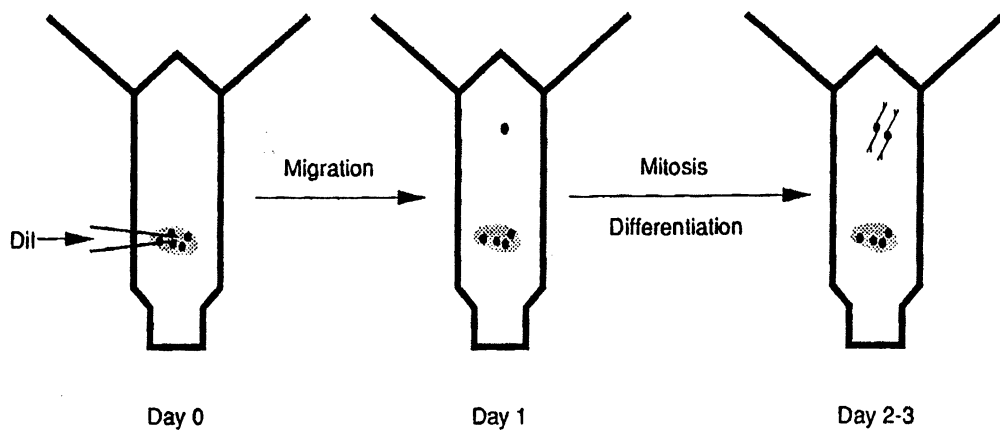


FIGURE 3 Schematic representation of nerve cell precursor migration as seen in a diI labeling experiment. DiI was injected into the ectoderm where it labeled a small patch of cells. All cell types are labeled but only nematocytes (not shown) and a few single interstitial cells migrate away from the site of injection. Single migrated interstitial cells divide and give rise to two nerve cells as shown. Migration occurs toward both the head and the foot.



FIGURE 4 Micrograph of two DiI labeled nerve cells similar to those shown schematically in Figure 3. The cell bodies are roughly 10  $\mu\text{m}$  in diameter.

tentatively conclude that nematocyte precursors and stem cells are not migratory. The latter conclusion is also supported by earlier observations that showed that interstitial cell clones grow as contiguous patches of cells; growing clones did not generate satellite clones due to migration of stem cells.<sup>22-24</sup> Thus, there is presently no evidence for stem cell migration in normal animals. In grafts between normal and interstitial cell-depleted tissue, however, extensive stem cell migration does occur and leads to rapid repopulation of the depleted tissue.<sup>9,21,24-27</sup>

#### NERVE CELL PRECURSOR MIGRATION OCCURS IN S PHASE

Although the cell cycle position of the migrating NVP has not been directly determined, several lines of evidence suggest that migration takes place at the beginning of the final cell cycle (see Figures 1 and 3). Observations of DiI-labeled NVP indicate that they usually migrate away from the injection site, stop, and then remain in this condition for up to a day before dividing and differentiating as nerve cells. Since the cell cycle of interstitial cells is about 1 day,<sup>28</sup> and the NVP spends up to 1 day at the final site before division, the migratory phase appears to be early in the cycle. This would place it in S phase, since hydra cells do not have a  $G_1$ <sup>28</sup> (see Figure 1). This conclusion is also supported by earlier observations that showed that nerve cell precursors do not change their position between the end of S phase and final differentiation.<sup>29</sup>

Summarizing the results presented, we can now define the NVP as a cell that arises in the body column and migrates to a final destination usually near the head or foot but also sometimes to sites within the body

column. Birth of the migrating cell occurs at or near the start of a final cell cycle. Such cells are in S phase and thus migration appears to occur in the S phase. Since commitment of NVP also occurs in the S phase, both commitment and migration appear to be closely linked, if not identical events. Completion of the cell cycle occurs at the final destination, followed by division and differentiation of both daughter cells as nerve cells. NVPs with these properties appear to be direct products of multipotent stem cells.

### INDUCTION OF NERVE CELL PRECURSORS BY TREATMENT WITH HEAD ACTIVATOR

Little is known about the signals regulating the formation of NVP in vivo. It has, however, been possible to generate large numbers of NVP by treating hydra with pM concentrations of a neuropeptide, the head activator.<sup>30–34</sup> Head activator treatment of whole animals induces formation of NVP throughout the body column. These NVP do not differentiate in intact hydra but do rapidly differentiate to nerve cells following explantation of tissue fragments or wounding of treated animals.<sup>34,35</sup> Untreated tissue has a background level of about 0.10 nerve cells per epithelial cell; head activator treatment for 12 to 18 hours followed by explantation of tissue fragments almost doubles this number.<sup>30,34</sup>

The ability to generate large numbers of NVP by head activator treatment has permitted investigation of their properties.<sup>33</sup> They are small interstitial cells with characteristic nuclear morphology and postmitotic G<sub>1</sub> nuclear DNA content; indeed, they frequently appear in pairs. Following explantation or wounding of treated tissue, nerve cell differentiation occurs within 5 hours. If the explantation or wounding signal does not occur within 24 hours after the head activator treatment, the NVP are no longer detectable when tissue fragments are explanted. Thus, NVP appear to be unstable.

There is still only limited information on the signals that control the differentiation of NVP in intact animals. One such signal is associated with bud formation. Berking<sup>36</sup> demonstrated that NVP migrate to the site of a prospective bud and accumulate there; initiation of bud evagination was followed by a synchronous wave of nerve cell differentiation within about 5 hours. The biochemical nature of the signal is presently unknown, but it appears to be short-range, since only NVP at the site of bud formation are induced to complete nerve cell differentiation. A functionally equivalent

signal must also be present near the head and foot since NVP differentiate in these regions as well.

### SUMMARY

Nerve cell differentiation in hydra is strikingly patterned. Recent work summarized here has now made it clear that this pattern of differentiation is due to migration of nerve cell precursors from the body column to sites of terminal differentiation in head and foot. This behavior is strikingly reminiscent of neural crest differentiation in vertebrate embryos.<sup>37,38</sup> Indeed, the similarity includes not only migration, but may also extend to the basic mechanism by which neurotrophic factors regulate the survival and differentiation of NVP.<sup>39</sup> Although there is no evidence for such neurotrophic factors in hydra at present, it is at least conceivable that the differentiation signals released by budding, explantation, or wounding include such factors.

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