these hormones in fibroblasts (*12, 13*). A biologically inactive analog of EGF has been described which retains its ability to bind but does not induce aggregation of receptors (*13*). Bivalent antibodies to EGF restore both the bioactivity and the morphological cross-linking (patch formation) of this derivative toward that observed with the native hormone. These studies suggest that microaggregation of receptors into groups of two or more may be essential for the biological responses of at least some hormones.

Most opiate receptors probably occur in the membrane of the synapse (*14*), and the size of the synapse is about 0.5 μm (*15*). It is possible that opiate receptors are densely distributed on postsynaptic membranes and that aggregation of the occupied receptors may be necessary for generating physiological effects. The fluorescent analog described in the present study may be useful in studying the mechanisms of receptor redistribution (*16*) and in determining precisely the localization of opiate receptors in the central and peripheral nervous systems.

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2. The abbreviations are as follows: Tyr, tyrosine; Gly, glycine; Phe, phenylalanine; Met, methionine; Leu, leucine; Ala, alanine; Lys, lysine; IC_{50}, the concentration of unlabeled ligand at which 50 percent of the maximum specific binding of labeled ligand is displaced; MEM, minimum essential medium; HAT, hypoxanthine, aminopterin, and thymidine; FBS, fetal bovine serum; EDTA, ethylenediaminetetraacetic acid.
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8. Binding of the rhodamine enkephalin to NG108 cells was visualized with an RCA silicon intensifier target TV camera (TC1030H) attached to a Zeiss Photomicroscope III equipped with epifluorescence. Video output was recorded on a Panasonic recorder (NV-8030) and displayed on a Hitachi TV monitor (VM-905AU), from which Polaroid photographs were taken.
9. The presence of physiological concentrations of calcium ions in cells treated with or without EDTA does not alter the appearance of clusters or the absence of internalization. Because of the dramatic reduction in the binding of enkephalin in the presence of 100 m*M* Na^+, the clusters cannot be detected. However, the nearly complete dissociation of cell-bound ^{125}I-labeled [D-Ala^2, D-Leu^5]enkephalin indicates that no internalization occurs in the presence of sodium ions (*5*).
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Neuronal Chemotaxis: Chick Dorsal-Root Axons Turn Toward High Concentrations of Nerve Growth Factor

Abstract. *Micropipettes containing 2 to 50 biological units of β nerve growth factor (NGF) were placed near growing axons of chick dorsal-root ganglion neurons in tissue culture. The axons turned and grew toward the NGF source within 21 minutes. This turning response to elevated concentrations of NGF appears to represent chemotactic guidance rather than a general enhancement of growth rate.*

Chemotaxis, the attraction of living protoplasm to a chemical substance, may help guide axons to their target tissues. Several studies in vitro (*1–3*) suggest that β nerve growth factor (NGF), a protein known to enhance axonal outgrowth from dorsal-root and sympathetic ganglia (*4*), is a chemotactic agent. These studies, however, leave unresolved the question of whether NGF actually guides axonal growth or simply enhances the survival or growth rate of axons that happen to be growing near the NGF source. We sought to distinguish between these possibilities by continuously observing the growth cones of chick dorsal-root axons that were exposed to a localized source of NGF. We found that these axons turn and grow toward the NGF source within 21 minutes, even if the background concentration of NGF is sufficient to support survival and rapid axonal growth.

Table 1. Turning response of dorsal-root ganglion axons to NGF.

Concentration of NGF (BU/ml)		Number of axons	
Background	Micropipette	Positive response*	No response†
1	50	40	0
1	1	0	40
1	1 (+ BSA‡)	0	5
1	1 (+ FCS§)	0	5
0	1	0	5
1	2	5	0

*Rate of turn, 3.3 ± 0.2 deg/min. †Rate of turn, 0.01 ± 0.14 deg/min. ‡Bovine serum albumin (0.1 mg/ml) added. §Fetal calf serum (2.5 μl/ml) added.

Lumbosacral dorsal-root ganglia from White Leghorn chick embryos 7 and 12 days of age were excised and placed onto glass cover slips coated with a mixture of collagen and poly-L-lysine (25:1 by weight). The explants were incubated in air at 34°C in 35-mm plastic petri dishes with nutrient medium similar to Ham's F-12 except buffered with 1,4-piperazinediethanesulfonic acid (Pipes). The medium was supplemented with 5 to 10 biological units (BU) of NGF per milliliter (*5, 6*). After 24 hours, the cover slips were placed in an observation chamber (34°C) and were viewed with an inverted phase-contrast microscope (×750).

A micropipette (tip diameter, 2 to 4 μm) filled with NGF (1 to 50 BU per milliliter of perfusion medium) was used as a localized NGF source. The tip of the micropipette was placed about 25 μm from the tip of a growth cone at approximately 45° to the axon's longitudinal axis (Fig. 1a) and slightly above the surface of the medium. A separate perfusion system added perfusion medium to one side of the observation chamber while continuously removing medium from the opposite side with a vacuum line. This system produced a flow of medium (25 ml/hour) past the axon in a direction opposite to the initial direction of axonal growth. The NGF, flowing from the micropipette at the rate of 1 to 2 μl/hour, was carried along with this background flow, producing a higher concentration of NGF on the side of the growth cone nearest the micropipette (this was initially determined with methylene blue).

Figure 1 shows a dramatic example of

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the effect of an NGF concentration gradient on the direction of axonal growth. The five photographs were taken over a 90-minute observation period during which successive repositionings of the NGF-filled micropipette resulted in an almost complete reversal of the axon's original direction of growth. Figure 1a shows the growth cone and the NGF-filled micropipette at the onset of perfusion. As the growing axon gradually turned toward the NGF source, the micropipette was moved to the position shown in Fig. 1b, and the growth cone grew toward the new position. Three subsequent repositionings (Fig. 1, c to e) resulted in a horseshoe-shaped pattern of axon growth (Fig. 1e).

In order to accurately measure the turning response of the axon to NGF, similar experiments were performed in which observation periods of shorter duration (30 minutes) were used and the lateral displacement of the cone tip was measured. Lateral displacement was measured as the shortest distance between the line described by the original axis of the axon and the new position of the growth cone. A lateral displacement of 20 μm within the 30 minutes of observation was considered a positive response (Table 1).

Dorsal-root axons bathed in a background solution containing NGF (1 BU/ml) were exposed to concentrated NGF (50 BU/ml) from a micropipette. All 40 axons tested turned and grew toward the NGF source (Table 1), exhibiting a lateral displacement of 20 μm in 9 to 21 minutes. In control experiments in which the micropipette contained the same concentration of NGF as the background, the axons showed only small random displacements (5 μm or less) toward or away from the micropipette and were scored as no response (Table 1). In ten of these control experiments, growth cones were observed for 2 hours. Lateral axonal displacements were less than 10 μm toward or away from the micropipette. No significant turning or lateral displacement was observed in these control axons even if the pipette was moved in the same pattern as for a positive response. Thus the turning of the axon toward elevated concentrations of NGF is not a response to pipette movement or movement of fluid from the pipette. Nor is the turning response associated with an increase in rate of growth, since the control and experimental axons grew at almost identical rates (88 ± 33 and 85 ± 39 μm/hour, respectively).

The turning response is not just a nonspecific attraction of growing dorsal-root axons to any protein source, since axons did not turn toward micropipettes containing bovine serum albumin or fetal calf serum (Table 1). But these axons are very sensitive to even low concentrations of NGF. We observed turning responses (20-μm lateral displacements) of axons toward micropipettes containing as little as 2 BU of NGF per milliliter, which was only 1 BU/ml above the background concentration (Table 1). However, axons did not turn toward a micropipette containing 1 BU of NGF per milliliter when the background contained no NGF.

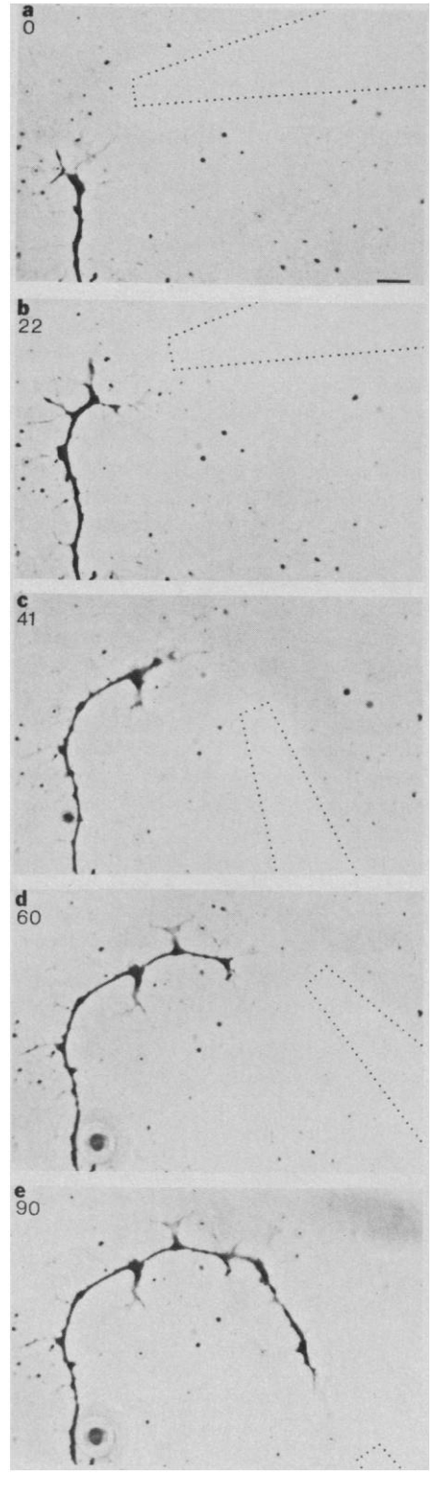

Fig. 1. Sequential photographs of a dorsal-root growth cone bathed in NGF (1 BU/ml) and exposed to an NGF gradient created by outflow from a micropipette containing NGF at 50 BU/ml. Numbers indicate time in minutes after the onset of perfusion by micropipette; dotted lines outline the micropipette in its successive placements. The growth rate for this axon was 72 μm/hour. After 90 minutes, the axon had grown 108 μm and had turned almost 160° relative to its original direction of growth. Scale bar, 10 μm.

In summary, chick dorsal-root axons rapidly (in 9 to 21 minutes) alter their direction of growth in response to an extracellular gradient of NGF. This response appears to result from chemotactic guidance rather than general enhancement of growth rate or survival. More extensive experiments are required to determine the relation between this chemotactic response and the previously observed growth of axons toward explants of target tissues or sources of concentrated NGF in longer-term experiments (*1-3, 7, 8*).

Since all dorsal-root axons that were bathed in low background concentrations of NGF (1 BU/ml) responded to an NGF concentration gradient, the chemotactic response to NGF that we measured does not account for the specificity with which different dorsal-root ganglion axons innervate different peripheral target tissues. Perhaps the response of different axons to NGF has quantitative differences too subtle to be detected. Also, other macromolecules analogous to NGF, or other mechanisms such as contact guidance (*9*), may help to guide axons and enable them to distinguish among different target tissues.

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