suggested that the similarities between the Old and New World hystricognaths might be pure parallelism (*8*), a suggestion later formalized taxonomically (*1, 9*). Studies of the admittedly great similarities between the living Old and New World hystricognaths caused several students to question this separation (*10, 11*), doubting whether it would have been possible to achieve such similarities as a result of parallelism. Finally, the recent renewed interest in continental drift has convinced some workers that late Eocene or early Oligocene phiomorphs would have been able to cross the South Atlantic on natural rafts, since the ocean would then have been so much narrower than it is at present (*2*). However, deep-sea drilling has shown (*12*) that the late Cretaceous South Atlantic was at least 3000 km wide, and (since the ocean in the late Eocene could not, on the basis of current theory, have been narrower than it was in the late Cretaceous) it has therefore been argued (*6*) that such trans-Atlantic migration could not have occurred.

For these reasons, the discovery of a fully hystricognathous fossil rodent in the middle (or late) Eocene of Texas is of the greatest importance. This specimen (Texas Memorial Museum No. 41372-179) from about 35 km north of the Big Bend National Park, in southwestern Texas, is a well-preserved lower jaw containing the alveolus of the premolar, all three molars, and the incisor. The angular process (Fig. 1) spreads laterally from the side of the mandible and descends well lateral of the alveolus of the incisor, in a fully hystricognathous condition. It is still too early to be sure of the special affinities of this specimen, but the structure of the cheek teeth is closer to that in some members of the predominantly North American Eocene family Sciuravidae than to that in any other rodent with which it has as yet been compared. It is impossible to determine whether or not this animal was hystricomorphous (although such is fundamentally the case in all other hystricognaths), since only a portion of the lower jaw is available.

This fossil is the first specimen of a fully hystricognathous Eocene rodent on record from anywhere in the world. The middle Eocene North American paramyid rodent *Reithroparamys* and some of its relatives show very incipient stages in hystricognathy (*10, 13*), but the gap between such incipiently hystricognathous forms and the fully hystricognathous Caviomorpha and Phiomorpha is such as to have permitted legitimate doubt about the actuality of any relationship. An Eocene hystricognath in Texas, however, suggests that the postulate of a pantropical subhystricognathous population, proposed recently (*6*), may well have been correct. This specimen also strengthens the evidence for the idea, proposed by Wood (*14*), that there was a very distinct late Eocene Middle American rodent fauna, the northern limits of which barely reached across the international boundary into what is now the southern United States, in southern California, in the Tierra Vieja area of west Texas, and now in the region of the Big Bend.

I anticipate that future investigators will discover rodents of the same general type, in the later Eocene of southwest Asia, properly placed to have been ancestral to the African phiomorphs.

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References and Notes

1. A. E. Wood and B. Patterson, *Bull. Mus. Comp. Zool. Harvard Univ.* **120**, 282 (1959).
2. R. Hoffstetter and R. Lavocat, *C. R. Hebd. Seances Acad. Sci., Paris, Ser. D* **271**, 172 (1970).
3. A. E. Wood, *Bull. Peabody Mus. Nat. Hist. Yale Univ.* **28**, 23 (1968).
4. R. Lavocat, "Les Rongeurs du Miocène d' Afrique Orientale," in *Fossil Vertebrates of Africa* (Academic Press, New York, in press).
5. H. Tobien, *Jahrb. Ver. "Freunde Univ. Mainz"* **1968**, 51 (1968).
6. A. E. Wood and B. Patterson, *Mammalia* **34**, 628 (1970).
7. A. E. Wood, *Evolution* **4**, 87 (1950).
8. G. G. Simpson, *Bull. Amer. Mus. Nat. Hist.* **85**, 1 (1945).
9. A. E. Wood, *J. Mammal.* **36**, 165 (1955).
10. S. O. Landry, *Univ. Calif. Publ. Zool.* **56**, 1 (1957).
11. T. V. Fischer and H. W. Mossman, *Amer. J. Anat.* **124**, 89 (1969); C. A. Woods, *Bull. Amer. Mus. Nat. Hist.*, in press.
12. A. E. Maxwell, R. P. Von Herzen, K. J. Hsü, J. E. Andrews, T. Saito, S. F. Percival, Jr., E. D. Milow, R. E. Boyce, *Science* **168**, 1047 (1970).
13. A. E. Wood, *Trans. Amer. Phil. Soc.* (new series) **52**, 1 (1962).
14. ———, *Bull. Tex. Mem. Mus.*, in press.
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Nerve Growth Factor: Stimulation of Regenerative Growth of Central Noradrenergic Neurons

Abstract. *The growth of new axonal sprouts was studied from transected, ascending noradrenergic axons into transplants of iris tissue in the caudal hypothalamus of the rat. A single intraventricular injection of nerve growth factor, given at the time of axonal damage, resulted in an increased formation and growth of new noradrenaline sprouts 7 days later. The effect seemed to be proportional to the administered dose of nerve growth factor.*

Nerve growth factor (NGF) is a potent stimulator of growth of peripheral, sympathetic and sensory neurons [for review, see (*1, 2*)]. It is most effective on developing or growing neurons. When administered in vivo NGF causes hypertrophy and hyperplasia of the ganglia as well as increased innervation of the viscera. Only sympathetic and embryonic sensory neurons are known to respond to NGF, which indicates that it has a high specificity with respect to the target cell. No effect of NGF on the central nervous system has thus far been detected (*1*).

A remarkable capacity for regenerative sprouting and growth has been demonstrated for transected axons of central monoamine neurons in the adult rat (*3–5*). Abundant sprouting developed from either mechanically or electrolytically severed preterminal axons, and this appeared primarily during the second and third week after the trauma. The sprouting fibers grew into the brain tissue surrounding the lesion, into the necrotic tissue within the lesion, into and along the walls of intracranial blood vessels, and into adjacent cranial nerves. The growth of the central catecholamine neurons was attracted and directed by transplants of peripheral tissue normally innervated by sympathetic noradrenergic nerves (*4*). Because of the similarities between central and peripheral noradrenergic neurons we have now tested the ability of NGF to stimulate this growth. The growth of regenerating central sprouts into transplants of peripheral tissue was found most suitable for this study since it allows the selective observation of newly formed nerve fibers. At 7 days after transplantation the sprouting catecholamine fibers are beginning to grow into the transplant, which made it possible to reliably detect and quantitate a stimulatory effect of NGF on the regenerative growth of central noradrenergic neurons.

Twenty-one adult female Sprague-Dawley rats (180 to 200 g) were used.

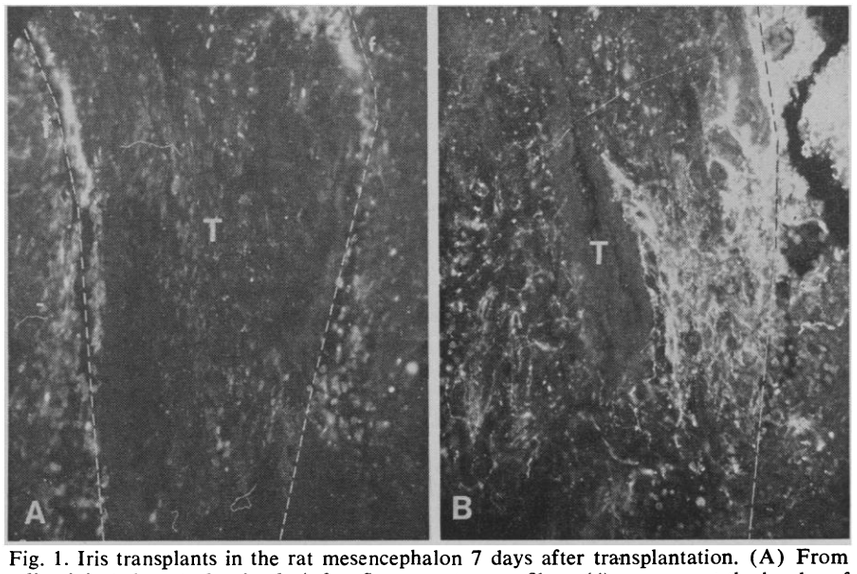

Fig. 1. Iris transplants in the rat mesencephalon 7 days after transplantation. (A) From saline-injected control animal. A few fluorescent nerve fibers (*f*) are seen at the border of the transplant (*T*). Note the absence of nerves within the transplant. The broken line indicates the outline of the transplant. (B) From NGF-treated animal. A single dose of NGF (4 μg/20 μl) was given intraventricularly at the time of transplantation. A large number of thin, varicose fluorescent nerve fibers have grown into the transplant (*T*). The broken line outlines the border of the transplant (× 110).

To rule out the possible involvement of peripheral sympathetic fibers, all animals were subjected to cranial sympathectomy through bilateral extirpation of the superior cervical ganglia. Four to 7 days after this operation, autologous transplantation of one iris to the region of the caudal hypothalamus and rostral mesencephalon was performed according to the technique described earlier (*4*). Essentially, this procedure is carried out as follows: one eye is removed, and the iris is dissected out and washed in saline; the iris is then lowered by freehand with a glass tube through the brain down to the desired position in the posterior hypothalamus. With the transplant in the desired position it will transect the so-called dorsal catecholamine tract that contains almost exclusively noradrenaline axons [see (*6*)]. In this way the transected noradrenaline axons will be in contact with the transplanted tissue. Immediately after the transplantation the control rats received saline (20 μl), and the experimental rats received NGF solution (20 μl), injected through a cannula placed stereotaxically into the right lateral ventricle of each rat. The β subunit of NGF was obtained from Wellcome Reagents Ltd. (Beckenham, Kent, England) and was given in three different concentrations of 4 μg/20 μl, 0.4 μg/20 μl, or 0.04 μg/20 μl. According to the in vitro test these doses corresponded approximately to a biological activity of 2000, 200, and 20 units of NGF, respectively.

All animals were decapitated under ether anesthesia 7 days after the operation. The caudal hypothalamus and mesencaphalon, including the transplant, were dissected out and immediately processed for fluorescence histochemistry of monoamines according to the Falck-Hillarp method [for technical details, see (*7*)]. Only those animals in which the transplant was situated in the desired position of the dorsal catecholamine tract were included in the material.

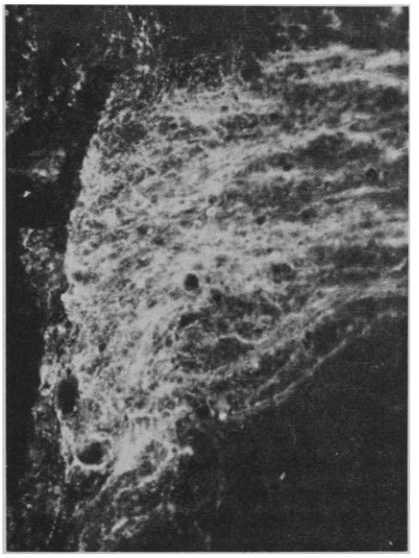

Fig. 2. Sagittal section through the dorsal catecholamine tract showing abundant sprouting of noradrenaline fibers at the border of the lesion (left). The animal was treated with a single dose of NGF (4 μg/20 μl) intraventricularly at the time of transplantation and was killed 7 days later (× 100).

In control animals 7 days after the operation, the transected axons in the dorsal catecholamine tract were to a large extent swollen and distorted and showed a strong accumulation of the characteristic green fluorescence of catecholamines. The axons could be traced up to the thin necrotic zone surrounding the transplant. Only a few thin, presumably growing fibers were seen in the necrotic zone; these thin fluorescent fibers were sometimes seen to run a short distance ventrally or dorsally on the surface of the iris transplant (Fig. 1A). Only occasionally were some fibers seen to grow into the tissue of the transplant, which was easily recognizable and clearly visible [see (*4*)].

The specimens from NGF-treated animals exhibited a picture at the dorsal catecholamine tract quite different from the controls. In the rats receiving the highest intraventricular dose (4 μg/20 μl), abundant thin fluorescent fibers, presumably newly formed sprouts, were noted at the proximal ends of the severed axons of the dorsal catecholamine tract (Fig. 2). These thin fibers were grouped into irregularly arranged bundles that could be traced into the necrotic zone surrounding the transplant and farther into the transplant (Fig. 1B). In the transplant, bundles of fibers were seen to grow in dorsal and ventral directions to cover a varying part of the dorsoventrally oriented iris transplant. In the animals given the medium dose of NGF (0.4 μg/20 μl) there was still a clear increase in the thin, sprouting fibers, but the growth was not as abundant as in the animals given the highest dose. After the lowest dose (0.04 μg/20 μl), the treated animals did not show a clear-cut difference from the controls. However, a considerable number of thin, growing fibers were regularly seen in the necrotic zone and also within a restricted area in the caudal part of the transplant, that is, the part facing the transected axons in the dorsal catecholamine tract.

A single intraventricular injection of NGF given at the time of axonal damage thus resulted in an increased formation and growth of new catecholamine sprouts 7 days later, as judged by the number of central noradrenaline fibers that had grown into the transplant. The effect appeared to be proportional to the administered dose of NGF. Previous studies have shown that the axons in the dorsal catecholamine tract are almost exclusively noradrenaline-containing (*6*).

Thus, the results obtained here demonstrate that NGF has a potent stimulatory effect on the regenerative sprouting and growth of severed central noradrenaline axons.

There have been few studies of the effect of NGF on regenerating neurons. With sensory neurons, Scott and Liu (*8*) found in the kitten that systemic injections of NGF caused an acceleration of the regenerative growth of the central process of the dorsal root ganglion cells. To our knowledge, observations on the effect of NGF on the regeneration of peripheral sympathetic neurons are lacking. Silberstein and co-workers (*9*) reported that NGF stimulated the reestablishment of a sympathetic nerve plexus in rat iris from superior cervical ganglion cells in vitro. From this it seems conceivable that NGF has a similar effect on the regenerative growth of central noradrenergic neurons and on the peripheral sympathetic and sensory neurons.

This is, to our knowledge, the first demonstration of an effect of NGF on central neurons, and it raises a number of interesting possibilities. For instance, our results suggest that NGF could be used to accelerate, increase the magnitude of, or improve the final result of, regeneration of central catecholamine neurons. It seems also possible that NGF is an endogenous, normally occurring physiological factor that is required for the normal development, maturation, and growth of certain central neuron systems, thus playing a role in central neurons similar to that in peripheral, sympathetic neurons.

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References and Notes

1. R. Levi-Montalchini and S. Cohen, *Ann. N.Y Acad. Sci.* **85**, 324 (1960).
2. R. Levi-Montalchini and P. U. Angeletti, *Physiol. Rev.* **48**, 534 (1968).
3. R. Katzman, A. Björklund, Ch. Owman, U. Stenevi, K. A. West, *Brain Res.* **25**, 579 (1971).
4. A. Björklund and U. Stenevi, *ibid.* **31**, 1 (1971).
5. A. Björklund, R. Katzman, U. Stenevi, K. A. West, *ibid.*, p. 21.
6. U. Ungerstedt, *Acta Physiol. Scand.* (Suppl.), 367 (1971); A. Björklund, B. Falck, U. Stenevi, *Brain Res.* **32**, 1 (1971).
7. B. Falck and Ch. Owman, *Acta Univ. Lundensis Sect. 2* **7**, 1 (1965).
8. D. Scott, Jr., and C. N. Liu, in *Mechanisms of Neural Regeneration*, M. Singer and J. P. Schade, Eds. (Elsevier, Amsterdam, 1964), p. 127.
9. S. D. Silberstein, D. G. Johnson, D. M Jacobowitz, I. J. Kopin, *Proc. Nat. Acad. Sci U.S.* **68**, 1121 (1971).
10. Supported by grants from NIH (NS 06701-05) and from the Swedish Medical Research Council (B71-14X-712-06A and B71-14X-56-07A).

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The Hill Plot and the Energy of Interaction in Hemoglobin

Abstract. *The Hill plot does not independently yield the average free energy of interaction per binding site, as has been proposed by Wyman, but rather the difference between the free energies of interaction on binding the first and last ligands. It is shown that additional data (or assumptions) and a model for cooperative behavior are required to obtain the average free energy of interaction.*

The concept of the Hill plot as a means of evaluating the average energy of interaction per binding site in cooperative ligand binding processes was first put forward by Wyman (*1*) and has since been employed by him and others (*2*) to study the nature of cooperative ligand binding in hemoglobin. If y is the fractional saturation of ligand and c_o is the concentration (or activity) of ligand, the equilibrium ligand binding behavior may be represented by a plot of log $[y/(1-y)]$ against log c_o, called the Hill plot. Wyman claims that this plot, independent of any model or additional data, yields the average Gibbs free energy of interaction per binding site (defined below). The purpose of this report is to demonstrate that, in fact, both additional data and a model for cooperative binding are required to obtain a value of the average free energy of interaction and, furthermore, that this value depends upon the model chosen.

The binding of oxygen to hemoglobin may be generally treated as four successive additions of one molecule of oxygen at a time. If we represent the equilibrium constant for the reaction $Hb(O_2)_{i-1} + O_2 \rightleftarrows Hb(O_2)_i$ as K_i, then the oxygen saturation y as a function of the oxygen partial pressure c_o is described by the well-known equation of Adair.

$$y = \frac{K_1 c_o + 2 K_1K_2c_o^2 + 3 K_1K_2K_3c_o^3 + 4 K_1K_2K_3K_1c_o^4}{4 (1 + K_oc_o + K_1K_2c_o^2 + K_1K_2K_3c_o^3 + K_1K_2K_3K_1c_o^4)} \quad (1)$$

The free energy of binding each successive molecule of ligand is given by

$$\Delta G_i = - RT \ln \gamma_i K_i$$

where R is the molar gas constant, T the absolute temperature, and $\gamma_1 \ldots \gamma_4$ are statistical factors equaling 1/4, 2/3, 3/2, and 4, respectively.

If we assume that the intrinsic free energy of ligand binding to a subunit unperturbed by interactions with neighbors, ΔG_o, is the same for all binding sites, then

$$\Delta G_i = \Delta G_o + \Delta G_i^I$$

where ΔG_i^I is the energy of interaction associated with binding ligand molecule i. The average Gibbs free energy of interaction per binding site, $\overline{\Delta G^I}$, is thus defined

$$\overline{\Delta G^I} \equiv \tfrac{1}{4} \sum_i \Delta G_i^I$$

We designate k_o the binding constant of a hypothetical noninteracting subunit, given by

$$\Delta G_o = - RT \ln k_o$$

The Adair equation may then be rewritten as

$$y = \frac{S_1X + 3S_2X^2 + 3S_3X^3 + S_4X^4}{1 + 4S_1X + 6S_2X^2 + 4S_3X^3 + S_4X^4} \quad (2)$$

where $X = k_oc_o$ and

$$S_j = \exp\left(\sum_{i=1}^{j} \Delta G_i^I / RT\right)$$

Equation 2 may be rearranged to yield

$$\frac{y}{1-y} = \frac{S_1X + 3S_2X^2 + 3S_3X^3 + S_4X^4}{1 + 3S_1X + 3S_2X^2 + S_3X^3} \quad (3)$$

It follows that

$$\lim_{X \to 0} \log\left(\frac{y}{1-y}\right) = \log S_1 + \log k_o + \log c_o \quad (4)$$

$$\lim_{X \to \infty} \log\left(\frac{y}{1-y}\right) = \log S_1 - \log S_3 + \log k_o + \log c_o \quad (5)$$

These two straight lines of slope 1 represent the asymptotic behavior of the Hill plot at limiting low and high ligand concentrations, respectively. The vertical distance D between them will be given by

$$D = \log S_1 - \log S_3 - \log S_1 = \frac{- \Delta G_1^I + \Delta G_1^I}{2.303\ RT} = \sqrt{2}\, N \quad (6)$$

where N is the normal distance between the two straight lines (Fig. 1). Thus, the unsupplemented Hill plot yields only the difference between the free energies of interaction on binding the first and last oxygen molecules. This is insufficient to determine $\overline{\Delta G^I}$.

The free energy of oxygen binding to a hypothetical noninteracting subunit in a tetramer, ΔG_o, cannot be measured directly. Its value may, however, be estimated by extrapolation from experimental data. For the purpose of this discussion we assume that the free en-