thought to be essential for the manufacture and use of stone tools.

The manual dexterity involved in the manufacture and use of stone tools not only involves a powerful thumb, but also other nuances of hand morphology that allow power in grasping and efficient positioning of the tool in the hand. Marzke of Arizona State University has outlined a number of important features (*10, 11*). Among these are a relatively long thumb in relation to the other fingers as well as the ability to spread and oppose the thumb and fingers as, for example, when grasping a small ball or using a hammer stone. This type of grip requires the palm to assume a cupped shape, which helps position the thumb and fingers around a spherical object. It is relevant that although A. *afarensis* lacks the stout thumb, it has both a relatively long thumb in relation to its other fingers and the necessary modifications on the index and middle finger side of the hand to allow partial cupping of the palm. Marzke concludes that although A. *afarensis* could not have grasped a hammer stone with all five fingers as modern humans can, it could have grasped it between its palm and its thumb, index, and middle fingers. *Australopithecus afarensis*, therefore, had hands that were more capable of tool use than those of living apes and would have occupied a half-way position in tool use between the stout-thumbed hominids and their more slender-thumbed antecedents.

There is no doubt that stout-thumbed hominids were anatomically more effective toolmakers and users than primates without this adaptation, but how much more effective were they? The suggestion that all early hominids subsequent to 2.5 million years ago might have occupied "cultural" niches and the implication that living primates as well as those hominids that lived before this date did not, must be clearly understood to be an inference drawn solely from the manual dexterity implied by the possession of stout thumbs. What a "cultural" niche means in this context is unclear. We are treading on dangerous ground if we jump to the conclusion that it means any more than a difference in manual dexterity that can be associated with the production and use of stone tools. Care should be taken not to overinterpret the stout-thumbed feature to suggest that it implies a major watershed in the intellectual, linguistic, or symbolic ability of our early ancestors. We must keep clearly in mind the distinction between those inferences that are firmly rooted in the evidence and those that may fall into the category of wishful thinking.

References

1. R. Susman, *Science* **265**, 1570 (1994).
2. W. H. Kimbel, D. C. Johanson, Y. Rak, *Nature* **368**, 449 (1994).
3. A. Hill, S. Ward, A. Deino, G. Curtis, R. Drake, *ibid.* **355**, 719 (1992)
4. F. Schrenk, T.G. Bromage, C.G. Betzler, U. Ring, Y. Juwayeyi, *ibid.* **365**, 833 (1994).
5. R. L. Susman, *Science* **240**, 781 (1988).
6. C. K. Brain, Ed., *Swartkrans: A Cave's Chronicle of Early Man* (Transvaal Museum, Pretoria, 1993).
7. E. Trinkaus and J. C. Long, *Am. J. Phys. Anthropol.* **83**, 419 (1990).
8. K. Gibson and T. Ingold, Eds., *Tools, Language, and Cognition* (Cambridge Univ. Press, Cambridge, 1993).
9. C. Boesch, P. Marchesi, N. Marchesi, B. Bruth, F. Joulian, *J. Hum. Evol.* **26**, 325 (1994).
10. M. W. Marzke, *ibid.* **12**, 197 (1983).
11. ———, and M. S. Shackley, *ibid.* **15**, 439 (1986).

Attractive Axon Guidance Molecules

Herwig Baier and Friedrich Bonhoeffer

Nerve cells are wired to other nerve cells over distances that are regularly more than a thousand times larger than their cell bodies. In many instances, these connections are unfailingly precise from the time they are formed during embryonic development. How do nerve cells find their partners? Santiago Ramòn y Ćajal (*1*) was one of the first to ask this question and to suggest a cellular mechanism. In embryonic nervous tissue, he observed amoeboid thickenings at the tips of what he interpreted correctly as elongating nerve processes (axons or dendrites). He called these thickenings "growth cones" and intuitively attributed to them a role in pathfinding and target recognition. He further speculated that substances released by the target tissue could lay a trace for the advancing growth cones by a mechanism similar to chemotaxis of whole organisms. In order to be able to navigate to the target, the growth cones would sniff out gradients of these chemotropic molecules and orient their migration accordingly.

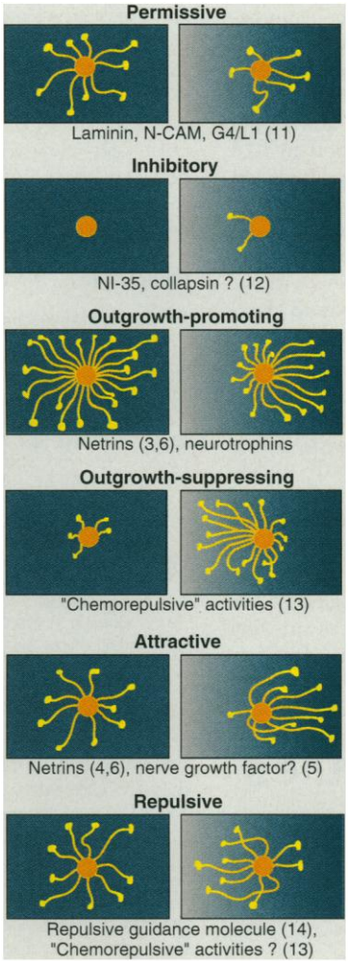
Although Cajal's notion of the growth cone as the essential player in the development of neuronal connections has found its way to textbooks, his further speculation, the chemoattraction hypothesis, has only recently been put on more solid ground. Evidence for the existence of chemoattraction has come from in vitro studies pioneered by Lumsden and Davies (*2*). In this type of assay, two pieces of tissue, one containing the target cells and the other giving rise to axons, are placed beside each other in a drop of collagen. The collagen matrix provides a suitable environment for axonal outgrowth and at the same time stabilizes the diffusion gradient of target-released factors by abolishing convection. If one of these factors is chemotropic, outgrowing axons turn toward the source of this factor, providing a straightforward assay of axon guidance. Such an assay then can be the starting point for biochemical purification and molecular cloning of guidance molecules.

This route has now been successfully followed by Marc Tessier-Lavigne and his co-workers at the University of California in San Francisco and at Columbia University (*3, 4*). The two molecules cloned, which they call netrins (after the Sanskrit "netr" meaning "guiding"), are the first chemotropic factors identified by their function. Other factors, like nerve growth factor, have been implicated in axon guidance in vitro (*5*), but a related function in vivo has remained obscure [see (*2*), for example].

In the new work (*3, 4*), the guidance of a population of axons in the spinal cord of chick and rat was examined. These axons originate from the so-called commissural neurons in the dorsal spinal cord and grow ventrally to the floor plate as an intermediate target. Here, their growth cones cross the midline and make a turn toward the brain. The initial phase of axon guidance to the floor plate can be reproduced in vitro by placing pieces of dorsal and ventral spinal cord into collagen. The ventral piece attracts commissural axons from the dorsal piece at the appropriate embryonic stages over a distance of a few hundred micrometers (*6*). This chemotropism is perfectly correlated with an outgrowth-promoting (trophic) effect on commissural axons in a much simpler assay: When a piece of dorsal spinal cord is exposed to floor plate–conditioned medium, there is a dramatic increase in the number and lengths of axons (*6*). The outgrowth assay was used to biochemically purify the activity, with the hope that tropic and trophic effects were caused by the same factor. It is now clear that taking this risk paid off.

Netrin-1 and netrin-2 are two novel secreted proteins of molecular weight 75,000 and 78,000 that are 72% identical to each other (*3*). They are for the most part membrane-associated, but also exist in soluble form. Netrin-1 is expressed solely by floor-plate cells, whereas netrin-2 transcripts are detected more widely and at lower levels in

The authors are at the Max-Planck-Institut für Entwicklungsbiologie, Post Office Box 2109, D-72011 Tübingen, Germany.



Mechanisms of axon guidance. As neuronal tissue sends out axons, the resulting outgrowth patterns depend on whether factors in the medium are presented in a homogeneous distribution (left column) or as a concentration gradient (right column). Permissive and inhibitory molecules determine whether axons can grow at all, in a dose-dependent fashion. Outgrowth-promoting molecules stimulate outgrowth by increasing number and lengths of axons. These factors preferentially accelerate and stabilize those axons pointing to and having reached higher concentrations of the factor, creating an effect that looks similar to directed outgrowth. Outgrowth-suppressing factors have the opposite function. Only attractive or repulsive molecules are capable of guidance proper, that is, of changing the growth direction of an individual growth cone. (This classification reflects our view and not necessarily the view of the authors cited.)

the ventral two-thirds of the spinal cord. Protein secretion and subsequent association with cell surfaces or the extracellular matrix may generate and stabilize a gradient in the tissue, which then directs axon growth toward the floor plate. Consistent with this scheme, gradients of recombinant netrins expressed in aggregates of a mammalian cell line (COS cells) induce turning of commissural axons toward the aggregate over approximately the same distance as does the floor plate (*4*). It is still unknown what the individual contributions of the two netrins are, since both are sufficient for axon guidance independent of the other.

The two netrins show a 50% homology to UNC-6, a secreted protein identified in the nematode *Caenorhabditis elegans* by mutational analysis (*7*). Strikingly, UNC-6 is required for circumferential guidance of growth cones, as well as migrating cells, in this species. In addition, the identification of sequences conserved among vertebrate netrins and UNC-6 has permitted the recent isolation of a *Drosophila* netrin gene (D-netrin), which is expressed in midline glial cells of the developing fly nervous system (*8*). Together, these factors form the UNC-6–netrin family of outgrowth-promoting (and presumably chemotropic) proteins. All of them are in part homologous to certain domains of laminin, a giant (880,000), heterotrimeric glycoprotein of the extracellular matrix. This points to one of the three laminin chains (probably the B2 chain) as the common evolutionary ancestor of the UNC-6–netrin family.

The netrin receptor on commissural axons is not known. In the nematode, UNC-5 is one of the UNC-6 receptors (*7*). Given the functional conservation of worm and vertebrate genes, an UNC-5–related molecule is the first choice to search for in chick and rat. Only sketchy information exists about the signal transduction pathways involved in axon guidance in general. On the part of the growth cone, intracellular signal amplification and contrast enhancement have been postulated to account for the enormous sensitivity needed to read a gradient of guidance molecules (*9*). With the purified factors now in hand, it will be possible to quantify this sensitivity by measuring the netrin gradients in vitro and in vivo.

Cloning of the netrins was accomplished by a functional biochemical approach that used a large dose of brute force [after all, 25,000 chick embryos have been used in total (*3*)]. Likewise, another functional strategy (namely, a mutant screen) identified UNC-6 in the nematode. Several other indirect strategies have been used to search for the netrins. One of them, based on an educated guess about the molelcular nature of these factors, tried to mimic the floor-plate effect on fiber outgrowth with known factors. A battery of neurotrophins, growth factors, extracellular matrix molecules (including laminin), and adhesion molecules were tested, but all of them failed to exert an effect comparable to floor plate–conditioned medium (*6*). Another seemingly elegant, but nonfunctional, approach picked up genes differentially expressed in floor plate versus dorsal spinal cord by using a subtractive complementary DNA library. Although an interesting floor plate–specific molecule was cloned in this screen, the axon guidance molecules did not show up (*10*). These two failures reflect the specificity and the low abundance of axon guidance molecules and may direct other research efforts in the field toward more functional approaches.

The figure proposes a conceptual framework for classifying guidance-related mechanisms, combining experimental evidence and theory. Although the picture is meant to be complete in a logical sense and to be consistent with published results, its terminology may be arguable or even controversial. "Adhesion," a dominant concept in the field for many years, is missing in the scheme. Adhesion is, of course, a prerequisite for anchoring a growth cone on a surface or in a matrix, but, for directed migration and guidance to occur, adhesion is not sufficient, because surface anchors have to be loosened and redirected continually. In our opinion, this remodeling of adhesive bonds results from, rather than causes, guiding forces. Indeed growth cones will not choose to migrate on more adhesive substrates (*11*), but are influenced by other cues. These cues may be permissive, outgrowth-promoting, or attractive (or their opposites; see figure).

Some factors seem to have very defined functions [like NI-35 (*12*)] (see figure), although too little is known about most to make a final statement. However, some molecules exert a combined effect, being say, both outgrowth-promoting and attractive, like the netrins (*4*). New factors are likely to show up with new properties, and new properties may be found for known factors. The value of our classification system should be judged by how smoothly new findings can be incorporated.

References

1. S. Ramòn y Cajal, *La Cellule* **9**, 119 (1892).
2. A. Lumsden and A. Davies, *Nature* **306**, 786 (1983).
3. T. Serafini *et al.*, *Cell* **78**, 409 (1994).
4. T. E. Kennedy, T. Serafini, J. R. de la Torre, M. Tessier-Lavigne, *ibid.*, p. 425.
5. R. W. Gundersen and J. N. Barrett, *Science* **206**, 1079 (1979).
6. M. Tessier-Lavigne, M. Placzek, A. G. S. Lumsden, J. Dodd, T. M. Jessell, *Nature* **336**, 775 (1988); M. Placzek, M. Tessier-Lavigne, T. M. Jessell, J. Dodd, *Development* **110**, 19 (1990).
7. E. M. Hedgecock, J. G. Culotti, D. H. Halls, *Neuron* **2**, 61 (1990).
8. K. Mitchell, J. Doyle, G. Tear, T. Serafini, T. E. Kennedy, C. Mirzayan, M. Tessier-Lavigne, C. S. Goodman, unpublished results.
9. F. Bonhoeffer and A. Gierer, *Trends Neurosci.* **7**, 378 (1984).
10. A. Klar, M. Baldassare, T. M. Jessell, *Cell* **69**, 95 (1992).
11. V. Lemmon, S. M. Burden, H. R. Payne, G. J. Elmslie, M. L. Hlavin, *J. Neurosci.* **12**, 818 (1992).
12. M. Schwab, *Trends Neurosci* **13**, 452 (1990); Y. Luo, D. Raible, J. A. Raper, *Cell* **75**, 217 (1993).
13. A. Pini, *Science* **261**, 95 (1993).
14. B. Stahl, B. Müller, Y. V. Boxberg, E. C. Cox, F. Bonhoeffer, *Neuron* **5**, 735 (11990); H. Baier and F. Bonhoeffer, *Science* **255**, 472 (1992).

15. We thank A. Gierer and M. Tessier-Lavigne for comments on the manuscript and the Tessier-Lavigne and Goodman labs for sharing unpublished results.