Biomedical Reviews 4: 95-102 (1995)

ISSN 1310-392X

DANCE ROUND

WE DANCE ROUND *IN A RING AND SUPPOSE,*
BUT THE SECRET SITS IN THE MIDDLE AND KNOWS.
ROBERT FROST

NEUROTROPIC ATTRACTION: FACTS AND FICTION

Egbert A.J.F. Lakke
Neuroregulation Group, Department of Physiology, University of Leiden, Leiden, The Netherlands

• Traumatic lesions to the spinal cord of adult individuals will cause degeneration of the severed distal axons, retraction of the severed proximal axons, scar formation in the damaged area, and eventually regenerative signs, which will occur along the retracted proximal axons. Growth cone-like appendages appear and regenerating axons enter the scar tissue. Within the relatively unstructured scar tissue the growth cones appear to lose their sense of direction, as well as their motive force, and progress ceases. The regenerative effort does not result in the establishment of any connection (1).

Although this synopsis of the regenerative process is very crude, it does highlight the three current approaches towards functional regeneration of the central nervous system. The structure of the intervening issue can be improved, for instance by interposing a nerve transplant or a tube filled with Schwann cells between proximal and distal stump (2,3). The motive force of the growth cones can be maintained by the addition of trophic factors, thus increasing the chance of growth cones to broach the scar barrier (4). A direction vector can be imposed on the growth cones by the local application of tropic factors (5).

The underlying assumption of all three approaches is, that once the gap is bridged by the regenerating growth cone, this growth cone will be able to navigate towards its appropriate target again, to reestablish a formerly present synaptic contact. This appears to be the case in the peripheral nervous system (6), and during development (7-10). In the peripheral nervous system, however, the number of necessary pathway choices is small, and paved paths are well possible (11,12). A paved path is a tract whose local environment is permissive to growth cone translocation along its longitudinal axis, and which is equipped with road-signs at each intersection. Reentry upon such a paved path, by coaptation of the severed stumps, or through a nerve transplant, will result in reestablishment of a connection, at least in the same effector or sensor area.

Within the central nervous system the number of possible targets is staggering, and the presence of paved paths much less likely, if not impossible. Even so the growth cones manage to navigate towards their targets in an orderly fashion, at least during development, resulting in the emergence of a hodologic pattern which is similar in different individuals. Here we will review the possible role of **neurotropic** factors during development.

A trophic factor, like nerve growth factor, increases the translocation velocity of the growth cone, and/or maintains a synaptic contact. If a nervous system explant is exposed *in vitro* to a point source of an appropriate trophic factor (inducing a concentration gradient of the trophic factor), those growth cones that are exposed to the highest concentration will grow fastest. The direction of growth cone translocation, however, will not be affected. Even so there will be a tendency for the generated axons to point towards the trophic factor source (13). Without the presence of a gradient, all growth cones will be affected equally.

A tropic factor, like netrin, directs growth cone translocation along an axis parallel to the concentration gradient of the tropic factor, either towards (14) or away (15) from the point of highest concentration. If a nervous system explant is exposed *in vitro* to a point source of an appropriate tropic factor (inducing a concentration gradient of the tropic factor), all growth cones will turn towards (or away from) the tropic factor source. In the absence of a gradient nothing happens.

Though the activity of tropic factors as a guidance mechanism during development was first proposed by Cajal (16) and reiterated by Sperry (17), it was not until 1983 that the first definite proof of chemotropic acitvity was obtained, in a model system employing co-culture of chick trigeminal ganglion and skin explants (18). Since then tropic factors were implicated in the development of spinal commissural (19), corticopontine (20), corticospinal (21), corticothalamic (22), and cerebellar andbrainstem commissural connections (23,24). In 1994, the structure of a tropic factor, dubbed netrin, was described for the first time (14,25).

It should be noted that the maximum possible radius of action of a neurotropic factor is short in relation to the distance between axonal source and the projection target (excepting the commissural connections). Neurotropic factors will probably be effective over a distance of at most 1 mm (26), i.e. neurotropic attraction could lure a growth cone out of the white matter of the spinal cord into the gray matter of the cord, but could not induce the descent of such growth cones from the brainstem into the spinal cord. A second guidance mechanism is necessary for the growth cones to first reach the immediate vicinity of the projection target.

Furthermore it should be noted that, though neurotropic factor secretion would nicely resolve the target aquisition problem in the case of a small spherical target, its secretion *per se* will lead nowhere in the case of larger targets; the afferent axons will attain the appropriate target, but the organization of the projection within the target can not be controlled. At the same time we do know that some of the projections in whose development neurotropic attraction plays a role are highly (somatotopically) organized in the adult. One could, of course, suppose the presence of many, subtly different neurotropic factors emanating from one target area (27), but this leads to the same problem as the paved path approach; to many different neurotropic factors will be needed to organize all of the central nervous system.

In the following section we will propose a guidance mechanism which employs neurotropic attraction in such a way that topically organized connections ensue, while at the same time employing only one neurotropic factor per projection. In formulating this hypothesis we have assumed a biological tendency to generate simple, yet robust solutions to complex problems (28).

Neurotropic attraction will serve as a means for each target neuron to attract the appropriate growth cones, which travel through his sphere of neurotropic influence. This sphere is finite, both in space and in time (29). Several variables are important in the determination of the topology of the projection. Since neurotropic attraction of a certain target is finite in time, the time of arrival of the appropriate growth cones in a target area is essential, especially if the target is large, like the spinal cord. Time of arrival of the growth cones in its turn is determined by the starting time of axonogenesis, the (average) growth cone velocity, and the distance to the target. Distance to target increases in time, since the nervous system itself increases in volume, due to the continued addition of cells. This also implies that axons elongate by two mechanisms simultaneously. The axon elongates by the deposition of continuous axonal tube in the area behind the growth cone (30), and by intercalated growth to accomodate stretch induced by growth of the surrounding tissue (31).

It is possible that stretch-induced elongation is additive to tip elongation. In this case early generated axons will always profit more than late generated axons (their relative length being longer), and consequently reach further down the projection axis after having grown for the same amount of time (provided that tip-elongation speed is the same). It is also possible that the sum of tip elongation and stretch-induced elongation is constant, limited by the maximum production rate of the building materials at the cell soma, and/or by the transport rate of these (32,33). In that case, too, the early growing axons will reach further down the projection axis after the same duration of growth. In both cases, the time-span between arrival of the first departed and the last departed axon from a certain (point) source would be larger than the time-span between departure of the first and the last axon from this (point) source. The increase is a function of the overall growth of the nervous system. This is an interesting property, since there might be a built-in size correction here. If overall size increases, both target size and time-spread of afferent arrivals are increased, though not necessarily in a linear fashion.

In the case of point sources, the time of departure of each axon coincides with the initiation of axonogenesis, but in real sources its size and the orientation of its axonogenic gradient relative to a common exit point, will determine the time of departure from this common exit point. The result is predictable in those cases in which the axonogenic gradient points away from the common exit point; the time-span between departure of the first and the last axon from such a source would be larger than the time-span between genesis of the first and the last axon from such source. For other orientations the result is unpredictable,

for lack of solid data. The direction of the axonogenic gradients through most sources appear to coincide with the direction of the neurogenic gradients (34). Since the target, like the source, is extended and extending in space, it becomes important when neurotropic attraction starts in each part of the target area, and what rules govern its termination. At present we know nothing of these phenomena. We will assume a few, obvious rules. First, we assume that the direction of the neurotropic-attraction initiation-gradient coincides with the direction of the neurogenic gradients. Since the birth time difference is a physical datum which discriminates cells (often in a spatially highly ordered fashion), it would be unexpectedly ineffective if the developmental machinery were not to exploit this difference. Second, we assume that neurotropic attraction ceases upon acquisition of an appropriate number of the appropriate afferent contacts. This assumption is not always necessary.

We will study two examples, which differ in the geometry of the target area: the corticospinal projection, a linear target, and the corticopontine projection, a hemispherical target (Fig.l). In both cases, the source area is the cortex, a convoluted sheet of cells, to whose axons the entrance to the pedunculus cerebri serves as the common exit point. The neurogenic gradient of the cortex is like a wave front that ripples away from a central area located anterolaterally. This wave front travels rostrad, mediad and caudad simultaneously, but the general direction is from rostral to caudal and from lateral to medial (35,36). The axonogenic gradient follows the same general direction (34, 37-39). Departing growth cones enter upon a beaten path (the corticofugal tract), and continue along it until attracted by an appropriate neurotropic lure, or until no further headway can be made. A beaten path is a tract whose local environment is permissive to growth cone translocation along its longitudinal axis (40,41).

Axons originating in the hindlimb region of the somatomotor cortex are the first to arrive in the spinal cord (42), followed by fibres from the forelimb region, from the frontal cortex and from the occipital cortex (43). Though, when comparing hindlimb and forelimb areas, the forelimb fibres arrive last in the spinal cord, they are the first to enter the spinal cord gray matter (Fig. 1). Subsequent ingrowth progresses smoothly along the cord, from rostral to caudal (44). While cervical ingrowth occurs, growth cones which have passed the cervical cord at an earlier stage continue caudad. We think this signifies that the spinal target neurons become neurotropic attractive in a rostrocaudal fashion, mimicking their generation gradient (45), and that the initiation of neurotropic attraction is timed to the arrival of (the last of) the forelimb region axons. The most proximal growth cone will be the first to react. The reaction is a perpendicular collateral sprout (46), which enters the gray matter and possibly acquires a target. The growth cone continues along the descending path, probably at a lower speed (33). If the initiation of neurotropic-attraction gradient were to sweep down the spinal cord in a rostrocaudal fashion, this would result in the attraction, at each sequential rostrocaudal level of the spinal cord, of the most recently arrived (most proximate) growth cones (Fig. 1). This will result in a projection in which the earliest arriving fibres will project the most distant along the axis of the target, and the latest arriving fibres the most proximate, as observed during the development of the corticospinal (44,47) and the rubrospinal (48,49) projections. Fibres from the frontal and occipital cortex, which arrive even later in the cord do descend for some distance along the spinal cord, but do not enter into the gray matter (46,50). Eventually, the spinal cord collaterals disappear (51,52). It is possible that the target neurons remain neurotropic attractive after afferent acquisition, but that the "spinal" factor is not appropriate for frontal and occipital cortical growth cones (53). It is also possible that they enter upon a barren country, in which all appropriate neurotropic activity has ceased, and is continually ceasing in front of them (54). While all these assumptions do construct an elegantly simple developmental algorithm, there is not a shred of evidence to support it, apart from the proven presence of a chemotropic factor. In culture experiments, the growth cone orients itself towards the target, instead of generating a collateral sprout (21).

For lack of evidence for a neurotropic-attraction initiation-gradient we decided to investigate a target with a more complex geometry, which received afferents from the same source (55), by the same process of collateral budding (39), and for which the presence of a chemotropic factor was proven in the same experimental paradigm (20).

The neurons of the basal pontine nuclei, a hemispherical mass of cells are generated and deposited inside-outwards (56,57). If the initiation of neurotropic-attraction gradient were to sweep along the neurogenic gradient (as in the spinal cord), we would expect ingrowth into the central area first, rather than into a rostral and caudal area as was described at that time (39). And indeed, we found that the ingrowth pattern closely adhered to the neurogenic pattern (58). Early arriving fibres enter central areas of the pontine nuclei, and progressively later arriving fibres enter progressively more superficial layers of the pons, avoiding the more central regions, resulting in separate rostral and caudal bundles of fibres entering the pons. Superimposed on this inside-out ingrowth pattern is the mediolateral arrangement of the corticofugal fibres. Medially located fibres enter the subjacent, and thus medial, pons, etc. Medially located fibres originate in the frontal cortex, laterally located fibres in the occipital cortex (59). Both of these occurences conform very well to the proposed model (Fig.l). In this case, however, the initiation of neurotropic attraction in the pons seems timed to the arrival of the first fibres rather than to the last.

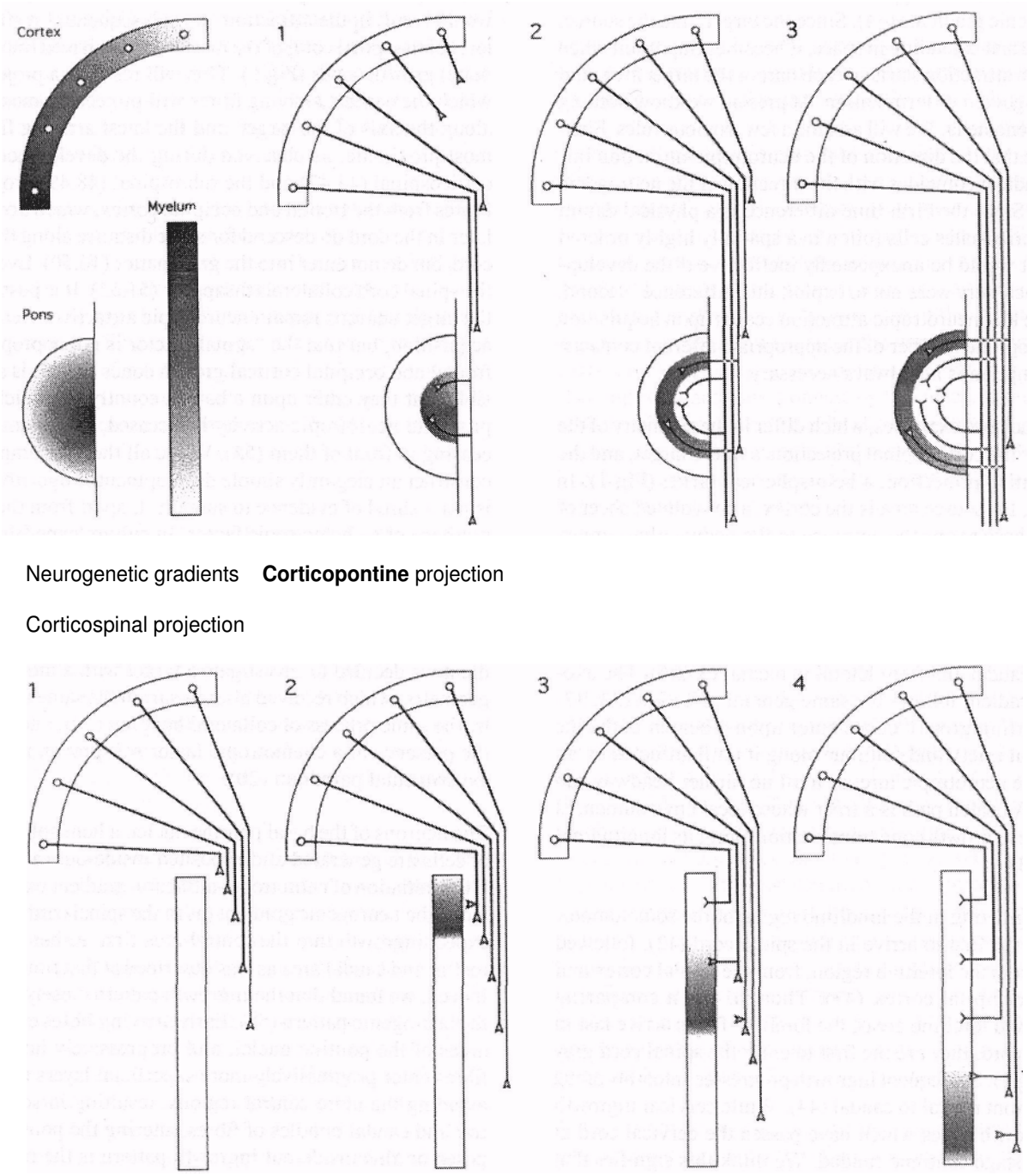

Figure 1. Neurogenic attraction: facts and fiction.

Neurogenic gradients: Idealized neurogenic gradients. The cerebral cortex is generated along a lateromedial and a rostrocaudal gradient (35,36); in transverse sections through the cortex only the lateromedial aspect of the gradient is visible. The pans is generated inside out (56,57). The myelum is generated along a rostrocaudal gradient (45), and at each level the ventral neurons are generated before the dorsal neurons.

We think a general rule can be derived which describes the relation between the projection geometry obtained and the neurogenic gradients of both source and target, and which is valid for all geometries (60). The predictions of this general rule unfortunately will have to be corrected for individual axonal growth speed, which is not necessarily the same for all source areas (61).

Whatever the validity of the proposed organization mechanism, it must be clear that the interaction of spatiotemporal gradients, whether derivatives from neurogenic gradients or not, must play a major role in the developmental machinery. Though this is by no means an original statement (see 57), not much is known about the dynamics of such gradients, nor for that matter on the dynamics of neurotropic factor secretion, nor on the volumetric growth of the nervous system. Essentially, our model holds that time-limited neurotropic attraction is a very convenient means to immobilize an otherwise temporospatial relation between a certain degree of maturity of the target neurons and the proximity of some susceptible growth cones. The geometry of the obtained projection pattern is however not dependent upon the neurotropic factor, but upon the relative geometry of source and target neurogenic gradients.

If this were true, and we think it is, this would also predict that not much benefit can be gained from the application of either tropic or trophic factors (whether two really exist as separate entities or not) into or distal to a lesion site. The real challenge in the orchestration of the regenerative process will lie in the recreation of appropriate temporospatial gradients, or in the exploitation of temporospatial gradient generated by the degenerative process (62). If gradients inherent to the degenerative process are to be exploited, then the regenerating axons will have to arrive as expedient as possible. To achieve this the application of exogenous neurotropic and neurotrophic factors will be an important tool.

REFERENCES

1. Voormolen JHC, de Beer FC, Marani E, Thomeer RTWM. Spinal cord regeneration: An experimental study in cats. *Rec Funct Nerv Syst* 1988; 13: 107-115

2. Bolin LM, Shooter EM. Characterization of a Schwann cell neurite-promoting activity that directs motoneuron axon outgrowth. *JNeurosci Res* 1994; 37: 23-35

3. Kirn DH, Connolly SE, Kline DG, Voorhies RM, Smith A, Powell M, Yoes T, *etal.* Labeled Schwann cell transplants versus sural nerve grafts in nerve repair. *J Neumsurg* 1994; 80: 254-260

4. Goudsmit E, Fliers E, Swaab DF. Testosterone supplementation restores vasopressin innervation in the senescent rat brain. *Brain Res* 1988; 473: 306-313

5. Hagg T, Varon S. Neurotropism of nerve growth factor for adult rat septal cholinergic axons *in vivo. Exp Neural* 1993; 119: 37-45

6. Hoffmann CF, Thomeer RT, Marani E. Reimplantation of ventral rootlets into the cervical spinal cord after their

Corticopontine projection: Idealized corticopontine development (adaptedfrom Leergaardet al [58]). Fibres from the cortexreach (each slice of) the pons in a graded way; laterally originating fibres first, medially originating fibres last. Around the time that the laterally originating fibres start to arrive, the target neurons in the central core of the pons begins to exert neurotropic attraction. They attract the most proximate growth cones only, the others being beyond their radius of action (frame 1). Neurotropic attractiveness spreads outward through the pons, and successively more external shells of target neurons become populated with afferents (frame 2,3). In this way the lateromedial axis of the cortex is projected from inside to outside into each slice of pons. The rostrocaudal axis of the cortex is projected onto the mediolateral axis of the pons (not illustrated). The only thing the corticofugal axons have to do is to grow down along the tract until attracted. The only thing the target cells have to do is to become neurotropic-attractive at a certain time after generation. This and only one neurotropic factor will suffice to organize the corticopontine projection during development.

Corticospinal projection: Idealized corticospinal development. Fibres from the somatosensory cortex reach the entrance to the spinal cord in a graded way; medially originating fibres first, laterally originating fibres last (frame I). Around the time that the laterally originating fibres start to arrive, the target neurons in the rostral cervical spinal cord begin to exert neurotropic attraction. They attract the most proximate growth cones only, the others being beyond their radius of action (frame 2). A wave of neurotropic attractiveness travels down the spinal cord, slowly overtaking even the most distal growth cones, and luring them into the spinal gray matter (frame 3,4). In this way the mediolateral axis of the somatomotor cortex is projected onto the rostrocaudal axis of the spinal cord. The only thing the corticofugal axons have to do is to grow down along the tract until attracted. The only thing the target cells have to do is to become neurotropic-attractive at a certain time after generation. This and only one neurotropic factor would suffice to organize the corticospinal projection during development.

avulsion: an anterior surgical approach. *Clin Neurol Neurosurg* 1993; (95 Suppl): 112S-118S

7. Bregman BS, Bernstein Goral H. Both regenerating and late-developing pathways contribute to transplant-induced anatomical plasticity after spinal cord lesions at birth. *Exp Neurol* 1991; 112: 49-63

8. DeSantis M, Berger PK, Laskowski MB, Norton AS. Regeneration by skeletomotor axons in neonatal rats is topographically selective at an early stage of reinnervation. *Exp Neurol* 1992; 116: 229-239

9. Shieh JY, Leong SK, Wong WC. Effect of spinal cord hemisection on rubrospinal neurons in the albino rat. / *Anat* 1985; 143: 129-141

10. Wang XM, Qin YQ, Xu XM, Martin GF. Developmental plasticity of reticulospinal and vestibulospinal axons in the North American opossum, Didelphis virginiana. / *Comp Neurol* 1994; 349: 288-302

11. Tang J, Landmesser L, Rutishauser U. Polysialic acid influences specific pathfmding by avian motoneurons. *Neuron* 1992; 8: 1031-1044

12. Tomaselli KJ, Doherty P, Emmett CJ, Damsky CH, Walsh FS, Reichardt LF. Expression of beta 1 integrins in sensory neurons of the dorsal root ganglion and their functions in neurite outgrowth on two laminin isoforms. / *Neurosci* 1993; 13:4880-4888

13. Letourneau PC. Chemotactic response of nerve fiber elongation to nerve growth factor. *Dev Biol* 1978; 66: 183-196

14. Kennedy TE, Serafini T, de la Torre JR, Tessier-Lavigne M. Netrins are diffusible chemotropic factors for commissural axons in the embryonic spinal cord. *Cell* 1994; 78: 425-435

15. Messersmith EK, Leonardo ED, Shatz CJ, Tessier-Lavigne M, Goodman CS, Kolodkin AL. Semaphorin III can function as a selective chemorepellent to pattern sensory projections in the spinal cord. *Neuron* 1995; 14: 949-959

16. Cajal SR. La retine des vertebres. *La Cellule* 1892; 9: 119-158

17. Sperry RW. Chemoaffmity in the orderly growth of nerve fiber patterns and connections. *Proc NatlAcad Sci USA* 1963; 50: 703-710

18. Lumsden AG, Davies AM. Earliest sensory nerve fibres are guided to peripheral targets by attractants other than nerve growth factor. *Nature* 1983; 306: 786-788

19. Bovolenta P, Dodd J. Guidance of commissural growth cones at the floor plate in embryonic rat spinal cord. *Development* 1990; 109: 435-447

20. Heffner CD, Lumsden AG, O'Leary DD. Target control of collateral extension and directional axon growth in the mammalian brain. *Science* 1990; 247: 217-220

21. Joosten EA, van der Ven PF, Hooiveld MH, ten Donkelaar HJ. Induction of corticospinal target finding by release of a diffusible, chemotropic factor in cervical spinal grey matter. *Neurosci Lett* 1991; 128: 25-28

22. Novak N, Bolz J. Formation of specific efferent connections in organotypic slice cultures from rat visual cortex cocultured with lateral geniculate nucleus and superior colliculus. *Eur J Neurosci* 1993; 5: 15-24

23. Shirasaki R, Tamada A, Katsumata R, Murakami F. Guidance of cerebellofugal axons in the rat embryo: Directed growth toward the floor plate and subsequent elongation along the longitudinal axis. *Neuron* 1995; 14: 961-972

24. Tamada A, Shirasaki R, Murakami F. Floor plate chemoattracts crossed axons and chemorepels uncrossed axons in the vertebrate brain. *Neuron* 1995; 14: 1083-1093

25. Serafini T, Kennedy TE, Galko MJ, Mirzayan C, Jessell TM, Tessier-Lavigne M. The netrins define a family of axon outgrowth-promoting proteins homologous to C. elegans UNC-6. *Cell* 1994; 78: 409-424

26. Tessier Lavigne M, Placzek M. Target attraction: are developing axons guided by chemotropism? *Trends Neurosci* 1991; 14: 303-310

27. Ullrich B, Ushkaryov YA, Sudhof TC. Cartography of neurexins: More than 1000 isoforms generated by alternative splicing and expressed in distinct subsets of neurons. *Neuron* 1995; 14: 497-507

28. Cherniak C. Component placement optimization in the brain. *J Neurosci* 1994; 14: 2418-2427

29. Lotto RB, Price DJ. The stimulation of thalamic neurite outgrowth by cortex-derived growth factors in vitro: The influence of cortical age and activity. *Eur J Neurosci* 1995; 7:318-328

30. Craig AM, Wyborski RJ, Banker G. Preferential addition

tion of newly synthesized membrane protein at axonal growth cones. *Nature* 1995; 375: 592-594

31. Bray D. Axonal growth in response to experimentally applied mechanical tension. *DevBiol* 1984; 102: 379-89

32. Ahmad FJ, Joshi HC, Centonze VE, Baas PW. Inhibition of microtubule nucleation at the neuronal centrosome compromises axon growth. *Neuron* 1994; 12: 271-80

33. van Veen MP, van Pelt J. Neuritic growth rate described by modeling microtubule dynamics. *Bull Math Biol* 1994; 56: 249-273

34. Bayer SA, Altaian J. Directions in neurogenetic gradients and patterns of anatomical connections in the telencephalon. *Prog Neurobiol* 1987; 29: 57-106

35. Hicks SP, D'Amato CJ. Cell migrations to the isocortex in the rat. *AnatRec* 1968; 160: 619-634

36. Smart IH. Histogenesis of the mesocortical area of the mouse telencephalon. *J Anat* 1984; 138 : 537-552

37. Erzurumlu RS, Jhaveri S. Emergence of connectivity in the embryonic rat parietal cortex. *CerebCortex* 1992; 2:336-352

38. Miller MW. Effect of prenatal exposure to alcohol on the distribution and time of origin of corticospinal neurons in the rat. / *Comp Neurol* 1987; 257: 372-382

39. O'Leary DD, Terashima T. Cortical axons branch to multiple subcortical targets by interstitial axon budding: implications for target recognition and "waiting periods". *Neuron* 1988; 1: 901-910

40. Joosten EAJ. Developmental expression of N-CAM epitopes in the rat spinal cord during corticospinal tract axon outgrowth and target innervation. *Brain Res Dev Brain Res* 1994; 78: 226-236

41. Lemmon V, Burden SM, Payne HR, Elmslie GJ, Hlavin ML. Neurite growth on different substrates: permissive versus instructive influences and the role of adhesive strength. *JNeurosci* 1992; 12: 818-826

42. Schreyer DJ, Jones EG. Topographic sequence of outgrowth of corticospinal axons in the rat: a study using retrograde axonal labeling with Fast blue. *Brain Res* 1988; 466: 89-101

43. Joosten EA, Gribnau A A, Dederen PJ. An anterograde tracer study of the developing corticospinal tract in the rat: three components. *Brain Res* 1987; 433: 121-130

44. Alisky JM, Swink TD, Tolbert DL. The postnatal spatial and temporal development of corticospinal projections in cats. *Exp Brain Res* 1992; 88: 265-76

45. Altman J, Bayer SA. The development of the rat spinal cord. *Adv Anat Embryol Cell Biol* 1984; 85: 1-166

46. Kuang RZ, Kalil K. Development of specificity in corticospinal connections by axon collaterals branching selectively into appropriate spinal targets. *J Comp Neurol* 1994; 344; 270-282

47. Miller MW. The origin of corticospinal projection neurons in rat. *Exp Brain Res* 1987; 67: 339-351

48. Lakke EA, Marani E. Prenatal descent of rubrospinal fibers through the spinal cord of the rat. / *Comp Neurol* 1991; 314: 67-78

49. Oudega M, Lakke EAJF, Marani E, Thomeer RTWM. Development of the rat spinal cord: Immuno- and enzymohistochemical approaches. *Adv Anat Embryol Cell Biol* 1993; 129: 1-164

50. Joosten EA, van Eden CG. An anterograde tracer study on the development of corticospinal projections from the medial prefrontal cortex in the rat. *Brain Res Dev Brain Res* 1989; 45: 313-319

51. Adams CE, Mihailoff GA, Woodward DJ. A transient component of the developing corticospinal tract arises in visual cortex. *Neurosci Lett* 1983; 36: 243-8

52. Oudega M, Varon S, Hagg T. Distribution of corticospinal motor neurons in the postnatal rat: Quantitative evidence for massive collateral elimination and modest cell death. *J Comp Neurol* 1994; 347: 115-126

53. Kuang RZ, Merline M, Kalil K. Topographic specificity of corticospinal connections formed in explant coculture. *Development* 1994; 120: 1937-1947

54. Joosten EAJ, Bar PR, Gispen WH, Bregman BS. Transient projections from rat occipital cortex are able to respond to a spinal target derived diffusible factor *in vitro*. *Neurosci Lett* 1993; 164: 85-88

55. Wiesendanger R, Wiesendanger M. The corticopontine system in the rat. I. Mapping of corticopontine neurons. *J Comp Neurol* 1982; 208: 215-226

56. Altman J, Bayer SA. Prenatal development of the cerebellar system in the rat: II. Cytogenesis and histogenesis of the inferior olive, pontine gray and the precerebellar

, reticular nuclei. *J Comp Neurol* 1978; 179: 49-76

57. Altman J, Bayer SA. Development of the precerebellar nuclei in the rat: IV. The anterior precerebellar extramural migratory stream and the nucleus reticularis tegmenti pontis and the basal pontine gray. *J Comp Neurol* 1987; 257: 529-552

58. Leergaard TB, Lakke EAJF, Bjaalie JG. Development of topographical organization in the corticopontine projection. A Dil and 3-D computer reconstruction study in the rat. *J Comp Neurol* 1995. In press

59. Glickstein M, Kralj HI, Legg C, Mercier B, Ramna Rayan M, Vaudano E. The organisation of fibres within the rat basis pedunculi. *Neurosci Lett* 1992; 135: 75-79

60. Kalay A, Parnas H, Shamir E. Neuronal growth via hybrid system of self-growing and diffusion based grammar rules. *Bull Math Biol* 1995; 57: 205-227

61. Davies AM. Intrinsic differences in the growth rate of early nerve fibres related to target distance. *Nature* 1989; 337: 553-555

62. Oudega M, Varon S, Hagg T. Regeneration of adult rat sensory axons into intraspinal nerve grafts: Promoting effects of conditioning lesion and graft predegeneration. *Exp Neurol* 1994; 129: 194-206

Received 23 August 1995
Accepted 15 September 1995

Address for correspondence:
Dr Egbert A. J.F. Lakke
Department of Physiology
University of Leiden
FOB 9604
NL-2300 RC Leiden
The Netherlands

Tel: 31 (71) 5276759
Fax: 31 (71) 5276782
E-mail: lakke@rullf2. leidenuniv. nl