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The trigeminal system: an advantageous experimental model for studying neuronal development

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Summary

The trigeminal system is a well-characterized sensory system that has been studied extensively in mammals and birds. The clear definition and easy accessibility of the components of this system throughout development have facilitated investigation of several fundamental aspects of neural development. This has led to important advances in our understanding of the mechanism of axonal guidance, the physiology of neurotrophic factors and the establishment and refinement of neural connections. The first convincing evidence for axonal guidance by chemotropism has been obtained. The site and timing of nerve growth factor (NGF) synthesis and NGF receptor expression have been elucidated, thereby clarifying the role of this neurotrophic factor in development. A novel concept in neurotrophic factor physiology has emerged: the survival of neurones that innervate two separate target fields is regulated by two different neurotrophic factors derived from the respective target fields. The development of somatotopic maps of the periphery in the central nervous system (CNS) is dependent on spatial information provided by the periphery. The transfer of this information from the periphery to the CNS is not simply achieved by the ordered growth and arrangement of the intervening sensory nerve fibres.

Key words: neurone, nerve growth factor, trigeminal ganglion, neurotrophic factor, axon.

Phases of neuronal development

At the cellular level, many populations of neurones in the vertebrate nervous system pass through the following phases of development.

(a) Differentiation from progenitor cells.

(b) Growth of axons to their target fields.

(c) Target field innervation. This phase extends throughout the period axons arrive in the target field. It is associated with the death of a proportion of the innervating neurones. Neuronal death begins shortly after the earliest axons reach their targets and ends shortly after the last axons arrive. This adjusts the number of innervating neurones to the requirements of their target fields and may play a role in the elimination of inappropriately connected neurones.

(d) Remodelling axon connections. After the phase of neuronal death, the connections of the remaining neurones undergo extensive modification. An initial excess of axon branches in the target field is removed and favourable connections are consolidated. This process transforms initially diffuse target field innervation patterns into precise topographical mappings of one region to another in the nervous system.

The trigeminal system and its advantages

The accessibility of populations of sensory neurones for experimental studies is one of the main reasons why much of our understanding of the cellular and molecular basis of neuronal development has come from studies of these neurones. The best-characterized developing sensory system which has provided extensive information on many aspects of neuronal development is the trigeminal system. This comprises two populations of first-order sensory neurones, the trigeminal ganglion and the trigeminal mesencephalic nucleus (TMN), their peripheral and central target fields and subsequent projections in the central nervous system (CNS). The trigeminal ganglion innervates mainly mechanoreceptors, thermoreceptors and nocioceptors in the face, oral cavity and nasal

cavity. Its central processes terminate on several groups of neurones in the brainstem which project to the somatosensory cortex *via* relay in the thalamus. The TMN innervates mainly proprioceptors in the muscles of mastication. Its central branches terminate on trigeminal motor neurones and several other groups of neurones in the brainstem.

The trigeminal system has been studied most extensively in developing mammals and birds. In each of these classes of vertebrates, the system offers particular advantages for investigating different facets of neuronal development. These species-related advantages and the general advantages of the trigeminal system are outlined below.

(a) Because of its comparatively large size, the trigeminal ganglion can be dissected from the embryo before it has innervated its target fields. This permits investigation in culture of the factors that influence sensory axon growth and guidance at the appropriate stage of development. Changes in sensory neurone physiology related to target field innervation can also be studied under exacting *in vitro* conditions.

(b) The maxillary and mandibular processes, which comprise most of the peripheral target field of the trigeminal ganglion, are clearly defined prior to and during the phase of innervation. This permits investigation of the influence of virgin target tissue on the guidance of early trigeminal nerve fibres in culture. Also, it is possible to measure changes in the levels of regulatory molecules (e.g. neurotrophic factors) in the target field at stages throughout innervation and to study how the synthesis of these molecules is controlled. Since the maxillary process is the most-densely innervated cutaneous target field in the mammalian embryo, these changes are likely to be most marked and easily measured in this location.

(c) The trigeminal system in rodents is an important and extensively studied experimental model for investigating the establishment of topographic neural projections. The characteristic pattern of whisker follicles on the snout is replicated in the brainstem, thalamus and somatosensory cortex by similar and functionally corresponding arrays of multineuronal units. Since the receptive field of this neural projection is situated in the skin, it is particularly easy to manipulate experimentally.

(d) The accessibility of both the trigeminal ganglion and TMN in chick embryos has provided the opportunity to study the specific neurotrophic factor requirements of these functionally distinct kinds of sensory neurones. Furthermore, the investigation of whether the survival of sensory neurones is regulated by different neurotrophic factors from their peripheral and central target fields has been facilitated by the availability of a homogeneous preparation of proprioceptive neurones from the TMN.

In the following sections, I shall consider the features and development of the trigeminal system in mammals and birds, describe the major experimental applications of this system, and outline the principal findings and conclusions of these studies. My objective is to provide an integrated anatomical, cellular and molecular account of neural development in a well-characterized experimental system.

The mammalian trigeminal system

An essential prerequisite for the design and interpretation of studies to understand the cellular and molecular basis of neural development is an accurate chronology of the phases of normal development of the experimental system under investigation. This has been obtained for the trigeminal ganglion and its innervation of the periphery from detailed light and electron microscopic studies of mouse embryos (Davies, Lumsden, Slavkin & Burnstock, 1981; Davies & Lumsden, 1984, 1986; Davies, 1987*a*,*b*). The time course of the principle developmental events is listed below and summarized in Fig. 1.

(a) The trigeminal ganglion becomes discernible by the ninth embryonic day (E9).

(b) The earliest nerve fibres emerge from the ganglion at E9·5 and the last fibres leave at E13. These fibres grow towards the periphery at a constant rate of 20 μm per hour.

(c) The earliest nerve fibres reach the epithelium of their peripheral target fields by E10·5 in the mandibular process and by E11 in the maxillary process. The last nerve fibres reach their targets just after E15.

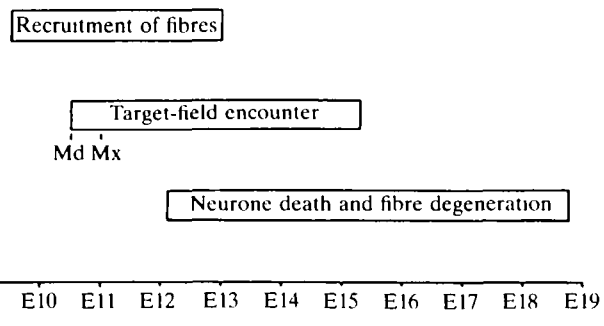


Fig. 1. Diagram showing the time course of the principal anatomical events in the development of the mouse trigeminal ganglion and its innervation of the periphery. The boxes indicate the timing and duration of (i) the stage of recruitment of nerve fibres to the trigeminal nerve; (ii) the period these fibres arrive in their peripheral target fields (the stage at which the earliest nerve fibres reach cutaneous epithelium in the mandibular process (Md) and in maxillary process (Mx) are indicated) and (iii) the period of neurone death and fibre degeneration.

(d) Over 50 % of the neurones are lost in a phase of cell death that extends from E13 to E19.

Axonal guidance

The attraction of axons to their target fields by specific chemotropic factors is one of several mechanisms that may account for the precision with which axons reach their targets in development. A useful *in vitro* method for investigating the operation of this mechanism is explant coculture of neural and target tissues. The majority of studies employing this method, however, have been conducted late in development using regenerating neurones and denervated target tissue (Ebendal & Jacobson, 1977). Although neurite outgrowth in these studies was more marked on the side of the neural explant facing the cocultured target tissue, it was neither confined to this location nor exclusively directed towards the target tissue but radiated from the entire perimeter of the explant. This pattern of neurite outgrowth is not consonant with chemotropism. It is due to a neurotrophic factor enhancing neuronal survival and regeneration; the effect being quantitatively greater on the side of the neural explant facing the source of this factor (the denervated target tissue). To avoid these complications and provide meaningful information on the mechanism of axonal guidance it is essential to carry out coculture experiments at the stage when axons are growing to their targets.

The feasibility of dissecting the trigeminal ganglion and its peripheral target field from the embryo prior to innervation has provided the opportunity to set up explant cocultures at the appropriate stage of development (Lumsden & Davies, 1983, 1986, 1987). In these early cocultures (Fig. 2), neurites grow directly from the trigeminal ganglion to its cutaneous target field (either the maxillary or mandibular process). Explants of other cutaneous target fields do not influence the growth of trigeminal neurites at this stage and neither the maxillary process nor the mandibular process influence the growth of neurites from other sensory ganglia. This suggests that the early trigeminal cutaneous target field produces a diffusible factor which specifically directs the growth early trigeminal neurites. This factor appears to be of epithelial origin as neurite outgrowth from early trigeminal ganglia cocultured with the isolated epithelial and mesenchymal components of its target field is exclusively epithelium-directed.

The importance of having carried out these cocultured experiments prior to target field innervation is demonstrated by the results of culturing the trigeminal ganglion with its cutaneous target field or other cutaneous targets later in development. In these cultures, neurites grow radially from the entire perimeter of the ganglion and unlike the specific target-directed neurite outgrowth observed in earlier cocultures, this undirected outgrowth is abolished by antiserum to nerve growth factor (NGF).

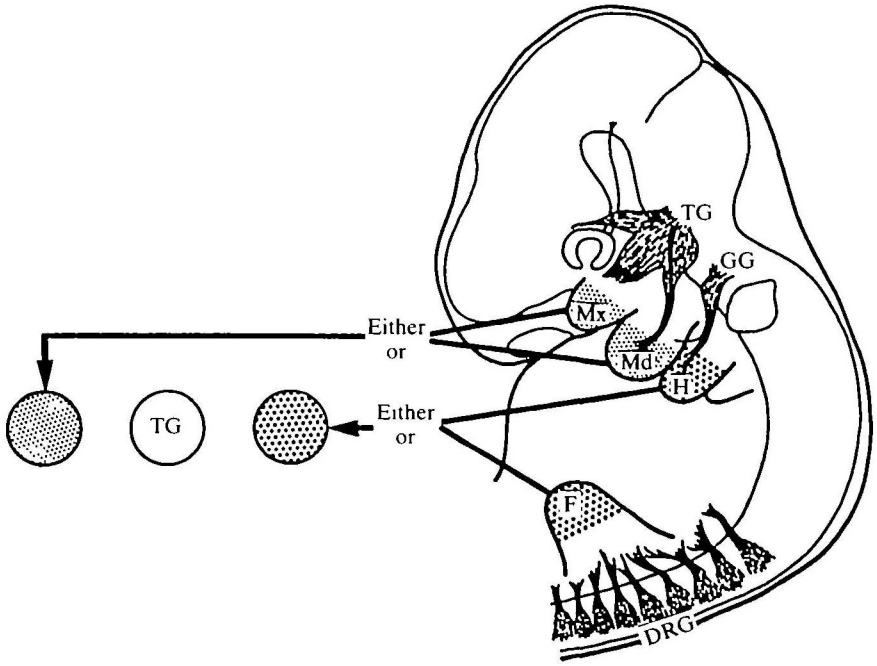
Chemotropism is not the only influence on the route taken by developing trigeminal nerve fibres. Punctate tracts of laminin extending for a short distance from the trigeminal ganglion into the mesenchyme in the centre of the maxillary and mandibular processes have been observed just before sensory fibres grow into these regions (Riggott & Moody, 1987; A. G. S. Lumsden & J. Cohen, unpublished findings). Since laminin is a very effective substratum for neurite growth *in vitro* it is likely that these tracts channel growing axons into distinct trunks in the proximal part of their course. This ensures that the fibres do not radiate directly from the ganglion to their target epithelium under the action of the chemotropic agent.

Clarifying the role of neurotrophic factors

The view that embryonic neurones depend for their survival on the supply of a trophic factor present in limiting amounts in their target field has received substantial support from work on NGF (for reviews, see Levi-Montalcini & Angeletti, 1968; Thoenen & Barde, 1980; Davies, 1987*b*). This protein promotes the survival of symapthetic and certain kinds of sensory neurones *in vitro* and prevents loss of these neurones *in vivo* if administered during the period of natural neuronal death. Anti-NGF antiserum administered during the same period eliminates these neurones. NGF in the target field binds to specific receptors on the innervating neurones and is conveyed to their cell bodies by axonal transport.

In addition to the evidence that NGF has a trophic action on neurones during the phase of target field innervation, there is less-rigorous evidence that it may subserve different functions in other phases of neuronal development. For example, studies of the effects of very high concentrations of NGF on the growth of late-embryonic or neonatal sensory and sympathetic axons have led to the widely held view that NGF attracts developing nerve fibres to their target fields by chemotropism (Levi-Montalcini, 1982).

To clarify the role of NGF in development, it is necessary to ascertain when and where it is synthesized and when NGF receptors are expressed. Application of sensitive assays for NGF, its messenger RNA and NGF receptors to the trigeminal system at closely staged intervals throughout development has resolved these basic issues (Davies *et al.* 1987*a*). NGF synthesis in the target field commences with the arrival of the earliest nerve fibres and NGF-receptor expression on trigeminal neurones begins when their fibres reach the target field. This demonstrates that



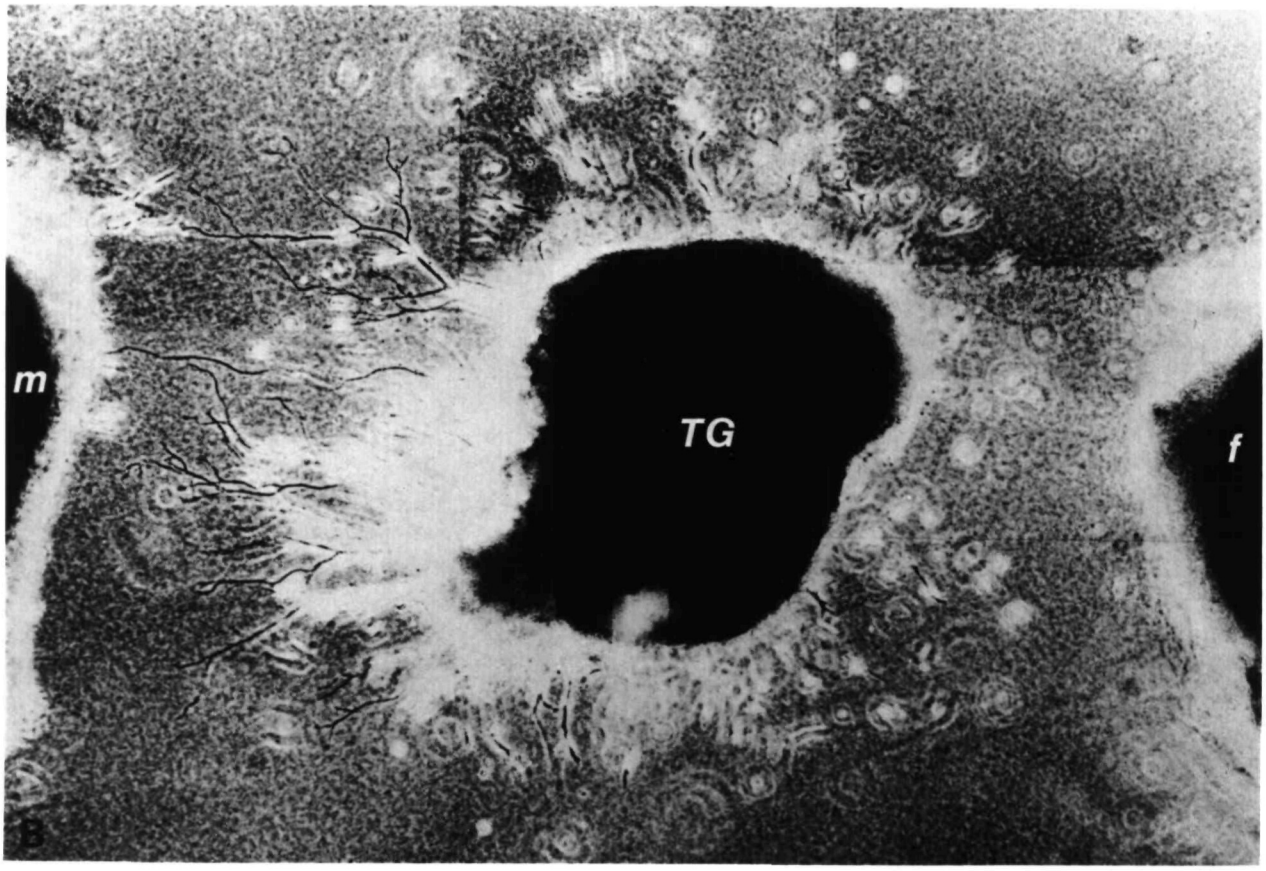


Fig. 2. (A) Camera lucida drawing of an E10 mouse embryo showing the location of the trigeminal ganglion (*TG*) and the major part of its peripheral target field (light stipple) composed of the maxillary process (*Mx*) and mandibular process (*Md*) of the first branchial arch. Two inappropriate cutaneous target fields used in coculture experiments are shown (dark stipple), the hyoid process of the second branchial arch (*H*) and the tip of the forelimb bud (*F*). The location of the adjacent sensory ganglion, the geniculate ganglion (*G*), and dorsal root ganglia innervating the limb bud (DRG) are also shown. The scheme for arranging explants in collagen coculture is illustrated to the left of the embryo. (B) Phase-contrast photomontage of an E10 coculture of the trigeminal ganglion (*TG*) with the maxillary process (*m*) and forelimb (*f*) after 48 h incubation showing neurites growing exclusively and directly towards their target field.

NGF does not attract sensory nerve fibres to their target fields during development.

Assay of NGF mRNA in isolated target field epithelium and mesenchyme together with localization by *in situ* hybridization (Fig. 3) has shown that its concentration correlates with innervation density (Davies *et al.* 1987*a*); it is highest in the epithelium (presumptive epidermis), lower in the subjacent mesenchyme (presumptive dermis) and lowest in the deep mesenchyme (presumptive subcutaneous tissue). These findings suggest that the local availability of NGF regulates innervation density. They also discount the view, based on studies of NGF immunoreactivity in the denervated iris, that NGF is synthesized exclusively by Schwann cells (Rush, 1984).

Establishment of somatic projections

Several findings suggest that development of the central representations of the whisker follicle pattern depends on spatial information from the periphery. Snout skin cultured prior to innervation is capable of generating the pattern, indicating that this is an intrinsic property of the skin. In development, the pattern appears in the skin before its sequential emergence in the brainstem, thalamus and cortex. Damage to whisker follicles or their nerve supply disrupts the establishment of the central patterns (for reviews see Durham & Woolsey, 1984; van der Loos & Welker, 1985). This raises the fundamental issue of the cellular basis of pattern transfer from the periphery to the CNS.

Contrary to the view of Erzurumlu & Killackey (1982, 1983), pattern transfer is not due to the maintenance of order in the developing maxillary nerve. Detailed reconstructions of this nerve during the early stages of whisker follicle development (Davies & Lumsden, 1986) have shown that there is no consistent pattern in the arrangement of nerve fibre fasciculi and no correspondence between the number and arrangement of fasciculi and the pattern of whisker follicles. Fasciculi merge and branch to form an intricate plexus in which nerve fibres do not remain in associated groups but freely mingle. This finding together with the occurrence of collateral branching in the early nerve raises the possibility that during development individual trigeminal neurones innervate not one but several follicles and that their central connections are also initially diffuse. Studies are currently in progress to ascertain whether early trigeminal connections are indeed diffuse and whether action potentials in developing trigeminal neurones are necessary for the emergence of the precise topographic projection.

The avian trigeminal system

In contrast to the mammalian system, most TMN neurones in birds are segregated within a discrete region of the midbrain which can be easily and cleanly dissected from surrounding structures. Because of their large size, these neurones can be separated from all other cells by differential sedimentation resulting in an essential pure preparation of proprioceptive neurones (Davies, 1986). This permits comparative developmental studies with the predominantly cutaneous sensory neurones of the trigeminal ganglion. In birds, the neural-crest-derived and placode-derived neurones of this ganglion are segregated into its dorsomedial (DM) and ventrolateral (VL) parts. These two groups of neurones are easily obtained by subdissection of the ganglion. In view of the marked size difference between DM and VL neurones (VL are larger), it is possible they innervate different classes of cutaneous receptors. The location of TMN, DM and VL neurones in the chick embryo is shown in Fig. 4.

Specificity of neurotrophic factors

In recent years, it has become apparent that NGF is one of several neurotrophic factors. To ascertain which kinds of neurones are supported by each of these factors, it is essential to study neuronal preparations which are homogeneous in terms of their function and the connections they make. Almost all of the work on the neurotrophic factor requirements of developing sensory neurones, however, has been done on dorsal root ganglion neurones (DRG) which are composed of a variety of cutaneous sensory, visceral sensory and proprioceptive neurones. Although DRG neurones are supported to varying extents by several different neurotrophic factors (Levi-Montalcini & Angeletti, 1968; Barde, Edgar & Thoenen, 1982; Barbin, Manthorp & Varon, 1984; Gurney, Heinrich, Lee & Yin, 1986), it has not been possible to show in these studies whether each factor addresses functionally distinct kinds of sensory neurones.

Several *in vitro* studies of the effects of NGF on the survival of DM and VL trigeminal neurones (Ebendal & Hedlund, 1975; Davies & Lumsden, 1983; Davies & Lindsay, 1984), nodose ganglion neurones (Lindsay & Rohrer, 1985) and the neurones of other avian cranial sensory ganglia (Davies & Lindsay, 1985) have led to the view that neural-crest-derived sensory neurones are dependent on NGF for survival whereas those of placodal origin are not. The recent demonstration, however, that TMN neurones do not survive in the presence of NGF (Davies, Lumsden & Rohrer, 1987*b*) indicates that NGF dependence is not an

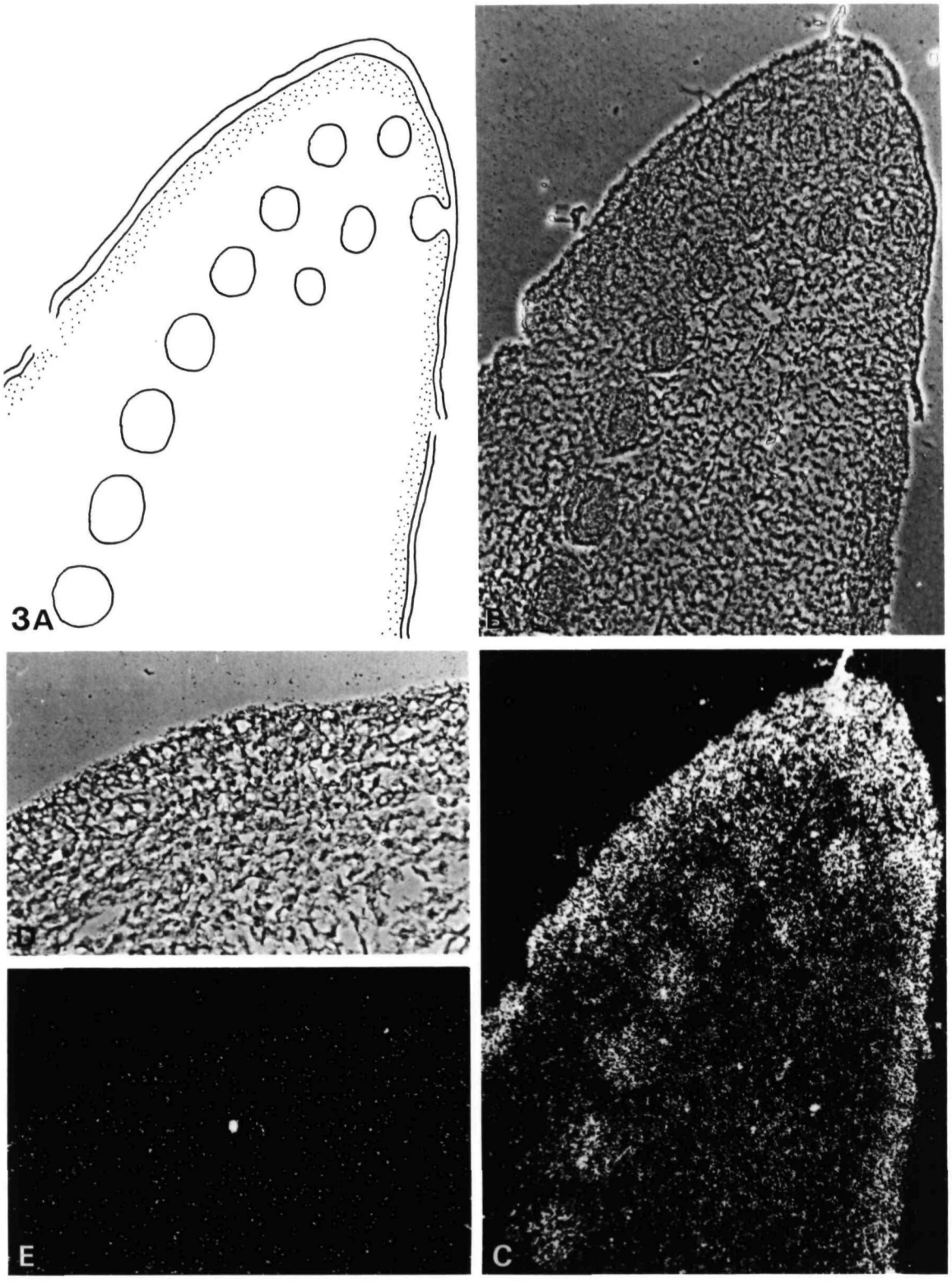


Fig. 3. A, B and C show a line diagram, phase-contrast photomicrograph and dark-field photomicrograph, respectively, of a section through an E13 whisker pad that was hybridized with a ^{35}S-labelled NGF cRNA probe to demonstrate the location of NGF mRNA. The line diagram shows the thickness of the surface epithelium (double lines), the location of the immediately subjacent mesenchyme (stipple) and the location of whisker follicles (circles) seen in B. The grain density in C is highest in relation to surface and follicular epithelium, it is lower in the subjacent mesenchyme and lowest in the deep mesenchyme. D and E are phase-contrast and dark-field views of a section through an E10 maxillary process (in which NGF mRNA is undetectable) hybridized with the same probe showing that the grain density over the section is very low. This demonstrates that there is negligible nonspecific binding.

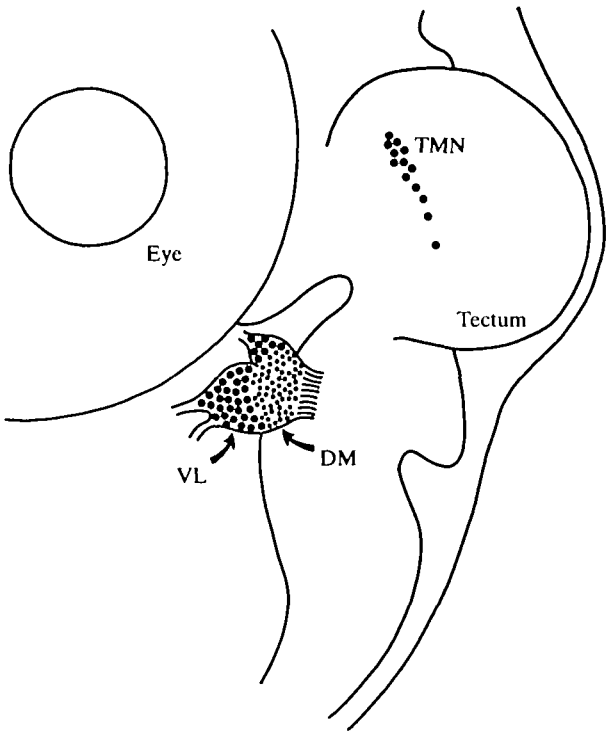


Fig. 4. Line diagram of part of the head of an E8 chick embryo to show the location of the populations of first-order sensory neurones of the trigeminal system: the TMN neurones in the midbrain and the segregation of large placode-derived VL neurones (large circles) and small neural crest-derived DM neurones (small circles) in the trigeminal ganglion.

invariable characteristic of neural-crest-derived sensory neurones. The survival of TMN neurones is supported by a factor present in skeletal muscle, the peripheral target tissue of these neurones (Davies, 1986). This factor has negligible trophic effects on either the neural-crest-derived or the placode-derived neurones of the trigeminal ganglion. These findings indicate that the trophic requirements of developing sensory neurones are not simply related to whether they are of neural crest or placodal origin but suggest that they are related to the kinds of structures or tissues they innervate.

The demonstration that about half the neurones in newborn rat DRG die after their central nerve fibres are cut (Yip & Johnson, 1984) suggests that at least some sensory neurones are dependent on trophic support from the CNS. The proposal that this is mediated by NGF is unlikely as NGF is undetectable in the central branches of DRG but is present in their peripheral branches (Korsching & Thoenen, 1985).

A likely candidate for a factor that mediates the trophic effect of the CNS on developing sensory neurones is brain-derived neurotrophic factor (BDNF, Barde *et al.* 1982). This promotes the survival of embryonic DRG neurones in culture but does not influence the survival of either sympathetic or parasympathetic neurones (Lindsay, Thoenen & Barde, 1985; Davies, Thoenen & Barde, 1986*a*). Comparative studies of the effects of BDNF on the survival of embryonic trigeminal neurones in culture have shown that whereas the great majority of TMN and VL neurones are supported by this factor, only a very small proportion of DM neurones survive (Davies *et al.* 1986*a*,*b*). This indicates that BDNF does not support the survival of all sensory neurones and suggests that BDNF-dependent and NGF-dependent sensory neurones are largely distinct.

The predominantly BDNF-insensitive DM neurones have been useful for investigating whether the CNS contains other neurotrophic factors for sensory neurones. The majority of DM neurones survive in cultures supplemented with an extract of mouse brain, and this effect is not abolished by anti-NGF antiserum (A.M.D., unpublished findings). This suggests that the CNS contains a neurotrophic factor for sensory neurons that is neither BDNF nor NGF. Purification of this factor will be necessary to determine whether it is specific for BDNF-insensitive neurones.

The specificity of neurotrophic factors from the periphery and CNS for trigeminal neurones is summarized in Table 1.

Cooperation of neurotrophic factors

Is the survival of neurones that innervate two target fields regulated by two different neurotrophic factors? The feasibility of obtaining a functionally homogeneous preparation of sensory neurones from the

Table 1. *Sensory function, embryonic derivation and presumed peripheral and central neurotrophic factor requirements of populations of first-order trigeminal neurones in the embryonic chick*

Population	Sensory function	Derivation	Neurotrophic from periphery	Neurotrophic factor from CNS
TMN	Proprioceptive	Neural crest	Novel factor present in skeletal muscle (distinct from NGF)	BDNF
DM trigeminal	Small diameter cutaneous sensory	Neural crest	NGF	Novel factor present in CNS (distinct from BDNF)
VL trigeminal	Large diameter cutaneous sensory	Placode	?	BDNF

TMN has permitted investigation of whether the survival of sensory neurones is regulated by separate neurotrophic factors from their peripheral and central target fields. The survival of the great majority of TMN neurones in culture is promoted by either of two neurotrophic factors: BDNF (present in the CNS) and a factor from skeletal muscle (Davies *et al.* 1986*b*). There is no additional survival in the presence of saturating levels of both factors, indicating that each neurone responds to both factors. The combined effect of both factors is additive at concentrations that promote half-maximal survival alone and is greater than additive at very low concentrations. This suggests that peripheral and central neurotrophic factors may potentiate each other at low, possibly physiological, concentrations. The finding that the responsiveness of TMN neurones to each factor is maximal during the period of natural neuronal death is further evidence that both factors cooperate in regulating sensory neurone survival during development.

Conclusions

Several aspects of neuronal development have been studied to advantage in the trigeminal system.

In the mouse, the first compelling evidence for the operation of chemotropic guidance of axons in the developing nervous system has been obtained. The trigeminal epithelium appears to be specified to attract its innervation by the production of a diffusible chemotropic agent to which early trigeminal neurites specifically respond. The restricted site and timing of synthesis of this agent preclude its characterization by conventional biochemical techniques. Molecular cloning of its messenger RNA by expression is, however, a possible approach.

The role of NGF in the early development of neurones has been clarified. The view that NGF attracts the nerve fibres of NGF responsive neurones to their targets must be abandoned as NGF is not synthesized in the target field prior to its innervation and NGF receptors are not detectable on early neurones at the stage when they are growing to their targets. The commencement of NGF synthesis and the expression of NGF receptors coincide with the onset of target field innervation. Although it appears that NGF receptor expression occurs as part of an intrinsic developmental programme in sensory neurones, it has yet to be determined whether or not NGF synthesis in the target field is induced by the innervating new fibres.

Contrary to the prevalent view, there is no consistent relationship between the dependence of sensory neurones on a particular neurotrophic factor and their derivation from neural crest or placode. Rather, the specific neurotrophic factor requirements of sensory neurones appear to be more closely related to the kinds of tissues and sensory receptors they innervate.

The cooperation of different neurotrophic factors from the periphery and CNS in regulating sensory neurone survival suggests a mechanism for selectively supporting those neurones which make appropriate connections in both target fields. If there are limiting concentrations of these factors, only neurones that make appropriate cellular associations in both target fields are able to procure sufficient trophic support to survive. In view of these findings in sensory neurones, it will be of interest to ascertain whether the survival of other kinds of neurones that innervate more than one target field is dependent on different neurotrophic factors from each of their target fields.

References

Barbin, G., Manthorp, M. & Varon, S. (1984). Purification of the chick eye ciliary neuronotrophic factor. *J. Neurochem.* **43**, 1468–1478.

Barde, Y.-A., Edgar, D. & Thoenen, H. (1982). Purification of a new neurotrophic factor from mammalian brain. *EMBO J.* **1**, 549–553.

Davies, A. M. (1986). The survival and growth of embryonic proprioceptive neurones is promoted by a factor present in skeletal muscle. *Devl Biol.* **115**, 56–67.

Davies, A. M. (1987*a*). The growth rate of sensory nerve fibres in the mammalian embryo. *Devlopment* **100**, 307–311.

Davies, A. M. (1987*b*). Molecular and cellular aspects of patterning sensory neurone connections in the vertebrate nervous system. *Development* **100**, 185–208.

Davies, A. M., Bandtlow, C., Heumann, R., Korsching, S., Rohrer, H. & Thoenen, H. (1987*a*). Timing and site of nerve growth factor synthesis in developing skin in relation to innervation and expression of the receptor. *Nature, Lond.* **326**, 353–358.

Davies, A. M. & Lindsay, R. M. (1984). Neural crest derived spinal and cranial sensory neurones are equally sensitive to NGF but differ in their response to tissue extracts. *Devl Brain Res.* **14**, 121–127.

Davies, A. M. & Lindsay, R. M. (1985). The avian cranial sensory ganglia in culture: Differences in the response of placode-derived and neural crest-derived neurones to nerve growth factor. *Devl Biol.* **111**, 62–72.

Davies, A. M. & Lumsden, A. G. S. (1983). Influence of nerve growth factor on developing dorso-medial and ventolateral neurons of chick and mouse trigeminal ganglia. *Int. J. devl Neurosci.* **1**, 171–177.

Davies, A. M. & Lumsden, A. G. S. (1984). Relation of target encounter and neuronal death to nerve growth factor responsiveness in the developing mouse trigeminal ganglion. *J. comp. Neurol.* **223**, 124–137.

DAVIES, A. M. & LUMSDEN, A. G. S. (1986). Fasciculation in the early mouse trigeminal nerve is not ordered in relation to the emerging pattern of whisker follicles. *J. comp. Neurol.* **253**, 13–24.

DAVIES, A. M., LUMSDEN, A. G. S. & ROHRER, H. (1987*b*). Neural crest-derived proprioceptive neurons expressed NGF receptors but are not supported by NGF in culture. *Neuroscience* **20**, 37–46.

DAVIES, A. M., LUMSDEN, A. G. S., SLAVKIN, H. C. & BURNSTOCK. G. (1981). Influence of nerve growth factor on the embryonic mouse trigeminal ganglion in culture. *Devl Neurosci.* **4**, 150–156.

DAVIES, A. M., THOENEN, H. & BARDE, Y.-A. (1986*a*). The response of chick sensory neurons to brain-derived neurotrophic factor. *J. Neurosci.* **6**, 1897–1904.

DAVIES, A. M., THOENEN, H. & BARDE, Y.-A. (1986*b*). Different factors from the central nervous system and periphery regulate the survival of sensory neurones. *Nature, Lond.* **319**, 497–499.

DURHAM, D. & WOOLSEY, T. A. (1984). Effects of neonatal whisker lesions on mouse central trigeminal pathways. *J. comp. Neurol.* **223**, 424–427.

EBENDAL, T. & HEDLUND, K.-O. (1975). Effects of nerve growth factor on the chick embryo trigeminal ganglion in culture. *Zoon* **3**, 33–47.

EBENDAL, T. & JACOBSON, C.-O. (1977). Tissue explants affecting extension and orientation of axons in cultured chick embryo ganglia. *Expl Cell Res.* **105**, 379–387.

ERZURMLU, R. S. & KILLACKEY, H. P. (1982). Order in the developing rat trigeminal nerve. *Devl Brain Res.* **3**, 305–310.

ERZURMLU, R. S. & KILLACKEY, H. P. (1983). Development of order in the rat trigeminal system. *J. comp. Neurol.* **213**, 365–380.

GURNEY, M. E., HEINRICH, S. P., LEE, M. R. & YIN, H. (1986). Molecular cloning and expression of neuroleukin, a neurotrophic factor for spinal and sensory neurons. *Science* **234**, 566–574.

KORSCHING, S. & THOENSEN, H. (1985). Nerve growth factor supply for sensory neurons: site of origin and competition with the sympathetic nervous system. *Neurosci. Letts.* **54**, 201–205.

LEVI-MONTALCINI, R. (1982). Developmental neurobiology and the natural history of nerve growth factor. *A. Rev. Neurosci.* **5**, 341–362.

LEVI-MONTALCINI, R. & ANGELETTI, P. U. (1968). Nerve growth factor. *Physiol. Rev.* **48**, 534–569.

LINDSAY, R. M. & ROHRER, H. (1985). Placodal sensory neurons in culture: Nodose ganglion neurons are unresponsive to NGF, lack NGF receptors but are supported by a liver-derived neurotrophic factor. *Devl Biol.* **112**, 30–48.

LINDSAY, R. M., THOENEN, H. & BARDE, Y.-A. (1985). Placode and neural crest-derived sensory neurons are responsive at early developmental stages to brain-derived neurotrophic factor. *Devl Biol.* **112**, 319–328.

LUMSDEN, A. G. S. & DAVIES, A. M. (1983). Earliest sensory nerve fibres are guided to peripheral targets by attractants other than nerve growth factor. *Nature, Lond.* **306**, 786–788.

LUMSDEN, A. G. S. & DAVIES, A M. (1986). Chemotrophic effect of specific target epithelium in the developing mammalian nervous system. *Nature, Lond.* **323**, 538–539.

LUMSDEN, A. G. S. & DAVIES, A. M. (1986). Chemotropic effect of specific target epithelium in the developing mammalian nervous system. *Nature, Lond.* **323**, 538–539.

LUMSDEN, A. G. S. & DAVIES, A. M. (1987). Chemotropic influence of specific target epithelium on the growth of embryonic sensory neurites. In *Epithelial mesenchymal interactions in neural development* (ed. J. R. Wolfe & J. Sievers). NATO–ASI *Series H5*, pp. 323–340.

RIGGOTT, M. J. & MOODY, S. A. (1987). Distribution of laminin and fibronectin along peripheral trigeminal axon pathways in the developing chick. *J. comp. Neurol.* **258**, 580–596.

Rush, R. A. (1984). Immunohistochemical localization of endogenous nerve growth factor. *Nature, Lond.* **312**, 364–367.

THOENEN, H. & BARDE, Y.-A. (1980). Physiology of nerve growth factor. *A. Rev. Physiol.* **60**, 284–335.

VAN DER LOOS, H. & WELKER, E. (1985). Development and plasticity of somatosensory brain maps. In *Development, Organization and Processing in Somatosensory Pathways* (ed. M. Rowe & N. D. Wilis), pp. 53–67. New York: Alan R. Liss.

YIP, H. K. & JOHNSON, E. M. (1984). Developing dorsal root ganglion neurons require trophic support from their central processes: Evidence for a role of retrogradely transported nerve growth factor from the central nervous system to the periphery. *Proc. natn. Acad. Sci. U.S.A.* **81**, 6245–6249.