CMLS, Cell. Mol. Life Sci. 59 (2002) 1787–1802
1420-682X/02/111787-16

CMLS Cellular and Molecular Life Sciences

Review

Sensing life: regulation of sensory neuron survival by neurotrophins

M. Kirstein and I. Fariñas*

Departamento de Biología Celular, Universidad de Valencia, Doctor Moliner 50, 46100 Burjassot (Spain),
Fax +34 96 3864372, e-mail: isabel.farinas@uv.es

Received 10 December 2001; received after revision 11 May 2002; accepted 13 May 2002

Abstract. Neurotrophins are a family of structurally and functionally related neurotrophic factors which, in mammals, include: nerve growth factor, brain-derived neurotrophic factor, neurotrophin-3 (NT-3), and NT-4/5. In addition to their canonical role in promoting neuronal survival, these molecules appear to regulate multiple aspects of the development of the nervous system in vertebrates, including neuronal differentiation, axon elongation and target innervation, among others. Actions of neurotrophins and of their receptors in vivo are being analyzed by loss-of-function or gain-of-function experiments in mice. Here, we review the phenotypes of the primary sensory system in these mutant mouse strains and the different strategies specifically involved in the regulation of neuronal survival by neurotrophins in this portion of the nervous system.

Key words. Neuronal survival; dorsal root ganglia; cranial ganglia; knockout; transgenic mice.

Introduction

Primary sensory neurons comprise morphologically and functionally heterogeneous groups of neurons in the peripheral nervous system (PNS) specialized in the transfer of different modalities of sensory information such as nociception, mechanoception and proprioception. Sensory neurons differ, albeit with a considerable overlap, in certain morphological traits, electrophysiological properties, and expression of neurotransmitters and neuropeptides [1]. The cell bodies of these different classes of pseudounipolar neurons are grouped in distinct cranial and spinal ganglia, and their unique emerging neurite bifurcates to innervate distinct types of specialized receptors in peripheral tissues and discrete areas of the central nervous system (CNS). Cranial ganglia involved in sensory perception include: the trigeminal ganglion (TRG), mainly composed of cutaneous sensory neurons supplying the skin of the face and oral cavity; the geniculate and petrosal ganglia, involved in gustatory perception; the auditory (otic) ganglia, comprising the vestibular ganglion involved in balance perception and cochlear (spiral) ganglion implicated in hearing; and the nodose ganglion, which conveys sensory visceral innervation from different organs. As a remarkable exception, proprioception in the head is mediated by sensory neurons of the trigeminal mesencephalic nucleus (TMN) located in the mesencephalon. In addition, spinal sensory neurons of different classes are grouped in a collection of paired ganglia along the spinal cord, the so-called spinal or dorsal root ganglia (DRG). They contain sensory neurons of different modalities that supply different types of sensory receptors in multiple body locations. Because of the precise anatomical location and limits of developing ganglia, sensory neurons are among the most accessible neuronal populations for experimental studies during development, both in vitro and in vivo. The number of neurons in each sensory ganglion appears to be rather constant and can be easily determined during development as well as in different experimental and genetic conditions. Moreover, developing

* Corresponding author.

sensory ganglia are advantageous for the study of neuron number establishment because peripheral neurons and their precursors, unlike their CNS counterparts, coexist in the ganglia all throughout development. Thus, sensory ganglia represent good models in which to analyze how cell numbers are controlled by secreted factors as well as by cell-cell interactions during development.

A remarkable feature of the developing vertebrate nervous system is that, for most populations, an excess of neurons is initially produced that is subsequently pruned down by apoptosis, at times mostly coincident with target innervation. Several decades ago, classical experiments of target ablation and addition in developing vertebrate embryos provided many examples where the process of naturally occurring cell death undergone by many neurons at specific developmental times appeared to be regulated by the target territories (reviewed in [2, 3]). Target areas are thought to produce restricted amounts of survival, or neurotrophic, factors, thus engendering a competition among innervating neurons that lead to survival of only a fraction of the initially generated neurons [2, 3]. The best known neurotrophic factors are those of the neurotrophin family which in mammals comprises several highly related polypeptides, both functionally and structurally: nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and NT-4/5. These factors appear to function as noncovalently associated homodimers that activate protein tyrosine kinases of the trk family of tyrosine kinase receptors, which includes three members: trkA, trkB and trkC. TrkA is the receptor for NGF, both BDNF and NT-4/5 activate trkB and NT-3 is the ligand for trkC and is also able to activate trkA and trkB although less efficiently [4–7] (fig. 1). These interactions, and the signal transduction pathways activated by them, appear to mediate the major cellular actions of the neurotrophins. Ligand binding to these receptors has been shown to activate several intracellular signaling pathways, some of which promote cell survival and others of which promote differentiation [5, 8]. In addition, all neurotrophins bind with comparable affinity to $p75^{NTR}$, a transmembrane glycoprotein distantly related to the tumor necrosis factor receptor family, whose function is still controversial and that will not be considered in the present review [4, 7].

In the sensory portion of the PNS neurotrophins play an essential role in the maintenance of a normal complement of neurons because it appears that virtually all sensory neurons require the presence of at least one neurotrophin during development. Following purification and cloning of the different neurotrophins, studies in vitro using explants or dissociated neuron-enriched cultures of peripheral sensory ganglia had suggested that each neurotrophin supports the survival of different, although overlapping, neuronal populations [9]. Expression studies using in situ hibridization to determine patterns of trk expression (see, for example [10, 11]) had also indicated some of the types of sensory neurons that were likely to be responsive to a particular neurotrophin in vivo. More recently, the effects of neurotrophins on sensory neuron survival in vivo have been analyzed in mouse strains in which neurotrophin and trk genes have been selectively inactivated and in mouse strains engineered to overexpress a particular neurotrophin under the control of a tissue specific promoter [12, 13] (fig. 1, 2; tables 1, 2). Because some neuronal populations lack distinguishing features in vitro, the analysis of these mutants has allowed the identification of neurons that are dependent on a particular neurotrophin based on their attributes such as innervation patterns, ter-

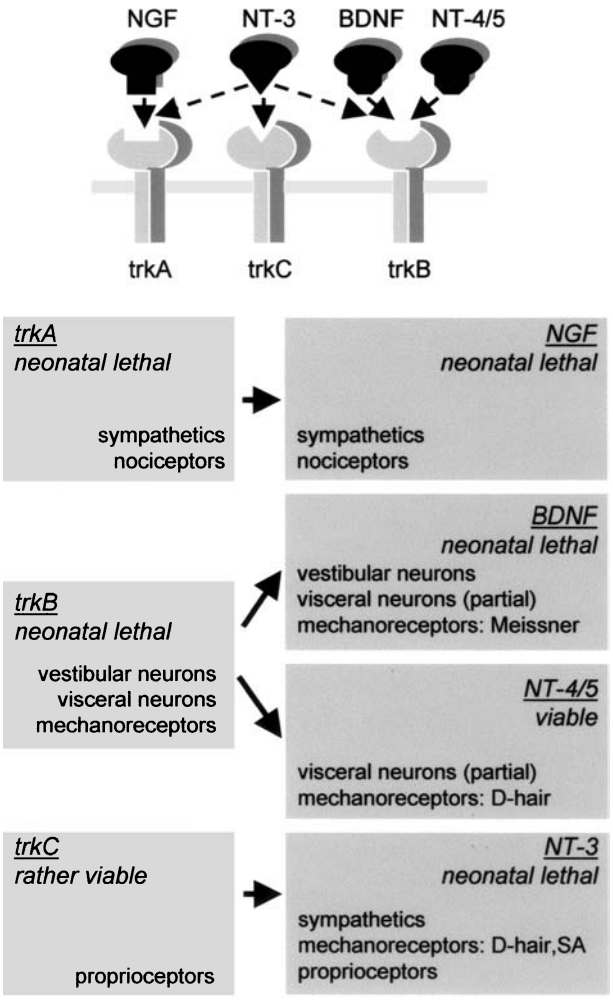

Figure 1. Ligand-receptor specificities and comparisons of phenotypes in mouse strains with targeted deletions in genes of the neurotrophin and trk families. (*Up*) Schematic drawing showing ligand-receptor specificities in the neurotrophin family. In mammals, NGF is the ligand for trkA, both BDNF and NT-4/5 can activate trkB, and NT-3 is the only ligand for trkC but it can also activate trkA and trkB in vitro and in vivo, at least in some cellular contexts. (*Bottom*) Phenotypes of mouse strains with genetic deficiencies in neurotrophin and trk receptor genes. Animal viability and relevant neuronal losses are indicated for comparisons between neurotrophin mutants and their corresponding trk mutants.

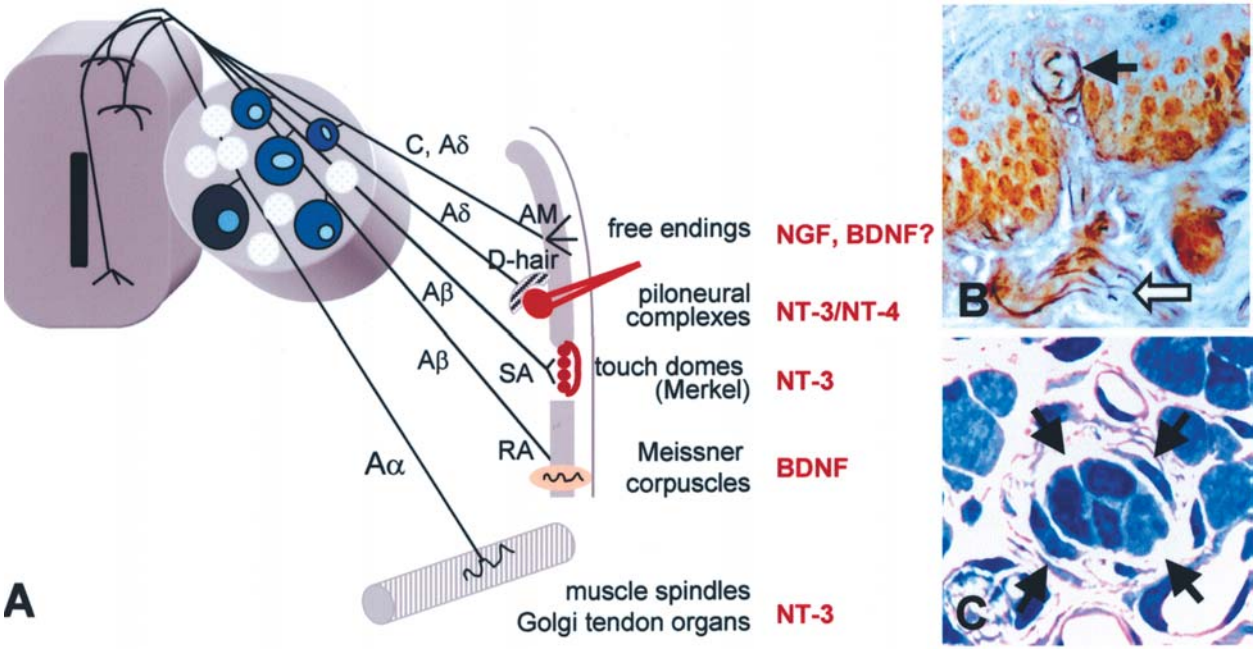

Figure 2. Neurotrophin dependencies of DRG neurons. (*A*) Schematic drawing that illustrates different DRG neuron subtypes, based on soma size, fiber diameter, electrophysiological properties and sensory receptors in skin and muscle. Trophic requirements, evidenced by loss of specific types of neurons in the different neurotrophin and neurotrophin receptor mutants, are indicated in red. (*B*) Example of silver-stained sensory axons in the dermis (open arrow) and of an innervated Meissner corpuscle (solid arrow) as seen in normal animals. BDNF and trkB mutants lack these structures in the digital non-hairy skin. (*C*) Example of a muscle spindle as seen in a cross section through the soleus muscle of a normal animal stained with toluidine blue. NT-3 and trkC mutants do not have muscle spindles at birth, an indication that proprioceptive neurons do not survive in the absence of trkC-mediated NT-3 signalling.

Table 1. Neuronal losses in the sensory system of neurotrophin- and neurotrophin receptor-deficient mice.

	trkA	NGF	trkB	BDNF	NT-4/5	BDNF/NT-4	trkC	NT-3	trkB/trkC	BDNF/NT3
Spinal										
Neuron loss	70–80%	70–80%	30%	35%	5%		20%	60%	40%	
Modality lost	nociceptive, thermoceptive, and AM mechanoreceptors		Meissner	Meissner	D-hair afferents		proprioceptive	proprioceptive, D-hair afferents and SA mechanoreceptors		
Timing										
E13	70–80%	70–80%	0%	0–30%	0%	0%	20%	60%	–	
P0	70–80%	70–80%	0%	0–30%	0%	0%	20%	60%	40%	
P15	nonviable	nonviable	30%	35%	–	–	20%	nonviable	nonviable	
adult	nonviable	nonviable	nonviable	nonviable	5%	–	20%	nonviable	nonviable	
Cranial										
TRG	70%	–	30%	30–45%	0%		20%	60%		
TMN	–	–	–	30–40%	10%		50%	60%		
Geniculate	–	–	95%	50%	50%		15–20%	30%	98%	65%
Vestibular	–	–	60–85%	85%	10%	100%	15–30%	20–30%	100%	100%
Cochlear	–	–	20%	10%	–	–	55–70%	85%	100%	100%
Nodose	–	–	95%	45–60%	55–60%	85–95%	15–20%	30–40%	95%	60–70%

Neuronal losses in the sensory system of mice carrying targeted mutations in genes coding for the different neurotrophins and their receptors. Available information on neuronal losses in double homozygous mutant mice is also included. Neuronal losses are expressed as the percentage of neurons lost in the mutant compared with wild-type controls. In the case of DRG, the modalities lost and the approximate timing for the deficit are indicated. TRG, trigeminal ganglion; TMN, trigeminal mesencephalic nucleus. See text for original references.

Table 2. Percentage of neurons in postnatal DRGs of neurotrophin transgenic mice relative to wild-type mice.

	K14-NGF	K14-BDNF	K14-NT-3	K14-NT-3×NT-3+/–	Myo-NT-3	Myo-NT-3×NT-3–/–
Neurons	200%	100%	140%	85%[a]	110%	45%[b]
trkA+ neurons	200%	n.s.	ND	ND	ND	ND
trkB+ neurons		160%	ND	ND	ND	ND
TrkC+ or Parv+ neurons		n.s.	increased	ND	150%	100%
Myelinated fibers	increased	100%	160%		increased	
Unmyelinated fibers	increased	75%	160%		ND	
Innervation enhanced		epidermal	dermal and epidermal	dermal and epidermal	muscle	muscle
Sensory receptors affected		Merkel cells, hair follicles Meissner corpuscles	Merkel cells hair follicles	Merkel cells hair follicles	muscle spindles	muscle spindles

Transgenes: K14, keratin 14 promoter; Myo, myogenin promoter.
[a] NT-3+/–: 70%.
[b] NT-3–/–: 40%. See text for original references.

minal endings, cytochemical markers and so on. Previous data on the role of neurotrophins in vivo had been obtained by injection of NGF-blocking antibodies (see [3] for a review). In this particular case, where the generation of specific antibodies had been possible, the deficits seen in the PNS of the immunologically deprived animals were coincident with the phenotypic consequences of the genetic deletion of NGF or trkA [14–17]. In the case of other neurotrophins, the study of null mice has rapidly provided a core of data comparable to that obtained previously for NGF. Analyses in the mutant strains have emphasized the apparent specificity of some neuronal types for a particular neurotrophin signaling pathway, although phenotypic comparisons among mice with different mutations and analysis of double mutations have revealed a considerable overlap in terms of requirements (table 1). Because most mutants die shortly after birth (fig. 1), the use of heterozygous animals, which show reduced levels of a particular neurotrophin or receptor, but normal development and viability, has proven extremelly useful in the analysis of some systems past birth [18, 19].

The neurotrophic hypothesis postulates that neurons become dependent on a particular neurotrophin when their axons come into contact with their final targets. Nevertheless, neurotrophins are expressed during development earlier than would be expected for factors implicated solely in the regulation of target-dependent neuronal survival [20, 22–24]. In the trigeminal system, BDNF and NT-3 are expressed in tissues through which trigeminal axons grow to the periphery [20, 25, 26], and analyses of the trophic requirements of cultured immature trigeminal neurons have indicated that these neurons may be dependent on these other neurotrophins before their axons have established contact with their final targets and become responsive to NGF [20, 21]. Accordingly, neuronal deficits are found in embryos lacking NT-3 or trkB at very early stages of development [25, 27, 28]. Moreover, deficits in DRG neurons seen in NT-3 and NGF mutants appear before neurons contact their final targets [23, 39]. In addition, some observations in vitro have suggested that neurotrophic factors can regulate the proliferation, survival and differentiation of certain neural and glial precursors, including sensory, sympathetic, motor, renal and enteric neural progenitor populations [29–38]. Therefore, neurotrophins could potentially influence the neuronal complement of a ganglion by regulating neuronal survival or by affecting precursor cell proliferation, differentiation and/or survival, or by multiple actions. In order to differentiate among these possibilities, it is necessary to obtain precise in vivo data on the timing of neurotrophin/trk signaling requirements and on the cell types affected by the neurotrophin deficiencies. However, some of the experiments addressed to answer this question have yielded controversial results [23, 25, 27, 28, 39–41]. During the development of the murine nervous system, many processes such as cell death, proliferation, neurogenesis and differentiation occur simultaneously, thereby complicating the interpretation of some of the experiments. Moreover, in the complex context of organismic development, the complete elimination of the product of one gene can result in indirect or compensatory changes that occur as a consequence of the primary effects of the mutation.

Next, we will review effects of neurotrophins on the regulation of neuron numbers in the sensory system. However, neurotrophins are known to regulate aspects of the development and maintenance of the nervous system

other than neuronal survival, including neuronal differentiation, axon elongation, synaptic function and plasticity among others [12]. Particularly, changes in the phenotypes of DRG sensory neurons are produced as a result of alterations in neurotrophin levels after the period of naturally occurring cell death as shown by studies using blocking antibodies or neurotrophin administration (see [41b] for a review). In the neurotrophin mutants, early death of some neurons precludes analyses of actions of these neurotrophins on subsequent aspects of their development. Some of these actions have been recently studied in the sensory system using an elegant strategy consistent in the analysis of neurotrophin actions on sensory neurons isolated from Bax mutants, in which cell death is prevented by the targeted inactivation of the proapoptotic gene [42], or in the analysis of sensory ganglia in Bax/NGF or Bax/trkA double homozygous mutants where normal numbers of neurons are present at birth but NGF-dependent neurons fail to differentiate properly [43]. This neurotrophin-regulated physiological plasticity can result in variations in the relative proportions of specific sensory neurons within a ganglion and may contribute to the final organization of a sensory ganglion.

Spinal sensory neurons: defining neurotrophin requirement specificity and timing at the cellular level

Spinal sensory neurons are highly heterogeneous. Each DRG innervates a full array of targets in the periphery, including skin, muscle and viscera. Individual DRG neurons supply specific types of sensory receptors in these tissues and organs, conveying information about position in space (proprioception), pain (nociception), distension or touch (mechanoception) and so on. DRG neurons vary widely in cell soma size and axon diameter, and some of these variations can be correlated with their specific functions, but nevertheless, there is considerable overlap [1]. In general, the largest neurons with thickly myelinated large-caliber axons and high-conduction velocities (A-α fibers) are proprioceptive and innervate sensory organs in muscle (muscle spindles) and joints (Golgi tendon organs, Pacinian corpuscles). On the other side of the spectrum, small DRG neurons have nonmyelinated, slowly-conducting, small-caliber axons (C-fibers) but can also have thinly myelinated axons (A-δ fibers) like some medium-sized neurons. Some of the neurons with C-fibers are nociceptors of different types (sensitive to noxious heat stimuli only, noxious mechanical stimuli only or polymodal) or can be activated by gentle mechanical stimuli. In the middle-range distribution, neurons with slowly conducting A-δ fibers generally innervate skin and can vary in physiological properties. Some medium-sized afferents have very low mechanical thresholds, that are activated by gentle stimuli, and adapt very rapidly to sustained stimuli (D-hair afferents). Others, however, innervate high-threshold mechanical receptors and adapt relatively slowly to maintained stimuli (HTMR afferents or A-mechanoreceptors, AMs). Also with medium-sized cell bodies, some DRG neurons have thickly-myelinated high-conducting fibers (A-β fibers) that innervate different skin receptors. Some of these afferents are slowly adapting (SA) and generally supply Merkel cells in touch domes. Some others are rapidly adapting (RA) afferents that innervate hair follicles and Meissner and Pacinian corpuscles (fig. 2).

DRG neurons express all trk receptors throughout development and during postnatal life [10, 24, 41]. In addition, cultures of embryonic DRG neurons show some survival in response to each of the four neurotrophins [9], and most mouse neurotrophin mutant strains show losses of DRG neurons to a variable extent (table 1). The different mutations result in relatively selective neuronal losses, as shown i) by a combination of morphological techniques, mostly the estimation of neuron numbers by cell counting and the analysis of peripheral and/or central projections as well as of specific cell markers, and ii) electrophysiologically, using in vitro skin-nerve preparations. The latter allow single-unit extracellular recordings from teased myelinated axons encoding different cutaneous modalities (for example touch, vibration), after mechanical stimulation of the hairy skin, and their classification into four separate categories: D-hair receptors, AMs, RA and SA mechanoreceptors [44]. This sophisticated analysis has been combined with the use of adult heterozygous animals, which do not show decreased viability but have reduced levels of a neurotrophin, and it has been especially useful in cases where neuronal populations could not be morphologically characterized because of the lack of adequate cytochemical markers [18, 19].

All DRG neurons derive from neural crest cells that delaminate from the neural tube and migrate away at embryonic day (E)9 and coalesce to form the first discernible ganglia at E10 in the mouse (fig. 3). After coalescence, neural crest-derived sensory-committed precursor cells have the potential to proliferate to increase their number and the potential to differentiate into neurons. Precursor proliferation and neurogenesis take place over the same developmental period, as cells that incorporate the thymidine analogue bromodeoxyuridine (BrdU) and cells that express markers of postmitotic neurons appear to coexist in the ganglia at stages immediately after ganglion coalescence. Birth-dating studies in the mouse have indicated that virtually all neurons are generated between E10 and E13 [45]. Accordingly, DRG neuron numbers increase progressively until E13, when the final number of neurons present at birth is reached and, therefore, developmental processes taking place prior to this stage are the primary determinants of the final size of the spinal ganglia [23]. DRG sensory neurons appear to initiate axon elongation

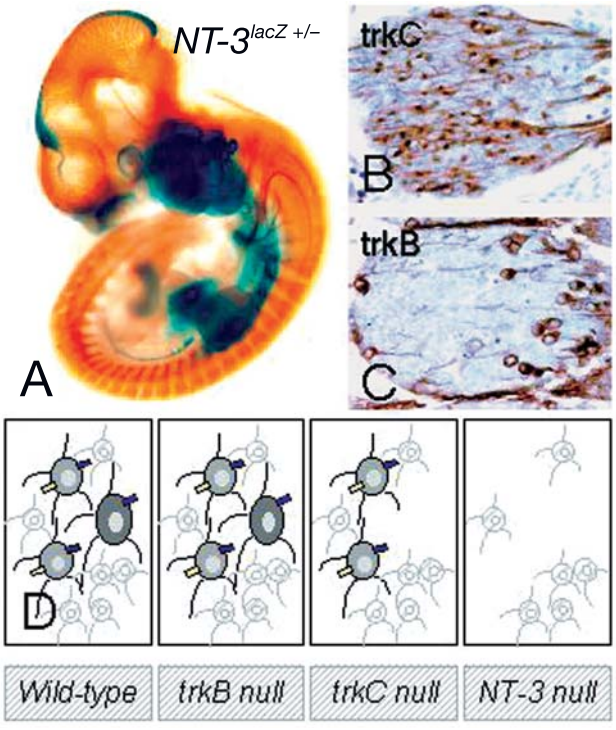

Figure 3. NT-3 signalling through trkB in DRG neurons. NT-3 is very highly expressed during early development, starting at E10, specially around the tips of the growing sensory axons as soon as these exit the DRGs, as can be seen in whole embryos carrying a copy of the *lacZ* gene into the NT-3 locus (*NT-3^{lacZ}*) doubly stained with X-gal (blue) and with antibodies to neurofilament (brown) (*A*). At E11, trkB and trkC proteins are expressed by DRG cells with clear neuronal morphology (*B* and *C*). (*D*) Schematic drawing that illustrates in a simplified manner the expression of trkB (yellow) and trkC (blue) receptors by DRG neurons and which neurons appear to be lost in different mutants (trkB, trkC and NT-3 knockouts) with respect to wild types.Most trkB-expressing neurons also express trkC. Both trkB and trkC appear to be functional, as neurons that express both receptors survive in mice in which either receptor is eliminated. However, trkC and trkC/trkB neurons are lost in the absence of NT-3, indicating that NT-3 signals through trkB and through trkC.

immediately after they are generated. In the mouse, sensory projections exit the ganglia at E10 (fig. 3), but many DRG neurons do not reach their final targets until late in embryogenesis. In the trunk, target tissues are innervated as early as E13, but target tissues in distal portions of the limbs are not innervated before E18 [23, 24]. Interestingly, cell death in DRGs of normal embryos peaks at E11 and E12 [23, 39] and, therefore, long before many sensory axons have reached their final destinations. Thus, primary modulation of the final number of neurons by cell loss appears to occur before neurons are actually exposed to target-derived neurotrophic factors. Patterns of neurotrophin expression during embryonic development and the deficits seen in certain mutants are in agreement with this early dependence from tissues other than final targets.

In early DRGs, cells immunolabeled with antibodies that specifically recognize trkA, trkB and trkC have a clear neuronal morphology, express neurofilament and do not incorporate the proliferation marker BrdU [41, 48]. Therefore, in spite of the high levels of messenger RNA (mRNA) for trkB and trkC previously observed in migrating neural crest cells, trk protein expression does not appear to precede neurogenesis. In chick embryos there is also strong evidence for the presence of trkC mRNA in migrating neural crest cells with neurogenic potential [35], but trkC protein has been observed only in few neural crest cells [46], suggesting that the same translational repression may be involved. Interestingly, in zebrafish trkC mRNA expression does not precede neurogenesis [47]. An interesting possibility is that there are high levels of trkB and trkC receptor mRNA expression (see, for instance [12]) whose translation might be repressed in precursor cells and initiated concomitantly with neuronal differentiation or under certain culturing conditions.

The expression patterns of the different trk proteins are highly dynamic at times of sensory neurogenesis. At E11, trkC appears to be expressed in a majority of neurons, but comparatively few neurons continue to express this receptor at E13. Conversely, while trkA-expressing cells are present in moderate numbers at E11, the vast majority of neurons at E13 express this receptor [41]. Classical birth-dating studies had shown that large neurons (presumably including the trkC-positive proprioceptive populations) are born earlier than small neurons (most of which express trkA) in the DRG, suggesting different waves of neurogenesis for DRG neurons [45]. The observation that the number of neurons expressing different trk receptors peak at different embryonic stages could be also interpreted as an indication that the generation of the trk-expressing populations follows an orderly neurogenic schedule as it has been recently shown for the TRG, using a combination of birth-dating BrdU labeling and neurofilament immunocytochemistry [48]. This type of study has indicated that while most trkB- and trkC-expressing neurons are generated between E10 and E11, the vast majority of trkA-expressing neurons are generated between E11 and E13. Similarly, analysis of trk expression in DRGs of mice carrying targeted mutations in genes coding for *Drosophila* atonal-related bHLH transcription factors *neurogenin-1* and *-2* has revealed that ngn2 is transiently required only for the differentiation of trkB- and trkC-positive neurons, whereas ngn1 is required later for most or all trkA-positive neurons and a fraction of the trkB- and trkC-positive ones [49]. All these data together suggest that changes in receptor expression and neurotrophin dependence could be explained by different waves of neurogenesis and that many neurons, at least in DRG and TRG, express only one receptor from the time they become postmitotic [48]. However, a certain fraction of neurons appear to switch neurotrophin dependence over time. Early-born trigeminal neurons, identified by BrdU incorporation at the earliest times of ganglion formation,

appear to go through a transient period of responsiveness to BDNF [21].

The different neurotrophins are also expressed in embryonic tissues in a dynamic fashion. NT-3 is the most abundantly expressed neurotrophin during embryogenesis. Analysis of mice carrying an insertion of a lacZ gene into the NT-3 locus has indicated that endogenous NT-3 expression is very high in the mesenchyme around the developing ganglia at E11 and surrounding the tips of the growing sensory axons all throughout development [23] (fig. 3). Thus, NT-3 expression domains change rapidly as axons grow distally. In the developing skin, for instance, NT-3 is high in the mesenchyme underlying the ectoderm at E11, but the expression switches at E13, concomitant with innervation of the presumptive epidermis, and NT-3 is found at high levels in the ectoderm, but not in the mesenchyme. Finally, NT-3 is found in the final target areas at the time of innervation [23] (fig. 4). In the skin, NT-3 is found in the basal layer of the developing epidermis and in hair follicles starting at E15, when these structures become innervated. In the muscle, NT-3 expression becomes restricted to myoblasts and developing muscle spindles [23, 76]. NGF is also present as early as E11, but its expression is restricted to the limb epidermis. It is, therefore, likely to be accessible to neurons that have already projected into the limb but not to other cells in the ganglion [24]. Less information is available in the case of BDNF and NT-4/5 although it appears that it is not expressed at detectable levels at times of gangliogenesis (see [70]).

Analyses of embryos carrying mutations in different neurotrophin genes have indicated that NGF and NT-3 play an early role in determining the final neuronal complement in DRGs, whereas BDNF does not appear to regulate neuronal survival until later (but see [50]). Numbers of DRG cells and neurons in all three mutants are indistinguishable from wild-type values at time of ganglion coalescence [23, 24, 39, 50; unpublished results], consistent with an apparent normal migration of trunk neural crest cells [23; unpublished observations]. At E13, when the full complement of neurons is basically produced in normal animals, mutants for NGF and NT-3, but not BDNF, show losses in the number of neurons that are basically those found in the corresponding mutants at birth, that is 70–80% reduction in the number of neurons in the absence of NGF and 60–70% loss in the absence of NT-3. This indicates that these neurotrophins play a crucial role in determining the final number of neurons during the most active period of neurogenesis, before final target encounter, when the full complement of DRG neurons is being generated in normal animals. In the next sections we will discuss the types of neurons that are lost in each mutant and how and when the deficits are being produced.

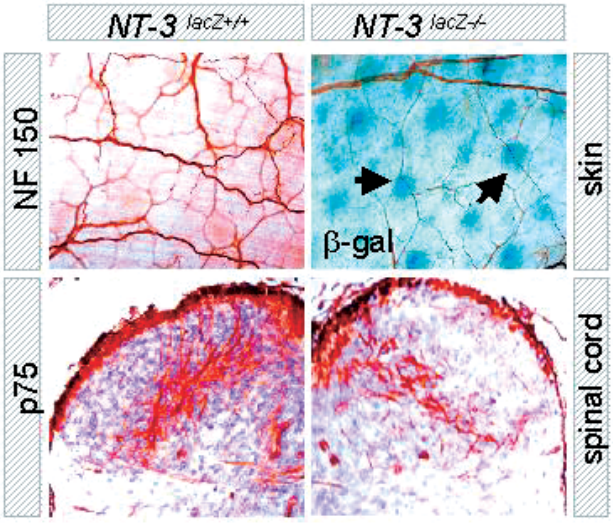

Figure 4. Prenatal cutaneous phenotype of NT-3 mutant mice. (*Upper panels*) Whole-mounted skin of E15 wild-type and *NT-3^{lacZ}* homozygous mutant mice reacted for beta-galactosidase and neurofilament (NF 150) immunostaining. Hair follicles express NT-3 at this developmental stage, coincident with their innervation by cutaneous sensory fibers. Innervation appears to be reduced in the mutants at this early time point. (*Lower panels*) Staining of sensory afferents to the dorsal horn of the spinal cord with antibodies to p75 in E15 wild-type and *NT-3^{lacZ}* homozygous mutant mice, as seen in cross-sections through the spinal cord counterstained with cresyl violet. Afferences to layer I and to layers III–IV are reduced in NT-3 mutants.

Different populations of small-diameter neurons are NGF sensitive during development

Both, in vivo injection of NGF-blocking antibodies and targeted deletion of the NGF or trkA genes, have helped to determine which types of sensory neurons are dependent on NGF in vivo (fig. 1, 2; table 1, 2). Although approximately 50% of DRG neurons express trkA in normal mice postnatally [51], more than 80% of all DRG neurons express this receptor during embryonic development [10, 24, 41]. Accordingly, around 70–80% of the DRG neurons are missing at birth in animals lacking this receptor or its ligand [14–17]. The neurons lost include all nociceptive neurons that mediate pain perception and neurons implicated in innocuous thermal and low-threshold mechanoreceptive stimuli perception [17]. In agreement with the loss of all nociceptors, DRGs are depleted of small-diameter neurons that express calcitonin gene-related peptide (CGRP) and substance P (SP), and projections to the most superficial layers of the spinal cord are lost [14–17]. Physiologically, animals deprived of NGF/trkA signaling during embryonic life have reduced sensitivity to painful stimuli at birth [14, 15, 17]. Nociceptive neurons have been shown to continually express trkA throughout embryonic development and during postnatal life [51]. The loss of the non-nociceptive population of trkA-dependent neurons is reflected in the elimination of the population of small neurons that specifically

bind the IB4 lectin in postnatal DRGs and of axonal projections to the inner portion of layer II in the spinal cord [17]. These neurons express trkA during prenatal development and are, therefore, lost following trkA signaling elimination, but stop expressing the receptor after birth. Postnatal neurotrophic requirements of these neurons are unknown, but IB4-positive/trkA-negative neurons in postnatal DRGs express c-ret, and some neonatal DRG neurons respond to GDNF in culture [52]. It is yet unknown whether these neurons depend exclusively on this neurotrophic factor in vivo or whether they depend on other members of the neurotrophin family as well.

Virtually, all DRG neurons lost in the NGF and trkA null mice appear to die before E14 [24; unpublished results]. Expression of NGF is seen in the skin of developing limbs, at a distance from the developing DRG, as early as E11, and therefore it is likely to be accessible to neurons that have already projected into the limb bud epidermis. But many NGF-dependent neurons are born before E14 and do not reach their final target territories until 5 days later [24]. In transgenic mice that selectively express NGF under the control of the keratinocyte-specific keratin 14 promoter (K-14-NGF), a construct that drives overexpression of NGF in skin starting at E11, a doubling in the total number of sensory neurons and in the number of trkA-positive neurons, along with significant increases in myelinated and nonmyelinated fibers in the skin, are found [53–55] (table 2). These results suggest that most NGF-dependent neurons require neurotrophic support before target encounter and that NGF availability is limited.

TrkB signaling by BDNF- and NT-4/5 regulate postnatal maintenance of distinct cutaneous sensory neurons

Initial reports on the characterization of mice deficient in trkB or BDNF indicated that approximately 30–35% of the DRG neurons were lost in these animals 2 weeks after birth [56–58] and that NT-4/5 deficient mice did not show any neuron deficits in the DRGs at birth [59, 60]. More recent work in the same strains indicates that neuronal deficits in trkB- and BDNF-deficient animals occur postnatally because there appears to be no deficit at birth [16, 61; unpublished observations]. Nevertheless, prenatal deficits in a different BDNF mutant strain of mice have been reported [50]. Yet normal numbers of DRG neurons have been reported in another strain, in which electrophysiological analysis of myelinated axons in nerve-skin preparations has indicated that BDNF specifically regulates the sensitivity, but not the number, of SA mechanoreceptors in hairy skin [19]. The differences may reflect variations in material preparation, in techniques employed for the analysis and/or in genetic background, but nonetheless the apparent discrepancies are, at the very least, puzzling. It is important to note that a phenotype characterized by a significant loss of neurons during the first 2 postnatal weeks has been found coincidentally in mice deficient in BDNF and in its receptor trkB in different laboratories. Comparative studies using different mutant strains will be necessary to rule out the possibility of genetic modifiers.

In our hands, 2-week-old mice with targeted deletions of either trkB or BDNF genes appear to lack Meissner corpuscles in the digital glabrous skin (fig. 2; table 1) along with a small fraction of myelinated axons in the digital nerves supplying the footpads [unpublished results]. The development of these sensory corpuscles requires sensory afferent innervation, and therefore their absence is a clear indication of a loss of a type of RA cutaneous afferents in the DRG of the mutant animals. A similar result has been reported for the palate of the same strain of trkB null mice [62]. As the small number of Meissner-innervating neurons cannot account for the entire neuronal deficit seen in the deficient DRG, other sensory modalities are likely to disappear in the absence of BDNF-dependent trkB signaling. Furthermore, BDNF-deficient animals have a significantly reduced number of myelinated axons in dorsal roots (ca. 35%) when compared with wild types [57]. Because approximately half of the sensory afferents are nonmyelinated and, therefore, not considered in dorsal root counts at the light microscope level, the fact that equal percentages of myelinated axons and of cell bodies are lost in BDNF null mice strongly suggests that nonmyelinated C-fibers are also lost. Accordingly, we found a decrease in the number of unmyelinated axons in dorsal roots of BDNF-deficient animals at 2 weeks after birth [unpublished results]. Transgenic lines that overexpress BDNF in the epidermis during embryogenesis show increased innervation to the skin, specifically to hair follicles, Meissner corpuscles and Merkel cells. Interestingly, however, this cutaneous overexpression of BDNF, unlike that of NGF or NT-3, does not increase the number of sensory neurons in the DRG [63] (table 2). This is likely to suggest that BDNF is acting after the period of naturally occurring cell death to maintain a normal complement of DRG neurons and that whereas a deficiency in BDNF production causes elimination of certain types of neurons, an excess of this neurotrophin in target areas, such as the skin, results in changes in innervation patterns but not in neuron numbers.

The lack of NT-4/5 also results in postnatal deficits in the number of DRG neurons. Small-diameter myelinated ($A\delta$) D-hair afferents are reduced in number in the absence of NT-4, starting 3 weeks after birth [64]. This is a very small number of neurons because D-hair receptors account for less than 5% of the total number of axons in nerves innervating the glabrous skin. Nevertheless, the results are in accordance with the idea that neurotrophins act on sub-

classes of neurons in a modality-specific way and that trkB signaling appears necessary for postnatal maintenance.

Proprioceptive neurons are dependent on NT-3-mediated trkC signaling before target encounter, and other neuronal types depend on trkC-independent NT-3 signalling

NT-3 activation of trkC has been shown to be essential for the survival of the relatively small (< 20%) population of large-diameter parvalbumin-positive DRG proprioceptive neurons, which supply stretch and tension receptors in muscle and joints and convey information about the position of limbs in space. Therefore, NT-3 and trkC-deficient mice show abnormal postures and movements, an absence of the Ia afferent projection of spinal proprioceptive neurons to motor neurons, and a failure of muscle spindles (fig. 2) and Golgi tendon organs to form [65–72]. Large-diameter DRG neurons, a neuronal population likely to include the proprioceptive neurons, are the first ones to be generated during development [45], and DRG neurons expressing trkC are depleted in NT-3-deficient embryos by E12 [66, 68, 70]. Because afferent innervation of the muscle is required for the induction of muscle spindles, the fact that the muscle is never innervated by presumptive proprioceptive afferents accounts for their absence in NT-3-deficient animals [73]. High-level expression of NT-3 in the mesenchyme surrounding the ganglion and around the tips of the early projecting sensory axons at these stages is seemingly responsible for maintaining proprioceptive neurons alive as soon as they are generated [23, 24]. The early neuronal deficiencies in proprioceptive neurons in NT-3 and trkC null mice and the availability of NT-3 in areas crossed by growing sensory axons indicate that NT-3 is needed before target innervation. NT-3 seems to be the only factor needed by spinal proprioceptive neurons, as large proprioceptive trkC-positive neurons can be selectively rescued in mice deficient in NT-3 by transgenic expression of NT-3 under a myogenin promoter, resulting in muscle-specific expression as early as E11 [74], but not by BDNF insertion into the NT-3 locus [70]. Studies using chick embryos injected with blocking antibodies to trkC suggest that spinal proprioceptive neurons require NT-3 after target innervation as well [75]. Consistent with the possibility of a dual NT-3 dependence period for proprioceptive neurons also in mice is the fact that NT-3 expression around growing axons at early developmental times is followed by NT-3 expression restricted to muscle spindles [23, 24, 76]. Not all proprioceptive neurons in the mouse, however, appear to depend on NT-3 for their survival, because some trigeminal mesencephalic proprioceptive neurons and some muscle spindles in jaw muscles are still present in NT-3 and trkC, but absent in BDNF or NT-4/5 null mice [77–79] (table 1).

Comparison between the phenotypes of the NT-3- and trkC-deficient animals (table 1; fig. 1) has revealed that many more DRG sensory neurons are lost in NT-3-deficient (~60–70%) [66–72] than in trkC-deficient mice (~20–30%) [65, 69, 70]. NT-3 appears to play an additional role in the survival of DRG neurons that do not project to muscle and the NT-3 deficiency results in deficits in nearly all DRG neuronal classes [72]. For instance, in addition to the lack of all trkC neurons, deletion of NT-3, but not trkC, results in deficits in specific cutaneous afferents, as seen in skin-nerve preparations and morphological analyses in the whisker pad: D-hair afferents and SA mechanoreceptors that innervate specialized Merkel cells in the touch domes of the hairy skin [18, 80]. Transgenic mice overexpressing NT-3 under the control of a keratin promoter show increased numbers of DRG neurons, enlargement of skin touch domes and increased numbers of associated Merkel cells [54]. Moreover, D-hair afferents are thought to terminate on hair follicles, and in keratin-14-driven NT-3 overexpressors the number of circular endings associated with piloneural complexes is enhaced [54]. It has been suggested that the NT-3 deficiency results in the loss of SA afferents to Merkel cells postnatally, as the number of Merkel cells per touch dome increases in wild types, but not in heterozygous and homozygous mutant mice, after birth [20]. However, it needs to be shown that the numbers of DRG neurons are reduced further than 60% after birth and, more importantly, that the number of touch dome units is normal in NT-3-deficient animals at the beginning of the postnatal period. Innervation of Merkel cells in hairy skin begins at E15, a time when NT-3 expression in the skin is restricted to hair follicles and basal keratinocytes [23] (fig. 4) and when some neurons in the DRG of NT-3 null mice begin to re-express trkC [50, 68; unpublished results]. In NT-3 mutant mice, skin innervation and projections to layers III and IV of the dorsal horn of the spinal cord are already reduced at E15 (fig. 4), suggesting that at least a fraction of the cutaneous afferents lost in the absence of NT-3 are lost before birth.

The striking discrepancy between the phenotypes of trkC- and NT-3-deficient mice strongly suggests that NT-3 acts on neurons through receptors other than trkC. Experiments have shown that neurons isolated from cranial ganglia of trkC-deficient embryos indeed respond to high doses of NT-3 in vitro [81]. However, it has been difficult to know whether such concentrations of neurotrophins are attained in vivo. We have shown that in addition to trkC-expressing neurons, trkB-expressing neurons are also lost in the DRGs of NT-3 mutants [41]. In early DRGs, neurons likely to be the proprioceptive ones only express trkC and account for those neurons lost in the absence of either NT-3 or trkC. Other early neurons express both trkC and trkB and are not lost in either trkC or trkB null mice but do not survive in the absence of NT-3, suggesting that NT-

3 is the endogenous survival factor for these neurons and that it can activate either receptor effectively [41]. Therefore, these trkB/trkC neurons are not lost in trkC mutants, providing an explanation for the discrepancy between the phenotype of the trkC and the NT-3 mutant mice and suggesting that NT-3-mediated activation of trkB is essential for the survival of these cells during normal development (see fig. 3). Accordingly, the loss of neurons in DRGs of double trkB/trkC mutant homozygotes appears to be larger than the sum of the deficits in the two single trk mutants [61]. In this context, a very elegant approach, consistent in the generation of mice in which BDNF replaces NT-3 (B/N mice) and, therefore, only trkB signaling is preserved, has given further support to the relevance of NT-3 signaling through the trkB receptor in the control of DRG neuron survival. In the absence of NT-3, BDNF can rescue a substantial number of DRG neurons during gangliogenesis [70]. Interestingly, the proprioceptive deficit caused by the NT-3 deletion is not rescued by BDNF in these mice [70]. Moreover, starting at E13, when most neurons express trkA and in spite of the presence of normal levels of NGF, a significant decline in the number of neurons is observed in the B/N mice, suggesting that NT-3 supports neuronal survival through trkA [70]. This is in agreement with the observation of apoptotic trkA-positive cells in the TRG of NT-3-deficient mice [48]. These results indicate that interactions of NT-3 with receptors other than trkC occur in vivo and that this may reflect production of relatively high levels of NT-3 during development.

Analyses of the dorsal root ganglia of NT-3-deficient mouse embryos [23, 24, 39, 50] have indicated that the deficits initially described in neonate animals develop very early during embryogenesis. A loss of DRG neurons is observed soon after ganglion coalescence (at around E11), at times when many sensory precursor cells are proliferating and neurons are being generated, and the deficit is complete by E13, before any of the sensory neurons have reached their final peripheral targets. Neuronal death is significantly elevated in the ganglia of NT-3 null embryos when compared with wild-type littermates [23, 39]. Neuronal death is also significantly enhanced in the DRGs of embryos that lack a functional trkC receptor at E11 [24]. This suggests that the absence of NT-3-mediated trkC signaling results in the loss of proprioceptive neurons, which are missing in both the trkC and NT-3 mutant mice at birth. As mentioned above, the loss of trkB/trkC-positive neurons [23], and possibly trkA [70], may account for the rest of the deficit seen in the NT-3, but not trkC, mutant. Effects of the NT-3 deficiency on either the survival or proliferation of precursor populations in vivo have been proposed. In embryos lacking NT-3, precursors appear to abandon the cell cycle and differentiate into neurons prematurely between E11 and E12, as suggested by a transiently enhanced neurogenesis along with a reduction in the numbers of proliferating cells without changes in their death or proliferation rates [23]. Other reports have suggested that NT-3 deficiency results in increased death of precursor cells that fail to differentiate and reenter the cell cycle [39, 40]. More work will be necessary to clarify these apparent discrepancies, but nonetheless, the data on trk receptor expression argue that the in vivo reported effects of the lack of NT-3 on sensory neuron precursors might be indirect, as these cells do not appear to express detectable levels of trk receptors. Moreover, putative NT-3 effects on precursor cells would need to be mediated by receptors other than trkC, as mice deficient in trkC signaling only lack proprioceptive neurons. Nonetheless, trkC is the neurotrophin receptor expressed by neural crest cells at the mRNA level [11].

NT-3 expression is notably high along the projection pathway of sensory axons in the way to their final targets (fig. 3) [23]. Later, the expression becomes restricted to areas known to be innervated by neurons that are dependent on NT-3 (fig. 4). Overexpression of NT-3 in the epidermis or in the muscle beginning at E11 produces significant enhancements of the sensory system with increases in the number of DRG neurons and in the number of trkC (or parvalbumin)-positive neurons along with specific increases in cutaneous or muscle innervation, respectively. The effect on the number of neurons is a likely result of a net increase in the amount of NT-3 in target areas. These results indicate that DRG neurons are dependent on their final targets for maintenance and that NT-3 levels in vivo are limiting. Overexpression experiments in mice have indicated that tissue-specific expression of NT-3 in NT-3 homozygous or heterozygous mutant mice rescues NT-3-dependent neurons that innervate areas of transgene expression but cannot rescue all neurons lost (table 2). The effect of the transgenic expression on the specific innervation is quite dramatic, but the number of DRG neurons is reduced with respect to NT-3 wild-type animals that express either transgene.

Gustatory neurons: same modality in different ganglia share a taste

Taste is mediated by sensory neurons located in the geniculate and petrosal ganglia. They innervate gustatory papilla in the dorsal surface of the tongue, complex structures composed of a core of connective tissue covered by an epithelial sheet in which discrete groups of around 50 spindle-shaped modified epithelial cells constitute the taste buds, chemosensory organs involved in perceiving chemical stimuli and in taste transduction. Three types of gustatory papilla (fungiform, circumvallate and foliate) are distinguished based on their form, number and spatial distribution and on their taste buds [82]. Fungiform papillae are located in the anterior tongue, while the circum-

vallate and follate papillae are located in the posterior tongue. Gustatory neurons in the geniculate ganglion innervate taste buds in fungiform papillae present in the anterior tongue, whereas neurons in the petrosal ganglion innervate taste buds in circumvallate and folliate papillae located in the posterior tongue [82]. In addition, the remaining lingual epithelium is innervated by somatosensory fibers originating in the trigeminal and petrosal ganglia.

Adult taste buds as well as developing gustatory epithelium express BDNF mRNA, whereas the nongustatory surrounding epithelium expresses NT-3 mRNA [83–85]. In agreement with their expression patterns, these neurotrophins appear to have distinct but complementary roles in supporting development of gustatory and somatosensory innervation of the tongue [84], and cranial ganglia supplying innervation to the tongue show neuronal losses in BDNF- and NT-3-deficient mice [57–59,66, 67]. Double BDNF/NT-4/5 homozygous mutants or trkB null mice lose ~90–95% of all geniculate ganglion neurons found in a wild-type animal [59–61, 86], indicating that virtually all geniculate neurons depend on trkB signaling for their survival during development. In BDNF- and in NT-4/5-deficient mice only ~50% of geniculate ganglion neurons are lost during development, suggesting that each neurotrophin acts on a separate population of gustatory neurons [57–60, 87]. The opposite effect is found in mice that overexpress BDNF or NT-4/5 under the control of the K14 promoter, in which the geniculate ganglion appears substantially larger than in control mice [88]. There is no clear available quantitative data on neuronal losses in the petrosal ganglion in neurotrophin mutants, because the petrosal and adjacent nodose ganglia are not well segregated in mice. Nevertheless, the nodose-petrosal complex is reduced in BDNF and NT-4/5 mutant mice by ~50% and virtually disappears in trkB signaling-deficient mice [57–61]. Small, but significant, neuronal losses are also found in the geniculate ganglion of mice lacking NT-3 or its receptor trkC [66, 67]. In NT-3 deficient mice gustatory papillae appear to be present in normal numbers, and their appearance is relatively normal [84]. Interestingly, some geniculate neurons are known to innervate taste buds in the soft palate, and although their neurotrophin requirement has not been thoroughly analyzed in the mutant strains, some evidence indicates that they are not innervated by BDNF-dependent neurons [88].

We have observed trkB immunoreactive neurons in the mouse geniculate ganglion as early as E11 [unpublished observations], and degeneration of ganglion cells is almost complete in trkB mutant mice before taste afferents reach the developing fungiform papillae in control animals [86]. In spite of the lack of innervation, taste buds in the mutants form initially. Eventually, however, the size of the fungiform papillae and their taste bud organization are altered in the mutant animals [86]. Accordingly, in postnatal mice lacking BDNF or NT-4/5, numbers of fungiform and circumvallate papillae, as well as of taste buds, are reduced, and the remaining ones have an altered morphology, reduced size and diminished numbers of taste buds [84,87, 89–91], presumably from prolonged denervation during morphogenesis in the absence of a normal complement of gustatory neurons. These results suggest that induction of gustatory papillae and taste bud formation during normal development in vivo occurs autonomously, but that sensory innervation is required for sustained growth and full differentiation of gustatory papillae and acquisition of a complete numerical complement of taste buds [82, 86]. Interestingly, the numbers of fungiform papillae and taste buds are also reduced in K14-BDNF and K-14-NT-4/5 overexpressing mice, because despite an increased number of sensory axons, these fail to innervate gustatory papillae [88].

Visceral sensory neurons: response diversity through one receptor

The nodose-petrosal sensory ganglion complex (NPG) is the major source of visceral afferent innervation to thoracic and abdominal viscera, and multiple neurotrophins are required to support a full complement of NPG visceral neurons (table 1). Null mutations in genes encoding BDNF, NT-3 or NT-4/5 result in a loss of 40–60% of neurons in the NPG [58–61, 66, 67, 92, 93]. In addition, virtually all visceral neurons in this sensory complex disappear in the absence of a catalytic trkB receptor [61] or in double BDNF/NT-4/5 homozygous mutant mice [60, 61, 92], indicating that nodose neurons are heterogeneous with respect to their response to trkB ligands and that BDNF and NT-4/5 act in a complementary nonredundant fashion on discrete populations of NPG neurons.

Many NPG neurons supply arterial vasculature and are implicated in the control of respiration but convey different modalities of sensory information. Dopaminergic chemoafferent neurons innervate glomus cells in the carotid body and respond to changes in arterial oxygen tension, whereas baroreceptor afferents innervate specialized regions of the cardiac outflow tract as free endings and respond to mechanical deformations caused by rapid changes in arterial blood pressure. In spite of subserving different sensory modalities, arterial chemoafferent and baroreceptor neurons share a common requirement for BDNF, but not NT-4/5 [92, 94]. BDNF is transiently expressed in baroreceptor targets and in the carotid body at the onset of sensory innervation [94]. At this time, most NPG neurons express high levels of trkB protein (see [48]) and are dependent on BDNF when cultured in vitro [94]. NT-4/5-sensitive nodose neurons belong to a different, tyrosine hydroxylase-negative, population of NPG

neurons. The selective deficits in the respiratory afferent system can explain the lethality of the BDNF and trkB mutations, and conversely, the lack of effect of the NT-4/5 deficiency on respiratory afferents can explain the viability of these mutation [92]. Several possibilities exist that can explain these differential responses to ligands that act on the same receptor. These neurotrophins could be expressed in different targets to which distinct populations of nodose neurons project. They could also be expressed in the same targets but at different times during development in coincidence with the expression of trkB selectively in certain neurons. Alternatively, the distinctive responses could be the result of a differential activation of trkB by the two ligands. In $trkB^{Shc/Shc}$ mice, which carry a point mutation into the juxtamembrane exon of the mouse trkB that disrupts binding of adaptor proteins to the Shc site, BDNF-, but not NT-4/5-, dependent neurons are preserved [95]. Activation studies in neurons isolated from these knockin mice indicate that BDNF can activate downstream survival signals more efficiently than NT-4/5 via a Shc site-independent pathway and suggests important differences in the way that these two ligands activate the same receptor [95].

Inner ear vestibular neurons: one neurotrophin balances the system

The inner ear develops from the otic placode that invaginates to form the otocyst by E9.5 [96]. Between E9.5 and E15.5, cochlear (spiral) and vestibular sensory neurons of the statoacustic ganglion originate from the antero-ventral aspect of the developing ear, migrate rapidly away and subsequently extend processes that will innervate the hearing and the balance regions of the inner ear as well as certain nuclei in the brainstem [96]. These otic (cochleovestibular) sensory neurons require BDNF and NT-3 for their survival [97]. Progressive differentiation of the inner ear neurons and innervated epithelia results in two segregated and specialized ganglia, the spiral or cochlear ganglion innervating the auditory epithelium of the cochlea and the vestibular ganglion, which supplies balance structures such as the saccule, utricle and semicircular canals. Expression studies have shown that all inner ear sensory epithelia express both neurotrophins, while otic sensory neurons express their receptors, trkB and trkC (see [98]). *BDNF* and *trkB,* but not *NT-4/5*, mutant mice have a deficit in balance due to a dramatic reduction in the number of vestibular neurons and the loss of all innervation to the semicircular canals [16, 57, 58, 61, 99–102]. This is probably the most remarkable case in the sensory system of a one-to-one relationship between a neurotrophin receptor signaling pathway and a particular ganglion. The complete loss of all sensory neurons innervating the semicircular canals in BDNF and trkB null mutants is consistent with the exclusive expression of BDNF in the sensory epithelia of these structures [98] and the expression of trkB by vestibular neurons [E. Huang, personal communication] during embryogenesis. The loss of balance afferents to the canals is very rapid, and within 2 days of the onset of fiber outgrowth (E12), projections and neurons are completely eliminated in the mutants. In other structures of the vestibular system, such as the saccule and the utricle, there is prominent NT-3 expression along with rather weak BDNF expression, as seen in mice with lacZ genes within the corresponding loci [98]. However, a prominent loss of utricular and saccular sensory afferents only results from the lack of BDNF. Even a single functional trkB allele in a trkC null background can rescue most innervation to these sensory epithelia, whereas loss of a functional trkC allele on a trkB null background only marginally adds to the already severe loss of vestibular sensory neurons (table 1) [103]. These results strongly emphasize the need for at least minimal levels of BDNF to support vestibular afferent neurons.

Inner ear auditory neurons: fine tuning of neurotrophin expression for neurons that hear all

Cochlear neurons represent the most remarkable example of how temporal and spatial patterns of neurotrophin expression finely regulate neuronal survival in vivo. The cochlea is a convoluted duct lined by an auditory epithelium which is innervated by neurons of the cochlear or spiral ganglion sitting in the center of the turning structure. The ganglion itself adopts a spiraled shape, and neurons in each turn project radially towards the epithelium in a highly organized and topographic fashion. Interestingly, despite the fact that neurons and hair cells originate from the initial placode, they show opposed temporal gradients of differentiation along the length of the cochlea during embryogenesis, and these countercurrents of maturation appear to have an important impact on how neurotrophins regulate the survival of neurons in a spatially organized structure.

Cochlear neurons appear to depend exclusively on two neurotrophins, BDNF and NT-3, for their survival during development. NT-3-deficient mice lose as much as 85% of the cochlear neurons, whereas absence of BDNF results in a very modest reduction of cochlear neurons [66, 67, 97–100]. Moreover, BDNF/NT-3 or trkB/trkC double homozygous mutants lose all cochlear neurons around birth [100, 101, 103]. It had been suggested that these deficits could be reconciled with a differential effect of BDNF and NT-3 on the two existing populations of cochlear neurons: the most abundant spiral type I, which innervate inner hair cells of the auditory epithelium, and the rare spiral type II, which innervate outer hair cells [100]. However, examination of whole projection patterns in BDNF and trkB ho-

mozygous mutants revealed the presence of innervated outer hair cells in the basal turn of the cochlea and a virtually complete loss of afferents to the apical turn both to outer and inner hair cells, more suggestive of a longitudinal, not a radial, effect of BDNF in spiral neuron survival [101]. Moreover, despite the initial observations in NT-3 and trkC mutants suggesting a complete loss of afferent innervation to inner hair cells [99, 100], visualization of all cochlear projections in whole-mounted cochleae indicated that NT-3 and trkC mutants lose all spiral neurons, including both types, in the basal turn [103, 104]. Thus, the lack of NT-3 or BDNF results invariably in losses of cochlear neurons with a clear spatial bias in the longitudinal direction. Despite the elegance and simplicity of the one-type of neuron/one-type of neurotrophin model, differences along the length of the cochlear epithelium most precisely characterize the cochlear phenotypes of the neurotrophin mutants.

During embryonic development all cochlear neurons appear to express both trkB and trkC and, therefore, can respond to either neurotrophin. However, the distributions of BDNF and NT-3 in the cochlear epithelium show very different longitudinal and radial developmental dynamics [98]. NT-3 in the cochlear epithelium is expressed by supporting, but not hair, cells, and therefore NT-3 expression is seen in the cochlear epithelium at any time during the longitudinal extension of the cochlea, which proceeds in a basal-to-apical direction [98]. In contrast, BDNF expression appears to be largely restricted to differentiated hair cells [98]. Because hair cell differentiation follows an apex-to-base direction [96], that is, opposite to cochlear growth, it follows that the earliest differentiated cochlear neurons project to a sensory epithelium that lacks mature BDNF-producing hair cells and, consequently, must be initially supported by NT-3 released from other epithelial cell types. Therefore, in NT-3 mutants basal turn neurons project to an area completely devoid of any neurotrophins and do not survive. This is not the case for other cochlear regions, where different levels of both BDNF and NT-3 are found at the time of innervation. As predicted by this interpretation, transgenic expression of BDNF under control of the NT-3 promoter rescues the initial innervation deficit, and therefore the basal turn of the cochlea in these animals is innervated [70]. Thus, the opposed temporal gradients of cochlear neuron and hair-cell differentiation [96] transform a temporal gradient of neurotrophin expression into a spatial gradient of innervation loss in the corresponding mutants.

In summary, analyses of mutant strains of mice with altered neurotrophin signaling indicate that limited availability of neurotrophins largely determines the final numbers of neurons in peripheral sensory neuron populations by regulating their survival during embryogenesis, starting at times prior to final target innervation. Direct effects of the lack of a given neurotrophin on neuronal survival can be followed by indirect compensatory changes that contribute to the final deficit, and therefore detailed analyses are needed to precisely determine the physiological essential role of any given factor. Analysis of aspects other than early dependence for survival are hampered in the mutants by loss of the responsive neurons. Finally, studies in systems such as the auditory suggest that study of the mechanisms involved in regulation of the dynamic expression of the various neurotrophins [105] will be essential to understand how neuronal survival is controlled, and how population sizes are determined during development.

Acknowledgements. Work in the authors' laboratory has been supported by grants from the Ministerio de Ciencia y Tecnología (SAF99-0119-C02-01 and 1FD97-2153).

1 Scott S. A. (1992) Sensory Neurons. Diversity, Development and Plasticity. Oxford University Press, New York
2 Oppenheim R. W. (1991) Cell death during development of the nervous system. Annu. Rev. Neurosci. **14:** 453–501
3 Levi-Montalcini R. (1987) The nerve growth factor 35 years later. Science **237:** 1154–1162
4 Dechant G. (2001) Molecular interactions between neurotrophin receptors. Cell Tiss. Res. **305:** 229–238
5 Huang E. J. and Reichardt L. F. (2001) Neurotrophins: roles in neuronal development and function. Annu. Rev. Neurosci. **24:** 677–736
6 Patapoutian A. and Reichardt L. F. (2001) Trk receptors: mediators of neurotrophin action. Curr. Opin. Neurobiol. **11:** 272–280
7 Lee F. S., Kim A. H., Khursigara G. and Chao M. V. (2001) The uniqueness of being a neurotrophin receptor. Curr. Opin. Neurobiol. **11:** 281–286
8 Kaplan D. R. and Miller F. D. (2000) Neurotrophin signal transduction in the nervous system. Curr. Opin. Neurobiol. **3:** 381–391
9 Davies A. M. (1994) The role of neurotrophins in the developing nervous system. J. Neurobiol. **25:** 1334–1348
10 Mu X,. Silos-Santiago I., Carroll S. L. and Snider W. D. (1993) Neurotrophin receptor genes are expressed in distinct patterns in developing dorsal root ganglia. J. Neurosci. **13:** 4029–4041
11 Tessarollo L., Tsoulfas P., Martin-Zanca D., Gilbert D. J., Jenkins N. A., Copeland N. G. et al. (1993) TrkC, a receptor for neurotrophin-3, is widely expressed in the developing nervous system and in non-neuronal tissues. Development **118:** 463–475
12 Reichardt L. F. and Fariñas I. (1997) Neurotrophic factors and their receptors: roles in neuronal development and function. In: Molecular Approaches to Neural Development, Cowan M. W., Jessell T. M., Zipurski L. (eds), Oxford University Press, New York
13 Fariñas I. (1999) Neurotrophin actions during the development of the peripheral nervous system. Microscopy Research and Technique **45:** 233–242
14 Crowley C., Spencer S. D., Nishimura M. C., Chen K. S., Pitts-Meek S., Armanini M. P. et al. (1994) Mice lacking nerve growth factor display perinatal loss of sensory and sympathetic neurons yet develop basal forebrain cholinergic neurons. Cell **76:** 1001–1011
15 Smeyne R.J., Klein R., Schnapp A., Long L. K., Bryant S., Lewin S. A. et al. (1994) Severe sensory and sympathetic neuropathies in mice carrying a disrupted Trk/NGF. Nature **368:** 246–248

16 Minichiello L., Piehl F., Vazquez E., Schimmang T., Hökfelt T., Represa J. and Klein R. (1995) Differential effects of combined trk receptor mutations on dorsal root ganglion and inner ear sensory neurons. Development **121:** 4067–4075
17 Silos-Santiago I., Molliver D. C., Ozaki S., Smeyne R. J., Fagan A. M., Barbacid M. et al. (1995) Non-trkA-expressing small DRG neurons are lost in trkA deficient mice. J Neurosci. **15:** 5929–5942
18 Airaksinen M. S., Koltzenburg M., Lewin G. R. Masu Y., Helbig C., Wolf E et al. (1996) Specific subtypes of cutaneous mechanoreceptors require neurotrophin-3 following peripheral target innervation. Neuron **16:** 287–295
19 Carroll P., Lewin G. R., Koltzenburg M., Toyka K. V. and Thoenen H. (1998) A role for BDNF in mechanosensation. Nat. Neurosci. **1:** 42–46
20 Buchman, V. L. and Davies. A. M. (1993) Different neurotrophins are expressed and act in a developmental sequence to promote the survival of embryonic sensory neurons. Development **118:** 989–1001
21 Enokido Y., Wyatt S. and Davies A. M. (1999) Developmental changes in the response of trigeminal neurons to neurotrophins: influence of birthdate and the ganglion environment. Development **126:** 4365–4373
22 Schecterson L. and Bothwell M. (1992) Novel roles for neurotrophins are suggested by BDNF and NT-3 mRNA expression in developing neurons. Neuron **8:** 449–466
23 Fariñas I., Yoshida C. K., Backus C. and Reichardt L. F. (1996) Lack of neurotrophin-3 results in death of spinal sensory neurons and premature differentiation of their precursors. Neuron **17:** 1065–1078
24 White F.A., Silos-Santiago I., Molliver D. C., Nishimura M., Phillips H., Barbacid M. et al. (1996) Synchronous onset of NGF and trkA survival dependence in developing dorsal root ganglia. J. Neurosci. **16:** 4662–4672
25 Wilkinson G. A., Fariñas I., Backus C., Yoshida C. K. and Reichardt L. F. (1996) Neurotrophin-3 is a survival factor in vivo for early mouse trigeminal neurons. J. Neurosci. **16:** 7661–7669
26 O'Conner R. and Tessier-Lavigne M. (1999) Identification of maxillary factor, a maxillary process-derived chemoattractant for developing trigeminal sensory axons. Neuron **24:** 165–178
27 ElShamy W. M. and Ernfors P. (1996) Requirement of neurotrophin-3 for the survival of proliferating trigeminal ganglion progenitor cells. Development **122:** 2405–2414
28 Piñón L. G. P., Minichiello L., Klein R. and Davies A M. (1996) Timing of neuronal death in trkA, trkB, and trkC mutant embryos reveals developmental changes in sensory neuron dependence on trk signalling. Development **122:** 3255–3261
29 Sieber-Blum M. (1991) Role of the neurotrophic factors BDNF and NGF in the commitment of pluripotent neural crest cells. Neuron **6:** 949–955
30 Kalcheim C., Carmeli C.and Rosenthal A. (1992) Neurotrophin 3 is a mitogen for cultured neural crest cells. Proc. Natl. Acad. Sci. USA **89:** 1661–1665
31 Averbuch-Heller L., Pruginin M., Kahane N., Tsoulfas P., Parada, L.F., Rosenthal A. et al. (1994) Neurotrophin 3 stimulates the differentiation of motoneurons from avian neural tube progenitor cells. Proc. Natl. Acad. Sci. USA **91:** 3247–3251
32 Chalazonitis A., Rothman T. P., Chen J., Lamballe F., Barbacid M. and Gershon M. D. (1994) Neurotrophin-3 induces neural crest-derived cells from fetal gut to develop in vitro as neurons or glia. J. Neurosci. **14:** 6571–6584
33 DiCicco-Bloom E., Friedman W. J. and Black I. B. (1993) NT-3 stimulates sympathetic neuroblast proliferation by promoting precursor survival. Neuron **11:** 1101–1111
34 Verdi J. M. and Anderson D. J. (1994) Neurotrophins regulate sequential changes in neurotrophin receptor expression by sympathetic neuroblasts. Neuron **13:** 1359–1372
35 Henion P. D., Garner A. S., Large T. H. and Weston J. A. (1995) trkC-mediated NT-3 signalling is required for the early development of a subpopulation of neurogenic neural crest cells. Dev. Biol. **172:** 602–613
36 Karavanov A., Sainio K., Palgi J., Saarma M., Saxen L. and Sariola H. (1995) Neurotrophin 3 rescues neuronal precursors from apoptosis and promotes neuronal differentiation in the embryonic metanephric kidney. Proc. Natl. Acad. Sci. USA **92:** 11279–11283
37 Memberg S. P. and Hall A. K. (1995) Proliferation, differentiation, and survival of rat sensory neuron precursors in vitro require specific trophic factors. Mol. Cell Neurosci. **6:** 323–335
38 Dutton R., Yamada T., Turnley A., Bartlett P. F. and Murphy M. (1999) Sonic hedgehog promotes neuronal differentiation of murine spinal cord precursors and collaborates with neurotrophin 3 to induce Islet-1. J. Neurosci. **19:** 2601–2608
39 ElShamy W. M. and Ernfors P. (1996) A local action of neurotrophin-3 prevents the death of proliferating sensory neuron precursor cells. Neuron **16:** 963–972
40 ElShamy W. M., Friedvall L. K. and Ernfors P. (1998) Growth arrest failure, G1 restriction point override and S phase death of sensory precursor cells in the absence of neurotrophin-3. Neuron **21:** 1003–1015
41 Fariñas I., Wilkinson G. A., Backus C., Reichardt L. F. and Patapoutian A. (1998) Characterization of neurotrophin and trk receptor functions in developing sensory ganglia: direct NT-3 activation of trkB neurons in vivo. Neuron **21:** 325–334
41b. Lewin G. and Barde Y.-A. (1996) Physiology of the neurotrophins. Ann. Rev. Neurosci. **19:** 289–317
42 Lentz S. I., Knudson C. M., Korsmeyer S. J. and Snider W. D. (1999) Neurotrophins support the development of diverse sensory axon morphologies. J. Neurosci. **9:** 1038–1048
43 Patel T. D., Jackman A., Rice F. L. Kucera J. and Snider W. D. (2000) Development of sensory neurons in the absence of NGF/TrkA signaling in vivo. Neuron **25:** 345–357
44 Koltzenburg M., Stucky C. L. and Lewin G. R. (1997) Receptive properties of mouse cutaneous sensory neurons. J. Neurophysiol. **78:** 1841–1850
45 Lawson S. N. and Biscoe T. J. (1979) Development of mouse dorsal root ganglia: an autoradiographic and quantitative study. J. Neurocytol. **8:** 265–274
46 Lefcort F., Clary D. O., Rusoff A. C.and Reichardt L. F. (1996) Inhibition of the NT-3 receptor TrkC, early in chick embryogenesis, results in severe reductions in multiple neuronal subpopulations in the dorsal root ganglia. J. Neurosci. **16:** 3704–3713
47 Martin S. C., Sandell J. H. and Heinrich G. (1998) Zebrafish trkC1 and trkC2 receptors define two different cell populations in the nervous system during the period of axonogenesis. Dev. Biol. **195:** 114–130
48 Huang E. J., Wilkinson G. A., Fariñas I., Backus C., Zang K., Wong S. L. et al. (1999) Expression of Trk receptors in the developing mouse trigeminal ganglion: in vivo evidence for NT-3 activation of TrkA and TrkB in addition to TrkC. Development **10:** 2191–2203
49 Ma Q., Fode C., Guillemot F. and Anderson D. J. (1999) Neurogenin1 and neurogenin 2 control two distinct waves of neurogenesis in developing dorsal root ganglia. Genes Dev. **13:** 1717–1728
50 Liebl D.J. , Tessarollo L., Palko M. E. and Parada L. F. (1997) Absence of sensory neurons before target innervation in brain derived neurotrophic factor-, neurotrophin3-, and trkC-deficient embryonic mice. J. Neurosci **17:** 9113–9121
51 McMahon S. B., Armanini M. P., Ling L. H. and Phillips H. S. (1994) Expression and coexpression of trk receptors in subpopulations of adult primary sensory neurons projecting to identified peripheral targets. Neuron **12:** 1161–1171

52 Molliver D. C., Wright D., Leitner M. L., Parsadanian A. Sh., Doster K., Wen D. et al. (1997) IB4-binding DRG neurons switch from NGF to GDNF dependence in early postnatal life. Neuron **19:** 849–861

53 Albers K. M., Wright D. E. and Davis B. M. (1994) Overexpression of nerve growth factor in epidermis of transgenic mice causes hypertrophy of peripheral nervous system. J. Neurosci. **14:** 1422–1432

54 Albers K. M., Perrone T. N., Goodness T. P., Jones M. E., Green M. A. and Davis B. M. (1996) Cutaneous overexpression of NT-3 increases sensory and sympathetic neuron number and enhances touch dome and hair follicle innervation. J. Cell Biol. **134:** 487–497

55 Goodness T. P., Albers K. M., Davis F. E. and Davis B. M. (1997) Overexpression of nerve growth factor in skin increases sensory neuron size and modulates Trk receptor expression. Eur. J. Neurosci. **8:** 1574–1585

56 Klein R., Smeyne R. J., Wurst W., Long L. K., Auerbach B. A., Joyner A. L. et al. (1993) Targeted disruption of the trkB neurotrophin receptor gene results in nervous system lesions and neonatal death. Cell **75:** 113–122

57 Jones K. R., Fariñas I., Backus C. and Reichardt L. F. (1994) Targeted disruption of the BDNFgene perturbs brain and sensory neuron development but not motor neuron development. Cell **76:** 989–999

58 Ernfors P., Lee K. F.and Jaenisch R. (1994) Mice lacking brain-derived neurotrophic factor develop with sensory deficits. Nature **368:** 147–150

59 Liu X., Ernfors P., Wu H. and Jaenisch R. (1995) Sensory but not motor neuron deficits in mice lacking NT4 and BDNF. Nature **375:** 238–241

60 Conover J. C., Erickson J. T., Katz D. M., Bianchi L. M., Poueymirou W. T., McClain J. et al. (1995) Neuronal deficits, not involving motor neurons, in mice lacking BDNF and or NT4. Nature **375:** 235–238

61 Silos-Santiago I., Fagan A. M., Garber M., Fritzsch B. and Barbacid M. (1997) Severe sensory deficits but normal CNS development in newborn mice lacking trkB and trkC tyrosine protein kinase receptors. Eur. J. Neurosci. **9:** 2045–2056

62 Ichikawa H., Matsuo S., Silos-Santiago I. and SugimotoT. (2000) Developmental dependency of Meissner corpuscles on trkB but not trkA or trkC. Neuroreport **11:** 259–262

63 LeMaster A. M., Krimm R. F., Davis B. M., Noel T., Forbes M. E., Johnson J. E. et al. (1999) Overexpression of brain-derived neurotrophic factor enhances sensory innervation and selectively increases neuron number. J. Neurosci. **19:** 5919–5931

64 Stucky C. L., DeChiara T., Lindsay R. M., Yancopoulos G. D. and Koltzenburg M. (1998) Neurotrophin 4 is required for the survival of a subclass of hair follicle receptors. J. Neurosci. **18:** 7040–7046

65 Klein R., Silos-Santiago I., Smeyne R. J., Lira S. A., Bambrilla R., Bryant S. et al. (1994) Disruption of the neurotrophin-3 receptor gene trkC eliminates Ia muscle afferents and results in abnormal movements. Nature **368:** 249–251

66 Fariñas I., Jones K. R. , Backus C., Wang X.-Y. and Reichardt L. F. (1994) Severe sensory and sympathetic deficits in mice lacking neurotrophin-3. Nature **369:** 658–661

67 Ernfors P., Lee K. F., Kucera J. and Jaenisch R. (1994) Lack of neurotrophin-3 leads to deficiencies in the peripheral nervous system and loss of limb proprioceptive afferents. Cell **77:** 503–512

68 Tessarollo L., Vogel K. S., Palko M. E., Reid S. W. and Parada L. F. (1994) Targeted mutation in the neurotrophin-3 gene results in loss of muscle sensory neurons. Proc. Natl. Acad. Sci. USA **91:** 11844–11848

69 Tessarollo L., Tsoulfas P., Donovan M. J., Palko M. E., Blair-Flynn J., Hempstead B. L. et al. (1997) Targeted deletion of all isoforms of the trkC gene suggests the use of alternate receptors by its ligand neurotrophin-3 in neuronal development and implicates trkC in normal cardiogenesis. Proc. Natl. Acad. Sci. USA **94:** 14776–14781

70 Coppola V., Kucera J., Palko M. E., Martinez-De Velasco J., Lyons W. E., Fritzsch B. et al. (2001) Dissection of NT3 functions in vivo by gene replacement strategy. Development **128:** 4315–4327

71 Tojo H., Kaisho Y., Nakata M., Matsuoka K., Kitagawa M., Abe T. et al. (1995) Targeted disruption of the neurotrophin-3 gene with lacZ induces loss of trkC-positive neurons in sensory ganglia but not in spinal cords. Brain Res. **669:** 163–175

72 Airaksinen M. S. and Meyer M. (1996) Most classes of dorsal root ganglion neurons are severely depleted but not absent in mice lacking neurotrophin-3. Neuroscience **73:** 907–911

73 Kucera J., Fan G., Jaenisch R., Linnarsson S. and Ernfors P. (1995) Dependence of developing group Ia afferents on neurotrophin-3. J Comp. Neurol. **363:** 307–320

74 Wright D. E., Zhou L., Kucera J. and Snider W. D. (1997) Introduction of a neurotrophin-3 transgene into muscle selectively rescues proprioceptive neurons in mice lacking endogenous neurotrophin-3. Neuron **19:** 503–517

75 Oakley R. A., Garner A. S., Large T. H. and Frank E. (1995) Muscle neurons require neurotrophin-3 from peripheral tissues during the perios of normal cell death. Development **121:** 1341–1350

76 Copray J. C. and Brouwer N. (1994) Selective expression of neurotrophin-3 messenger RNA in muscle spindles of the rat. Neuroscience **63:** 125–1135

77 Kucera J., Fan G., Walro J., Copray S., Tessarrollo L. and Jaenisch R. (1998) Neurotrophin-3 and trkC in muscle are nonessential for the development of mouse muscle spindles. Neuroreport **9:** 905–909

78 Matsuo S., Ichikawa H., Silos-Santiago I., Arends J. J., Henderson T. A., Kiyomiya K. et al. (2000) Proprioceptive afferents survive in the masseter muscle of trkC knockout mice. Neuroscience **95:** 209–216

79 Fan G., Copray S., Huang E. J., Jones K., Yan Q., Walro J. et al. (2000) Formation of a full complement of cranial proprioceptors requires multiple neurotrophins. Dev. Dyn. **218:** 359–370

80 Fundin B. T., Silos-Santiago I., Ernfors P., Fagan A. M., Aldskogius H., DeChiara T. M. et al. (1997) Differential dependency of cutaneous mechanoreceptors on neurotrophins, trk receptors, and p75 LNGFR. Dev. Biol. **190:** 94–116

81 Davies A. M., Minichiello L. and Klein R. (1995) Developmental changes in NT3 signalling via TrkA and TrkB in embryonic neurons. EMBO J. **14:** 4482–4489

82 Mistretta C.M. and Hill D. L. (1995) Development of the taste system. In: Handbook of Olfaction and Gustation, pp. 635–668, Doty R. L. (ed.), Marcel Dekker, New York

83 Nosrat C. A., Ebendal T. and Olson L. (1996) Differential expression of brain-derived neurotrophic factor and neurotrophin 3 mRNA in lingual papillae and taste buds indicates roles in gustatory and somatosensory innervation. J. Comp. Neurol. **376:** 587–602

84 Nosrat C. A., Blomlof, ElShamy W. M., Ernfors P. and Olson L. (1997) Lingual deficits in BDNFand NT-3 mutant mice leading to gustatory and somatosensory disturbances, respectively. Development **124:** 1333–1342

85 Nosrat C. A. and Olson L. (1998) Changes in neurotrophin-3 messenger RNA expression patterns in the prenatal rat tongue suggest guidance of developing somatosensory nerves to their final targets. Cell Tissue Res. **292:** 619–623

86 Fritzsch B., Sarai P. A., Barbacid M. and Silos-Santiago I. (1997) Mice with a targeted disruption of the neurotrophin receptor trkB lose their gustatory ganglion cells early but do develop taste buds. Int. J. Dev. Neurosci. **15:** 563–576

87 Liebl D. J., Mbiene J. P. and Parada L. F. (1999) NT4/5 mutant mice have deficiency in gustatory papillae and taste bud formation. Dev. Biol. **213:** 378–389

88 Ringstedt T., Ibanez C. F. and Nosrat C. A. (1999) Role of brain-derived neurotrophic factor in target invasion in the gustatory system, J. Neurosci. **19:** 3507–3518
89 Krimm R. F., Miller K. K., Kitzman P. H., Davis B. M. and Albers K. M. (2001) Epithelial overexpression of BDNF and NT4 disrupts targeting of taste neurons that innervate the anterior tongue. Dev. Biol. **232:** 508–521
90 Oakley B., Brandemihl A., Cooper D., Lau D., Lawton A. and Zhang C. (1998) The morphogenesis of mouse vallate gustatory epithelium and taste buds requires BDNF-dependent taste neurons. Brain Res. Dev. Brain Res. **105:** 85–96
91 Mistretta C. M., Goosens K. A., Fariñas I. and Reichardt L.F. (1999) Alterations in size, number, and morphology of gustatory papillae and taste buds in BDNF null mutant mice demonstrate neural dependence of developing taste organs. J. Comp. Neurol. **409:** 13–24
92 Erickson J. T., Conover J. C., Borday V., Champagnat J., Barbacid M., Yancopoulos G. D. et al. (1996) Mice lacking brain-derived neurotrophic factor exhibit visceral sensory neuron losses distinct from mice lacking NT4 and display a severe developmental deficit in control of breathing. J. Neurosci. **16:** 5361–5371
93 Erickson J. T., Brosenitsch T. A. and Katz D. M. (2001) Brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor are required simultaneously for survival of dopaminergic primary sensory neurons in vivo. J. Neurosci. **21:** 581–589
94 Brady R., Zaidi S. I., Mayer C. and Katz D. M. (1999) BDNF is a target-derived survival factor for arterial baroreceptor and chemoafferent primary sensory neurons. J. Neurosci. **19:** 2131–2142
95 Minichiello L., Casagranda F., Tatche R. S., Stucky C. L., Postigo A., Lewin G. R. et al. (1998) Point mutation in trkB causes loss of NT4-dependent neurons without major effects on diverse BDNF responses. Neuron **21:** 335–345
96 Fritzsch B., Barald K. and Lomax M. (1998) Early embryology of the vertebrate ear. In: Development of the Auditory System, Springer Handbook of Auditory Research, pp. 80–145, Rubel E. W., Popper A. N., Fay R. R. (eds), Springer, New York
97 Fritzsch B., Silos-Santiago I., Bianchi L. and Fariñas I. (1997) The role of neurotrophic factors in regulating inner ear innervation. Trends Neuro. Sci. **20:** 159–165
98 Fariñas I., Jones K. R., Tessarollo L., Vigers A. J., Huang E., Kirstein M. et al. (2001) Spatial shaping of cochlear innervation by temporally-regulated neurotrophin expression. J. Neurosci. **21:** 6170–6180
99 Ernfors P., van de Water T., Loring J. and Jaenisch R. (1995) Complementary roles of BDNF and NT-3 in vestibular and auditory development. Neuron **14:** 1153–1164
100 Schimmang T., Minichiello L., Vazquez E., San Jose I., Giraldez F., Klein R. et al. (1995) Developing inner ear sensory neurons require TrkB and TrkC receptors for innervation of their peripheral targets. Development **121:** 3381–3391
101 Bianchi L. M., Conover J. C., Fritzsch B., De Chiara T., Lindsay R. M. and Yancopoulos G. D. (1996) Degeneration of vestibular neurons in late embryogenesis of both heterozygous and homozygous BDNF null mutant mice. Development **122:** 1965–1973
102 Fritzsch B., Silos-Santiago I., Smeyne R., Fagan A. M. and Barbacid M. (1995) Reduction and loss of inner ear innervation in trkB and trkC receptor knockout mice: a whole mount DiI and scanning electron microscopic analysis. Aud. Neurosci. **1:** 401–417
103 Fritzsch B., Barbacid M. and Silos-Santiago I. (1998) The combined effects of trkB and trkC mutations on the innervation of the inner ear. Int. J. Dev. Neurosci. **16:** 493–505
104 Fritzsch B., Fariñas I. and Reichardt L. F. (1997) Lack of NT-3 causes losses of both classes of spiral ganglion neurons in the cochlea in a region specific fashion. J. Neurosci. **17:** 6213–6225
105 Patapoutian A., Backus C., Kispert A. and Reichardt L. F. (1999) Regulation of neurotrophin-3 expression by epithelial-mesenchymal interactions: the role of Wnt factors. Science **283:** 1180–1183