Nerve Growth Factor and Nociception: From Experimental Embryology to New Analgesic Therapy

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Abstract

Nerve growth factor (NGF) is central to the development and functional regulation of sensory neurons that signal the first events that lead to pain. These sensory neurons, called nociceptors, require NGF in the early embryo to survive and also for their functional maturation. The long road from the discovery of NGF and its roles during development to the realization that NGF plays a major role in the pathophysiology of inflammatory pain will be reviewed. In particular, we will discuss the various signaling events initiated by NGF that lead to long-lasting thermal and mechanical hyperalgesia in animals and in man. It has been realized relatively recently that humanized function blocking antibodies directed against NGF show remarkably analgesic potency in human clinical trials for painful conditions as varied as osteoarthritis, lower back pain, and interstitial cystitis. Thus, anti-NGF medication has the potential to make a major impact on day-to-day chronic pain treatment in the near future. It is therefore all the more important to understand the precise pathways and mechanisms that are controlled by NGF to both initiate and sustain mechanical and thermal hyperalgesia. Recent work suggests that NGF-dependent regulation of the mechanosensory properties of sensory neurons that signal mechanical pain may open new

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G.R. Lewin and B.D. Carter (eds.), *Neurotrophic Factors*, Handbook of Experimental Pharmacology 220, DOI 10.1007/978-3-642-45106-5_10,

mechanistic avenues to refine and exploit relevant molecular targets for novel analgesics.

Keywords
NGF • Hyperalgesia • Pain • Inflammation • Mechanotransduction • STOML3 • Sensitization • TRP channel

1 Introduction

Nerve growth factor (NGF) is the founding member of the neurotrophin family. In the last 20 years the link between the biology of NGF and pain has been well established (Heppenstall and Lewin 2000; Pezet and McMahon 2006; Mantyh et al. 2011). At present, there are at least five major pharmaceutical companies running clinical trials of humanized antibodies designed to sequester NGF for the treatment of pain in conditions as varied as osteoarthritis, lower back pain, and interstitial cystitis (Cattaneo 2010; Lane et al. 2010; Evans et al. 2011; Brown et al. 2012, 2013). The first example of an NGF sequestering drug is Tanezumab a humanized monoclonal antibody that potently binds NGF developed by Rinat/Pfizer (Lane et al. 2010). Although the eventual success of an NGF-based drug for pain therapy is far from certain at the present time, the key role played by NGF signaling in pain is not in doubt. In this review we will provide an overview of how the study of NGF graduated from the province of embryologists to be the one of the most exciting drug targets for chronic pain in recent years. Since an NGF signaling axis is undoubtedly important in the etiology of pain, it is important to understand how NGF functions in the context of nociception and above all in the context of inflammatory hyperalgesia. Here we will primarily review the mechanistic basis of how NGF functions in nociception and chronic pain. The further understanding of NGF biology will be extremely important for understanding how best to manipulate NGF signaling to effectively treat chronic pain.

2 Experimental Embryology Leads the Way

In a classic series of experiments performed by Rita Levi-Montalcini and her collaborator Victor Hamburger the activity that was to be identified as NGF was studied using chicken embryos (Hamburger 1993). They described a process, now termed programmed cell death, whereby an overabundance of neurons generated during development, is reduced in number by apoptosis during critical periods. The fact that many, but not all, neurons die during such critical periods raised the question of what are the factors that keep the remaining neurons alive. From these types of experiments came the key insight that led to the eventual identification of NGF. Experiments using limb ablation, or the grafting of supernumerary limbs in embryos during critical stages of development, showed that the number of

surviving motor neurons, sympathetic ganglion neurons, or sensory neurons was dependent on the size of the peripheral target. Hamburger and Levi-Montalcini postulated that some target-derived survival factor synthesized in limiting amounts was responsible for preventing many of the neurons from undergoing programmed cell death. Bueker made the serendipitous discovery that injection of a mouse sarcoma tumor cell line into chick embryos could mimic the survival promoting effects of increased target size (Bueker and Hilderman 1953; Cohen 2008). These experiments eventually led to the identification of a source of this as yet unknown growth factor, namely, the mouse submaxillary gland. Stanley Cohen used this biochemical source to purify NGF and was able to use this purified protein material to generate rabbit polyclonal antibodies which bind to NGF (Cohen 1960; Levi-Montalcini and Booker 1960). This enabled Levi-Montalcini to carry out the first function blocking experiments, which addressed the endogenous function of NGF in the mouse. Thus, the injection of NGF binding antibodies into newborn mice led to a dramatic loss of sympathetic neurons showing that these neurons require NGF for their survival. The antibody approach taken by Levi-Montalcini was based on the idea that sequestration of endogenous NGF by high-affinity antibodies will prevent NGF binding to its endogenous receptors to prevent cell death or promote nerve fiber growth. It is worth noting that therapeutic interventions for the treatment of pain now being pursued 50 years later are based on this very same idea.

The availability of antibody tools to manipulate the endogenous levels of NGF allowed researchers to address the functional consequences of NGF sequestration. Initially, efforts focused on identifying precisely which neuronal populations depend on NGF for survival and when. It is through such experiments and later genetics that we know that NGF is required for the survival of sympathetic ganglion neurons and a large proportion of embryonic sensory neurons that are destined to become nociceptors (Ruit et al. 1990, 1992; Crowley et al. 1994). It appears that both sympathetic and sensory neurons largely lose their absolute dependency on NGF for survival in the postnatal period (Ruit et al. 1990, 1992).

In the 1980s and 1990s the main focus of developmental biologists was the question of whether these neurons required the neurotrophins to live, or otherwise in their absence to die, normally through an active apoptotic program (Lewin and Barde 1996). For example, it was known that all sensory neurons that express the high affinity NGF receptor trkA during embryonic development require NGF to survive, but it is also now clear that this population is not phenotypically homogenous (Crowley et al. 1994; Marmigère and Ernfors 2007). Recently, it has become possible using a nice genetic trick to examine the influence of NGF signaling in the embryo without the confounding effects of cell death. Thus mice lacking the cell death regulator Bax were generated on a genetic background in which the gene encoding NGF was also deleted; in the absence of Bax, neurons cannot execute an apoptotic program and remain alive in the absence of NGF (Patel et al. 2000; Luo et al. 2007). One key finding of such experiments is that NGF signaling is not required for long-distance axonal growth in the embryo, but is required for the terminal branch formation in the skin. However, there are other phenotypic characteristics of developing nociceptors that also require NGF signaling, for example, the expression of nociceptor-specific ion channels like the

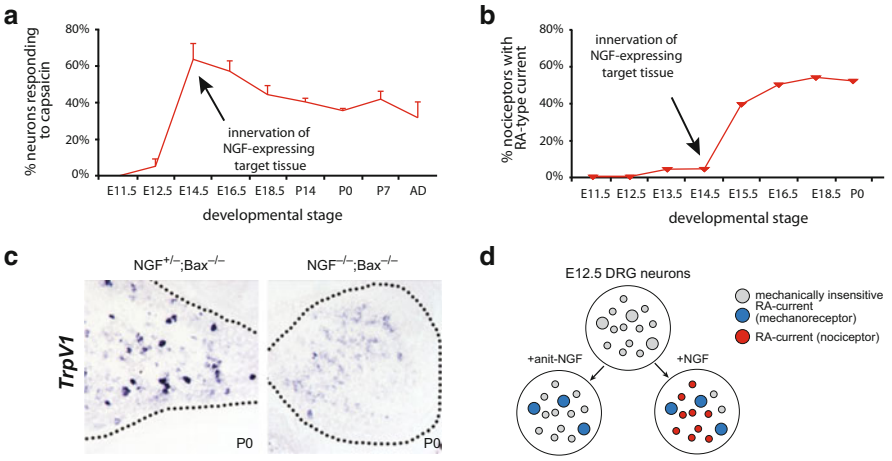

Fig. 1 NGF controls the expression of Trpv1 and mechanically gated ion channels in DRGs during embryonic development. (**a**) and (**b**) show the proportions of nociceptors that respond to the TRPV1 agonist capsaicin (**a**) and to mechanical stimulation (**b**) plotted as a function of developmental stage [data from (Hjerling-Leffler et al. 2007; Lechner et al. 2009)]. Note, both mechanosensitivity and capsaicin sensitivity are acquired at E14.5 when the peripheral projections begin to innervate NGF-expressing target tissues. (**c**) Trvp1 in situ hybridization in DRGs. Note in the absence of NGF (NGF−/−; Bax−/−), TrpV1 is not expressed in DRG neurons (Luo et al. 2007). (**d**) NGF is required for the acquisition of mechanotransduction currents in vitro. DRG neurons from E12.5 Bax−/− mice acquire RA-type mechanotransduction currents when cultured in the presence of NGF, but remain mechano-insensitive in the absence of NGF signaling (anti-NGF) see (Lechner et al. 2009)

capsaicin-activated ion channel TRPV1 and TRPM8 a menthol-gated channel involved in cold sensing (Luo et al. 2007) (Fig. 1a). Functional experiments using calcium imaging techniques also indicated that embryonic sensory neurons begin to respond to capsaicin at embryonic stages coinciding with the innervation of NGF-rich target tissues (Hjerling-Leffler et al. 2007) (Fig. 1a). The vast majority of nociceptors are primarily sensitive to mechanical stimuli and many possess fast activated mechanosensitive currents that are probably the functional basis of their mechanosensitivity (Hu and Lewin 2006; Wetzel et al. 2007). We thus asked when this mechanotransduction apparatus appears during development and if its appearance is regulated by target innervation or neurotrophins (Lechner et al. 2009) (Fig. 1b, d). Interestingly, one key finding of our study was that there are several waves of mechanotransduction induction in the sensory lineage with the first born, low-threshold mechanoreceptors (trkC population) acquiring mechanosensitive currents as soon as they innervate their peripheral targets (Fig. 1b). However, this process appears to be independent of growth factors and is probably regulated by an as yet unknown genetic program, possibly involving C-Maf genes (Lechner et al. 2009; Wende et al. 2012). In contrast, the vast majority of trkA-positive sensory neurons innervate their targets later and here it appears that target-derived NGF is absolutely required for the induction of mechanosensory competence (Lechner et al. 2009) (Fig. 1d). Thus the

physiological properties of developing sensory neurons that are essential for their adult function may already be specified by neurotrophin signaling in the early embryo (Fig. 1).

Despite the fact that NGF is not required for the continued survival of adult sensory neurons, it continues to be synthesized in the peripheral targets into adulthood. Indeed it has long been noted that the levels of NGF in the target correlate very well with the density of sympathetic and sensory innervation (Korsching and Thoenen 1983; Shelton and Reichardt 1984; Lewin and Barde 1996). Studies in the 1980s already showed that it is primarily neuropeptide containing nociceptive sensory neurons in the adult that respond to NGF (Lewin and Barde 1996). Thus the neuropeptide content, primarily substance P and calcitonin gene-related peptide (CGRP), of sensory neurons innervating tissues high in NGF, such as the skin, was observed to be high compared to tissues low in NGF (McMahon et al. 1989). Indeed, Lindsay and Harmar demonstrated that NGF directly upregulates the substance P content of adult sensory neurons (Lindsay and Harmar 1989). TrkA receptor expression is a feature of all developing nociceptors in the embryo, but its expression is extinguished in postnatal, small diameter, non-peptidergic nociceptors (Molliver et al. 1997). The high-affinity trkA receptor is the primary NGF signaling receptor and is co-expressed in neuropeptide-positive nociceptors in adults. In mature animals the peripheral tissue could be shown to influence the chemical composition of sensory afferents. Thus in experiments where a cutaneous nerve was rerouted to the NGF-poor skeletal muscle and a muscle nerve was rerouted to NGF-rich skin, the substance P content changed to match that characteristic of the new target, e.g., muscle nerve innervating skin now had a high substance P content (McMahon and Gibson 1987; McMahon et al. 1989). What was even more striking was the fact that the central connectivity of muscle afferents that had been redirected to skin now resembled that of normal skin afferents (Lewin and McMahon 1991). These results led us to carry out the first serious test of the idea that a neurotrophic factor could regulate synaptic strength in the nervous system. We decided to artificially raise the levels of NGF in the skeletal muscle, in this case the gastrocnemius muscle, by chronically pumping NGF into the muscle for a period of 14 days. By making extracellular recordings from spinal dorsal horn neurons we knew that only very few of these neurons receive strong synaptic drive from afferents innervating skeletal muscle. However, after exposure to NGF skeletal muscle afferents showed a huge increase in their ability to excite dorsal horn neurons and this increase was very large when compared to effects of muscle afferents innervating the contralateral, untreated muscle (Lewin et al. 1992b). This was in all probability the very first demonstration that a neurotrophic factor can modulate synaptic strength. Shortly afterwards, an elegant and more direct proof of this idea came from the lab of Moo Ming Poo, which showed that both neurotrophin-3 (NT-3) and brain-derived neurotrophic factor (BDNF) can increase the strength of neuromuscular synapses in vitro, with a surprisingly fast time course in the range of seconds (Lohof et al. 1993). Together these studies provided the foundation of a huge and important area of study, namely, how neurotrophins regulate synapses and synaptic strength in the nervous system (see chapters 9 and 16 from Bai Lui et al. and Boyce and Mendell).

3 NGF and Hyperalgesia: The Linchpin Theory 1993

Hyperalgesia is defined as an increase in the felt intensity of a noxious stimulus, usually following an injury or an inflammatory process. Secondary hyperalgesia is the area of hypersensitivity surrounding an injured area that is not due to peripheral sensitization of the primary afferent, as the afferents that innervate the secondary area cannot be directly sensitized by the injury. This type of hyperalgesia is thought to be due to sensitization of central circuits to afferent input coming from nearby the initial injury site (Treede et al. 1992; Lewin and Moshourab 2004). During the 1980s, it was becoming increasingly clear that the neurobiological basis of secondary hyperalgesia was to a large extent dependent on a phenomenon termed central sensitization (Woolf 1983; McMahon and Wall 1984; Cook et al. 1987; McMahon et al. 1993). Thus strong activation of nociceptors leads to a rapid and long-lasting plasticity at synapses between primary sensory neurons and dorsal horn neurons, and this long-lasting change in synaptic strength can sustain hyperalgesia. Hyperalgesia is induced following injury or inflammation, but can also be produced after skin application of substances that activate or sensitize nociceptors. A classic example of algogen-induced heat and mechanical hyperalgesia is that following the application of capsaicin to the skin (LaMotte et al. 1991). While working on the role of NGF in determining the phenotypic identity of nociceptors in Lorne Mendell's lab (Ritter et al. 1991; Lewin et al. 1992a), Amy Ritter and Gary Lewin noted that rats that had been exposed to daily injections of NGF were behaviorally more sensitive to mechanical and heat stimuli than untreated animals. These observations led them to make a more systematic study of the effects of NGF on nociceptive behaviors in the rat. To their surprise a single systemic injection of NGF (1 mg/kg body weight) produced profound heat and mechanical hyperalgesia, which lasted for several days. Interestingly, heat and mechanical hyperalgesia appeared to be mechanistically distinct as heat hyperalgesia appeared within minutes, whereas mechanical hyperalgesia first became apparent around 7 h after the injection, becoming maximal and sustained at 24 h (Lewin et al. 1993). The fact that a single molecule, NGF, could set into train a series of rapid functional changes with all the hallmarks of hyperalgesia normally seen after sterile inflammation raised the obvious question of whether NGF was necessary for inflammatory hyperalgesia. This question was particularly pertinent in light of data published by Donnerer and colleagues in 1992 showing that NGF was upregulated in the sciatic nerve following inflammation of the skin (Donnerer et al. 1992). It was now an obvious step to use blocking antibodies in vivo to show whether an inflammation-dependent rise in NGF was a necessary first step in producing hyperalgesia. After obtaining preliminary data using NGF blocking antibodies, a new model of inflammatory hyperalgesia was proposed where NGF represents a linchpin molecule that provides the key humoral link between inflammation and the nociceptive sensory neurons that initiate and sustain heat and mechanical hyperalgesia (Lewin and Mendell 1993). The key features of this model are shown in Fig. 2, highlighting the areas of progress that have been made since the discovery that NGF is necessary for inflammatory hyperalgesia. Soon after we reported that NGF could induce

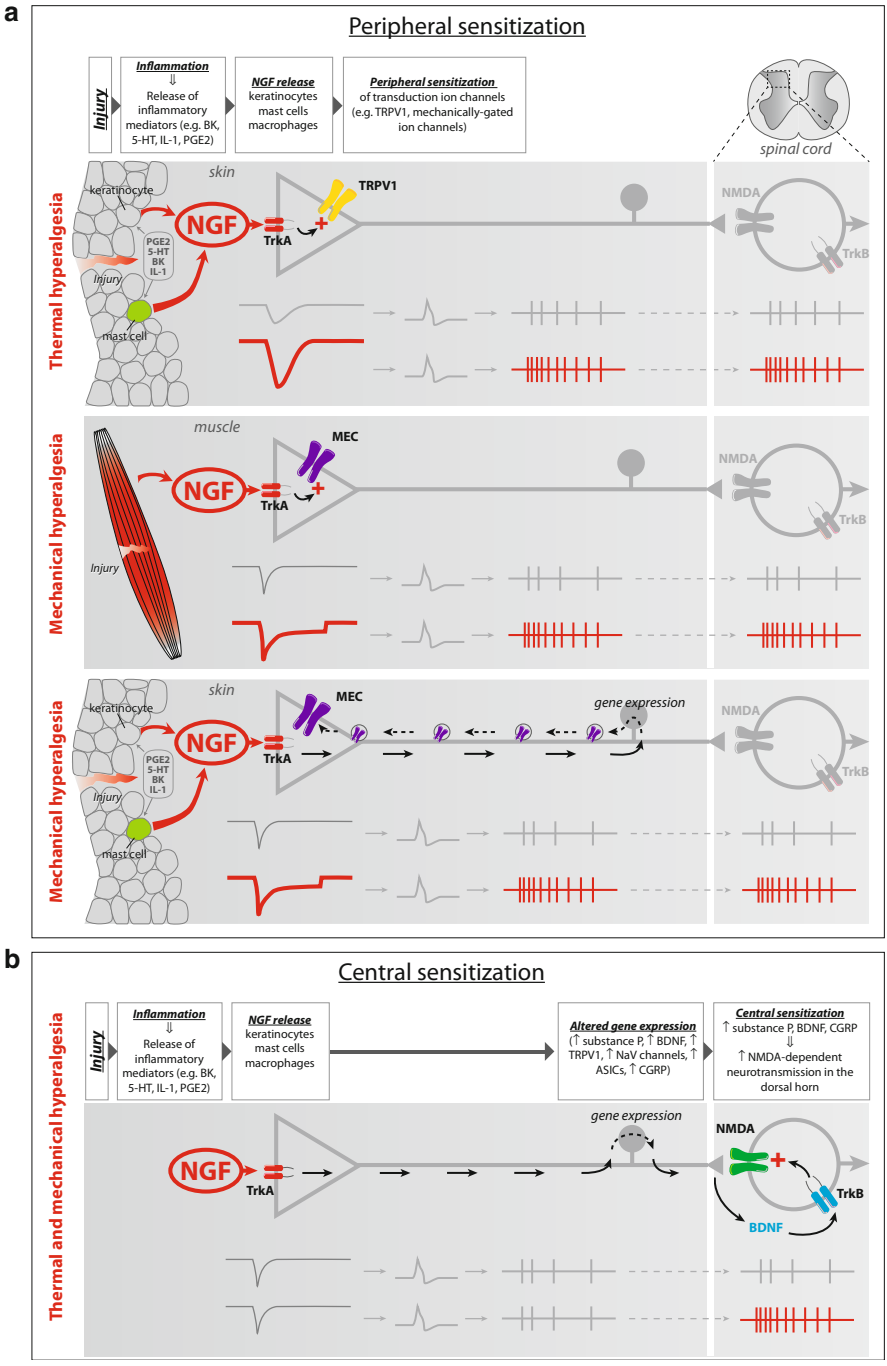

Fig. 2 Mechanisms of peripheral and central sensitization. (**a**) peripheral sensitization may result from posttranslational modifications (*top* and *middle panel*) or from increased gene expression and

hyperalgesia, several groups tested whether NGF blocking antibodies could ameliorate or block heat and mechanical hyperalgesia following inflammation. The first two reports showed that both the heat and mechanical hyperalgesia that follow a complete Freund's adjuvant-induced inflammation could be ameliorated by the administration of NGF blocking antibodies (Lewin et al. 1994; Woolf et al. 1994). Later on, the use of improved molecular tools to sequester NGF, namely, trkA-IgG fusion proteins that specifically bind endogenous NGF, was also shown to be capable of ameliorating heat and mechanical hyperalgesia associated with a carrageenan-evoked inflammation model in rats (McMahon et al. 1995). The key finding that blockade of NGF pain signaling in inflammatory conditions, where NGF is elevated, has a major analgesic effect has now been repeated in many models (Pezet and McMahon 2006; Mantyh et al. 2011).

In 1993, Lewin and Mendell proposed a mechanistic model illustrating the various ways in which increased NGF could produce heat and mechanical hyperalgesia following inflammation. One key feature of this model was the idea that the mechanisms that underlie the NGF-dependent heat hyperalgesia are distinct from those that underlie the mechanical hyperalgesia (Lewin and Mendell 1993; Lewin et al. 1994). We supposed that an important difference was that NGF is capable of inducing extremely rapid changes in the peripheral terminals of C-fibers that sensitizes them to noxious heat stimuli. Mechanical hyperalgesia on the other hand seemed to require the induction of changes in gene expression that eventually leads to central sensitization that maintains mechanical hyperalgesia (Lewin and Mendell 1993; Lewin et al. 1994). In the last 20 years much progress has been made in elucidating the molecular mechanisms that underlie peripheral NGF-dependent heat hyperalgesia. Progress has also been made in understanding NGF-dependent mechanical hyperalgesia and new data indicate that both central and peripheral mechanisms may be important, the molecular basis of which is just beginning to be unraveled.

4 NGF-Dependent Heat Hyperalgesia: Molecular Mechanisms

The availability of NGF in the skin was shown early on to regulate the number of C-fibers that respond to noxious heat. Thus, decreasing NGF levels with blocking antibodies reduced the number of C-fibers that respond to heat and raised NGF levels increased the number of heat-sensitive C-fibers (Lewin and Mendell 1994). These early experiments demonstrated that the molecular basis of noxious heat

Fig. 2 (continued) the insertion of additional mechanically gated ion channels in the plasma membrane of the peripheral nerve terminal (*bottom*). (**b**) NGF signaling induces the release of substance P, BDNF, and CGRP from the central terminals of sensory neurons, which sensitize NMDA receptors in second-order projection neurons resulting in the strengthening of synaptic transmission in the spinal dorsal horn—i.e., central sensitization

transduction was itself a target of regulation by NGF. The regulation of noxious heat transduction in single C-fibers in an inflammatory pain model was also shown to be dependent on NGF (Koltzenburg et al. 1999). The very rapid NGF-induced heat hyperalgesia was shown to be partly mediated by NGF-induced mast cell degranulation, which can in turn release more NGF (Mazurek et al. 1986; Lewin et al. 1994; Andreev et al. 1995). However, subsequent studies have emphasized that most of the rapid heat sensitization initiated by NGF takes place in the nociceptor. An important advance in the field was the discovery that a subpopulation of isolated sensory neurons possesses an ionic inward current directly activated by noxious heat sometimes referred to as I_{heat} (Cesare and McNaughton 1996). The I_{heat} inward current could also be sensitized by algogens like bradykinin and recording from isolated cells has proved to be a useful model to study molecules involved in nociceptor sensitization (Cesare and McNaughton 1996; Cesare et al. 1999). There was great excitement in the field when the capsaicin-gated ion channel TRPV1 was cloned by Julius and colleagues and shown to be gated by heat with an activation threshold similar to that of $I_{heat} \sim 43$ °C (Caterina et al. 1997). Thus the capsaicin receptor and the noxious heat transduction channel appeared to be one and the same thing. It was thus very striking when Mendell and Shu showed that a single short exposure of isolated sensory neurons to NGF (as well as NT-4) greatly potentiated the capsaicin current amplitude measured minutes later (Shu and Mendell 1999). Nerve growth factor-induced heat hyperalgesia was later found to be dependent on the presence of the TRPV1 ion channel as NGF-induced hyperalgesia is not found in TRPV1$^{-/-}$ mice (Chuang et al. 2001); the persistence of NGF-induced heat hyperalgesia in p75$^{-/-}$ mice demonstrates that trkA is probably the necessary receptor for downstream sensitization (Bergmann et al. 1998). The present consensus is that the TRPV1 ion channel is a noxious heat-gated ion channel present in many polymodal, noxious heat-sensitive C-fibers, but its presence does not appear to be necessary for these neurons to respond to noxious heat in vivo (Woodbury et al. 2004). Recent studies have implicated new heat-activated ion channels such as anoctamin-1, a calcium-activated chloride channel, and the TRP channel TRPM3 as being required for heat transduction in nociceptors (Vriens et al. 2011; Cho et al. 2012). However, it is not yet known if NGF-dependent heat hyperalgesia and nociceptor sensitization are dependent on either anoctamin-1 or TRPM3.

The absolute requirement for TRPV1 for NGF-dependent heat hyperalgesia and nociceptor sensitization has led many workers to use increased TRPV1 activity as a molecular surrogate for sensitization. Thus capsaicin has often been used, rather than heat, to activate TRPV1. Initial work using rat DRG neurons identified PKA as responsible for the sensitization brought about by NGF (Shu and Mendell 1999), but later work demonstrated that although protein kinase activity was involved in producing sensitization, it was PKC and PI3K that were responsible (Bonnington and McNaughton 2003). Differences in the sensitization protocol used and the recording method (whole-cell electrophysiology vs. calcium imaging) have been suggested to explain the differences in the results obtained. Whereas PKC acts predominantly via direct phosphorylation of TRPV1 (Numazaki et al. 2002), the

PI3K pathway has multiple steps: following trkA autophosphorylation at Tyr760, PI3K is activated, which in turn activates Src kinase, a non-receptor tyrosine kinase that subsequently phosphorylates Tyr200 on TRPV1 resulting in translocation to the plasma membrane and increased membrane expression (Zhang et al. 2005; Stein et al. 2006). An alternative explanation for NGF-induced heat hyperalgesia has been built on the observation that mutated trkA, which is unable to activate phospholipase C (PLC), fails to mediate NGF-induced sensitization, which the authors suggested was due to the action of PLC liberating TRPV1 from PIP2 inhibition being prevented; antibodies to PIP2 also evoked TRPV1 sensitization (Chuang et al. 2001). However, it has been argued that NGF can exert all its effects in a PIP2-independent manner (Zhang and McNaughton 2006) and later studies have shown that direct application of PIP2 actually potentiates TRPV1 (Stein et al. 2006). The study by Stein and colleagues has, however, recently been challenged by the finding that in artificial liposomes TRPV1 activation by both heat and capsaicin is inhibited by a variety of phosphoinositide lipids interacting with the C terminus of TRPV1 (Cao et al. 2013). Moreover, the authors show that activation threshold is not altered by channel number and therefore conclude that although NGF-dependent increased membrane expression of TRPV1 may account for some of the thermal hypersensitivity observed, it cannot explain decreases in thermal threshold. NGF-induced heat hyperalgesia is rapid in onset in vivo, but is also very long lasting and it has been suggested that NGF can also enhance TRPV1 expression levels via the Ras-MAPK pathway (Ji et al. 2002), which could contribute to the more persistent heat hyperalgesia in the presence of NGF. It should, however, be noted that there is good evidence that persistent heat hyperalgesia following inflammation or NGF elevation may also be dependent on central sensitization (Fig. 2).

The fact that TRPV1 is necessary for sensitization, but not for the transduction of noxious heat by nociceptors, is an important fact that requires further investigation (Woodbury et al. 2004; Koerber et al. 2010). It may be that freshly phosphorylated TRPV1 protein or newly inserted TRPV1 molecules in turn directly interact with candidate heat-gated channels, like anoctamin-1 or TRPM3, to produce sensitization. Alternatively, TRPV1 may itself have a signaling function that is required for the sensitization of heat transduction. In order to answer these questions a definitive identification of the molecule(s) necessary for heat transduction will be required. The signaling pathways that converge onto TRPV1 from trkA activation also appear to be engaged by other growth factor receptors such as c-Ret together with its co-receptors GFRα2 and GFRα3 (Stucky et al. 2002; Malin et al. 2006) that are preferentially activated by neurturin and artemin, respectively (Baloh et al. 2000; Bespalov and Saarma 2007). Neurturin signaling in particular may be like NGF, in the sense that it regulates the number of heat-sensitive neurons amongst the subpopulation of isolectin B4 (IB4)-positive sensory neurons that in the adult lack trkA receptors (Molliver et al. 1997; Stucky and Lewin 1999; Stucky et al. 2002). The receptor tyrosine kinase c-Kit is the receptor for stem cell factor (SCF) and was recently found to be expressed by a subpopulation of noxious heat-sensitive nociceptors (Milenkovic et al. 2007). It was shown that SCF/c-Kit

signaling is necessary to maintain nociceptor heat sensitivity and SCF can, like NGF, sensitize I_{heat} and produce a rapid, but short lasting, heat hyperalgesia in a TRPV1-dependent manner (Milenkovic et al. 2007). Interestingly, in the case of GDNF-like ligands and SCF where heat sensitization has been reported, mechanical hyperalgesia was absent [but see (Albers et al. 2006)].

5 Mechanisms of NGF-Dependent Mechanical Hyperalgesia

Mechanical hyperalgesia is the symptom that most concerns patients with painful conditions caused by inflammation or injury. It was thus very striking to observe that a short burst of elevated NGF can be sufficient to induce mechanical hyperalgesia that can last for days or even weeks in rodents and humans (Lewin et al. 1993; Petty et al. 1994). Systemic or local injection of NGF is unlikely to lead to sustained trkA activation because this small polypeptide would be rapidly degraded by extracellular proteases after injection. Thus, a pulse of NGF is sufficient to set in train a series of events that sustain mechanical hyperalgesia, often for days. Early pharmacological experiments already indicated that long-lasting NGF-induced heat hyperalgesia, but not mechanical hyperalgesia, is sustained by a central sensitization that requires NMDA receptors (Lewin et al. 1994) (Fig. 2). NGF can produce long-lasting changes in gene expression in adult sensory neurons and the first genes shown to be controlled by NGF were substance P and CGRP (Lindsay and Harmar 1989). Release of neuropeptides from sensory neurons may modulate the strength of spinal cord synapses (Seybold 2009); however, mice with a targeted mutation of the tachykinin-1 gene coding for the substance P peptide do not show deficits in inflammation-induced mechanical hyperalgesia (Cao et al. 1998). Thus considering that NGF is required for inflammation-induced mechanical hyperalgesia, it appears to be unlikely that substance P is a major central mediator. In contrast, studies on mice lacking a second major neuropeptide, CGRP expressed in trkA-positive sensory neurons (Molliver et al. 1997), have indicated broad deficits in inflammatory hyperalgesia including mechanical hyperalgesia (Salmon et al. 2001). One unusual rodent species, the naked mole rat, completely lacks both substance P and CGRP in cutaneous nociceptors, but exhibits a similar degree of mechanical hyperalgesia following complete Freund's adjuvant to that seen in mice (Park et al. 2008). Interestingly, however, NGF injected into naked mole rats does not produce heat hyperalgesia and this may be due to the presence of a hypo-functional trkA receptor in this species (Park et al. 2008; Smith et al. 2012).

Neurotrophins were traditionally thought of as being produced by the targets of sensory neurons, but it became apparent from developmental studies that many sensory neurons actually express and produce neurotrophins (Ernfors et al. 1990). It was therefore striking, when it was discovered that BDNF is normally produced by a subset of trkA-positive nociceptors and that the number of trkA neurons making this factor is dramatically increased by increased NGF (Apfel et al. 1996; Michael et al. 1997). Indeed, BDNF could be shown to be released by activity in sensory neurons and its release is enhanced by elevated NGF levels that follow

inflammation (Balkowiec and Katz 2000; Lever et al. 2001). Thus, increased peripheral NGF leads to increased production and release of BDNF from the central synapses of nociceptors in the spinal cord, which may be critical for certain central sensitization events, especially those involving NMDA receptors (Kerr et al. 1999). Direct electrophysiological evidence demonstrating that BDNF can rapidly potentiate transmission at synapses formed by nociceptors was provided by Mendell and colleagues (Garraway et al. 2003). The effects of mature BDNF on spinal synapses are rapid and probably occur via both pre- and postsynaptic trkB receptors and the potentiation observed is because of phosphorylation of NMDA receptor subunits (Kerr et al. 1999; Heppenstall and Lewin 2001; Garraway et al. 2003) (Fig. 2).

One complication of examining the central sensitization effects of BDNF is that this factor is also produced within the brain and spinal cord. Furthermore, the production and release of BDNF may be controlled by many factors. For example, it has been proposed that, when activated, spinal microglia cells may release BDNF, which in turn can modulate the excitability of dorsal horn neurons. The modulation of the anion gradient in lamina I projection neurons, possibly via the modulation of KCC2 (a potassium chloride co-transporter), can lead to a shift in the reversal potential for anions like chloride which makes normally hyperpolarizing inputs from inhibitory interneurons either ineffective or even depolarizing (Coull et al. 2005). This type of BDNF effect is thought to be particularly relevant for sustaining neuropathic pain. Other work, notably from Mendell's group, has also shown how BDNF can have highly synapse-specific effects in the spinal cord (Mendell and Arvanian 2002).

Global deletion of the BDNF gene leads to early postnatal lethality which has made the study of BDNF's role in the adult nervous system more difficult (Carroll et al. 1998). Nevertheless, studies using isolated spinal cords from young neurotrophin gene mutant mice have shown that the plasticity of ventral root potentials, which reflects C-fiber drive flexion reflexes, is selectively attenuated in the absence of BDNF, but not in the absence of NT-4 (Heppenstall and Lewin 2001). A systematic examination of pain-related behaviors in BDNF heterozygote mutant mice also indicated that even reduced gene dosage of this important factor can lead to deficits in acute noxious heat sensitivity and reduced pain behaviors, e.g., in the formalin test (MacQueen et al. 2001). An elegant genetic study using mice in which the BDNF gene was selectively deleted in nociceptive sensory neurons showed that BDNF is required for normal heat hyperalgesia following inflammation (Zhao et al. 2006). Although the authors did not definitively address the question of whether NGF-induced mechanical hyperalgesia depends on sensory neuron-derived BDNF, direct injection of NGF into skeletal muscle did not provoke mechanical hyperalgesia in this model, which in common with other studies suggests that elevated muscle NGF provokes central sensitization (Lewin et al. 1992b; Zhao et al. 2006). In summary, it seems that at least a proportion of the sustained heat hyperalgesia initiated by NGF may be sustained by central sensitization driven by BDNF and subsequent phosphorylation of postsynaptic NMDA receptors (Lewin et al. 1994; Zhao et al. 2006). However, it remains unclear whether the long-lasting mechanical hyperalgesia initiated by increased NGF is primarily dependent on peripheral or central mechanisms.

The long-lasting changes initiated by NGF may also affect the electrical properties of the primary afferent axons that transfer noxious information to the central nervous system. Nociceptors possess an array of voltage-gated sodium channels (Na_Vs) that are in some cases selectively expressed in these cells and strongly implicated in painful conditions. Nociceptors possess TTX-resistant and TTX-sensitive Na_Vs, which are carried primarily by $Na_V1.7$, $Na_V1.8$, and $Na_V1.9$ channels (Momin and Wood 2008); the modulation of such channels has been proposed to play a role in sensitization processes (England et al. 1996; Gold et al. 1996). Action potential initiation, voltage threshold, and sustained firing are dependent on the activation properties of Na_V channels (Blair and Bean 2002). It is therefore of interest that the availability of NGF can indeed modulate the action potential shape of nociceptors, both in culture as well as in vivo. Nociceptors have unusually broad action potentials with a prominent hump on their falling phase (Lechner et al. 2009). It is possible to identify nociceptors in cultures of adult sensory neurons that do not respond to NGF, as these can be live stained with fluorescently conjugated IB4. Interestingly, the density of TTX-sensitive sodium currents is actually less in NGF-sensitive nociceptors compared to IB4-positive NGF-insensitive neurons, which also display broader action potentials (Stucky and Lewin 1999). In vivo experiments have shown that chronically increasing the availability of NGF is associated with a broadening of the action potentials of identified Aδ nociceptors; conversely NGF deprivation is associated with a narrowing of the action potential in the same neurons (Ritter and Mendell 1992; Fang et al. 2005). The expression of TTX-resistant Na_Vs can be regulated by NGF and so it is conceivable that changes in action potential properties partly result from such regulation (Fjell et al. 1999). Genetic ablation of different Na_V genes in the sensory ganglia offers an opportunity to more directly assess their relative contributions to NGF-dependent sensitization events. Using mutant $Na_V1.8$ mice it was shown that the induction of NGF-dependent heat hyperalgesia requires the presence of $Na_V1.8$ channels (Kerr et al. 2001). However, heat hyperalgesia following carrageenan inflammation was only moderately delayed after genetic ablation of $Na_V1.8$ (Akopian et al. 1999) and was not affected in mice in which $Na_V1.8$ was inhibited in a cell autonomous manner (Stürzebecher et al. 2010). It is known that TTX-resistant Na_V currents can be measured very close to the spike initiation zone of peripheral nociceptors (Brock et al. 1998). It is therefore possible that a TRPV1-dependent sensitization process takes place in animals with ablated or attenuated $Na_V1.8$ channels, but that the increased activity of heat-sensitive nociceptors is not relayed to the CNS. The $Na_V1.7$ sodium channel plays an important role in setting the action potential threshold as well as amplifying subthreshold depolarization's to bring these neurons to fire (Dib-Hajj et al. 2013). Genetic ablation of this channel in mice and nonsense mutations in humans lead to a profound loss of pain sensation (Nassar et al. 2004; Cox et al. 2006; Momin and Wood 2008). NGF-dependent heat hyperalgesia is also essentially absent in mice with a sensory neuron-specific deletion of the SCN9A gene encoding $Na_V1.7$ channels (Nassar et al. 2004). Mechanical pain behavior is strongly attenuated in mice lacking $Na_V1.7$ in sensory neurons, which is consistent with a critical role for

this channel in sustaining nociceptor AP propagation (Nassar et al. 2004; Minett et al. 2012). There is, however, as yet only little direct evidence that the primary consequence of $Na_V1.7$ loss is an attenuation of the ability of somatic C-fibers to conduct action potentials (Wilson et al. 2011). For example there are, as yet, no reports in which this issue has been directly addressed using electrophysiological methods in somatic C-fibers; however, shRNA-mediated knockdown of $Na_V1.7$ in the vagus nerve has demonstrated a loss of sustained firing (Muroi et al. 2011). $Na_V1.7$ is an a important channel in olfactory sensory neurons (OSNs) and here it appears to be primarily required for the transfer of sensory information from OSN to second-order neurons in the olfactory bulb (Weiss et al. 2011). This has led to speculation that the primary mechanism leading to the spectacular loss of pain phenotypes in humans lacking $Na_V1.7$ channels is a block of information transfer from primary afferent C-fibers at their central synapses in the dorsal horn (Black et al. 2012; Minett et al. 2012). If the expression or subcellular distribution of $Na_V1.7$ channels is controlled by NGF availability (Gould et al. 2000; Diss et al. 2008), then it is conceivable that anti-NGF drugs work in an $Na_V1.7$-dependent manner.

A key difference between NGF-induced heat and mechanical hyperalgesia is the often radically different times courses that these phenomena display. Pure NGF-dependent hyperalgesia has in the last few years been increasingly studied in human subjects, as the injection of small amounts of NGF into the muscle or skin offers an excellent model for both short- and long-term sensitization, whilst bypassing inflammatory processes. During the first phase I safety trials of recombinant human NGF (rhNGF), it was quickly realized that human subjects experienced local soreness as well as a very long-lasting deep tissue hyperalgesia or myalgia following rhNGF injection (Petty et al. 1994). In this first human study a dose-dependent myalgia and hyperalgesia was observed to last for up to 7 weeks following a single injection. As in animal models, the mechanisms by which NGF produces mechanical hyperalgesia in humans will probably differ between very early phases and later phases following a transient increase in NGF. One early study noted signs of mechanical hyperalgesia within 6 h of an injection of rhNGF into the skin (Dyck et al. 1997). However, later studies using the same approach in humans showed that hyperalgesia, as measured using pressure pain threshold or pinprick sensitivity, first appears after 7 days and peaks 21 days after an intradermal rhNGF injection (Rukwied et al. 2010, 2013; Obreja et al. 2011a; Weinkauf et al. 2012, 2013). This discrepancy could be explained by spillover of injected NGF into underlying muscle tissue in humans, as well as in animal models. Thus, pronounced hyperalgesia has been noted following injection of rhNGF into human muscles or muscle fascia (Svensson et al. 2003, 2008; Andersen et al. 2008; Deising et al. 2012), but this hypersensitivity differs in several important respects from the NGF-induced mechanical hyperalgesia observed in the skin. First, mechanical hyperalgesia is observed within a few hours of the injection and the pressure pain hypersensitivity extends well beyond the area of the initial injection (Svensson et al. 2003, 2008; Andersen et al. 2008; Deising et al. 2012). As a rule, the muscle hypersensitivity following rhNGF injection is also observed to subside within a few

days of the NGF injection, in marked contrast to the very long-lasting hyperalgesia that follows a skin injection. In the skin model the available studies have noted that the mechanical hyperalgesia remains strictly restricted to the area of the initial rhNGF injection (Rukwied et al. 2010; Obreja et al. 2011a; Weinkauf et al. 2012), a strong indicator that a peripheral sensitization process may be involved (Treede et al. 1992; Lewin and Moshourab 2004).

In animal models a systemic injection of NGF provoked mechanical hyperalgesia, which first appears between an hour and several hours after the injection and persists for days (Lewin et al. 1993, 1994; Thompson et al. 1995). One group has, however, claimed to observe mechanical hyperalgesia minutes after the injection (Malik-Hall et al. 2005). As in humans, local skin injection of NGF in rats also provokes a localized mechanical hyperalgesia that persists for days (Mills et al. 2013). It appears that elevated NGF in skeletal muscle can sensitize muscle afferents to mechanical stimuli, but the evidence from human and animal studies suggests that secondary hyperalgesia is a prominent feature of this model, which involves central sensitization (Lewin et al. 1992b; Hoheisel et al. 2007, 2013). The observation that elevated NGF in the skin does not appear to provoke secondary mechanical hyperalgesia suggests that nociceptor sensitization plays a prominent role in this model. In general, it has been remarkably difficult to convincingly demonstrate nociceptor sensitization to mechanical stimuli in a variety of inflammatory models as conflicting results have been published (Andrew and Greenspan 1999; Lewin and Moshourab 2004; Milenkovic et al. 2008; Lennertz et al. 2012), Indeed, initial studies failed to detect prominent mechanical sensitization of nociceptors after acute or long-term NGF exposure (Lewin et al. 1993, 1994; Lewin and Mendell 1994; Obreja et al. 2011b).

The UV-B sunburn model is an interesting system to study peripheral mechanisms of mechanical hyperalgesia, as there is convincing evidence that central mechanisms do not play a prominent role in this model (Bishop et al. 2009, 2010). Recordings from nociceptors innervating UV-B-sensitized skin have demonstrated alterations in their firing rates to suprathreshold mechanical stimulation (Bishop et al. 2010). However, although some fiber types like C-fiber mechanonociceptors lacking noxious heat sensitivity (C-Ms) showed increased suprathreshold responses to intense mechanical stimulation, other fiber types like A-δ mechanonociceptors displayed reduced responses (Bishop et al. 2010). The complex changes in coding properties of different nociceptor subclasses in the UV-B model raise the possibility that mechanical hyperalgesia may be signaled to the spinal circuits by altered patterns of afferent activation dispersed across two or more nociceptor classes. Clear, direct evidence that cutaneous nociceptors are sensitized to mechanical stimuli after exposure to elevated NGF in vivo has been missing, until recently (Hirth et al. 2013). Many nociceptors are polymodal, meaning that they are activated by more than one modality of noxious stimulus, e.g., C-fibers activated by noxious mechanical and heat stimuli are termed C-mechanoheat units (C-MH). Using this classification scheme it is possible to record the following additional types of nociceptors in human skin using microneurography techniques: C-mechanosensitive (C-M), C-mechanosensitive and cold (C-MC), C-mechanosensitive heat and cold (C-MHC), C-mechano-insensitive and

heat-insensitive (C-MiHi), C-mechanosensitive and heat (C-MH), C-fiber heat only (C-H), and finally C-low-threshold mechanoreceptors (C-LT) (Lewin and Moshourab 2004). Broadly, the same types of nociceptors have been recorded in the skin of rats and mice (Lewin and Mendell 1994; Koltzenburg et al. 1997), but there appear to be consistent species differences, particularly in the incidence of each fiber type. In particular, C-MiHi fibers, identified in subhuman primates as mechanically insensitive afferents (MIAs), appear to be rare in rodents (Handwerker et al. 1991; Meyer et al. 1991; Kress et al. 1992; Lewin and Mendell 1994), but are relatively common in human hairy skin (Schmidt et al. 1995; Weidner et al. 1999). Several studies have strongly implicated C-MiHi fibers in peripheral sensitization processes; thus these fibers can rapidly acquire mechanosensitivity when stimulated with strong algogens. Recent studies by Schmelz and colleagues have shown that C-MiHi units are also observed in the skin of the pig, which they have claimed may be a more suitable animal model for human nociceptors (Obreja and Schmelz 2010). One feature of C-MiHi fibers recorded in humans and in pigs is that they display a very strong and prominent activity-dependent slowing of their conduction velocity (Weidner et al. 1999; Obreja et al. 2011b; Hirth et al. 2013). Thus, the higher the firing rate the longer it takes for the action potentials to reach the first spinal synapses. Strikingly, cutaneous NGF elevation in pigs selectively reduced the magnitude of activity-dependent slowing, as well as reducing the number of conduction failures at a moderate stimulation frequency of 2 Hz (Obreja et al. 2011a, b). The authors have named this phenomenon axonal sensitization as it is postulated that reduced slowing and more reliable following of electrical stimuli could underpin mechanical hyperalgesia. Moreover patients experienced more pain when cutaneous electrical stimuli were employed at the height of the hyperalgesia induced by local intradermal injection of rhNGF. The more reliable initiation and propagation of action potentials in nociceptors under these circumstances may be physiologically relevant as electrical stimulation could be seen as analogous to the driving depolarization produced by opening of transduction channels. However, the same authors failed to find very marked signs of nociceptor sensitization to natural mechanical stimuli in initial studies (Obreja et al. 2011a, b). It is clear that the "axonal sensitization" that they observed is probably caused by changes in the distribution or physiological properties of ion channels that regulate conduction. Obvious candidates are $Na_V1.7$ and $Na_V1.8$, which have indeed been implicated as targets of NGF signaling (Fjell et al. 1999; Gould et al. 2000; Fang et al. 2005; Diss et al. 2008). Nevertheless, there are other channels that regulate membrane excitability in nociceptors that could also be targets of NGF in this model, for example, hyperpolarization-activated cyclic nucleotide-gated cation channels like HCN2 (Emery et al. 2011; Mazo et al. 2013).

In a very recent study Hirth and colleagues actually provide good evidence for local nociceptor sensitization that is robust only 21 days after the initial NGF injection in a pig model (Hirth et al. 2013). Essentially, the authors show that at this point a significant and large proportion of formerly C-MiHi fibers are now very sensitive to mechanical stimuli; however, the suprathreshold coding properties of these fibers were not examined. There are a couple of interesting features of these findings, one is that the extremely long period of time it apparently takes before

sensitization of nociceptors is overt following local NGF exposure. Second, why does it takes so long for NGF-mediated signaling to induce an unmasking of mechanosensitivity whereby acute exposure to strong algogens can unsilence C-Mis with a very rapid time course (Schmidt et al. 1995). The human psychophysical data is clear about the fact that acute elevation of NGF in muscle, as opposed to skin, can produce a rapid sensitization, but even here there is little data to indicate why this may be the case. In one study in rats, NGF was injected directly into the muscle and led to an apparent activation of C-fibers afferents in the muscle, but did not lead to an acute sensitization of muscle C-fibers to mechanical stimuli (Hoheisel et al. 2005). In common with the innervation of the viscera (McMahon and Koltzenburg 1990), normal skeletal muscle is innervated by a large number of C-fibers that are insensitive to mechanical stimuli (Jankowski et al. 2013). It is not clear at the present time whether NGF can also lead to unmasking of mechanosensitivity in deep tissue nociceptors such as those innervating skeletal muscle (Fig. 2).

Although sensitization of nociceptors to mechanical stimuli has been observed and studied for many years, the molecular basis of the sensitization process is poorly understood. It has long been thought that one mechanism underlying sensitization may be the induction of excitability changes in nociceptor axons as has been discussed above. However, it is difficult to argue that such a sensitization process should be specific to mechanical stimuli as is often observed. The molecular mechanisms by which nociceptors actually detect mechanical stimuli are only just beginning to be unraveled and it is this transduction process that is likely to be a target for inflammatory factors like NGF. Mechanical stimuli are likely transduced directly at the sensory endings of nociceptors and this process probably involves the direct gating of a mechanosensitive ion channel by force or displacement (Hu et al. 2006). There are enormous technical challenges to overcome before it is possible to make direct recordings of mechanosensitive currents at the endings of nociceptors in situ. However, acutely cultured sensory neurons possess mechanosensitive ion channels that are directly gated by mechanical stimuli (McCarter et al. 1999; Drew et al. 2004, 2007; Hu and Lewin 2006; Lechner et al. 2009; Hu et al. 2010). It is now clear that there are at least two, and maybe three, biophysically distinct mechanosensitive conductances present in sensory neurons (Poole et al. 2011). Mechanosensitive currents in sensory neurons have been classified according to their inactivation kinetics: currents that inactivate very rapidly ($\tau_1 < 5$ ms) are termed rapidly adapting, RA-type; intermediately adapting ($\tau_1 < 50$ ms); and IA-type and slowly adapting (no adaptation during a 230-ms stimulus), SA-type. In the mouse the RA-type currents are sodium selective with a linear current–voltage relation and reversal potential >30 mV (Hu and Lewin 2006; Lechner et al. 2009). The RA-type current was not blocked by ruthinium red, but displays much slowed kinetics in the presence of benzamil, a broad range ENaC/Deg family channel blocker (Hu and Lewin 2006). The slowly adapting current is found exclusively in nociceptors, is a nonselective conductance, and appears much later in the development of sensory neurons (Hu and Lewin 2006; Lechner

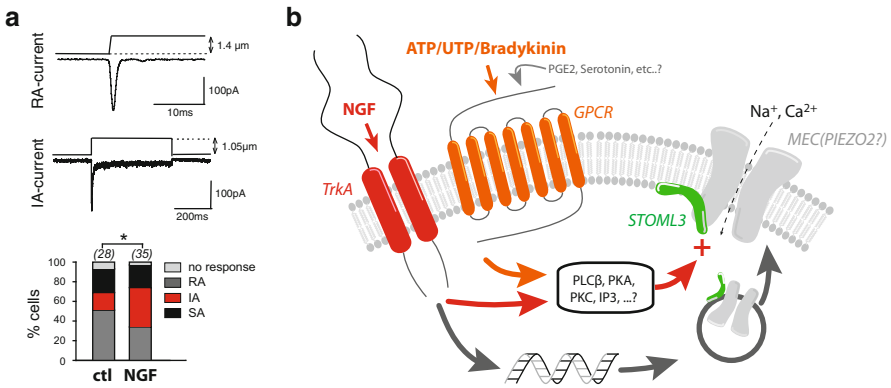

Fig. 3 Sensitization of mechanotransduction currents. (**a**) NGF increases the amplitude and slows the inactivation kinetics of RA- and IA-type currents, reproduced from (Lechner et al. 2009). (**b**) illustrates possible signaling cascades that may underlie the sensitization of mechanotransduction currents. NGF- and bradykinin-induced sensitization requires activation of PKA and PKC (Di Castro et al. 2006). NGF-induced sensitization was further shown to require transcription of new channels (Di Castro et al. 2006)

et al. 2009; Hu et al. 2010). There is now solid evidence that mechanosensitive currents found in cultured sensory neurons are indeed the in vitro counterparts of the transduction current in vivo. Thus manipulations that abolish or reduce the activity of mechanosensitive currents in vitro, such as removal of the essential mechanotransduction protein STOML3 or toxin-mediated block of these channels, also block mechanosensitivity in vivo (Drew et al. 2007; Wetzel et al. 2007; Hu et al. 2010). Agents that sensitize C-fibers in vivo, such as high concentrations of ATP, also rapidly and selectively sensitize the RA- and IA-type currents found in nociceptors (Lechner and Lewin 2009). Thus, within a few seconds of activation of the Gq-coupled $P2Y_2$ receptors by UTP or ATP, the amplitude of RA-type and IA-type currents was elevated and the inactivation time slowed so that each mechanical stimulus evoked a larger charge transfer through transduction channels. This effect leads to a clear increase in mechanically evoked action potential firing both in vitro and in vivo (Lechner and Lewin 2009). The principal sensitization mechanism via $P2Y_2$ receptor activation was to increase the charge transfer by slowing RA- and IA-type current inactivation kinetics; interestingly very similar effects of exposure to NGF have been reported for mechanosensitive currents in sensory neurons (Di Castro et al. 2006; Lechner et al. 2009). However, in contrast to the G-protein-mediated effects of UTP, the NGF effects required several hours to appear to be mediated by protein kinase C and may be due to the insertion of new mechanosensitive channels into the membrane (Di Castro et al. 2006). It is of course difficult to study the detailed molecular mechanism of such effects when the identities of the mechanosensitive channel(s) are unknown (Fig. 3).

Models of mechanotransduction have been very well developed in the *Caenorhabditis elegans* nematode worm model as here most of the molecular

players have been identified using reverse genetic approaches (Lewin and Moshourab 2004; Arnadóttir and Chalfie 2010; Poole et al. 2011; Geffeney and Goodman 2012). Interestingly, in worm touch receptors the ion channel is composed of the MEC-4 and MEC-10 proteins, which are worm orthologs of the acid sensing ion channels (ASICs, all members of the ENaC/Deg family) and the ASIC proteins have also been implicated as regulators of mechanosensitivity in sensory neurons. Thus deletion of the *ASIC2* and *ASIC3* genes, but not the *ASIC1* gene, leads to clear deficits in the mechanosensitivity of cutaneous mechanoreceptors and nociceptors (Price et al. 2000, 2001; Page et al. 2004; Moshourab et al. 2013). However, it appears very unlikely that ASIC subunits are in fact necessary for the formation of a mechanosensitive current in DRG neurons as these appear unaltered following *ASIC* gene deletion (Drew et al. 2004; Lechner et al. 2009). However, another *mec* gene identified in *C. elegans* is the stomatin domain protein MEC-2, which has at least two functional orthologs in mammals, stomatin and STOML3 (stomatin-like protein 3) (Lapatsina et al. 2012a). Both MEC-2 and STOML3 are required for the normal function of mechanotransduction in *C. elegans* and in the mouse, respectively (O'Hagan et al. 2005; Wetzel et al. 2007; Moshourab et al. 2013). Mutant mice lacking the *Stoml3* gene have severe deficits in mechanoreceptor and nociceptor function in that a large proportion of these cutaneous sensory neurons are mechanically insensitive. Indeed a much larger proportion of thinly myelinated nociceptors innervating the hairy skin lack mechanosensitivity in STOML3 mutant mice, a phenotype that is reminiscent of the mechanically insensitive nociceptors identified in normal human and pig skin (Weidner et al. 1999; Hirth et al. 2013). Stomatin-domain proteins like STOML3 and stomatin modulate the proton gating of ASIC2 and ASIC3 proteins and some of the structural motifs of the stomatin domains required for this modulation were recently identified (Price et al. 2004; Brand et al. 2012; Lapatsina et al. 2012b). In this context, it is interesting that deletion of *stomatin* or *stoml3* genes, together with the *Asic3* or *Asic2* genes, leads to a dramatic loss of mechanosensitivity in nociceptors, especially those with thinly myelinated Aδ axons (Moshourab et al. 2013). Although the ASIC proteins probably do not form part of the mechanotransducer, their presence or absence together with stomatin-domain proteins in sensory endings could be a molecular substrate to regulate mechanosensitivity in so-called "silent" nociceptors. The expression of ASIC proteins in sensory neurons is in fact controlled in part by neurotrophin signaling (Mamet et al. 2002; McIlwrath et al. 2005). It has been shown that pro-inflammatory mediators, including NGF, are involved in upregulating ASIC mRNAs and that NGF moderately increases the density of ASIC currents in cultured sensory neurons (Mamet et al. 2002). At the present time, however, it is not clear whether the presence of any of the ASIC proteins in the DRG is required for full-blown NGF-induced hyperalgesia. Acid is itself a potent activator and modulator of muscle nociceptors (Mense 2009), and ASIC3 proteins play a prominent role in muscle hyperalgesia (Sluka et al. 2003). In humans it was recently shown that acid-induced pain is significantly enhanced, even up to 14 days after a single injection of NGF into the muscle fascia of the back. The time course of the enhanced acid pain roughly paralleled the course of the mechanical

hyperalgesia (Deising et al. 2012). The parallel nature of mechanical and acid hypersensitivity in the muscle fascia model could mean that ASIC3, together with stomatin-domain proteins (Moshourab et al. 2013), is involved in regulating the mechanosensitivity of muscle nociceptors, but as yet there is no direct evidence to support this speculation. It is thought that ASIC3 and TRPV1 are the main ion channels that drive nociceptor activation after exposure to physiological tissue acidity observed after inflammation (Smith and Lewin 2009). Recently, we have shown that acid-evoked depolarization via TRPV1 and ASICs is potently counteracted by proton inhibition of Na_Vs, in particular $Na_V1.7$ in nociceptors (Smith et al. 2011). The inhibition of $Na_V1.7$ in nociceptors from naked mole-rats is so potent that it can abolish both the acid-induced activation of nociceptors and the accompanying sensitization of nociceptors to mechanical stimuli (Smith et al. 2011). Since NGF may also regulate $Na_V1.7$, and its presence can put a break on acid nociception, it is conceivable that a cell-specific regulation of this channel might contribute physiological differences between the acid sensitivity of cutaneous and deep tissue nociceptors.

Recently, two proteins were identified as bona fide stretch-activated ion channels, Piezo1 and Piezo2, and are widely expressed in both neuronal and non-neuronal tissues, as well as in sensory neurons (Coste et al. 2010, 2012). RNAi-mediated knockdown of Piezo2 in sensory neurons has implicated this stretch-activated channel as contributing to RA-type mechanosensitive currents (Coste et al. 2010). However, Piezo2 currents are nonselective and when measured in N2a neuroblastoma cells they are blocked by ruthenium red, both features not matching those of native sensory neuron RA currents (Hu and Lewin 2006; Lechner et al. 2009; Coste et al. 2010). Genetic evidence that Piezo1 or 2 are pore-forming mechanotransduction channels in sensory neurons is, however, still lacking.

There is a highly controversial literature on the possible involvement of the mustard oil-activated Trp channel TRPA1 in mechanotransduction (Patel et al. 2010; Nilius et al. 2012). The TRPA1 channel undoubtedly plays an important role governing the chemosensitivity of nociceptive afferents and is required for normal inflammatory pain behaviors in mice (Bautista et al. 2006; Kwan et al. 2006; Macpherson et al. 2007; McNamara et al. 2007). Recent studies have implicated TRPA1 as a contributor to mechanosensitive conductances found in sensory neurons (Vilceanu and Stucky 2010; Brierley et al. 2011); however, although these studies show a diminution of mechanosensitive channel activity, it is very difficult to differentiate between direct and indirect effects of TRPA1 gene deletion or pharmacological blockade. This is especially the case for TRPA1 which is a calcium-permeable ion channel, which itself can also be activated by the elevation of intracellular calcium (Zurborg et al. 2007). Thus, since mechanosensitive channels are calcium permeable it is possible that ion fluxes generated by transducing currents could be rapidly amplified by activating TRPA1 channels (Brierley et al. 2011). Similarly to ASIC proteins there is some evidence that TRPA1 channels are regulated by NGF availability (Malin et al. 2011), and deletion of the TRPA1 gene leads to complex changes in the mechanosensitivity of identified C-fiber afferents innervating the hairy skin (Kwan et al. 2009). There is solid

pharmacological evidence that TRPA1 blockade can prevent the moderate sensitization of C-fibers to suprathreshold mechanical stimulation following complete Freund's adjuvant inflammation (Lennertz et al. 2012). However, it is unclear if the presence of TRPA1 channels is required for NGF-induced mechanical hyperalgesia.

6 Cell Biology of Long-Lasting Sensitization Induced by NGF

The cell biology of DRG sensory neurons is unusual; these neurons accomplish two fundamentally different tasks at their central and peripheral endings that are separated by an enormous distance. Synaptic transmission and precise connectivity are established at the spinal cord end and transduction is accomplished at specialized endings in the periphery. In between, located about two-thirds of the distance between these points is the cell body, which must provide specialized proteins, membranes, and organelles that are sometimes differentially distributed between the peripheral and central branch (García-Añoveros et al. 2001). The retrograde and local signal transduction events initiated by NGF have been studied for decades and it is clear that NGF can exert some effects locally in the periphery and many effects are transported and propagated to the cell body via the so-called signaling endosome (Campenot and MacInnis 2004). However, in the periphery of sensory axons there exists a robust and stable transduction apparatus equipped to transduce mechanical signals in different ways in different sensory subtypes. Indeed, there are now examples of ion channel proteins that are specifically targeted to the peripheral endings of specific mechanoreceptor types, e.g., the potassium channel KCNQ4 in rapidly adapting mechanoreceptors (Heidenreich et al. 2012). How is this exquisite spatial and functional segregation achieved? The transport of proteins involved in the transduction and transformation of sensory signals at the peripheral endings of sensory neurons is very poorly understood, but represents a clear potential target for NGF modulation of afferent mechanosensitivity. Since STOML3 is the only protein known to participate directly in fast mechanotransduction it was of interest to examine how this membrane protein is trafficked within sensory neurons. We found that STOML3 is localized to a highly mobile and molecularly distinct transport vesicle within cultured sensory neuron axons (Lapatsina et al. 2012b). These vesicles are capable of co-transporting the related stomatin-domain protein, stomatin, together with each of the ASIC family members found in the DRG. Members of the Rab GTPase family of protein are involved in controlling the organization and identity of different membranous compartments within cells and neurons. For example, the Rab5 and Rab7 proteins are localized to signaling endosomes that are thought to retrogradely transport neurotrophin signals from the periphery to the cell body (Deinhardt et al. 2006). Interestingly, the STOML3 containing vesicles are not part of the signaling endosome pool as they are Rab5 negative, but are Rab11 positive. Rab11-positive vesicles have been characterized as composing a slowly recycling endocytic compartment and may be transported predominantly anterogradely in sensory neurons (Ascaño et al. 2009;

Eva et al. 2010). Indeed gain- or loss-of-function Rab11 mutants radically change vesicle behavior, but these compartments still contain STOML3 (Lapatsina et al. 2012b). The STOML3 vesicle is obviously enriched in proteins that are destined to function in transduction at the peripheral endings of sensory neurons and so we have proposed to name these vesicles "transducosomes." Indeed uncoupling of the "transducosome" from microtubules leads to rapid incorporation into the plasma membrane with an accompanying increase in acid-gated currents (Lapatsina et al. 2012b). Ex vivo recordings from sensory afferents innervating the skin have demonstrated that transduction of mechanical stimuli at the peripheral endings of sensory neurons is very stable for many hours in the absence of a connection to the cell body. Indeed early nerve injury experiments provided evidence that anterogradely transported proteins are first incorporated into cut endings to confer mechanosensitivity at a speed which is consistent with their transport distally via fast axonal transport (Koschorke et al. 1994). The stability of the transduction complexes at sensory endings is likely to be a function of three main factors: the number of "transducosomes" that arrive per unit of time, the propensity of such vesicles to fuse with the membrane and deliver functional transduction proteins, and finally the stability of existing transduction complexes. If this model is correct it is obvious that the ability of a sensory neuron to become sensitized to mechanical stimuli or indeed to become newly mechanically sensitive can be regulated at the levels of vesicle transport, fusion, or endocytosis of or recovery of spent transduction complexes. It is clear from the time course of fast mechanical hyperalgesia (hours) that local action of NGF might regulate the steps outlined above, but the molecular details are still completely unclear. Long-lasting mechanical hyperalgesia could be sustained by signals that are carried by signaling endosomes to initiate a cell body response, which may or may not include new gene expression, but would change the transduction process via the transport of novel, perhaps modulatory, subunits to the mechanotransducer. We recently identified a large extracellular tether protein that appears to be required for efficient and fast transduction in mechanoreceptors and many nociceptors (Hu et al. 2010). It is obvious that the transport of this protein could provide a way to "unsilence" nociceptors, but this hypothesis can only be tested once the identity of this protein is known.

7 The NGF Nexus of Pain

It is clear that NGF elevation that accompanies inflammation initiates a complex series of events, some of which are local and fast and others are global and long lasting. Anti-NGF therapy is remarkably effective in a broad variety of pain conditions ranging from muscle pain to bone cancer pain (Mantyh et al. 2010, 2011; Jimenez-Andrade et al. 2011). This remarkable efficacy of anti-NGF probably arises through the broad range of molecular events that are set into motion by elevated NGF levels in a variety of different tissues. In this review we have concentrated on molecular targets in the sensory innervation of skin and skeletal

muscle, but there is now abundant evidence that NGF may influence post-inflammatory events in other deep tissues. It follows that the diverse molecular changes initiated by NGF all serve to promote hyperalgesia, and we have discussed many individual examples in this review. Although both heat and mechanical hyperalgesia may be sustained, at least in part by synaptic changes in the spinal cord, there is increasing evidence that peripheral mechanisms that are very long lasting could also be specific targets of NGF signaling. For example, sensory mechanotransduction itself may be controlled by NGF signaling in a cell-specific manner. The molecular dissection of such effects will depend on identifying more of the key molecular players in mechanotransduction. It should also be noted that more knowledge on the downstream targets of NGF could eventually lead to the development of next generation pharmaceuticals that target these downstream players directly without the need to alter NGF availability.

Acknowledgments The authors' work was supported by grants from the Deutsche Forschungsgemeinshaft collaborative research centers 665 and 958 and a senior European Research Council grant. EStJS was supported by fellowship from the Alexander von Humboldt foundation.

References

Akopian AN, Souslova V, England S, Okuse K, Ogata N, Ure J, Smith A, Kerr BJ, McMahon SB, Boyce S, Hill R, Stanfa LC, Dickenson AH, Wood JN (1999) The tetrodotoxin-resistant sodium channel SNS has a specialized function in pain pathways. Nat Neurosci 2:541–548

Albers KM, Woodbury CJ, Ritter AM, Davis BM, Koerber HR (2006) Glial cell-line-derived neurotrophic factor expression in skin alters the mechanical sensitivity of cutaneous nociceptors. J Neurosci 26:2981–2990

Andersen H, Arendt-Nielsen L, Svensson P, Danneskiold-Samsøe B, Graven-Nielsen T (2008) Spatial and temporal aspects of muscle hyperalgesia induced by nerve growth factor in humans. Exp Brain Res 191:371–382

Andreev NY, Dimitrieva N, Koltzenburg M, McMahon SB (1995) Peripheral administration of nerve growth factor in the adult rat produces a thermal hyperalgesia that requires the presence of sympathetic post-ganglionic neurones. Pain 63:109–115

Andrew D, Greenspan JD (1999) Mechanical and heat sensitization of cutaneous nociceptors after peripheral inflammation in the rat. J Neurophysiol 82:2649–2656

Apfel SC, Wright DE, Wiideman AM, Dormia C, Snider WD, Kessler JA (1996) Nerve growth factor regulates the expression of brain-derived neurotrophic factor mRNA in the peripheral nervous system. Mol Cell Neurosci 7:134–142

Arnadóttir J, Chalfie M (2010) Eukaryotic mechanosensitive channels. Annu Rev Biophys 39: 111–137

Ascaño M, Richmond A, Borden P, Kuruvilla R (2009) Axonal targeting of Trk receptors via transcytosis regulates sensitivity to neurotrophin responses. J Neurosci 29:11674–11685

Balkowiec A, Katz DM (2000) Activity-dependent release of endogenous brain-derived neurotrophic factor from primary sensory neurons detected by ELISA in situ. J Neurosci 20: 7417–7423

Baloh RH, Enomoto H, Johnson EM Jr, Milbrandt J (2000) The GDNF family ligands and receptors – implications for neural development. Curr Opin Neurobiol 10:103–110

Bautista DM, Jordt S-E, Nikai T, Tsuruda PR, Read AJ, Poblete J, Yamoah EN, Basbaum AI, Julius D (2006) TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents. Cell 124:1269–1282

Bergmann I, Reiter R, Toyka KV, Koltzenburg M (1998) Nerve growth factor evokes hyperalgesia in mice lacking the low-affinity neurotrophin receptor p75. Neurosci Lett 255:87–90
Bespalov MM, Saarma M (2007) GDNF family receptor complexes are emerging drug targets. Trends Pharmacol Sci 28:68–74
Bishop T, Ballard A, Holmes H, Young AR, McMahon SB (2009) Ultraviolet-B induced inflammation of human skin: characterisation and comparison with traditional models of hyperalgesia. Eur J Pain 13:524–532
Bishop T, Marchand F, Young AR, Lewin GR, McMahon SB (2010) Ultraviolet-B-induced mechanical hyperalgesia: a role for peripheral sensitisation. Pain 150:141–152
Black JA, Frézel N, Dib-Hajj SD, Waxman SG (2012) Expression of Nav1.7 in DRG neurons extends from peripheral terminals in the skin to central preterminal branches and terminals in the dorsal horn. Mol Pain 8:82
Blair NT, Bean BP (2002) Roles of tetrodotoxin (TTX)-sensitive Na+ current, TTX-resistant Na+ current, and Ca2+ current in the action potentials of nociceptive sensory neurons. J Neurosci 22:10277–10290
Bonnington JK, McNaughton PA (2003) Signalling pathways involved in the sensitisation of mouse nociceptive neurones by nerve growth factor. J Physiol 551:433–446
Brand J, Smith ESJ, Schwefel D, Lapatsina L, Poole K, Omerbašić D, Kozlenkov A, Behlke J, Lewin GR, Daumke O (2012) A stomatin dimer modulates the activity of acid-sensing ion channels. EMBO J 31:3635–3646
Brierley SM, Castro J, Harrington AM, Hughes PA, Page AJ, Rychkov GY, Blackshaw LA (2011) TRPA1 contributes to specific mechanically activated currents and sensory neuron mechanical hypersensitivity. J Physiol 589:3575–3593
Brock JA, McLachlan EM, Belmonte C (1998) Tetrodotoxin-resistant impulses in single nociceptor nerve terminals in guinea-pig cornea. J Physiol 512(Pt 1):211–217
Brown MT, Murphy FT, Radin DM, Davignon I, Smith MD, West CR (2012) Tanezumab reduces osteoarthritic knee pain: results of a randomized, double-blind, placebo-controlled phase III trial. J Pain 13:790–798
Brown MT, Murphy FT, Radin DM, Davignon I, Smith MD, West CR (2013) Tanezumab reduces osteoarthritic hip pain: results of a randomized, double-blind, placebo-controlled phase 3 trial. Arthritis Rheum 65(7):1795–1803
Bueker ED, Hilderman HL (1953) Growth-stimulating effects of mouse sarcomas I, 37, and 180 on spinal and sympathetic ganglia of chick embryos as contrasted with effects of other tumors. Cancer 6:397–415
Campenot RB, MacInnis BL (2004) Retrograde transport of neurotrophins: fact and function. J Neurobiol 58:217–229
Cao YQ, Mantyh PW, Carlson EJ, Gillespie A-M, Epstein CJ, Basbaum AI (1998) Primary afferent tachykinins are required to experience moderate to intense pain. Nature 392:390–394
Cao E, Cordero-Morales JF, Liu B, Qin F, Julius D (2013) TRPV1 channels are intrinsically heat sensitive and negatively regulated by phosphoinositide lipids. Neuron 77:667–679
Carroll P, Lewin GR, Koltzenburg M, Toyka KV, Thoenen H (1998) A role for BDNF in mechanosensation. Nat Neurosci 1:42–46
Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D (1997) The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389:816–824
Cattaneo A (2010) Tanezumab, a recombinant humanized mAb against nerve growth factor for the treatment of acute and chronic pain. Curr Opin Mol Ther 12:94–106
Cesare P, McNaughton P (1996) A novel heat-activated current in nociceptive neurons and its sensitization by bradykinin. Proc Natl Acad Sci USA 93:15435–15439
Cesare P, Dekker LV, Sardini A, Parker PJ, McNaughton PA (1999) Specific involvement of PKC-epsilon in sensitization of the neuronal response to painful heat. Neuron 23:617–624
Cho H, Yang YD, Lee J, Lee B, Kim T, Jang Y, Back SK, Na HS, Harfe BD, Wang F, Raouf R, Wood JN, Oh U (2012) The calcium-activated chloride channel anoctamin 1 acts as a heat sensor in nociceptive neurons. Nat Neurosci 15:1015–1021

Chuang HH, Prescott ED, Kong H, Shields S, Jordt SE, Basbaum AI, Chao MV, Julius D (2001) Bradykinin and nerve growth factor release the capsaicin receptor from PtdIns(4,5)P2-mediated inhibition. Nature 411:957–962

Cohen S (1960) Purification of a nerve-growth promoting protein from the mouse salivary gland and its neuro-cytotoxic antiserum*. Proc Natl Acad Sci USA 46:302–311

Cohen S (2008) Origins of growth factors: NGF and EGF. J Biol Chem 283:33793–33797

Cook AJ, Woolf CJ, Wall PD, McMahon SB (1987) Dynamic receptive field plasticity in rat spinal cord dorsal horn following C-primary afferent input. Nature 325:151–153

Coste B, Mathur J, Schmidt M, Earley TJ, Ranade S, Petrus MJ, Dubin AE, Patapoutian A (2010) Piezo1 and Piezo2 are essential components of distinct mechanically activated cation channels. Science 330:55–60

Coste B, Xiao B, Santos JS, Syeda R, Grandl J, Spencer KS, Kim SE, Schmidt M, Mathur J, Dubin AE, Montal M, Patapoutian A (2012) Piezo proteins are pore-forming subunits of mechanically activated channels. Nature 483:176–181

Coull JAM, Beggs S, Boudreau D, Boivin D, Tsuda M, Inoue K, Gravel C, Salter MW, De Koninck Y (2005) BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. Nature 438:1017–1021

Cox JJ, Reimann F, Nicholas AK, Thornton G, Roberts E, Springell K, Karbani G, Jafri H, Mannan J, Raashid Y, Al-Gazali L, Hamamy H, Valente EM, Gorman S, Williams R, McHale DP, Wood JN, Gribble FM, Woods CG (2006) An SCN9A channelopathy causes congenital inability to experience pain. Nature 444:894–898

Crowley C, Spencer SD, Nishimura MC, Chen KS, Pitts-Meek S, Armanini MP, Ling LH, McMahon SB, Shelton DL, Levinson AD (1994) Mice lacking nerve growth factor display perinatal loss of sensory and sympathetic neurons yet develop basal forebrain cholinergic neurons. Cell 76:1001–1011

Deinhardt K, Salinas S, Verastegui C, Watson R, Worth D, Hanrahan S, Bucci C, Schiavo G (2006) Rab5 and Rab7 control endocytic sorting along the axonal retrograde transport pathway. Neuron 52:293–305

Deising S, Weinkauf B, Blunk J, Obreja O, Schmelz M, Rukwied R (2012) NGF-evoked sensitization of muscle fascia nociceptors in humans. Pain 153:1673–1679

Di Castro A, Drew LJ, Wood JN, Cesare P (2006) Modulation of sensory neuron mechanotransduction by PKC- and nerve growth factor-dependent pathways. Proc Natl Acad Sci USA 103:4699–4704

Dib-Hajj SD, Yang Y, Black JA, Waxman SG (2013) The Na(V)1.7 sodium channel: from molecule to man. Nat Rev Neurosci 14:49–62

Diss JKJ, Calissano M, Gascoyne D, Djamgoz MBA, Latchman DS (2008) Identification and characterization of the promoter region of the Nav1.7 voltage-gated sodium channel gene (SCN9A). Mol Cell Neurosci 37:537–547

Donnerer J, Schuligoi R, Stein C (1992) Increased content and transport of substance P and calcitonin gene-related peptide in sensory nerves innervating inflamed tissue: evidence for a regulatory function of nerve growth factor in vivo. Neuroscience 49:693–698

Drew LJ, Rohrer DK, Price MP, Blaver KE, Cockayne DA, Cesare P, Wood JN (2004) Acid-sensing ion channels ASIC2 and ASIC3 do not contribute to mechanically activated currents in mammalian sensory neurones. J Physiol 556:691–710

Drew LJ, Rugiero F, Cesare P, Gale JE, Abrahamsen B, Bowden S, Heinzmann S, Robinson M, Brust A, Colless B, Lewis RJ, Wood JN (2007) High-threshold mechanosensitive ion channels blocked by a novel conopeptide mediate pressure-evoked pain. PLoS One 2:e515

Dyck PJ, Peroutka S, Rask C, Burton E, Baker MK, Lehman KA, Gillen DA, Hokanson JL, O'Brien PC (1997) Intradermal recombinant human nerve growth factor induces pressure allodynia and lowered heat-pain threshold in humans. Neurology 48:501–505

Emery EC, Young GT, Berrocoso EM, Chen L, McNaughton PA (2011) HCN2 ion channels play a central role in inflammatory and neuropathic pain. Science 333:1462–1466

England S, Bevan S, Docherty RJ (1996) PGE2 modulates the tetrodotoxin-resistant sodium current in neonatal rat dorsal root ganglion neurones via the cyclic AMP-protein kinase A cascade. J Physiol 495(Pt 2):429–440

Ernfors P, Wetmore C, Olson L, Persson H (1990) Identification of cells in rat brain and peripheral tissues expressing mRNA for members of the nerve growth factor family. Neuron 5:511–526

Eva R, Dassie E, Caswell PT, Dick G, Ffrench-Constant C, Norman JC, Fawcett JW (2010) Rab11 and its effector Rab coupling protein contribute to the trafficking of beta 1 integrins during axon growth in adult dorsal root ganglion neurons and PC12 cells. J Neurosci 30:11654–11669

Evans RJ, Moldwin RM, Cossons N, Darekar A, Mills IW, Scholfield D (2011) Proof of concept trial of tanezumab for the treatment of symptoms associated with interstitial cystitis. J Urol 185:1716–1721

Fang X, Djouhri L, McMullan S, Berry C, Okuse K, Waxman SG, Lawson SN (2005) TrkA is expressed in nociceptive neurons and influences electrophysiological properties via Nav1.8 Expression in rapidly conducting nociceptors. J Neurosci 25:4868–4878

Fjell J, Cummins TR, Fried K, Black JA, Waxman SG (1999) In vivo NGF deprivation reduces SNS expression and TTX-R sodium currents in IB4-negative DRG neurons. J Neurophysiol 81:803–810

García-Añoveros J, Samad TA, Zuvela-Jelaska L, Woolf CJ, Corey DP (2001) Transport and localization of the DEG/ENaC ion channel BNaC1alpha to peripheral mechanosensory terminals of dorsal root ganglia neurons. J Neurosci 21:2678–2686

Garraway SM, Petruska JC, Mendell LM (2003) BDNF sensitizes the response of lamina II neurons to high threshold primary afferent inputs. Eur J Neurosci 18:2467–2476

Geffeney SL, Goodman MB (2012) How we feel: ion channel partnerships that detect mechanical inputs and give rise to touch and pain perception. Neuron 74:609–619

Gold MS, Reichling DB, Shuster MJ, Levine JD (1996) Hyperalgesic agents increase a tetrodotoxin-resistant Na+ current in nociceptors. Proc Natl Acad Sci USA 93:1108–1112

Gould HJ 3rd, Gould TN, England JD, Paul D, Liu ZP, Levinson SR (2000) A possible role for nerve growth factor in the augmentation of sodium channels in models of chronic pain. Brain Res 854:19–29

Hamburger V (1993) The history of the discovery of the nerve growth factor. J Neurobiol 24: 893–897

Handwerker HO, Kilo S, Reeh PW (1991) Unresponsive afferent nerve fibres in the sural nerve of the rat. J Physiol 435:229–242

Heidenreich M, Lechner SG, Vardanyan V, Wetzel C, Cremers CW, De Leenheer EM, Aránguez G, Moreno-Pelayo M, Jentsch TJ, Lewin GR (2012) KCNQ4 K(+) channels tune mechanoreceptors for normal touch sensation in mouse and man. Nat Neurosci 15:138–145

Heppenstall PA, Lewin GR (2000) Neurotrophins, nociceptors and pain. Curr Opin Anaesthesiol 13:573–576

Heppenstall PA, Lewin GR (2001) BDNF but not NT-4 is required for normal flexion reflex plasticity and function. Proc Natl Acad Sci USA 98:8107–8112

Hirth M, Rukwied R, Gromann A, Turnquist B, Weinkauf B, Francke K, Albrecht P, Rice F, Hägglöf B, Ringkamp M, Engelhardt M, Schultz C, Schmelz M, Obreja O (2013) Nerve growth factor induces sensitization of nociceptors without evidence for increased intraepidermal nerve fiber density. Pain 154(11):2500–2511

Hjerling-Leffler J, Alqatari M, Ernfors P, Koltzenburg M (2007) Emergence of functional sensory subtypes as defined by transient receptor potential channel expression. J Neurosci 27:2435–2443

Hoheisel U, Unger T, Mense S (2005) Excitatory and modulatory effects of inflammatory cytokines and neurotrophins on mechanosensitive group IV muscle afferents in the rat. Pain 114:168–176

Hoheisel U, Unger T, Mense S (2007) Sensitization of rat dorsal horn neurons by NGF-induced subthreshold potentials and low-frequency activation. A study employing intracellular recordings in vivo. Brain Res 1169:34–43

Hoheisel U, Reuter R, de Freitas MF, Treede R-D, Mense S (2013) Injection of nerve growth factor into a low back muscle induces long-lasting latent hypersensitivity in rat dorsal horn neurons. Pain 154(10):1953–1960

Hu J, Lewin GR (2006) Mechanosensitive currents in the neurites of cultured mouse sensory neurones. J Physiol 577:815–828

Hu J, Milenkovic N, Lewin GR (2006) The high threshold mechanotransducer: a status report. Pain 120:3–7

Hu J, Chiang L-Y, Koch M, Lewin GR (2010) Evidence for a protein tether involved in somatic touch. EMBO J 29:855–867

Jankowski MP, Rau KK, Ekmann KM, Anderson CE, Koerber HR (2013) Comprehensive phenotyping of group III and IV muscle afferents in mouse. J Neurophysiol 109:2374–2381

Ji R-R, Samad TA, Jin S-X, Schmoll R, Woolf CJ (2002) p38 MAPK activation by NGF in primary sensory neurons after inflammation increases TRPV1 levels and maintains heat hyperalgesia. Neuron 36:57–68

Jimenez-Andrade JM, Ghilardi JR, Castañeda-Corral G, Kuskowski MA, Mantyh PW (2011) Preventive or late administration of anti-NGF therapy attenuates tumor-induced nerve sprouting, neuroma formation, and cancer pain. Pain 152:2564–2574

Kerr BJ, Bradbury EJ, Bennett DLH, Trivedi PM, Dassan P, French J, Shelton DB, McMahon SB, Thompson SWN (1999) Brain-derived neurotrophic factor modulates nociceptive sensory inputs and NMDA-evoked responses in the rat spinal cord. J Neurosci 19:5138–5148

Kerr BJ, Souslova V, McMahon SB, Wood JN (2001) A role for the TTX-resistant sodium channel Nav 1.8 in NGF-induced hyperalgesia, but not neuropathic pain. Neuroreport 12:3077–3080

Koerber HR, McIlwrath SL, Lawson JJ, Malin SA, Anderson CE, Jankowski MP, Davis BM (2010) Cutaneous C-polymodal fibers lacking TRPV1 are sensitized to heat following inflammation, but fail to drive heat hyperalgesia in the absence of TPV1 containing C-heat fibers. Mol Pain 6:58

Koltzenburg M, Stucky CL, Lewin GR (1997) Receptive properties of mouse sensory neurons innervating hairy skin. J Neurophysiol 78:1841–1850

Koltzenburg M, Bennett DL, Shelton DL, McMahon SB (1999) Neutralization of endogenous NGF prevents the sensitization of nociceptors supplying inflamed skin. Eur J Neurosci 11:1698–1704

Korsching S, Thoenen H (1983) Nerve growth factor in sympathetic ganglia and corresponding target organs of the rat: correlation with density of sympathetic innervation. Proc Natl Acad Sci USA 80:3513–3516

Koschorke GM, Meyer RA, Campbell JN (1994) Cellular components necessary for mechano-electrical transduction are conveyed to primary afferent terminals by fast axonal transport. Brain Res 641:99–104

Kress M, Koltzenburg M, Reeh PW, Handwerker HO (1992) Responsiveness and functional attributes of electrically localized terminals of cutaneous C-fibers in vivo and in vitro. J Neurophysiol 68:581–595

Kwan KY, Allchorne AJ, Vollrath MA, Christensen AP, Zhang D-S, Woolf CJ, Corey DP (2006) TRPA1 contributes to cold, mechanical, and chemical nociception but is not essential for hair-cell transduction. Neuron 50:277–289

Kwan KY, Glazer JM, Corey DP, Rice FL, Stucky CL (2009) TRPA1 modulates mechanotransduction in cutaneous sensory neurons. J Neurosci 29:4808–4819

LaMotte RH, Shain CN, Simone DA, Tsai EF (1991) Neurogenic hyperalgesia: psychophysical studies of underlying mechanisms. J Neurophysiol 66:190–211

Lane NE, Schnitzer TJ, Birbara CA, Mokhtarani M, Shelton DL, Smith MD, Brown MT (2010) Tanezumab for the treatment of pain from osteoarthritis of the knee. N Engl J Med 363: 1521–1531

Lapatsina L, Brand J, Poole K, Daumke O, Lewin GR (2012a) Stomatin-domain proteins. Eur J Cell Biol 91:240–245

Lapatsina L, Jira JA, Smith ESJ, Poole K, Kozlenkov A, Bilbao D, Lewin GR, Heppenstall PA (2012b) Regulation of ASIC channels by a stomatin/STOML3 complex located in a mobile vesicle pool in sensory neurons. Open Biol 2:120096

Lechner SG, Lewin GR (2009) Peripheral sensitisation of nociceptors via G-protein-dependent potentiation of mechanotransduction currents. J Physiol 587:3493–3503

Lechner SG, Frenzel H, Wang R, Lewin GR (2009) Developmental waves of mechanosensitivity acquisition in sensory neuron subtypes during embryonic development. EMBO J 28:1479–1491

Lennertz RC, Kossyreva EA, Smith AK, Stucky CL (2012) TRPA1 mediates mechanical sensitization in nociceptors during inflammation. PLoS One 7:e43597

Lever IJ, Bradbury EJ, Cunningham JR, Adelson DW, Jones MG, McMahon SB, Marvizón JC, Malcangio M (2001) Brain-derived neurotrophic factor is released in the dorsal horn by distinctive patterns of afferent fiber stimulation. J Neurosci 21:4469–4477

Levi-Montalcini R, Booker B (1960) Destruction of the sympathetic ganglia in mammals by an antiserum to a nerve-growth protein. Proc Natl Acad Sci USA 46:384–391

Lewin GR, Barde YA (1996) Physiology of the neurotrophins. Annu Rev Neurosci 19:289–317

Lewin GR, McMahon SB (1991) Dorsal horn plasticity following re-routeing of peripheral nerves: evidence for tissue-specific neurotrophic influences from the periphery. Eur J Neurosci 3: 1112–1122

Lewin GR, Mendell LM (1993) Nerve growth factor and nociception. Trends Neurosci 16:353–359

Lewin GR, Mendell LM (1994) Regulation of cutaneous C-fiber heat nociceptors by nerve growth factor in the developing rat. J Neurophysiol 71:941–949

Lewin GR, Moshourab R (2004) Mechanosensation and pain. J Neurobiol 61:30–44

Lewin GR, Ritter AM, Mendell LM (1992a) On the role of nerve growth factor in the development of myelinated nociceptors. J Neurosci 12:1896–1905

Lewin GR, Winter J, McMahon SB (1992b) Regulation of afferent connectivity in the adult spinal cord by nerve growth factor. Eur J Neurosci 4:700–707

Lewin GR, Ritter AM, Mendell LM (1993) Nerve growth factor-induced hyperalgesia in the neonatal and adult rat. J Neurosci 13:2136–2148

Lewin GR, Rueff A, Mendell LM (1994) Peripheral and central mechanisms of NGF-induced hyperalgesia. Eur J Neurosci 6:1903–1912

Lindsay RM, Harmar AJ (1989) Nerve growth factor regulates expression of neuropeptide genes in adult sensory neurons. Nature 337:362–364

Lohof AM, Ip NY, Poo MM (1993) Potentiation of developing neuromuscular synapses by the neurotrophins NT-3 and BDNF. Nature 363:350–353

Luo W, Wickramasinghe SR, Savitt JM, Griffin JW, Dawson TM, Ginty DD (2007) A hierarchical NGF signaling cascade controls Ret-dependent and Ret-independent events during development of nonpeptidergic DRG neurons. Neuron 54:739–754

Macpherson LJ, Xiao B, Kwan KY, Petrus MJ, Dubin AE, Hwang S, Cravatt B, Corey DP, Patapoutian A (2007) An ion channel essential for sensing chemical damage. J Neurosci 27: 11412–11415

MacQueen GM, Ramakrishnan K, Croll SD, Siuciak JA, Yu G, Young LT, Fahnestock M (2001) Performance of heterozygous brain-derived neurotrophic factor knockout mice on behavioral analogues of anxiety, nociception, and depression. Behav Neurosci 115:1145–1153

Malik-Hall M, Dina OA, Levine JD (2005) Primary afferent nociceptor mechanisms mediating NGF-induced mechanical hyperalgesia. Eur J Neurosci 21:3387–3394

Malin SA, Molliver DC, Koerber HR, Cornuet P, Frye R, Albers KM, Davis BM (2006) Glial cell line-derived neurotrophic factor family members sensitize nociceptors in vitro and produce thermal hyperalgesia in vivo. J Neurosci 26:8588–8599

Malin S, Molliver D, Christianson JA, Schwartz ES, Cornuet P, Albers KM, Davis BM (2011) TRPV1 and TRPA1 function and modulation are target tissue dependent. J Neurosci 31:10516–10528

Mamet J, Baron A, Lazdunski M, Voilley N (2002) Proinflammatory mediators, stimulators of sensory neuron excitability via the expression of acid-sensing ion channels. J Neurosci 22: 10662–10670

Mantyh WG, Jimenez-Andrade JM, Stake JI, Bloom AP, Kaczmarska MJ, Taylor RN, Freeman KT, Ghilardi JR, Kuskowski MA, Mantyh PW (2010) Blockade of nerve sprouting and neuroma formation markedly attenuates the development of late stage cancer pain. Neuroscience 171: 588–598

Mantyh PW, Koltzenburg M, Mendell LM, Tive L, Shelton DL (2011) Antagonism of nerve growth factor-TrkA signaling and the relief of pain. Anesthesiology 115:189–204

Marmigère F, Ernfors P (2007) Specification and connectivity of neuronal subtypes in the sensory lineage. Nat Rev Neurosci 8:114–127

Mazo I, Rivera-Arconada I, Roza C (2013) Axotomy-induced changes in activity-dependent slowing in peripheral nerve fibres: role of hyperpolarization-activated/HCN channel current. Eur J Pain 17:1281–1290

Mazurek N, Weskamp G, Erne P, Otten U (1986) Nerve growth factor induces mast cell degranulation without changing intracellular calcium levels. FEBS Lett 198:315–320

McCarter GC, Reichling DB, Levine JD (1999) Mechanical transduction by rat dorsal root ganglion neurons in vitro. Neurosci Lett 273:179–182

McIlwrath SL, Hu J, Anirudhan G, Shin J-B, Lewin GR (2005) The sensory mechanotransduction ion channel ASIC2 (acid sensitive ion channel 2) is regulated by neurotrophin availability. Neuroscience 131:499–511

McMahon SB, Gibson S (1987) Peptide expression is altered when afferent nerves reinnervate inappropriate tissue. Neurosci Lett 73:9–15

McMahon SB, Koltzenburg M (1990) Novel classes of nociceptors: beyond Sherrington. Trends Neurosci 13:199–201

McMahon SB, Wall PD (1984) Receptive fields of rat lamina 1 projection cells move to incorporate a nearby region of injury. Pain 19:235–247

McMahon SB, Lewin GR, Anand P, Ghatei MA, Bloom SR (1989) Quantitative analysis of peptide levels and neurogenic extravasation following regeneration of afferents to appropriate and inappropriate targets. Neuroscience 33:67–73

McMahon SB, Lewin GR, Wall PD (1993) Central hyperexcitability triggered by noxious inputs. Curr Opin Neurobiol 3:602–610

McMahon SB, Bennett DL, Priestley JV, Shelton DL (1995) The biological effects of endogenous nerve growth factor on adult sensory neurons revealed by a trkA-IgG fusion molecule. Nat Med 1:774–780

McNamara CR, Mandel-Brehm J, Bautista DM, Siemens J, Deranian KL, Zhao M, Hayward NJ, Chong JA, Julius D, Moran MM, Fanger CM (2007) TRPA1 mediates formalin-induced pain. Proc Natl Acad Sci USA 104:13525–13530

Mendell LM, Arvanian VL (2002) Diversity of neurotrophin action in the postnatal spinal cord. Brain Res Brain Res Rev 40:230–239

Mense S (2009) Algesic agents exciting muscle nociceptors. Exp Brain Res 196:89–100

Meyer RA, Davis KD, Cohen RH, Treede RD, Campbell JN (1991) Mechanically insensitive afferents (MIAs) in cutaneous nerves of monkey. Brain Res 561:252–261

Michael GJ, Averill S, Nitkunan A, Rattray M, Bennett DL, Yan Q, Priestley JV (1997) Nerve growth factor treatment increases brain-derived neurotrophic factor selectively in TrkA-expressing dorsal root ganglion cells and in their central terminations within the spinal cord. J Neurosci 17:8476–8490

Milenkovic N, Frahm C, Gassmann M, Griffel C, Erdmann B, Birchmeier C, Lewin GR, Garratt AN (2007) Nociceptive tuning by stem cell factor/c-Kit signaling. Neuron 56:893–906

Milenkovic N, Wetzel C, Moshourab R, Lewin GR (2008) Speed and temperature dependences of mechanotransduction in afferent fibers recorded from the mouse saphenous nerve. J Neurophysiol 100:2771–2783

Mills CD, Nguyen T, Tanga FY, Zhong C, Gauvin DM, Mikusa J, Gomez EJ, Salyers AK, Bannon AW (2013) Characterization of nerve growth factor-induced mechanical and thermal hypersensitivity in rats. Eur J Pain 17:469–479

Minett MS, Nassar MA, Clark AK, Passmore G, Dickenson AH, Wang F, Malcangio M, Wood JN (2012) Distinct Nav1.7-dependent pain sensations require different sets of sensory and sympathetic neurons. Nat Commun 3:791

Molliver DC, Wright DE, Leitner ML, Parsadanian AS, Doster K, Wen D, Yan Q, Snider WD (1997) IB4-binding DRG neurons switch from NGF to GDNF dependence in early postnatal life. Neuron 19:849–861

Momin A, Wood JN (2008) Sensory neuron voltage-gated sodium channels as analgesic drug targets. Curr Opin Neurobiol 18:383–388

Moshourab RA, Wetzel C, Martinez-Salgado C, Lewin GR (2013) Stomatin-domain protein interactions with acid sensing ion channels modulate nociceptor mechanosensitivity. J Physiol 591:5555–5574

Muroi Y, Ru F, Kollarik M, Canning BJ, Hughes SA, Walsh S, Sigg M, Carr MJ, Undem BJ (2011) Selective silencing of NaV1.7 decreases excitability and conduction in vagal sensory neurons. J Physiol 589:5663–5676

Nassar MA, Stirling LC, Forlani G, Baker MD, Matthews EA, Dickenson AH, Wood JN (2004) Nociceptor-specific gene deletion reveals a major role for Nav1.7 (PN1) in acute and inflammatory pain. Proc Natl Acad Sci USA 101:12706–12711

Nilius B, Appendino G, Owsianik G (2012) The transient receptor potential channel TRPA1: from gene to pathophysiology. Pflugers Arch 464:425–458

Numazaki M, Tominaga T, Toyooka H, Tominaga M (2002) Direct phosphorylation of capsaicin receptor VR1 by protein kinase Cepsilon and identification of two target serine residues. J Biol Chem 277:13375–13378

O'Hagan R, Chalfie M, Goodman MB (2005) The MEC-4 DEG/ENaC channel of Caenorhabditis elegans touch receptor neurons transduces mechanical signals. Nat Neurosci 8:43–50

Obreja O, Schmelz M (2010) Single-fiber recordings of unmyelinated afferents in pig. Neurosci Lett 470:175–179

Obreja O, Kluschina O, Mayer A, Hirth M, Schley M, Schmelz M, Rukwied R (2011a) NGF enhances electrically induced pain, but not axon reflex sweating. Pain 152:1856–1863

Obreja O, Ringkamp M, Turnquist B, Hirth M, Forsch E, Rukwied R, Petersen M, Schmelz M (2011b) Nerve growth factor selectively decreases activity-dependent conduction slowing in mechano-insensitive C-nociceptors. Pain 152:2138–2146

Page AJ, Brierley SM, Martin CM, Martinez-Salgado C, Wemmie JA, Brennan TJ, Symonds E, Omari T, Lewin GR, Welsh MJ, Blackshaw LA (2004) The ion channel ASIC1 contributes to visceral but not cutaneous mechanoreceptor function. Gastroenterology 127:1739–1747

Park TJ, Lu Y, Jüttner R, Smith ESJ, Hu J, Brand A, Wetzel C, Milenkovic N, Erdmann B, Heppenstall PA, Laurito CE, Wilson SP, Lewin GR (2008) Selective inflammatory pain insensitivity in the African naked mole-rat (Heterocephalus glaber). PLoS Biol 6:e13

Patel TD, Jackman A, Rice FL, Kucera J, Snider WD (2000) Development of sensory neurons in the absence of NGF/TrkA signaling in vivo. Neuron 25:345–357

Patel A, Sharif-Naeini R, Folgering JRH, Bichet D, Duprat F, Honoré E (2010) Canonical TRP channels and mechanotransduction: from physiology to disease states. Pflugers Arch 460:571–581

Petty BG, Cornblath DR, Adornato BT, Chaudhry V, Flexner C, Wachsman M, Sinicropi D, Burton LE, Peroutka SJ (1994) The effect of systemically administered recombinant human nerve growth factor in healthy human subjects. Ann Neurol 36:244–246

Pezet S, McMahon SB (2006) Neurotrophins: mediators and modulators of pain. Annu Rev Neurosci 29:507–538

Poole K, Lechner SG, Lewin GR (2011) The handbook of touch: the molecular and genetic basis of touch. Springer, New York, NY

Price MP, Lewin GR, McIlwrath SL, Cheng C, Xie J, Heppenstall PA, Stucky CL, Mannsfeldt AG, Brennan TJ, Drummond HA, Qiao J, Benson CJ, Tarr DE, Hrstka RF, Yang B, Williamson RA,

Welsh MJ (2000) The mammalian sodium channel BNC1 is required for normal touch sensation. Nature 407:1007–1011
Price MP, McIlwrath SL, Xie J, Cheng C, Qiao J, Tarr DE, Sluka KA, Brennan TJ, Lewin GR, Welsh MJ (2001) The DRASIC cation channel contributes to the detection of cutaneous touch and acid stimuli in mice. Neuron 32:1071–1083
Price MP, Thompson RJ, Eshcol JO, Wemmie JA, Benson CJ (2004) Stomatin modulates gating of acid-sensing ion channels. J Biol Chem 279:53886–53891
Ritter AM, Mendell LM (1992) Somal membrane properties of physiologically identified sensory neurons in the rat: effects of nerve growth factor. J Neurophysiol 68:2033–2041
Ritter AM, Lewin GR, Kremer NE, Mendell LM (1991) Requirement for nerve growth factor in the development of myelinated nociceptors in vivo. Nature 350:500–502
Ruit KG, Osborne PA, Schmidt RE, Johnson EM Jr, Snider WD (1990) Nerve growth factor regulates sympathetic ganglion cell morphology and survival in the adult mouse. J Neurosci 10:2412–2419
Ruit KG, Elliott JL, Osborne PA, Yan Q, Snider WD (1992) Selective dependence of mammalian dorsal root ganglion neurons on nerve growth factor during embryonic development. Neuron 8:573–587
Rukwied R, Mayer A, Kluschina O, Obreja O, Schley M, Schmelz M (2010) NGF induces non-inflammatory localized and lasting mechanical and thermal hypersensitivity in human skin. Pain 148:407–413
Rukwied RR, Main M, Weinkauf B, Schmelz M (2013) NGF sensitizes nociceptors for cowhage- but not histamine-induced itch in human skin. J Invest Dermatol 133:268–270
Salmon A-M, Damaj MI, Marubio LM, Epping-Jordan MP, Merlo-Pich E, Changeux J-P (2001) Altered neuroadaptation in opiate dependence and neurogenic inflammatory nociception in αCGRP-deficient mice. Nat Neurosci 4:357–358
Schmidt R, Schmelz M, Forster C, Ringkamp M, Torebjörk E, Handwerker H (1995) Novel classes of responsive and unresponsive C nociceptors in human skin. J Neurosci 15:333–341
Seybold VS (2009) The role of peptides in central sensitization. Handb Exp Pharmacol 194:451–491
Shelton DL, Reichardt LF (1984) Expression of the beta-nerve growth factor gene correlates with the density of sympathetic innervation in effector organs. Proc Natl Acad Sci USA 81:7951–7955
Shu X, Mendell LM (1999) Nerve growth factor acutely sensitizes the response of adult rat sensory neurons to capsaicin. Neurosci Lett 274:159–162
Sluka KA, Price MP, Breese NM, Stucky CL, Wemmie JA, Welsh MJ (2003) Chronic hyperalgesia induced by repeated acid injections in muscle is abolished by the loss of ASIC3, but not ASIC1. Pain 106:229–239
Smith ESJ, Lewin GR (2009) Nociceptors: a phylogenetic view. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 195:1089–1106
Smith ESJ, Omerbašić D, Lechner SG, Anirudhan G, Lapatsina L, Lewin GR (2011) The molecular basis of acid insensitivity in the African naked mole-rat. Science 334:1557–1560
Smith ESJ, Purfürst B, Grigoryan T, Park TJ, Bennett NC, Lewin GR (2012) Specific paucity of unmyelinated C-fibers in cutaneous peripheral nerves of the African naked-mole rat: comparative analysis using six species of Bathyergidae. J Comp Neurol 520:2785–2803
Stein AT, Ufret-Vincenty CA, Hua L, Santana LF, Gordon SE (2006) Phosphoinositide 3-kinase binds to TRPV1 and mediates NGF-stimulated TRPV1 trafficking to the plasma membrane. J Gen Physiol 128:509–522
Stucky CL, Lewin GR (1999) Isolectin B(4)-positive and -negative nociceptors are functionally distinct. J Neurosci 19:6497–6505
Stucky CL, Rossi J, Airaksinen MS, Lewin GR (2002) GFR alpha2/neurturin signalling regulates noxious heat transduction in isolectin B4-binding mouse sensory neurons. J Physiol 545:43–50
Stürzebecher AS, Hu J, Smith ESJ, Frahm S, Santos-Torres J, Kampfrath B, Auer S, Lewin GR, Ibañez-Tallon I (2010) An in vivo tethered toxin approach for the cell-autonomous inactivation of voltage-gated sodium channel currents in nociceptors. J Physiol 588:1695–1707

Svensson P, Cairns BE, Wang K, Arendt-Nielsen L (2003) Injection of nerve growth factor into human masseter muscle evokes long-lasting mechanical allodynia and hyperalgesia. Pain 104:241–247

Svensson P, Wang K, Arendt-Nielsen L, Cairns BE (2008) Effects of NGF-induced muscle sensitization on proprioception and nociception. Exp Brain Res 189:1–10

Thompson SW, Dray A, McCarson KE, Krause JE, Urban L (1995) Nerve growth factor induces mechanical allodynia associated with novel A fibre-evoked spinal reflex activity and enhanced neurokinin-1 receptor activation in the rat. Pain 62:219–231

Treede RD, Meyer RA, Raja SN, Campbell JN (1992) Peripheral and central mechanisms of cutaneous hyperalgesia. Prog Neurobiol 38:397–421

Vilceanu D, Stucky CL (2010) TRPA1 mediates mechanical currents in the plasma membrane of mouse sensory neurons. PLoS One 5:e12177

Vriens J, Owsianik G, Hofmann T, Philipp SE, Stab J, Chen X, Benoit M, Xue F, Janssens A, Kerselaers S, Oberwinkler J, Vennekens R, Gudermann T, Nilius B, Voets T (2011) TRPM3 Is a nociceptor channel involved in the detection of noxious heat. Neuron 70:482–494

Weidner C, Schmelz M, Schmidt R, Hansson B, Handwerker HO, Torebjörk HE (1999) Functional attributes discriminating mechano-insensitive and mechano-responsive C nociceptors in human skin. J Neurosci 19:10184–10190

Weinkauf B, Obreja O, Schmelz M, Rukwied R (2012) Differential effects of lidocaine on nerve growth factor (NGF)-evoked heat- and mechanical hyperalgesia in humans. Eur J Pain 16:543–549

Weinkauf B, Main M, Schmelz M, Rukwied R (2013) Modality-specific nociceptor sensitization following UV-B irradiation of human skin. J Pain 14:739–746

Weiss J, Pyrski M, Jacobi E, Bufe B, Willnecker V, Schick B, Zizzari P, Gossage SJ, Greer CA, Leinders-Zufall T, Woods CG, Wood JN, Zufall F (2011) Loss-of-function mutations in sodium channel Nav1.7 cause anosmia. Nature 472:186–190

Wende H, Lechner SG, Cheret C, Bourane S, Kolanczyk ME, Pattyn A, Reuter K, Munier FL, Carroll P, Lewin GR, Birchmeier C (2012) The transcription factor c-Maf controls touch receptor development and function. Science 335:1373–1376

Wetzel C, Hu J, Riethmacher D, Benckendorff A, Harder L, Eilers A, Moshourab R, Kozlenkov A, Labuz D, Caspani O, Erdmann B, Machelska H, Heppenstall PA, Lewin GR (2007) A stomatin-domain protein essential for touch sensation in the mouse. Nature 445:206–209

Wilson MJ, Yoshikami D, Azam L, Gajewiak J, Olivera BM, Bulaj G, Zhang M-M (2011) μ-Conotoxins that differentially block sodium channels NaV1.1 through 1.8 identify those responsible for action potentials in sciatic nerve. Proc Natl Acad Sci USA 108:10302–10307

Woodbury CJ, Zwick M, Wang S, Lawson JJ, Caterina MJ, Koltzenburg M, Albers KM, Koerber HR, Davis BM (2004) Nociceptors lacking TRPV1 and TRPV2 have normal heat responses. J Neurosci 24:6410–6415

Woolf CJ (1983) Evidence for a central component of post-injury pain hypersensitivity. Nature 306:686–688

Woolf CJ, Safieh-Garabedian B, Ma Q-P, Crilly P, Winter J (1994) Nerve growth factor contributes to the generation of inflammatory sensory hypersensitivity. Neuroscience 62:327–331

Zhang X, McNaughton PA (2006) Why pain gets worse: the mechanism of heat hyperalgesia. J Gen Physiol 128:491–493

Zhang X, Huang J, McNaughton PA (2005) NGF rapidly increases membrane expression of TRPV1 heat-gated ion channels. EMBO J 24:4211–4223

Zhao J, Seereeram A, Nassar MA, Levato A, Pezet S, Hathaway G, Morenilla-Palao C, Stirling C, Fitzgerald M, McMahon SB, Rios M, Wood JN, London Pain Consortium (2006) Nociceptor-derived brain-derived neurotrophic factor regulates acute and inflammatory but not neuropathic pain. Mol Cell Neurosci 31:539–548

Zurborg S, Yurgionas B, Jira JA, Caspani O, Heppenstall PA (2007) Direct activation of the ion channel TRPA1 by Ca2+. Nat Neurosci 10:277–279