Introduction

Sensory neurons enable us to sense and perceive the environment which is a prerequisite for triggering appropriate behavior. The first and essential step in this process is the transduction of the physical energy of the stimuli into trains of nerve impulses. Nerve fibers that have received the information about the inner or outer environment are wired up to the central nervous system where sensory information is used for appropriate reaction, conscious or unconscious.

Sensory receptors have different morphologies and organization but they all extract the same elementary information from a given stimulus: modality, intensity, duration and location. The stimulus, whatever its modality, is always converted to an electrical signal, the receptor potential, and eventually, to action potential; the intensity and the duration of the stimulus are coded in electrical signals. Different sense organs produce distinctive subjective sensations and sensation evoked by a stimulus depends upon the type of sense organ that has been stimulated. Recognition by the central nervous system (CNS) of the modality of the stimulus and its position depend on the nature of the sensory ending and its anatomical location.

There are several types of energies that impinge on our senses – chemical, thermal, mechanical and electromagnetic. Sensory receptors are adapted to transduce a particular stimulus, known as the adequate stimulus. Therefore, detecting and transducing mechanical energy enables us to perceive touch, sound, and position. Chemical stimuli are perceived as smell and taste, electromagnetic energy is tranduced into perception of light and colors. Although certain forms of energy are specific for certain senses, common to all of them is that, if their intensity is above a certain threshold, they can evoke the sensation of pain. There were two opposing theories on the nature of the pain. Pattern theory proposed that pain results from a pattern of intense activity of neurons that can encode subtle sensations such as warmth and fine touch (Sinclair, 1955). The specificity theory, on the other hand, proposed that pain is a sensation conveyed via unique anatomical structures (Melzack and Wall, 1965). The existence of a population of sensory neurons in the skin that were silent until stimuli were intense enough to threaten or cause tissue damage was demonstrated (Bessou and Perl, 1969; Burgess and Perl, 1967; McCleskey and Gold, 1999). The existence of sensory neurons that respond to intense stimuli was postulated and those neurons were

termed nociceptors (Sherrington, 1906). Nociceptors are the first cells in the series of neurons that lead to the sensation of pain. Nociception is synonymous with the response of peripheral sensory neurons to the presence of threatening or damaging stimuli and it involves the integration and modulation of peripheral input to cause a sensation that is perceived centrally as pain.

Cutanous primary sensory neurons

Primary sensory neurons represent the interface between the external environment and the central nervous system. To detect and transfer particular features of the stimuli that impinge upon the body to the CNS they have highly specialized adaptations that include the heterogeneous expression of a broad number of ion channels, receptors, neurotransmitters and neuromodulators as well as the establishment of highly ordered patterns of innervations of the peripheral target and central neurons (Woolf, 1996).

Skin represents the main somatic sense organ. Cutaneous sense organs comprise of wide range of various receptors responsible for detecting mechanical, thermal or noxious stimuli applied to the body surface. Somatic sensory reception is conveyed by neurons whose cell bodies are located in dorsal root ganglia (DRG). Specialized sensory endings of those cells are embedded in different layers of dermis and epidermis while the central process of the sensory neuron arborizes to form synapses in the dorsal horn of spinal cord (Figure 1).

One can distinguish three types of nerve cell fibers that originate in DRG and innervate the skin. They are classified according to their conduction velocity (CV) which depends upon their axon diameter and the degree of myelination. Aβ fibers are thickly myelinated and have large cell bodies; their CV in mice is higher than 10 m/s. Aδ fibers are thinly myelinated, have medium size cell bodies and have a CV in the range of 1 to 10 m/s. C fibers are unmyelinated, have a CV lower than 1 m/s, small cell bodies and they are the largest group of primary afferent neurons innervating skin. The majority of sensory neurons in DRG can detect mechanical stimuli. Physiologically, two distinct kinds of responses to mechanical stimuli are in principle possible, either afferent fibers discharge only during the dynamic phase of stimulus application, i.e. the movement of a mechanical stimulus, or they fire during the static maintenance of a mechanical stimulus (Figure 1).

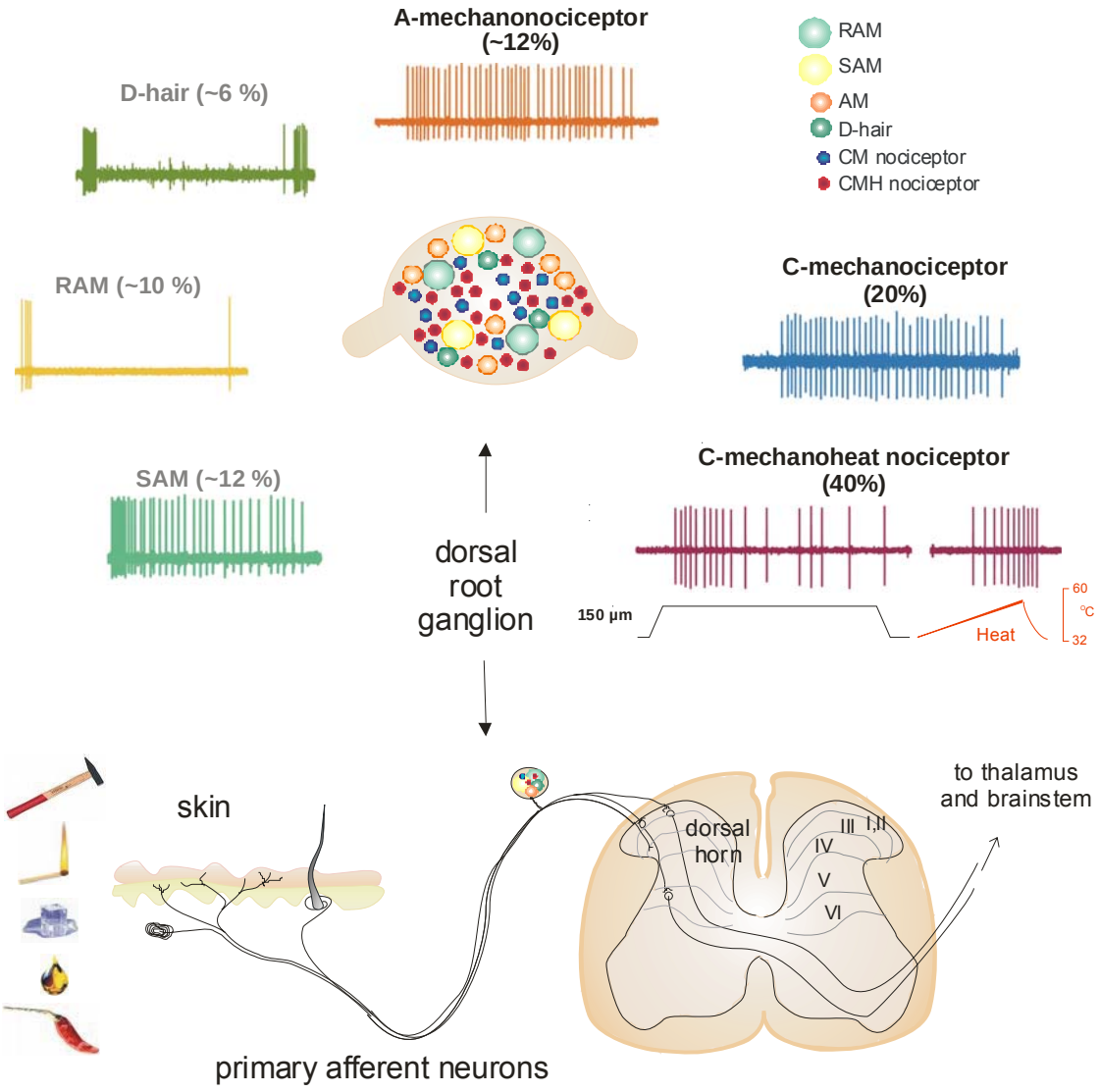

Figure 1. Mechanoreceptors of mouse skin innervated by the saphenous nerve
Cutaneous peripheral nerve endings terminate in different dermal and epidermal layers. They are specialized to detect mechanical, thermal and chemical stimuli. A schematic drawing of a DRG containing myelinated and unmyelinated neurons with distinct cell diameters is shown in the center. The central process of the sensory neuron arborizes to form synapses in the dorsal horn of spinal cord. Typical response properties of different mouse mechanoreceptors from the saphenous nerve to a standardized ramp and hold indentation stimulus at 150µm is shown and the response of distinct types of fibers is depicted in color corresponding to the cell body. The approximate incidence of the mechanoreceptor class is indicated next to its name.

Receptors that are preferentially excited during movement are rapidly adapting; there are two types, rapidly adapting mechanoreceptors (RAMs) among Aβ and down hair low threshold mechanoreceptors (D-hairs) among Aδ fibers. Those receptors responding to

both phases of stimulation or just to the static phase are referred to as slowly adapting: low threshold slowly adapting mechanoreceptors (SAMs) in Aβ range and high threshold A-mechanonociceptors (AMs) in Aδ range. Besides detecting mechanical stimuli, a small percentage of slowly adapting A fibers are able to detect thermal stimuli, like cooling or warming. However, the vast majority of temperature sensitive fibers are among unmyelinated fibers.

Cutaneous nociceptors

Nociceptive neurons detect noxious heat, cold, mechanical or chemical stimuli. On the basis of their CV, nociceptive neurons are known to be small diameter slowly conducting unmyelinated C fibers and larger, more rapidly conducting, thinly myelinated Aδ fibers mechanonociceptor (AMs) (Koerber, 1992). There are also implications that up to 20% of the Aβ fibers could be nociceptive (Djouhri and Lawson, 2004).

AMs are known as mechanonociceptors since they primarily have responses to high threshold mechanical stimuli. A subpopulation, around 25% of these fibers, also respond to heat stimulation, and a lower percentage to cold stimuli (Koltzenburg et al., 1997).

C-fibers represent the major subpopulation of nociceptors and make up 60 to 70% of sensory afferents in DRG that innervate the skin. The free nerve endings innervate different layers of the epidermis and dermis, and make connections in a highly topographic fashion within the substancia gelatinosa of the spinal cord, predominantly lamina I and II. There is a great biochemical and functional diversity among C fibers.

Most C-fiber nociceptors are so-called polymodal receptors that respond to both noxious mechanical and thermal stimuli. They are referred to C-mechanoheat fibers (CMH). These fibers can be activated or sensitized to thermal stimuli by a wide range of exogenously applied algesic chemicals such as bradykinin, prostaglandins, and capsaicin (Lewin and Moshourab, 2004). A substantial number of C-fiber nociceptors are also present that respond to mechanical but not thermal stimuli, C-mechanonociceptors (CM). In addition, one can distinguish C-mechanocold (CMC), C-mechanoheatcold (CMHC) and C-fibers that respond just to heat (CH). A group of C-fibers, around 10% in rodent skin, display little or no sensitivity to mechanical or thermal stimulation, C-mechano insensitive heat insensitive (CMiHi) (Handwerker et al., 1991). They are also known as sleeping nociceptors and their important feature is that upon sensitization with algogenes, like capsaicin, some of these neurons can within minutes

become responsive to tonic pressure and heat stimuli (Schmidt et al., 1995). Another group of C-fibers could be found, in high proportion in cat but are rare in rodents, C-fiber low threshold mechanoreceptor (CLT). They are also present in substantial numbers in humans and it is postulated that they mediate nondiscriminitive touch (Olausson et al., 2002).

C-fibers can also be divided on the basis of their peptide content and their mode and site of termination within the spinal cord. One group of C-fibers express: receptors for glial-cell-derived neurothophic factor (GDNF), fluoride-resistant acid phosphatase (FRAP), an IB_4 lectin binding site, and $P2X_3$ purine receptors. These fibers terminate almost exclusively within the inner lamina II of the dorsal horn with a glomerular type of synaptic ending. The other group of fibers express the high affinity nerve growth factor (NGF) receptor, TrkA, and synthesize peptides such as supstance P and caltitonin gene related peptide (CGRP). These fibers terminate more superficially within the dorsal horn, lamina I and outer lamina II (Molliver et al., 1995). It is common to classify neurons as peptidergic and non-peptidergic based on their binding of IB_4 although considerable colocalisation for supstance P or CGRP and IB_4 or FRAP has been reported (Orozco et al., 2001).

Transduction of noxious stimuli

The afferent sensory nerve terminal is unique in its capacity to detect a range of physical and chemical stimuli and also to reset stimulus threshold in the context of tissue injury or disease (Caterina and Julius, 1999). Innocuous stimuli like light touch or gentle warming evoke different sensations compared to damaging pressure or heat. Although the nature of the physical stimuli is same, the threshold for activation of nociceptive neurons is higher compared to those of neurons that signal in the innocuous range. The molecular mechanisms involved in the transduction of different sensory modalities in the mammalian skin are still largely unknown. Nevertheless, a great number of specific receptors, ion channels and modulators putatively involved in transduction processes have been identified. While some of the molecules involved in transduction might be the same for both innocuous and noxious stimuli of the same modality, there are those that are specific just for nociception. They include ion channels that detect and transduce exclusively high threshold nociceptive stimuli, those that determine specific excitability

properties of nociceptors, and also proteins involved in modulation of the nociceptive system (Figure 2).

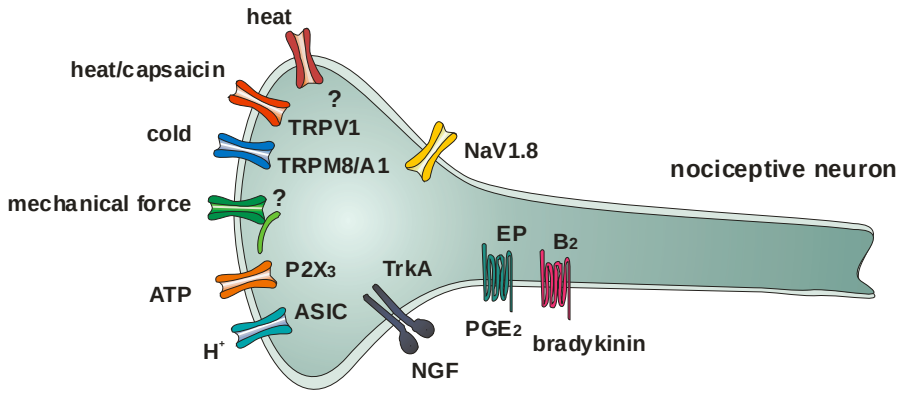

Figure 2. Schematic drawing of different molecules involved in transduction and modulation of nociceptive stimuli

Chemical activation

Many chemicals agents produce pain when applied to the skin. In many cases, the physiological pain induced by these agents results from tissue injury and is therefore indirect. Inflammatory mediators are numerous: bradykinin, prostaglandins, leukotrienes, serotonin, histamine, substance P, thromboxanes, platelet-activating factor, adenosine and ATP, protons and free radicals (Marchand et al., 2005).

Two substances are known to have acute nociceptive effect independent of inflammation or naturopathic state, capsaicin and hystamine. Capsaicin produces intense burning pain that lasts for several minutes when injected into the skin (Baumann et al., 1991; Ringkamp et al., 2001). Its pungent affect is mediated by the TRPV1 ion channel, which is activated by both noxious heat and protons. When administered to the skin histamine produces long lasting itch. It produces a vigorous, long-lasting response via activation of subpopulation of mechanically insensitive fibers (Schmelz et al., 1997). Histamine probably activates nociceptors via the H_1 receptor located on the peripheral terminals (Ikoma et al., 2006).

Of great interest is nociception initiated by protons, as a result of tissue acidosis. Protons can be released from damaged cells and they are able to activate and modulate

certain ion channels expressed in DRG neurons. Members of the acid sensing ion channel (ASIC) family are expressed by nociceptors and have been proposed to be the principal acid sensor (Waldmann et al., 1997). However, capsaicin sensitive currents and heat currents mediated through TRPV1 are sensitised by protons (Tominaga et al., 1998).

Mechanotransduction

Mechanosensitivity is an almost ubiquitous attribute of sensory neurons in the DRG whether they respond to innocuous or noxious stimuli (Lewin and Moshourab, 2004). Recent attempts to elucidate the molecular basis of mechanotransduction in mammals led to the identification of several candidate genes encoding mechanically gated ion channels, ion channel subunits and regulatory proteins. So far the primary candidate molecules for a role in mammalian mechanotransduction are members of the transient receptor potential TRP (transient receptor potential) and DEG/ENaC (degenerin/epithelial Na channel) families of ion channels. These assumptions are based on identification of mechanosensors in two invertebrates, the nematode *C.elegans* and the fruit fly *Drosophila*.

Mutagenesis screens for touch insensitivity in *C.elegans* identified numerous mutations assigned to the *mec* (mechanosensory insensitive) genes belonging to DEG/ENaC family. The *mec* genes are required for generation of touch cells, specification of touch cell fate (*mec-3*) and the function of mechanotransduction complex, which comprises of extracelular matrix proteins, cytoskeletal proteins and ion channel subunits. The transduction channel in *C.elegans* is a complex of two subunits MEC-4 and MEC-10 and two accessory subunits MEC-2 and MEC-6 (O'Hagan et al., 2005). A mammalian homolog of MEC-2, stomatin-like protein 3 (SLP3) seems to be essential for the mechanotransduction in a subset (35%) of cutaneous touch receptors (Wetzel et al., 2007). ASIC2 and ASIC3, homolog to MEC-4, localize to the peripheral terminals of putative touch receptors (Lumpkin and Bautista, 2005). Disrupting ASIC2 in mice revealed impairment specific to RAMs which displayed reduced sensitivity, while disrupted ASIC3 led to increased firing rate of RAM (Moshourab and Lewin, submitted) (Price et al., 2000). However, mechanically gated currents in sensory neurons in ASIC3 and ASIC2/ASIC3 double knockout mice are not different to *wt* (Drew et al., 2004).

Interestingly, the mechanosensitivity of C-fiber nociceptors is impaired in the absence of ASIC3 (Moshourab and Lewin, manuscript in prep).

Drosophila NOMPC (TRPN1) mutants show defects in hearing, touch and propriception. TRPN1 homolog in *C.Elegans, Trp4*, is expressed in mechanosensory neurons and involved in stretch-receptor-mediated proprioception (Li et al., 2006). TRPN1 is expressed in mechanosensory hair cells in some fish and amphibians. The only mammalian TRP channel with an extended ankyrin domain like TRPN1 is TRPA1 and it is expressed in nocicptors and hair cells (Vollrath et al., 2007). The TrpA homolog in *Drosophila*, *painless*, is required for both thermal and mechanical nociception, but not for sensing light touch (Tracey et al., 2003).However, $TrpA^{-/-}$ mice show no deficits in acute touch sensitivity and have normal auditory responses (Bautista et al., 2006). In contrast, TRPA1 is crucial for the hypersensitivity to both touch and heat that accompanies skin inflammation by mustard oil as well as the heat hypersensitivity caused by bradykinin (Lumpkin and Caterina, 2007). TRPV isoforms in *C.Elegans* OSM-9 and OCR-2 colocalize in polymodal sensory neurons and might form heteromeric transduction channels (Kahn-Kirby and Bargmann, 2005). Polyunsaturated fatty acids seem to be active upstream of TRPV family channels in sensory transduction, maybe as modulators or by providing an adequate lipid environment (Kahn-Kirby et al., 2004). Two members

of the TRPV family, NAN and IAV in *Drosophila* colocalize in sensory cilia and they are essential for sound evoked neuronal activity in auditory antene (Gong et al., 2004). Mammalian TRPV4, homolog of OSM-9, can be activated by hypotonic solutions and it is expressed in large diameter sensory neurons and Merkel cells. However, disrupting *Trpv4* expression in mice has only modest effect on acute mechanosensory threshold, although a significantly reduced response to harmful stimuli caused by pressure was observed (Suzuki et al., 2003). TRPV1 is required for osmosensation in hypothalamic neurons and stretch evoked reflex in bladder, but does not participate in mechanotransductin in skin (Birder et al., 2002).

The subtle mechanosensory defects in knockout mice suggest that there are other candidates still to be identified and that many types of channel work in concert to control the sensitivity of touch and pain receptors (Lumpkin and Caterina, 2007).

Thermosensation

Thermosensitive neurons detect temperature either in the noxious range or in the innocuous range. For most humans 42^{o}C is the point perceived as a shift from innocuous warmth to noxious heat (Hardy et al., 1952). Innocuous cool is defined as temperatures between 30^{o}C and 15^{o}C, whereas temperatures below 15^{o}C are in general perceived as noxious cold (Benham et al., 2003). Thermosensation seems in most cases to be carried out by the direct activation of thermally-gated ion channels in the surface membrane of sensory neurons.

To date most temperature sensitive ion channels identified are members of the TRP family. TRPV1 was the first heat-activated channel to be identified from the rodent DRG cDNA library by using a calcium-imaging based expression assay (Caterina et al., 1997). TRPV1 is activated by noxious heat (>42^{o}C) with a Q_{10} value greater than 20 and it is also directly gated by capsaicin, strong acidic conditions, ethanol, and a variety of endogenous lipids (Tominaga et al., 1998; Trevisani et al., 2002). Cultured sensory neurons from TRPV1$^{-/-}$ mice displayed complete loss of capsaicin and thermal sensitivity. However, recordings from the skin nerve preparation showed that heat sensitive C-fibers are still functional in these mice, although in a lower proportion and with reduced heat evoked discharge. Furthermore, the other studies failed to detect any differences in the heat responses of C-fibers between *wt* and TRPV1$^{-/-}$ mice (Caterina et al., 2000; Zimmermann et al., 2005). TRPV1$^{-/-}$ mice display very mild if any behavioral deficits in acute thermal sensation (Davis et al., 2000). Also, intracellular recordings made from IB$_4$+ nociceptors lacking TRPV1 and TRPV2 indicate that they have normal temperature thresholds and response properties (Woodbury et al., 2004). These findings suggest that TRPV1 is not needed for heat detection, but it is necessary for inflammation induced thermal hyperalgesia (Davis et al., 2000). The other TRP channel proposed to sense noxious heat was TRPV2 which has an activation threshold of 52^{o}C. It is found in sensory neurons of all sizes with a higher expression in medium to large size neurons (Caterina et al., 1999; Ma, 2001). However, there is currently no direct evidence that TRPV2 acts as a thermosensor *in vivo*. TRPV3 responds to innocuous warm stimuli, 34-39^{o}C, and in rodents it is not expressed in DRG cells but in kertinocytes (Peier et al., 2002). However, TRPV3$^{-/-}$ mice show significantly reduced sensitivity to warmth and to noxious stimulus (55^{o}C) suggesting the role of skin keratinocytes in warmth transduction (Moqrich et al., 2005). TRPV4 is also activated by physiological

ranges of temperatures (24-34^{o}C) and TRPV4$^{-/-}$ mice do not have altered behavior to the hot plate test (Suzuki et al., 2003).

TRPM8 and TRPA1 are proposed to be the major ion channels sensing cool and cold temperatures, respectively. TRPM8 respond to cooling (<25^{o}C) and TRPA1 is activated by temperatures below 17^{o}C. However, cold sensation is little affected in TRPA1$^{-/-}$ mice; although females showed less sensitivity in a cold plate test (0^{o}C), males did not (Kwan et al., 2006). TRPM8$^{-/-}$ mice exhibit reduced avoidance to cold temperatures, but have normal nociceptive responses to subzero temperatures (Dhaka et al., 2007). Recent data show that a substantial population of sensory neurons responding to cold stimuli does not express either TRPM8 or TRPA1, suggesting the existence of another molecule that function as cold sensor (Babes et al., 2006).

The thermo TRPs have the unique properties that their temperature sensitivities of activation and deactivation are highly asymmetrical which results in the current passing through them being strongly dependent upon temperatures (Voets et al., 2004). The majority of TRPs are also expressed in skin cells in combination with neurotransmitters and their receptors, which implies a possible role of keratinocytes as thermosensors (Chung et al., 2004). The other mechanisms suggested to be involved in thermotransduction include TREK-1 channels which keep the neuron close to its resting potential, but close at cooler temperature (Maingret et al., 2000). Recently it was also shown that $Na_v1.8$ null mutant mice show negligible responses to noxious cold and mechanical stimulation at low temperatures and that $Na_v1.8$ might be critical molecule for the perception of pain at cold temperatures (Zimmermann et al., 2007).

Great progress has been made in understanding of the mechanisms of temperature sensation since the discovery of thermo-TRPs. These TRP channels undoubtedly participate in peripheral termosensation, particularly in hypersensitivity states. Nevertheless, there seem to be other, as yet undiscovered, molecules working as acute thermal sensors *in vivo*.

Growth factors and sensory neuron function

Neurotrophins are a family of growth factors critical for the development and function of the nervous system of vertebrates. Many seem to be active in a very restricted time period during development, whereas others act throughout the whole lifespan. There

are five highly conserved neurotrophins: NGF, BDNF (brain derived neurotrophic factor), NT3 (neurotrophin 3), NT4/5 and NT6 (expressed only in fish). Two cellular receptors bind neurothrophins: the low affinity p75 receptor which belongs to the tumor necrosis factor (TNF) superfamily, and high affinity tropomyosin receptor kinase (Trk) receptors. The three Trk receptors subtypes TrkA, TrkB and TrkC are highly conserved, mainly differing only in their extracellular domains and each can be activated with one or more neurothrophins. The Trk receptors are tyrosine kinase receptors expressed as dimers with or without p75 (Dechant, 2001).

During development neurotrophins support the survival of neuronal subpopulations that express appropriate receptors. TrkA primarily binds NGF, TrkB binds both BDNF and NT4 initiating different signaling cascades, and the preferable receptor for NT3 is TrkC. Loss of one of neurotrophins or its preferred high affinity receptor can lead to the loss of one or more functionally defined types of sensory neurons (Snider, 1994). Neutrophin receptors are differentially expressed in the central and peripheral nervous system; therefore different members of the neurotrophin family have distinct, but sometimes overlapping neuroprotective actions. Neurtrophins are produced by target tissues and act on receptors expressed on the terminals of sensory neurons. The activation of Trk receptors activates several intracellular signaling cascades including internalization of the Trk-receptor complex that consequently exerts transcriptional control over the neuron. The precursor forms of neurotrophins, pro-NGF and pro-BDNF, may themselves have independent biological activity and activate distinct transduction pathways (Pezet and McMahon, 2006).

Neurotrophins are essential for supporting the survival of peripheral neurons during the period of target innervation. While being dependent on a survival basis on one or more neurotrophic factors in early embryonical stages, during development sensory neurons differentiate into more subtypes that start to display phenotypic dependence. NGF has a number of roles in the development of neuronal and non-neuronal cells. During development NGF promotes the survival and maturation of several populations of neurons that express TrkA and p75. In adults, the focus of NGF signaling shifts away from the regulation of neuronal survival to the regulation of neuronal phenotype and function.

Sensory modality dependence on NGF

NGF was the first growth factor to be identified as a factor with the property of enhancing growth and differentiation in sensory and sympathetic nerve cells (Levi-Montalcini and Angeletti, 1968). NGF has long been known to promote the survival, axonal growth and differentiation of cultured sensory neurons (Greene and Shooter, 1980). NT3/TrkC signaling is the first required for the development of neural crest derived neurons, but sensory neurons derived from embryos 12 days on require NGF for survival (Davies, 1994). During embryonic life virtually all small diameter sensory neurons express TrkA. Mice that are homozygous for a null mutation in the NGF gene are born with virtually no sympathetic neurons and a loss of more than 70% of DRG neurons (Crowley et al., 1994). This cell loss is restricted to small and medium size cells and animals fail to respond to noxious mechanical stimuli (Crowley et al., 1994). There is a large similarity between defects in the peripheral nervous system of NGF and TrkA deficient mice suggesting that the trophic effect of NGF is mediated trough its high affinity receptor. *TrkA*$^{-/-}$ mice lose 70 to 90% of DRG cells (Smeyne et al., 1994). These animals display severe sensory defects including no reaction to noxious mechanical and heat stimuli (60^{o}C). It has been shown that BAX, a member of the caspase family of proteases, plays a key role in natural occurring neuronal apoptosis in the developing peripheral nervous system in the absence of NGF/TrkA signaling (Deckwerth et al., 1996). During the postnatal period the trophic factor sensitivity of sensory neurons changes and the expression of TrkA is downregulated. In the neonate rodent TrkA is found on 70 to 80% of neurons and declines over the first two weeks after birth to its adult distribution of 40% (Molliver and Snider, 1997). This is not the case with p75 which remains at a constant level of expression in around 50% of cells (Bennett et al., 1996). A fraction of nociceptive neurons switch their neurotrophic factor dependence from NGF by upregulating the expression of Ret, a signaling subunit of the receptor complex for members of the glial cell line-derived neurotrophic factors (GDNF) family. All nociceptive neurons initially express TrkA and later on the transcription factor Runx1, which is needed to switch some of the nociceptors to GDNF proteins responsive neurons. The fraction of developing nociceptors that loses TrkA maintains expression of transcription factor Runx1 and gains Ret expression, while the other fraction of sensory neurons loses Runx1 and retains TrkA as they mature (Chen et al., 2006). The expression of these two receptors, TrkA and c-Ret, determines two subpopulations of nociceptors in

adulthood: peptidergic (TrkA and CGRP positive) and nonpetidergic (Ret and IB_4 positive). (Chen et al., 2006)It is recently shown that the maturation of nonpeptidergic DRG neurons and expression of Ret, its coreceptors GFRα1 and GFRα2, and several other genes characteristic of nonpeptidergic neurons is controld by NGF (Luo et al., 2007).

The early postnatal period is crucial for the fine sensory modality tuning of nociceptors. The expression of TrkA or c-Ret receptor complex determines the involvement of these neurons in development of inflammatory or neuropathic pain, respectively (Snider and McMahon, 1998). IB_4 positive and negative neurons are functionally different in that IB_4-negative cells generate larger heat evoked currents (I_{heat}), but smaller TTXr Na^+ current (Stucky and Lewin, 1999). In contrast, considering that there is a little overlap between TrkA expressing IB_4-positive neurons, another study failed to find any difference in the magnitude of I_{heat} between neurons responsive and unresponsive to NGF (Galoyan et al., 2003). However, there might be considerable overlap between IB_4 and CGRP and TrkA expressing neurons (Kashiba et al., 2001). The postnatal survival of IB_4-positive neurons depends on GDNF signalling and the noxious heat response function of these neurons is regulated by neurturin-GFRα2 signalling (Molliver et al., 1997; Stucky et al., 2002). GFRα3 neurons are particularly interesting because 80% of these neurons coexpress Ret and TrkA and are therefore responsive to artemin and NGF (Elitt et al., 2006).

During the first postnatal weeks the availability of NGF influences the phenotype of nociceptive neurons in adulthood. NGF function in vivo has been studied utilizing inhibition of application of NGF. Elimination of NGF by functionally blocking antibody (anti-NGF) starting from the second day up to the middle of second postnatal week does not lead to the cell death, but does lead to a change in the distribution of phenotypes of primary sensory neurons in rat. AM nociceptors, approximately half of them, undergo a phenotypic switch whereby they are converted to a low threshold D-hairs (Lewin et al., 1992; Ritter et al., 1991). Also, the mechanical threshold of remaining AMs is increased, while there is no change in behavioral sensitivity to mechanical stimuli (Ritter et al., 1993). C-fiber nociceptors are also affected by this treatment. The proportion of C-MHs, among subclasses of C fibers, is reduced to 10% compared to 28% in control group and they are replaced by low threshold CM fibers (Lewin and Mendell, 1994). In contrast, treatment with NGF, in the same period of rat life, increases the relative proportion of C-MH fibers. These data indicate the importance of NGF for the development of responsiveness to noxious heat.

Aims

There is a great diversity among C fiber nociceptors, and a numerous molecules, including receptors, ion channels and transcription factors are known to be expressed in just subpopulations of C fibers. Such molecules are generally expressed in a partially overlapping or mutually exclusive fashion. However, direct evidence about correlation between particular markers and the modality of nociceptors or their physiological role is not evident.

As described, NGF has great impact on nociceptive phenotype of primary sensory neurons. Deprivation of NGF signaling during the postnatal development has been shown to lead to phenotypic switch, influencing both myelinated and unmyelinated nociceptors. To identify genes involved in nociception, and possibly markers for certain physiologically distinct subpopulation of nociceptors, NGF signaling during early postnatal life was targeted in this study. Using the mouse as an experimental animal further allows for the investigation of plausible nociceptive genes function in gene targeted mutants. The involvement of NGF in shaping of nociceptive phenotype could be revealed by detailed physiological analysis of cutaneous primary sensory neurons after postnatal treatment with functionally blocking NGF antibody. One would predict that temporary block of NGF signaling during the critical developmental period would induce permanent changes in the physiology of nociceptors, underlined by the altered gene expression. Differential gene expression after the treatment might originate in the altered abundance of afferents of a certain modality. Also, NGF may regulate the expression of a specific protein or group of proteins that normally confer noxious heat sensitivity on sensory neurons. The identification of genes differently expressed after the anti-NGF treatment could be performed by the employment of expression gene chip arrays. This screen tends toward recognition of candidate genes specific for subtypes of the primary sensory neurons, particularly unmyelinated nociceptors. *In situ* hybridization might reveal the specific expression pattern of some of the differentially expressed genes. Genes specifically expressed in subpopulations of DRG neurons might define the distinct functional properties of primary sensory receptors. Depending upon the availability of gene targeted mice, the effect of disruption of some of the candidate genes might be analyzed as well. The functional analysis of genes with altered expression level after the anti-NGF treatment could confirm the effectiveness of the screening approach and strengthen the hypothesis of the involvement of the other NGF regulated genes in mechanical or thermal nociception.

Until now, numerous molecules are implicated in the transduction of nociceptive stimuli. However, the data are not always fully substantiated and suggest that there are some other, as yet unrevealed, molecules involved in this process. I hope to get insight into what they could be by using this screen.

Prior to the screen for genes whose expression level in adulthood is determined by the availability of NGF during postnatal development, the functional properties of cutaneous mechanoreceptors and mechanonociceptors using *in vitro* skin nerve preparation would be studied in detail. The employment of computer controlled stimulator should enable to precisely determine the onset of the stimulus and therefore resolve the time period needed for the transduction of the stimulus into the electrical signal. The eventual dependence of physiological properties on temperature might give the insight in the nature of transduction process. Recording at two different temperatures might reveal eventual discrepancies between mechanoreceptor and nociceptor transduction mechanisms.