

## Nerve Growth Factor, Sensitizing Action on Nociceptors

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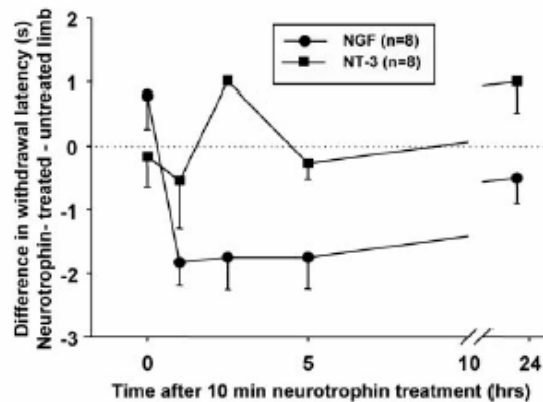
### Definition

The response of the nociceptive system can be sensitized by exposure to a ► **neurotrophin** molecule called ► **nerve growth factor** (NGF). This sensitization has 2 components, one peripheral due to an enhanced response to nociceptive stimuli, and the other central due to increased action of nociceptive impulses in the dorsal horn.

### Characteristics

Nerve Growth Factor is a member of a family of molecules called neurotrophins. Neurotrophins are best known for their function during development, specifically in promoting axonal growth and in assuring cell survival. Cells affected selectively by NGF express a specific receptor tyrosine kinase called ► **trkA** to which NGF binds. Nociceptors express trkA, which makes them sensitive to NGF during development (reviewed in Lewin and Mendell 1993; Mendell et al. 1999). Recently, however, a postnatal role for NGF has been established. Administration of NGF to an animal results in enhanced responsiveness to noxious stimulation (► **hyperalgesia**), which is partly due to direct sensitization of nociceptive afferents, i.e. peripheral sensitization. In addition, exposure of the receptive field of sensory neurons to NGF and other sensitizing agents, elicits changes in the cell body in the dorsal root ganglion that increase the central effect of sensory impulses, a phenomenon known as central sensitization. Several findings have established the involvement of endogenous NGF in sensitizing the subsequent response to nociceptive inputs after injury (reviewed in Lewin and Mendell 1993; Mendell et al. 1999). First, is the upregulation of NGF in skin and other peripheral tissues after inflammatory injury. A second is the demonstration that administration of exogenous NGF can elicit hyperalgesia. The third is the finding that inflammatory pain can be significantly reduced by interfering with endogenous NGF action, using either an antibody to NGF or an immunoadhesin (trkA-IgG) which sequesters endogenous NGF.

The time course of hyperalgesia elicited by systemically administered NGF (1  $\mu$ g/g) has revealed 2 phases of the response, an initial thermal component beginning just a few minutes after NGF administration, and a later one beginning several hours after NGF administration that includes mechanical hyperalgesia (Lewin et al. 1994). The early response can also be elicited by local injection



**Nerve Growth Factor, Sensitizing Action on Nociceptors, Figure 1** Administration of NGF to the foot of the rat makes the affected paw hyperalgesic to noxious heat as measured by a reduced latency to withdrawal from a fixed thermal stimulus. The ordinate represents the mean difference in the latency of response of the affected limb compared to the contralateral limb (negative value implies that it took less time for the thermal stimulus to reach noxious threshold on the treated foot than on the untreated foot). NGF treatment gave a rapid and consistent thermal hyperalgesia lasting at least 1 day. NT-3 produced no change in response to noxious heat. (Adapted from Shu et al. 1999).

tions of NGF into the periphery (Fig. 1), suggesting that exogenous NGF directly sensitizes thermal nociceptive afferents but not high threshold mechanoreceptors (Shu et al. 1999). These confirm the results of previous recordings from individual nociceptors using a ► **skin-nerve preparation**. In these experiments, it has been found that the response to noxious heat is sensitized, measured as a decrease in threshold, whereas there is no systematic change in the threshold to mechanical stimulation (Rueff and Mendell 1996). This suggests that mechanical hyperalgesia is of central origin (Lewin et al. 1994; see below) although the possibility of a peripheral contribution by increased discharge of high threshold mechanoreceptors is not ruled out by currently available data.

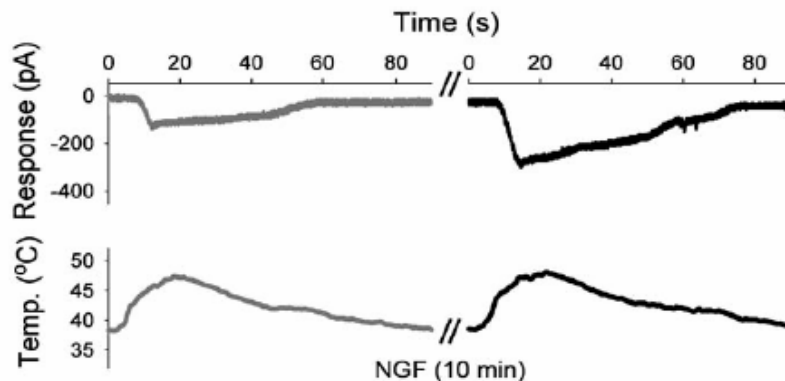
NGF has also been shown to operate as a sensitizing agent in visceral structures such as the bladder or the gut. As in skin, there is upregulation of NGF message and protein in painful inflammatory conditions, brought on by diseases such as interstitial cystitis or in an experimental model of ulcers (e.g. Lamb et al. 2004). Administration of NGF to the visceral periphery results in enhanced afferent activity. Experimental models of arthritis are also characterized by release of NGF into the synovial fluid, indicating a role in joint hyperalgesia (Manni et al. 2003).

A difficulty in determining the mechanism of NGF action from these experiments arises from the multiplicity of cell types in the peripheral target tissues that express trkA (the high affinity receptor for NGF) or that release NGF. Many of these cells are non neural, and are believed to interact closely in the inflammatory cascade. For example, ► **mast cells** are known to express trkA

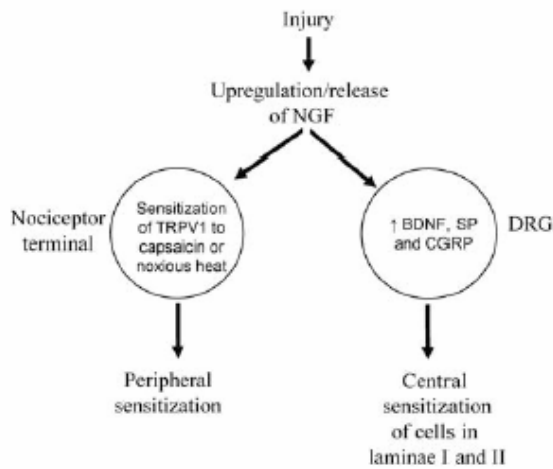
and to release NGF after injury, and ► **keratinocytes** have been shown to release NGF in response to histamine produced by mast cells (reviewed in Mendell et al. 1999). Degranulation of mast cells can diminish the sensitizing action of exogenous NGF and other inflammatory mediators (Lewin et al. 1994). In order to investigate the effect of NGF directly on nociceptors, small diameter cells acutely dissociated from DRG have been studied in culture. The assumption in carrying out such experiments is that the cell body in culture expresses the same receptors as the peripheral terminals *in situ*. A problem with this approach is that the original target (skin, muscle, viscera) can only be identified if it is prelabeled with a dye transported to the ganglion from the target tissue. However, this still leaves the identity of the receptor type (e.g. for skin: polymodal nociceptor, mechanical nociceptor, D-Hair, etc.) to be determined, since unique molecular identifiers are not yet available at this level of resolution. NGF is now recognized as an inflammatory mediator with a sensitizing action similar to that associated with other inflammatory mediators such as prostaglandin and bradykinin. The sensitizing effect of NGF has been examined most extensively on the response to capsaicin, which is now known to signal via the recently cloned ► **TRPV1 receptor** (also known as VR1). This receptor can also be activated by physiological stimuli, specifically noxious heat and low pH (rev. in Caterina and Julius 2001). Normally, the TRPV1-mediated response studied in isolated cells is smaller to the second of 2 capsaicin or noxious heat stimuli (i.e., exhibits tachyphylaxis) that are separated by as much as 10 or 15 min (Galoyan et al. 2003; Shu and Mendell 1999). However, in the presence of NGF (100 ng/ml), tachyphylaxis does not occur in most cells; rather the second response is larger than the first, i.e. it is sensitized (Shu and Mendell 1999) (Fig. 2). These same studies have revealed that the initial response to noxious heat or capsaicin is larger on the average in the presence of NGF than in its absence. Sensitization by NGF is not accompanied by any systematic change in threshold temperature (Galoyan et al. 2003), unlike sensitization measured in the skin-nerve prepara-

tion (Rueff and Mendell 1996). Thus NGF-induced sensitization is not a property of nociceptors alone; other cells in the skin (keratinocytes, mast cells, etc.) are likely to contribute significantly. It is important to note that administration of NGF alone does not elicit any response from the cell; it merely sensitizes the response evoked by noxious heat or capsaicin. Immunohistochemical analysis of these cells reveals that the ability of NGF to sensitize these responses is strongly correlated with expression of *trkA* (Galoyan et al. 2003), indicating that sensitization to noxious heat by NGF involves an interaction between the ► **trkA receptor** and the TRPV1 receptor. Chuang et al. (2001) have demonstrated that activation of *trkA* disinhibits TRPV1 via action of phospholipase C (PLC) leading to a reduced level of PIP2 which, at normal levels, maintains a tonic level of inhibition of TRPV1. NGF also sensitizes the response of nociceptors by increasing their membrane gain, as determined by an enhanced action potential firing in response to an imposed current (Zhang et al. 2002). This occurs as a result of augmentation of a TTX-resistant  $\text{Na}^+$  current known to be expressed in nociceptors. An additional factor underlying this enhanced response to depolarization is inhibition of a  $\text{K}^+$  current. NGF mediates these actions on membrane gain by activating the ► **p75 receptor**, rather than *trkA* which is responsible for enhancing the inward current through TRPV1. The p75 receptor is coupled to the sphingomyelin signaling pathway, and exposure to ► **ceramide**, an independent intermediate of this signaling pathway, mimics the effect of NGF on membrane gain. Experiments with independent expression of p75 and TRPV1 in heterologous cells suggest that the p75 receptor is unlikely to be crucial for sensitization of the response of TRPV1 to capsaicin (Chuang et al. 2001). However, some modulatory effect of p75 on the response of *trkA* is not ruled out by these experiments.

Thus, NGF can sensitize the response of nociceptors to noxious heat both by enhancing the response of the noxious heat sensitive receptor via *trkA*, and by amplifying the gain of the membrane via the p75 receptor, in effect sensitizing the response of the receptor as well as



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**Figure 2** Response of small diameter DRG cell in acute cell culture to noxious heat stimulation. Note that the response to the second pulse of heat (bottom traces) measured 10 min after the initial response in the continuous presence of NGF (100 ng/ml) during the 10 min interval was a larger inward current (top traces) measured in perforated patch clamp mode. This sensitization is never observed under control conditions. (Adapted from Galoyan et al. 2003).



**Nerve Growth Factor, Sensitizing Action on Nociceptors, Figure 3** Schematic diagram illustrating some effects of NGF in causing peripheral sensitization by direct action on nociceptive terminals and indirect central sensitization by upregulating peptides such as brain derived neurotrophic factor (BDNF), substance P (SP) and calcitonin gene related peptide (CGRP).

enhancing the gain of the impulse encoder. Longer term exposure to NGF also induces changes in the **P2X3 Receptor** composition of sensory neurons. Thus NGF can influence the response of these neurons to ATP which is released by non neural cells after damage or noxious stimuli (Scholz and Woolf 2002).

The central action of nociceptors is also sensitized by inflammatory stimuli including NGF (Scholz and Woolf 2002). NGF has not been shown to have any direct effect on spinal neurons in the superficial dorsal horn that are involved in transmitting nociceptive signals (Kerr et al. 1999). Rather, exposure of the peripheral terminals to NGF results in internalization of the NGF-trkA complex and transport to the cell body, where it stimulates upregulation of several peptides including substance P, CGRP and another neurotrophin, **brain derived neurotrophic factor (BDNF)**. These peptides are released into the dorsal horn (e.g., Lever et al. 2001) where they can rapidly sensitize the response of dorsal horn neurons in lamina II to subsequent inputs (Garraway et al. 2003). They can also elicit changes in gene expression that are pronociceptive (long term central sensitization, Scholz and Woolf 2002).

Together, these studies indicate that the role of NGF in eliciting sensitization of nociceptors is complex with both direct peripheral and indirect central components (Fig. 3).

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## Nerve Inflammation

- ▶ Inflammatory Neuritis

## Nerve Injury

- ▶ Retrograde Cellular Changes after Nerve Injury

## Nerve Lesion

### Definition

Lesion to/damage of a peripheral nerve.

- ▶ Causalgia, Assessment