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Publications
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You HJ, Mørch CD, Arendt-Nielsen L (2004) Electrophysiological characterization of facilitated spinal withdrawal reflex to repetitive electrical stimuli and its modulation by central glutamate receptor in spinal anesthetized rats. Brain Research, 1009: 110-119

You HJ, Mørch CD, Chen J, Arendt-Nielsen L (2003) Role of central NMDA versus non-NMDA receptor in spinal withdrawal reflex in spinal anesthetized rats under normal and hyperexcitable conditions. Brain Research, 981: 12-22

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Table of Content

Preface

This Ph.D.-thesis consists of a literature review and three research papers. Study I was carried out at the Orofacial Laboratory at University of Toronto, Canada January 2004 to July 2004. Studies II and III were carried out at Center for Sensory-Motor Interaction at Aalborg University, in the period from 2002 – 2006.

Acknowledgement

List of abbreviations

AMH: mechanically and heat-sensitive A-fiber nociceptors
C1:   first cervical
C2:   second cervical
C2/C3:   the neck
CMH: mechanically and heat-sensitive C fiber nociceptors
COR: cornea
DH: dorsal horn
EMG: electromyography
Fos-LI: fos-like immunoreactivity
HRP: horseradish peroxidase
MAS: masseter
NWR: nociceptive withdrawal reflex
PAG: periaqueductal gray
PAW: the forepaw
RF: mechano-receptive field
SHO: shoulder
SLN: superior laryngeal nerve
TMJ: temporomandibular joint
UCC: upper cervical spinal cord
V1:   ophthalmic
V2:   maxillary
V3:   mandibular
Vc: subnucleus caudalis
VPL: ventroposterolateral nucleus
VPM: ventroposteromedial nucleus
XII: hypoglossal

1 Introduction

Pain has been defined as "an unpleasant sensory and emotional experience with actual or potential tissue damage, or described in terms as such" (Merskey and Bogduk, 1994). It has also been emphasized that pain is a personal experience that depends on genetic differences, past experience, anxiety, expectation etc. However, specific aspects of pain can be conceptualized (Loeser and Melzack, 1999).

The difference between the concepts of nociception and the perception of pain must be appreciated. Nociception is in general thought of as the detection of actual or potential tissue damage by transducers in the periphery and the transmission of the information through the nervous system. Perception of pain, on the other hand, is not necessarily evoked by a nociceptive input and nociceptive input does not necessarily evoke pain. In e.g. phantom limb pain the nociceptors are transected but years after the amputation 50 % of the patients still report pain (Melzack, 1990). Pain may be categorized according to the persistence as transient, acute or chronic. Transient pain is evoked by activity in the nociceptors and prompts the individual to avoid the noxious stimulation. Acute pain is evoked by a substantial insult to the tissue, but may cease before the tissue is completely healed. Chronic pain outlasts the healing of the injury and may persist for months and years (Loeser and Melzack, 1999).

1.1 The Nociceptive system

The stimulation that may cause tissue damage is said to be noxious and the primary afferent fibers that mediate nociception are termed nociceptors (Willis, 1985). Several different stimulation modalities can activate the nociceptive system and cause a painful sensation. Thermal stimulation, either heat over 43 – 45 ℃, or cold at 3 – 20 ℃ (Franz and Iggo, 1968), strong mechanical stimulation, and chemical stimulation by analgesic substances may activate the nociceptive system and cause pain. Noxious stimulation depolarizes free nerve endings of the afferent fibers in the tissue. How this depolarization occurs is not well understood, however several trans-membrane proteins have been shown to cause depolarization of the nerve membrane, e.g. heat-sensitive vanilloid receptors, (Caterina et al., 1997), cold-sensitive menthol or icilin receptors (Montell, 2003), mechano-sensitive osmotic receptors (Liedtke et al., 2000) etc. If the depolarization is strong enough to generate action potentials, these travel through the nociceptive nerve fiber, to the central nervous system.

The afferent fibers are classified according to their conduction velocity, which basically reflects the thickness and degree of myelinization of the fibers. Afferent fibers innervating the skin are Aα/β-fibers which are thick myalinated fast conducting (36 – 120 m/s), Aδ-fibers which are thin myalinated slow conducting fibers (4 – 36 m/s) and C- fibers that are thinner unmyalinated even slower conducting fibers (0.4 – 2.0 m/s) (Burgess and Perl, 1973). Nociceptive information is mainly transmitted by Aδ- and C-fibers, but not all Aδ- and C-fibers are mediating nociceptive information as some respond to cold, warm or tactile stimualtion. Afferent fibers innervating muscle tissue are termed group I/II fibers corresponding to Aα/β-fibers, group III fibers corresponding to Aδ-fibers and group IV fibers corresponding to C-fibers. Nociceptive heat sensitive afferents in primate have been classified into three types based on their response properties to heat stimuli. Two types of mechanically and heat-sensitive A-fiber nociceptors (AMH) nociceptors have been reported. Type I AMHs are relatively insensitive to heat stimuli (threshold >53 °C) but respond with a long latency (10 s) (Treede et al., 1995;Treede et al., 1998). Type II AMHs are sensitive to heat (threshold ~46 °C) and respond briskly with a short latency (0.12 s) (Treede et al., 1995;Treede et al., 1998). The third group consists of mechanically and heat-sensitive C fiber nociceptors (CMH) (Tillman et al., 1995) responding to warm stimulation (threshold 41 °C) with a short response latency (0.10 s). High-threshold mechanoreceptive nociceptors do not respond to heat stimuli (Treede et al., 1995).

The nucleus of the afferent fibers innervating the somatic tissue of the body is located in the dorsal root ganglion and project to the dorsal horn (DH) of the spinal cord. Most afferents supplying the craniofacial tissue have their cell nucleus located in the Gasserian (trigeminal) ganglion but the nucleus of some of the craniofacial proprioceptors of the muscles and joints have their nucleus located in the trigeminal mesencephalic nucleus (Sessle, 2000). Thus, sensory information is mediated through afferent nerve fibers through the spinal or Gasserian ganglion into the spinal cord or brainstem. Here, the primary afferent fibers may ascend or descend and give off collaterals to terminate more rostral or caudal in the spinal cord or brainstem. The primary afferent fibers of the spinal cord ganglion terminate mainly in the DH. The DH is a laminated structure where the Aδ- and C- primary afferents mainly terminate in laminae I, II, V, and VI (Willis, 1985). Likewise, the Aδ- and C- primary afferents of the Gasserian ganglion terminate in the trigeminal brainstem sensory nuclear complex (Sessle, 2000). The trigeminal brainstem sensory nuclear complex is

usually subdivided into the principal sensory nucleus and the spinal tract nucleus, which is further divided into the oralis, interpolaris, and caudalis subnuclei. The most caudal of these, the subnucleus caudalis (Vc), resembles the laminated structure and function of the spinal cord DH, and is for therefore often termed the medullary DH. The medullary DH is thought to play a major role in processing the craniofacial nociception (Hu et al., 1981;Sessle, 2000). The upper cervical spinal cord (UCC) includes the first cervical (C1) and second cervical (C2) spinal segment (Hu et al., 2005). The DH of the UCC constitutes a transmission zone between the Vc and the rest of the spinal cord. The nociceptive primary afferents synapse onto second order interneurons of the spinal and medullary DH. The interneurons are classified according to their cutaneous response properties into three main types. The two nociceptive types of neurons are the nociceptive specific (NS) neurons that only respond to noxious stimuli and the wide dynamic range (WDR) neurons that respond to both noxious and non-noxious stimuli, with an increasing discharge rate as the intensity of the stimulus increases (Price et al., 1976;Hu et al., 1981). The third non-nociceptive type is the low-threshold mechanoreceptive neurons that respond to non-noxious stimuli only. The NS neurons are driven by nociceptive afferents whereas both nociceptive and non-nociceptive afferents converge onto the WDR neurons. The medullary and UCC DH receive afferent information from the trigeminal nerve, from several of the cranial nerves, and from the upper cervical nerves (Hu et al., 2005). This extensive convergence is a feature that distinguishes the medullary and UCC DH from the lower spinal DH, where convergence is more sparse, though it is recognized (Sessle et al., 1986;Hoheisel et al., 1993;Mense, 1994).

Some nociceptive neurons in the spinal cord and trigeminal nucleus send axons that project to various brain regions. In the medulla, ascending nociceptive projections terminate in the reticular formation (Peschanski and Besson, 1984;Lima, 1990). Many spinal and trigeminal nociceptive neurons also project to structures in the midbrain, especially the periaqueductal grey (PAG) and adjacent regions (Yezierski, 1988).

The thalamus is the main target of ascending nociceptive pathways (Willis, 1985). Many nociceptive neurons in the spinal cord project to the ventroposterolateral nucleus (VPL), and those in the trigeminal nucleus project to the ventroposteromedial nucleus (VPM) of the thalamus (Giesler et al., 1976;Peschanski and Besson, 1984). Nociceptive information is transmitted from the thalamus to the cerebral cortex. The somatotopically precise input arriving in the VPL and VPM is

conveyed specifically to the primary somatosensory cortex, while nociceptive input reaching the intralaminar nuclei is transmitted to several cortical areas (Willis, 1985).

The noxious afferent information may also result in a withdrawal movement of the stimulated limb. Such nociceptive withdrawal reflex (NWR) is a spinal reflex that protects the body from possible traumatic insults. The reflex is mediated through a polysynaptic connection in the spinal cord that activates motor neurons in the ventral horn and ultimately a set of muscles. The muscles are activated in such a way that the movement most likely will move the insulted tissue from the potential harm. The NWR has played a major role in the study of the nociceptive physiology and pain since their description was presented by Sherrington (1910). Sherrington (1910) described the NWR as a flexion of the ipsilateral limb and an extention of the contralateral limb following cutaneous or deep nerve stimulation of the frog, cat, and dog. Several years later the NWR was studied in humans (Eklund et al., 1959;Kugelberg et al., 1960;Grimby, 1963). The NWR has been proposed as a tool in the study of pain as several studies have shown a correlation between the NWR size and the intensity of pain caused by the stimulation eliciting the NWR (Willer, 1977;Willer and Bussel, 1980;Garcia-Larrea et al., 1989) (Study II).

The NWR is modulated by several sources of afferent input e.g. skin, muscles, tendons and joint as well as supra-spinal control (Schomburg, 1990). The afferents mediating this sensory information was termed 'flexor reflex afferents' and are characterized as the afferents that may elicit the flexion reflex (Lundberg, 1979). This idea was based upon spinal interneuron recordings showing convergence from different afferents excitatory and inhibitory post synaptic potentials (Hongo et al., 1966). Therefore, the NWR does not always reflect the perception of pain intensity, but reflects the convergence of afferent input onto neurons in the reflex pathway. For instance, during walking the NWRs are dependent on the stage of the gait cycle (Spaich et al., 2004), and in an upper limb grapping task the NWRs are dependent on the state of motion (Serrao et al., 2006). In animal studies, it has been shown that there is a correlation between the DH neurons and the single motor units after noxious electrical stimulation (Jankowska and Roberts, 1972a;Jankowska and Roberts, 1972b;Brink et al., 1983;Morgan, 1998;You et al., 2003). This indicates that the flexor afferents converge before or at the DH neurons that were recorded in these studies. In the orofacial area, the general protective reflex in the perioral area is the jaw opening reflex (Dubner et al., 1978) and the blink reflex that protects the eye (Ellrich et al., 1997a). The jaw-opening reflex is often evaluated as

exteroceptive suppression, also called a silent period, of the electromyography (EMG) activity during sustained voluntary contraction of the masseter (MAS) or temporalis muscle. The jaw-opening is rarely recorded directly in human studies because opening of the jaws is attained by gravitational pull in the lower jaw and activation of the jaw opening muscles (e.g. the digastric muscle) that are difficult to record from. Electrically evoked silent periods are usually divided into a short latency and long latency reflex (Cruccu et al., 1987). It has been argued that the long latency jaw-opening reflex is a nociceptive reflex, although not purely nociceptive (Ellrich et al., 1997b). The DH neuron recordings and NWR recordings prove a valuable window into the nociceptive physiology and sensory convergence at the level of the spinal cord.

1.2 Aim of the Ph.D. project
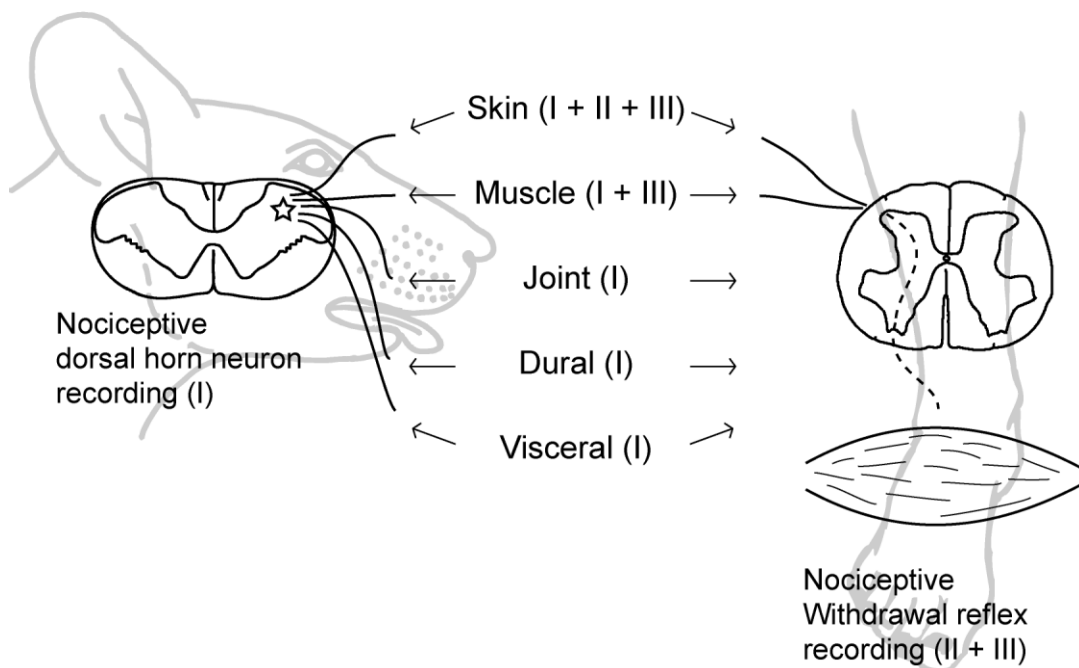
Figure 1. Schematic illustration of convergence in the sensory system investigated in the Ph.D. thesis. In study I nociceptive first cervical (C1) dorsal horn (DH) neurons of the rat was recorded, after stimulation of the Cutaneous, Muscle, joint, Dural, and visceral stimulation. In study II a new method to elicit nociceptive withdrawal reflexes (NWR) by radiant heat was presented. In study III the modulation of heat evoked NWRs by muscle stimulation was studied.

The aim of this Ph.D. project was to investigate the sensory convergence in human and animal experimental settings (Figure 1). Therefore, the convergence pattern of several cutaneous, deep, dural and visceral afferent sources was investigated in an electrophysiological study of nociceptive C1 DH neurons in rats (study I). Similar electrophysiological studies of nociceptive DH neurons are not feasible in human studies. Therefore, two studies of the NWR were performed (study II and III). Study II was set up to develop a novel approach to evoke NWRs by a natural and nociceptive specific heat stimulus, and to characterize the stimulus response-relations and the organization of these NWRs. Transient muscle pain was applied as conditioning stimulus of the NWR at different time intervals to investigate a possible facilitation of the NWR by muscle afferents caused by a convergence of cutaneous and muscle afferents (study III).

2 Animal models of trigeminal and upper cervical convergence

Animal models are often used in research of the nociceptive system (Le Bars et al., 2001). Though direct inquiry of possible pain is not available, important information about the nervous system still can be achieved; information that is not accessible in human experimental settings. In the following, some animal models that have been used to evaluate sensory convergence are discussed. Emphasis will be on sensory convergence in the craniofacial area, because sensory convergence and central sensitization has been proposed as the neurophysiologic basis for the spread and referral of pain in several craniofacial disorders (Sessle, 2000) such as temporomandibular disorder (Dworkin et al., 1990), whiplash (Munglani, 2000), angina pectoris (cardiac pain) (Foreman, 1999;Foreman, 2000), and headache (Bogduk, 2001;Bartsch and Goadsby, 2003b). Sensory convergence is pronounced in the craniofacial area as afferent information from both the trigeminal and cervical area projects both to the Vc and the UCC.

2.1 Anatomical investigation of afferent fibers

One approach to study sensory convergence is to investigate the projection of afferent input to the central nervous system. Generally, sensory information is processed at specific locations in the central nervous system in a somatotopic manner. Hence, adjacent afferent sources are processed at adjacent locations in the sensory cortex and a map of the peripheral tissue is described in the sensory cortex accordingly. This map is possibly better known as the homunculus man. A similar pattern is observed in the spinal and medullary DH.

2.1.1 Horseradish peroxidase

Transganglionic transport of horseradish peroxidase (HRP) has been introduced as a method to trace the projection of afferent nerves and thus to ascertain the central representation of peripheral receptive fields (Grant et al., 1979;Mesulam and Brushart, 1979). HRP injected to the trigeminal or C2 ganglions produced labeling in the C1 DH (Pfaller and Arvidsson, 1988), and a systematic study of the oral and facial nerves confirmed an 'onion-like' representation of the facial skin in the trigeminocervical complex, where rostral areas are represented rostrally in the subnucleus interpolaris, and more caudal areas are represented more caudally in the UCC (Shigenaga et al., 1986). HRP tracing of the corneal afferents (van Ham and Yeo, 1996), MAS afferents (Dessem and Luo, 1999), and the superior laryngeal nerve (SLN) afferents (Nomura and Mizuno, 1983) showed projection to the C1 DH. This indicated a convergence of several trigeminal and cervical afferent sources in the C1 DH. The HRP technique labels a broad spectrum of afferent fiber thicknesses, and not specifically the nociceptive afferents. Therefore, a method that labels nociceptive neurons more specifically may be preferred.

2.1.2 Fos-like immunoactivity

Fos-like immunoreactivity (Fos-LI) was introduced by Hunt et al. (1987) as a method to stain neurons involved in processing nociceptive input. After a noxious stimulation the immediate early gene c-*fos* is expressed in neurons responding to the stimulation (Morgan and Curran, 1989). The c-*fos* gene encodes the Fos protein that regulates "downstream" expression of other genes, most likely the preprodynorphin gene (Draisci and Iadarola, 1989;Hunter et al., 1995). The following increase in dynorphin probably induces antinociceptive action.

The Fos-LI staining method has been used extensively and proven a valuable tool in the investigation of the nociceptive system. Fos-LI neurons in the spinal cord DH and medullary DH are in general located in laminae I-II and V-VI and X (Hunt et al., 1987). However, the specific location of Fos-LI neurons depends on the stimulated afferent source. In general a somatotopic organization has been shown in the spinal and medullary DH. But, an overlap of Fos-LI neurons has been shown in the C1 DH, where stimulation of both trigeminal and cervical afferent sources produced Fos-LI. Noxious stimulation of the skin innervated by any of the 3 main branches of the trigeminal nerve, have shown that Fos-LI cells are located mainly in lamina I and V of the C1 DH (Strassman and Vos, 1993;Zhou et al., 1999). Furthermore, noxious corneal stimulation has shown

Fos-LI in the Vc/C1 transition (Lu et al., 1993) and in the C1 (Strassman and Vos, 1993;Meng and Bereiter, 1996), mainly in the lateral part of lamina I. Noxious mechanical dural stimulation revealed Fos-LI mainly in laminae I and V of the C1 DH (Strassman et al., 1994), and electrical stimulation of the dura revealed Fos-LI mainly in lateral part of laminae I and II (Kaube et al., 1993). Noxious stimulation of deep structures in the trigeminal and cervical area also evoked Fos-LI in the C1 DH by noxious stimulation of the trapezius and splenius muscles (Kalezic et al., 2004), the temporomandibular joint (TMJ) (Hathaway et al., 1995;Zhou et al., 1999), the MAS muscle (Imbe et al., 1999), the tongue (Strassman and Vos, 1993), and the hypoglossal (XII) nerve (Bereiter et al., 2000) evoked Fos-LI mainly in the laminae I and V. These findings indicate a substantial overlap of afferent nociceptive input to the C1 DH (Figure 2). Although the Fos-LI displays activity at single cell resolution it is not possible to derive if the noxious afferent input converges on common neurons or if the neurons are simply located in the same general areas of the C1 DH without any functional overlap.
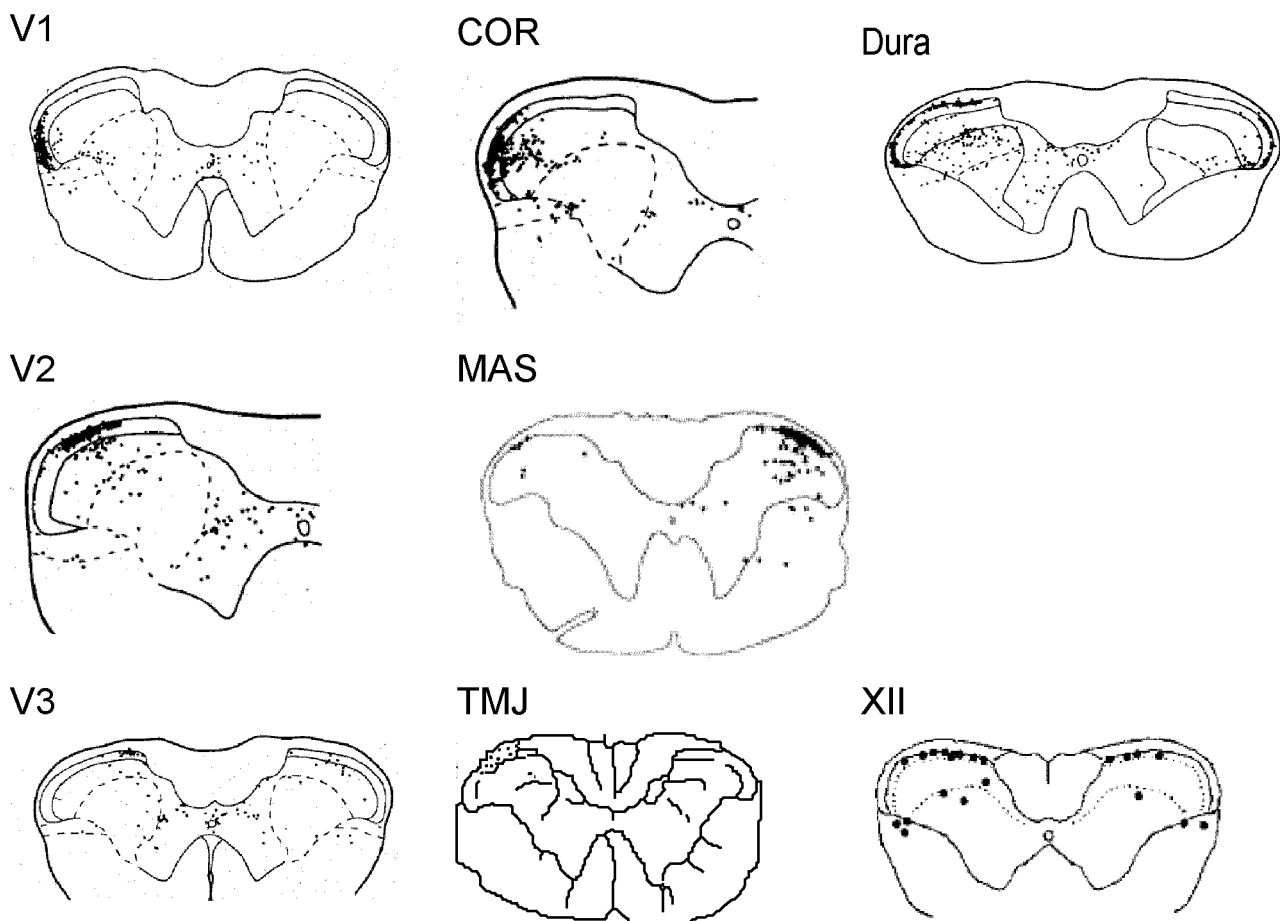
Figure 2. Neurons in the C1 spinal cord exhibited Fos-like immunoreactivity following noxious stimulation of skin areas innervated by the ophthalmic (V1), maxillary (V2), and mandibular (V3) branch of the trigeminal

nerve, the cornea (COR) (Strassman and Vos, 1993), dura (Strassman et al., 1994), the masseter (MAS) muscle (Imbe et al., 1999), the temporomandibular joint (TMJ) (Zhou et al., 1999), and hypoglossal (XII) (Bereiter et al., 2000) showing a substantial afferent convergence. The figure illustrates an overlap of afferent fibers from e.g. V1, dura, and COR in the lateral aspects of the lamina I. Comparisons between the studies should be done with caution as different stimulation modalities were used; complete Freund's adjuvant, thermal, mechanical pinch and electrical stimulation.

Fos-LI has proved to be a strong tool in the study of nociception, but some caution should be taken in the interpretation of the results. Some of the neurons in the pain pathway, although activated, do not show Fos-LI and all stained neurons are not necessarily nociceptive neurons (Harris, 1998). Anaesthetics and sedatives are known to be potent inhibitors of *fos* induction under certain circumstances (Hunt et al., 1987;Sonnenberg et al., 1989), and the distribution of Fos-LI depends on the modality of the noxious stimulation (Lima et al., 1993).

Anatomical investigation of afferent projection such as the HRP and Fos-LI to the spinal cord and not the least the upper cervical part, have provided valuable and detailed descriptions of activated areas. However, the anatomical studies cannot reveal possible convergence onto single neurons; only indicate an overlap in areas activated by afferent input. Furthermore, the anatomical studies cannot provide short-lasting dynamic information about the activity in the nervous system. Electrophysiological studies can provide such information, though it is more cumbersome to apply.

2.2 Electrophysiological indications of sensory convergence in the C1 DH

Electrophysiological studies have shown afferent convergence onto DH neurons throughout the spinal cord (Hoheisel and Mense, 1990) and medullary DH (Sessle et al., 1986). In the UCC afferent input from both trigeminal and cervical areas has been shown. Many of the UCC neurons receive a wide range of convergent input form afferent sources such as the skin (Hu et al., 2005), the cornea (COR) (Meng et al., 1997), and also deep structures such as the tongue, innervated by the XII nerve (Hu et al., 2005), the TMJ (Cairns et al., 2001a). Furthermore, electrophysiological studies have shown dural input to the UCC (Burstein et al., 1998;Yamamura et al., 1999;Malick et al., 2000;Bartsch and Goadsby, 2003b). Neurons activated by visceral stimulation have been recorded in the UCC (Foreman, 1999), as electrical stimulation of the phrenic nerve fibers above

the heart (Razook et al., 1995;Chandler et al., 1998), the vagal and sympathetic fibers (Chandler et al., 1996) and the SLN which is a branch of the vagal nerve, (Hu et al., 1981;Chandler et al., 1996), and to intrapericardial injections of algogenic chemicals (Qin et al., 2001) have been shown to evoke responses in neurons in the UCC.

Although, these studies have shown afferent convergence onto common interneurons in the UCC, only 2 or 3 afferent sources have been investigated in each study. A systematical investigation of input form several afferent sources onto interneurons in the in rostral part of the medullary DH of the cat has been reported (Sessle et al., 1986). Study I of the present thesis aimed at a systematic investigation of the convergent afferent input from several cutaneous, deep, dural, and visceral afferent sources onto nociceptive C1 DH neurons in the rat.

2.2.1 Stimulation and recording of C1 DH neurons

In study I, neurons were recorded from the C1 DH and electrolytic lesions confirmed the loci to be within the C1 DH according to the anatomical landmarks (Molander et al., 1989). The neurons were classified as nociceptive WDR or NS according to their cutaneous response properties (Price et al., 1976;Hu et al., 1981;Hu, 1990). Gentle mechanical stimulation (< 5 mg) was applied to the COR and dura, and noxious mechanical stimulation (~ 100 g) was applied to the TMJ (Cairns et al., 2001c). The sizes of the cutaneous mechano-receptive field (RF) were compared before and after the electrical stimuli were applied to evaluate possible expansion of the RF. Electrical stimulation was applied to 6 cutaneous sites; above the eye innervated by the ophthalmic trigeminal branch (V1), below the eye innervated by the maxillary trigeminal branch (V2), posterior to the corner of the mouth innervated by the mandibular trigeminal branch (V3), posterior to the ear in the area of the second and third cervical dermatomes (C2/C3), the shoulder (SHO), and the forepaw (PAW). Furthermore, electrical stimulation was applied to the COR, the dura after a partial craniotomy, the exposed C2 nerve, the XII nerve, SLN, the TMJ, and the MAS muscle. The responsiveness to stimulation of these afferent sources was assessed (Figure 3).
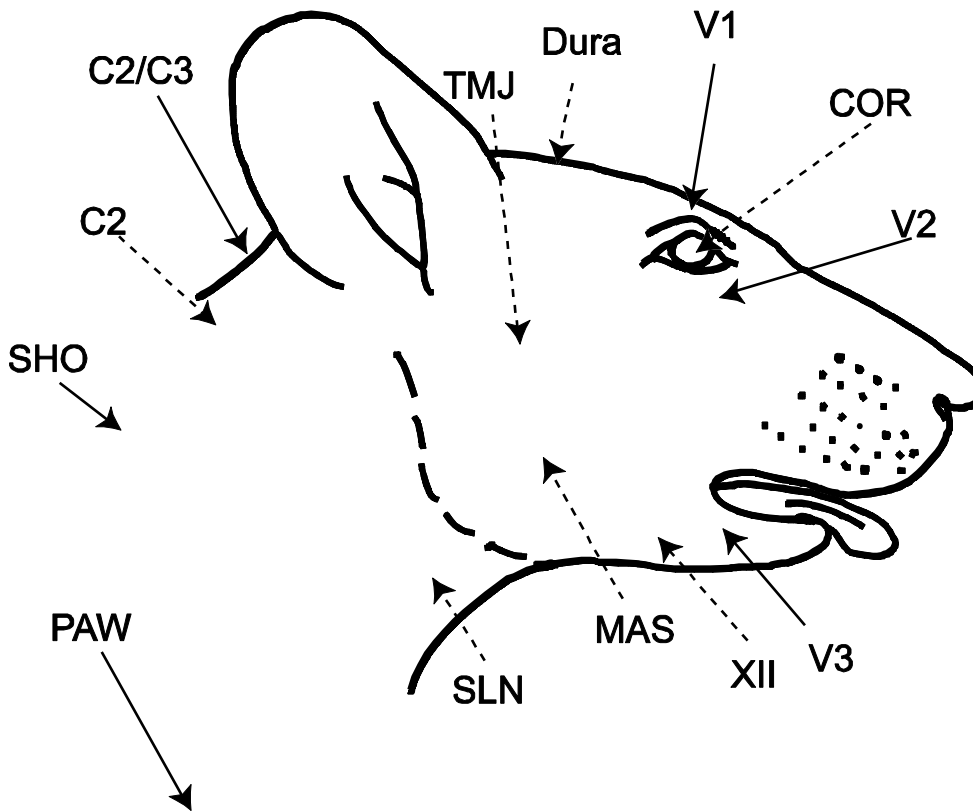
Figure 3. Electrical stimulation was applied to 6 cutaneous sites (solid arrows); above the eye innervated by the ophthalmic trigeminal branch (V1), below the eye innervated by the maxillary trigeminal branch (V2), posterior to the corner of the mouth innervated by the mandibular trigeminal branch (V3), posterior to the ear in the area of the second and third cervical dermatomes (C2/C3), the shoulder (SHO), and the forepaw (PAW). Furthermore, electrical stimulation was applied to 7 non-cutaneous sites (dashed arrows); the cornea (COR), the dura, the exposed second cervical (C2) nerve, the hypoglossal (XII) nerve, superior laryngeal nerve (SLN), the temporomandibular joint (TMJ), and the masseter (MAS) muscle.

Glutamate (0.5 M, 10 µl, pH 7.0) was injected to the tongue, MAS muscle, neck muscle (splenius cervicis), or intrapericardially, or dripped onto the dura. Glutamate has been shown to have an algesic effect (Yu et al., 1996;Cairns et al., 2001c). Furthermore, towards the end of some experiments, 0.5 ml 3.5% acetic acid was slowly (~5 s) injected intraperitoneally.

2.2.2 Cutaneous afferent input

Several electrophysiological studies have reported C1 DH neurons with cutaneous RF that includes the facial region and the C2 and C3 dermatomes (Burstein et al., 1998;Yamamura et al., 1999;Malick et al., 2000;Foreman, 2000) and outside the trigeminal and cervical areas (Smith et al., 1991;Chandler et al., 1996;Chandler et al., 1998;Clement et al., 2000). However, no neurons were

found with RF outside the craniofacial area and only few neurons responded to electrical stimulation of the SHO and PAW in Study I (Table 1).

2.2.3 Corneal afferent input

Neurons in the C1 DH have been shown to respond to noxious corneal stimulation and that corneal sensitive neurons also have cutaneous RF (Meng et al., 1997;Hirata et al., 1999;Malick et al., 2000;Hirata et al., 2004)(study I). Evidently, study I found a significant correlation between the responsiveness to COR and V1 stimulation, which confirmed previous studies describing neurons receiving both V1 and COR input (Meng et al., 1997;Hirata et al., 1999;Malick et al., 2000;Hirata et al., 2004).

2.2.4 Dural afferent input

Electrophysiological studies have indicated an even distribution throughout the C1 DH of neurons responding to dural stimulation (Burstein et al., 1998;Malick et al., 2000) (study I), though neurons in the Vc responding to dural stimulation seems to concentrate in the ventrolateral area (Burstein et al., 1998).

Patients with primary headaches often report pain that involves the front of the head; the V1 nerve branch territory. However, the pain may in due time exceeds the trigeminal territory to the back of the head which is innervated by the greater occipital nerve (Anthony, 1992;Bartsch and Goadsby, 2003b). Study I showed correlation between V1 and Dura, which is in accordance with previous studies showing neurons responding to both dural and cutaneous stimulation of the V1 (Burstein et al., 1998;Yamamura et al., 1999;Bartsch and Goadsby, 2002;Bartsch and Goadsby, 2003a). Convergence along with sensitization of central neurons in Vc (Burstein et al., 1998), in C1 (Study I), and in C2 (Bartsch and Goadsby, 2002) provide a neurophysiologic basis for the clinical phenomenon of spread and referred pain by which pain originating from an affected tissue is perceived as originating from a distant area (Ruch, 1947). In cervicogenic headache, pain spreading to the back of the head may be originating from the structures innervated by the C1 to C3 spinal nerves, and include the upper cervical synovial joints, the upper cervical muscles, the C2-3 disc, the vertebral and internal carotid arteries, and the dura mater of the upper spinal cord, and posterior cranial fossa (Bogduk, 2001). This was supported by study I showing similarities in responsive areas of the C1 DH following stimulation of the dura and C2/C3.

2.2.5 Deep afferent input

C2 nerve

A wide spread distribution of neurons responding to stimulation of the C2 nerve and injection of glutamate to the splenius cervicis muscle (innervated by the posterior primary rami of the inferior cervical spinal nerves) evoked responses in nociceptive C1 DH neurons (Study I).

TMJ

Studies have shown that injection of mustard oil into the TMJ evoked Fos-LI mainly in the superficial layers of the C1 DH but also few neurons in deeper layers (Hathaway et al., 1995;Zhou et al., 1999), whereas electrophysiological recordings showed a more widespread distribution throughout the C1 DH (study I). Study I showed correlation between TMJ and V2, although the TMJ is innervated by the maseteric and auticulotemporal branches of the V3 nerve (Davidson et al., 2003).

MAS muscle

More than half of the neurons responded to intra muscular electrical stimulation of the MAS muscle, whereas only one third responded to microinjection of glutamate into the MAS muscle (study I). It has been shown that injection of glutamate into the MAS muscle activated muscle afferents in rats, and that the same concentration evoked a painful sensation in humans (Cairns et al., 2001b). Anatomical studies have shown that the noxious stimulation of MAS projects to the intermedial part of lamina I of the C1 DH (Imbe et al., 1999;Dessem and Luo, 1999), whereas the present electrophysiological study found a more widespread distribution of MAS sensitive nociceptive C1 DH neurons (study I). The responsiveness to V3 cutaneous and MAS muscle stimulations was found to be correlated and their response pattern to C-fiber input were also similar (Study I). This complied with the fact that the MAS muscle is innervated by the masseteric branch of the V3 nerve.

XII nerve

In accordance to anatomical studies (Strassman and Vos, 1993;Carstens et al., 1995;Bereiter et al., 2000), study I showed neurons responding mainly to C-fiber stimulation of the XII nerve in the medial part of the C1 DH. Microinjection of glutamate to the tongue only rarely evoked responses in the C1 DH neurons (7% in both WDR and NS neurons). Furthermore, it has been shown that

electrical XII nerve trunk stimulation evoked more Fos-LI lamina I and II of the UCC than intramuscular tongue injection of mustard oil, thus indicating other afferent than the tongue musculature afferents travel in the XII nerve (Bereiter et al., 2000). Therefore, the low response rate found by glutamate injection of the tongue musculature may be caused both by few tongue glutamate receptors, and that tongue muscle afferents are only a subpopulation of the XII nerve. The responsiveness to stimulation of the XII nerve and SLN was significantly correlated and the difference in response patterns was small. The SLN innervates the larynx and the pharynx whereas the XII nerve innervates mainly the tongue, however these nerves seems to evoke similar responses in the C1 DH (study I).

2.2.6 Visceral afferent input

Previous electrophysiological studies have shown C1 DH neurons responding to electrical stimulation of SLN (Hu et al., 1981;Chandler et al., 1996). Approximately one third of the C1 DH neurons responded to stimulation of the SLN (Study I). Previous studies of neurons in the Vc showed even lower proportions (Hu et al., 1981).

Although, HRP tracing of left inferior cardiac nerve did not show projection more rostral than C8 (Kuo et al., 1984), stimulation of the vagal nerve afferents originating from the heart or other visceral structures, and cardiopulmonary sympathetic afferent fibers (Chandler et al., 1996) and the phrenic nerve (Razook et al., 1995;Chandler et al., 1998) evoked responses in the C1 DH neurons. However, phrenic fibers innervating diaphragm or abdominal structures either did not affect the C1 DH or to a lesser degree than phrenic input arising from thoracic structures (Razook et al., 1995;Chandler et al., 1998). Intrapericardial injections of algogenic chemicals has been shown to activate neurons in the C1 DH (Qin et al., 2001), however intrapericardial injections of glutamate did not evoke responses in the C1 DH neurons (study 1), indicating that glutamate receptors in the pericardial tissue either are not present or not sufficient numerous to evoke responses in the C1 DH neurons. Angina pectoris is often described as retrosternal crushing, burning, or squeezing characteristic pain, which may radiate to the throat, neck, or ulnar aspect of the left arm, sometimes reaching to the little finger. Less often, it radiates to the neck and jaw (Foreman, 1999). Stimulation of the phreneic, vagal or sympathetic afferent fibers and chemical stimulation of the heart excites spinothalamic tract neurons in the UCC segments of monkeys (Chandler et al., 1996;Chandler et al.,

1998) and rats (Razook et al., 1995;Qin et al., 2001), indicating that these neurons may be the neural basis for the projection of pain to the neck and the jaw areas.

2.2.7 Stimulus-Response relations

Neurons in the C1 DH responded to mechanical and electrical stimulation applied to the skin and to electrical stimulation of at least one of the deep, dural, and visceral sources (Study I, table 1). Study I showed stimulus-response relationship between mechanical stimulation applied to the center of the cutaneous RF for both the WDR and NS neurons (Study I, figure 4) showing the capability of the neurons to encode sensory information into the noxious range.

Electrical stimulation normalized to the activation threshold showed a double logarithmic relationship with similar correlation coefficients for all afferent sources (Figure 4). Therefore, the nociceptive neurons in the C1 DH are capable of encoding afferent information from cutaneous, deep, dural, and visceral sources.
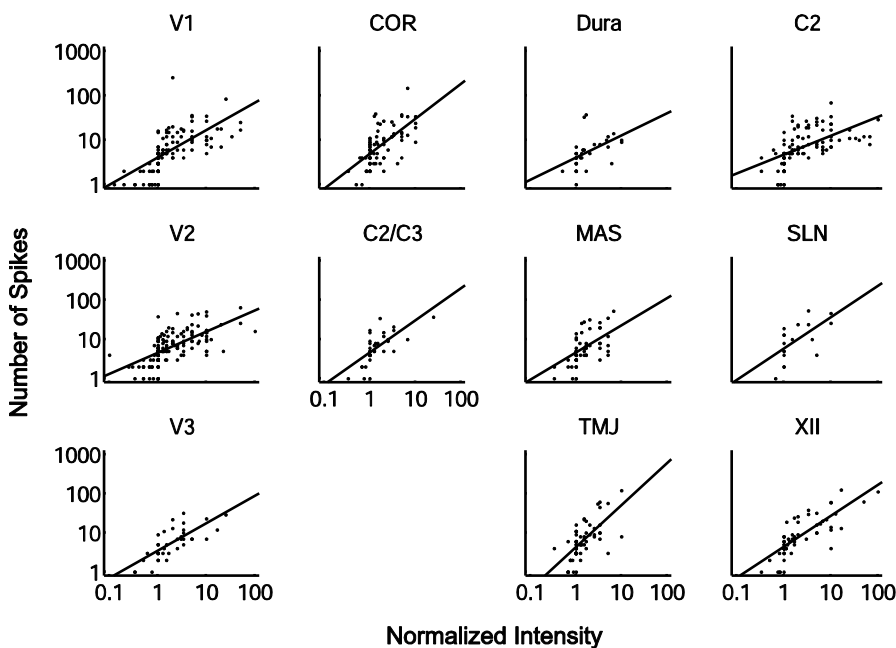
Figure 4. Stimulus response functions for electrical stimulation of the 6 cutaneous and the 7 non-cutaneous sites. The stimulation intensity was normalized to activation threshold of A- and C- fibers. The number of spikes to 5 stimuli was considered the response. The activation threshold was set to at least 3 responses to 5 electrical pulses; therefore some neurons did have few responses below threshold. The stimulus response relation could be approximated by a double logarithmic function. Linear regression of the double logarithmic transformed data showed a linear increase (OneWay ANCOVA, P < 0.001). The correlation coefficient was 0.68, 0.65, 0.76, 0.68, 0.64, 0.50, 0.61, 0.79, 0.67, 0.62, and 0.62 for stimulation of the V1, V2, V3, C2/C3, COR, dura, C2, XII, TMJ, MAS and SLN, respectively.

2.2.8 Afferent convergence in the craniofacial area.

Electrophysiological studies of the rostral Vc in cats showed a substantial afferent convergence onto nociceptive neurons (Sessle et al., 1986). Sessle et al., (1986) showed a larger number of afferent sources converged onto WDR than onto NS neurons, whereas study I did not find such difference between WDR and NS neurons in the C1 DH neurons. The reason for the difference between these two studies may be species differences but also the difference between the rostral Vc and the C1 DH.

Electrophysiological studies have shown that neurons in the UCC responding to deep, visceral, or dural afferent sources also have cutaneous RFs in the craniofacial area. These neurons may be the neurophysiological basis for various painful conditions where pain from one afferent source is also perceived as originating from distant cutaneous, deep, or visceral tissue. In temporomandibular disorder, pain originating from the TMJ or other deep adjacent areas may spread and be perceived from other facial areas innervated by all three branches of the trigeminal nerve (Dworkin et al., 1990). Afferent convergence from blood vessels and skins may play an important role in headache that often manifested with referral pain to the extracranial skin tissue in periorbital or temporal region (Piovesan et al., 2003;Bartsch and Goadsby, 2003b). Angina pectoris may result from nociceptive cardiac afferent input to UCC neurons and may lead to pain sensation in the neck and jaw areas (Foreman, 1999;Foreman, 2000).

2.2.9 Lack of afferent input caudal to the cervical area

Study I showed that neurons in the C1 DH responded to afferent input from several trigeminal and cervical afferent sources. All neurons responded to cutaneous stimulation of the facial skin in the area posterior to the whiskers and generally anterior to the ear. None of the neurons has cutaneous RF posterior to the ear. Electrical stimulation readily evoked responses from the cutaneous V1, V2, V3 and C2/C3 areas, but rarely from SHO and PAW. Likewise, intraperitoneal injection of ascetic acid or intrapericardial microinjection of glutamate did not evoke responses in any of the tested neurons. Therefore, it seemed that although nociceptive C1 DH neurons received a wide range of afferent input these sources it did not include input caudal to the cervical areas, which was in agreement with studies by Hu et al. (2005). However, several previous studies found afferent input from areas caudal of the cervical region activating neurons in the C1 (Yezierski and Broton, 1991;Smith et al., 1991;Razook et al., 1995;Villanueva et al., 1996;Chandler et al., 1996;Chandler

et al., 1998;Ness et al., 1998;Chandler et al., 1999;Qin et al., 2001;Qin et al., 2004). There are several possible reasons for differences between these studies and the findings by Hu et al. (2005) and Study I. The first reason could be species differences since Hu et al. (2005) and study I used rats whereas some of earlier studies used cats (Smith et al., 1991) or monkeys (Chandler et al., 1996;Chandler et al., 1998;Chandler et al., 1999). However, this possibility seems an unlikely explanation; although the rat has a limited and unclear C1 dermatomal representation, several of the earlier electrophysiological studies (Hathaway et al., 1995;Razook et al., 1995;Burstein et al., 1998;Yamamura et al., 1999;Malick et al., 2000;Qin et al., 2001;Qin et al., 2004) were carried out in rats. Another possible explanation is that the neuronal sample in some of the earlier studies also included recordings at sites deeper than laminae I–V of the C1 DH, and sometimes included neurons in the ventral horn (Qin et al., 2001;Qin et al., 2004), lateral reticular formation (Ness et al., 1998) including the subnucleus reticularis dorsalis (Villanueva et al., 1996), and lateral cervical nucleus (Yezierski and Broton, 1991). Many neurons in these deep locations typically have RFs including half or the whole body (Yezierski and Broton, 1991;Villanueva et al., 1996;Ness et al., 1998), whereas Vc and C1 DH neurons in lamina I–V have much more restricted RFs (Yamamura et al., 1999;Malick et al., 2000). The third explanation may be the use of antidromic activation as the searching stimulus in some of the earlier studies that led to the inclusion neurons with large RF in the sample. Some of these neurons were indeed recorded outside the DH region (Smith et al., 1991;Chandler et al., 1996;Chandler et al., 1998;Ness et al., 1998;Chandler et al., 1999;Qin et al., 2001;Qin et al., 2004).

2.2.10 Neuron classification based on deep, dural and visceral input

Classification of the responsiveness of nociceptive DH neurons is usually based on the response properties to cutaneous stimulation (Price et al., 1976;Hu et al., 1981;Hu, 1990). Study I revealed a substantial afferent input from other structures to C1 DH neurons. It therefore seemed obvious to inquire if there were any natural clustering based on the response properties to deep, dural and visceral stimulation. Principal component transformation (Hotelling, 1933) and k-means clustering of the responsiveness revealed two possible groups of neurons. Neurons in group I had in general a lower responsiveness and contained more NS neurons and fewer WDR neurons than group II. This indicates that the neurons can be classified according to their deep, dural and visceral responsiveness properties, and that the cutaneous response properties only to some extend were reflected in this clustering.

2.3 Short summary

Nociceptive neurons in the C1 DH receive excitatory afferent input from trigeminal and cervical cutaneous areas, but only sparse afferent input from cutaneous areas caudal to the C2/C3 dermatome. Furthermore, the nociceptive neurons receive convergent input from cutaneous, corneal, deep, dural and visceral sources. Electrical stimulation of several craniofacial structures has reviled a stimulus-response relationship indicating that these neurons were capable of encoding noxious information from several afferent sources. This indicates that the nociceptive neurons in the C1 DH may play an important role in nociceptive integration.

3 Human models of spinal cord convergence

Although animal experiments provide great insight into the physiology and pathology of nociception the knowledge cannot always be translated directly to the physiology and pathology of humans. For instance, it is not readily investigated if nociceptive responses in e.g. DH neurons in fact would cause a painful sensation had the animal been awake. Single firings in single second order DH neurons are most likely insufficient to evoke a conscious perception of pain. Similar problems are evident in all animal studies although pain behavior can be studied. Therefore, experimental methods to investigate the healthy human nociceptive physiology as well as pathologies of patients are important in order to understand the physiology and treat the pathology better. Although some of the methods used in animal experiments obviously can not be applied in human experiments there are several ways to investigate the nociception in humans. Especially, inquiries about the pain intensity (e.g. VAS) and quality (e.g. McGill questioner) are possible, if nociception leads to pain. It should be kept in mind that such inquiries are entirely subjective, as pain is subjective. Furthermore, careful instructions must be given to the subjects as to ensure the validity of the results. Less subjective methods to study the nociceptive system of humans may be desirable. For such purpose several imagining and electrophysiological methods are available. To study sensory convergence at the spinal level the NWR is especially interesting as a window into nociceptive processing at the spinal level.

3.1 NWR evoked by natural stimulation

Most investigations on NWR have used electrical stimulation to activate afferent responses (Sandrini et al., 2005). The electrical stimulation can be applied to the surface of the skin in order to activate the fibers innervating the skin and subcutaneous layers. In animal studies, the nerve can be

exposed by dissection and stimulated directly. Electrical stimulation enables a reliable and transient stimulation, where the parameters such as intensity, pulse width, repetitions and timing are easily controlled. The advantages of electrical stimulation are numerous, but some disadvantages are also evident. It is difficult to determine which afferents are activated. It is especially difficult to avoid activating non-nociceptive cutaneous afferents; therefore, electrical stimulation can not be assumed to be purely nociceptive. In addition, the electrical stimulation bypasses the receptor endings, so inquiries about the transduction mechanisms cannot be made. Furthermore, the afferents are activated simultaneously by the electrical pulse. This does probably not resemble natural stimulation where the afferent barrage is not synchronized. Therefore, the sensory information arriving at the spinal cord DH may not resemble natural stimulation, though the NWR is a natural response to a noxious stimulation.

3.1.1 Mechanical stimulation

Mechanical stimulation has been used as a natural stimulation to evoke NWR in animal studies (Le Bars et al., 2001). Unlike the manual pinch or pressure algometry often used in animal studies transient time-locked stimulation must be used to evoke NWR in human subjects. Some methods to activate the nociceptive system in a transient and time-locked manner have been proposed, but have some deficits. High-energy ultrasound stimulation is able to activate the nociceptive system, but it is not known whether the energy is transmitted as mechanical or heat energy (Gavrilov et al., 1977). Impact with a small metal slug has also been proposed (Kohlloffel et al., 1991), but there is no evidence that it will evoke NWR. The photoacoustic effect, where short laser pulses are converted into sound waves in the tissue may be a possible method to apply a transient activation of the mechanoreceptors (Doukas and Kollias, 2004), however, substantial parts of the energy may be converted into heat, and again there is no evidence that the stimulus can evoke NWR.

3.1.2 Heat stimulation

Heat is another stimulation modality used to activate the nociceptive system (Julius and Basbaum, 2001). Thermodes have been used to apply contact heat stimulation (Nielsen and Arendt-Nielsen, 1998;Arendt-Nielsen and Chen, 2003), but the mechanical aspect of contact heat has been shown to alter the perception of pain (Svensson et al., 1997). More importantly the temperature rise-time is too long to evoke NWR, therefore radiant heat stimulation may be preferable to contact heat stimulation (Baumgartner et al., 2005). However, temperature at the skin surface can be controlled by advanced thermodes (Chen et al., 2001;Casey, 2006). Such a temperature control is rarely

provided during laser stimulation, though a system has been developed (Meyer et al., 1976). Therefore it is important to control the delivered energy and the background temperature of the skin as variation in background temperature may lead to wrong conclusions (Tjolsen et al., 1988).

As a transient stimulation profile is necessary in order to evoke the NWR the temperature increase of the skin must be fast. High intensity stimulation is generally needed to allow investigations of transient and time critical responses (e.g. NWR, evoked cortical potentials, reaction time etc.). Although Hardy (1953) presented a method to evoke reflexes in one paraplegic subject by a focused light bulb. Experimental setups using high intensity light bulbs, such as a xenon lamp, may provide shot exposure of heat stimulation at high intensity using a shutter to restrict the stimuli. However, the broad-spectered visible light is significantly reflected from the skin surface, and blackening of the skin may be necessary (Andersen et al., 1994). Laser systems are able to deliver very high intensity radiation in a narrow frequency range, and in well defined areas. Therefore, laser stimulation seems to be suitable for this purpose (Plaghki and Mouraux, 2003). Each laser is characterized by the wavelength of the radiated light. Several different lasers are now commercially available even at high power (~100 W) e.g. diode laser in the visible wavelength range, solid state lasers such as the YAG and YAP lasers, and gas laser such as the CO_2-lasers in the infrared range.

When laser light is emitted to the skin some of the light will be reflected while the rest is transmitted through the surface. The proportions of transmitted light depend on the wavelength of the light. The transmitted light is then absorbed in the skin layer or transmitted through the layer. The energy of the absorbed light is converted into heat and thus contributed to an increase in temperature. The transmitted light will be passed directly on to deeper layers or scattered along the path (Hardy et al., 1956). The light will reemerge form the surface if scattering of the light bend the path more that 90º, and thus add to reflection. In a small volume of the skin the intensity of the transmitted light I_T can be expressed as a function of the *absorption coefficient, μ,* as

$$I_T = I_0 \exp(-\mu x),$$

where I_0 is the intensity of the light entering the volume and x is the length of the path that the light travels through the volume[1]. In general, μ is a material constant that depends on the wavelength of the light (Hardy and Muschenheim, 1934). The skin is inhomogeneous and therefore μ is variable through the skin for light in the visible and near infrared range (< 2 μm). In the infrared range the

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[1] If the volume is small, x is the dimension of the volume as scattering will be neglectable.

light is almost exclusively absorbed in water and since the water content is approximately homogeneous in the skin below the striatum corneum μ can be approximated to be constant. Furthermore, in the infrared range the human skin acts almost as a perfect black body (Hardy, 1934), and therefore none of the incident radiation is reflected from the surface.

The CO_2-laser emits light at a wavelength of 10.6 µm. At this wavelength μ is approximately 200cm^{-1} (Haimi-Cohen et al., 1983). From the equation it is seen that the transmission is reduced to 1/e (37%), at a depth of 50µm. Therefore, most of the energy is absorbed in the superficial layers reasonably close to the nociceptors that are located approximately 200 ± 170 µm below the surface (Tillman et al., 1995). For these reasons the CO_2-laser, as introduced by Mor and Carmon (1975), has proven suitable for the study of pain (Arendt-Nielsen and Bjerring, 1988a;Arendt-Nielsen and Bjerring, 1988b). However, solid state lasers such as thulium-YAG (wavelength 2.01µm; $\mu = 28$cm^{-1}) (Spiegel et al., 2000) or YAP (wavelength 1.34µm; $\mu < 20$cm^{-1}) (Iannetti et al., 2004) may be more suitable for activation of nociceptors as the energy from these lasers is absorbed closer to the nociceptors (Baumgartner et al., 2005). However, assuming that μ is constant throughout the skin for infrared absorption most energy will be absorbed in the superficial parts of the skin according to the equation above disregarding the value of μ. For review of heat stimulation please refer to Plaghki and Mouraux (2003)

Models for heat transfer during and after radiation of infrared pulses have been developed and tested (Buettner, 1951;Hendler et al., 1958), and has been applied to infrared laser stimulation (Haimi-Cohen et al., 1983;Bromm and Treede, 1983).

3.1.3 Heat evoked NWR

Heat evoked NWRs have been studies in animal models (e.g. (Burke et al., 1971;Behrends et al., 1983;Schomburg and Steffens, 1986). However, NWR are not easily evoked by heat stimulation in human subjects, and therefore the reports of heat evoked NWR in healthy human volunteers are few. Half a century ago Hardy (1953) presented a study where NWRs were evoked in one paraplegic subject by radiant heat. Several years later, Willer et al. (1979) reported a study where the heat was applied to the lateral edge of the dorsum of the foot (sural nerve territory) and the NWR was assessed as the EMG recorded from the tibialis anterior and biceps femoris muscles. Although an Argon laser was used, the authors suggested that a CO_2-laser would have been preferable had it been available (Willer et al., 1979). Campbell et al. (1991) reported a study where

they applied radiant heat to the dorsal forearm using a tungsten halogen projector lamp and assessed NWR responses as the EMG response recorded from the biceps muscle. In study II a method was developed to apply radiant heat supplied by a CO_2-laser to a large area and thereby activating a large number of afferents which utilized spatial summation (Nielsen and Arendt-Nielsen, 1997). Furthermore, the laser beam was rapidly moved during stimulation in a pattern creating a 'top-hat-like' spatial stimulation intensity profile in contrast to the Gaussian profile delivered by unfocused or expanded CO_2-laser beams (Figure 5). The Gaussian profile has a maximum where high temperature may cause skin damage (Arendt-Nielsen and Chen, 2003). By stimulation with the top-hat-like profile high temperatures may be achieved in a larger area without causing skin damage and still activate a sufficient amount of nociceptors to elicit a NWR response. It has been suggested that power densities over $0.35W/mm^2$ should be avoided (Beydoun et al., 1993). However, this estimation is done for Gaussian profiles and may be higher for top-hat-like spatial intensity profiles.
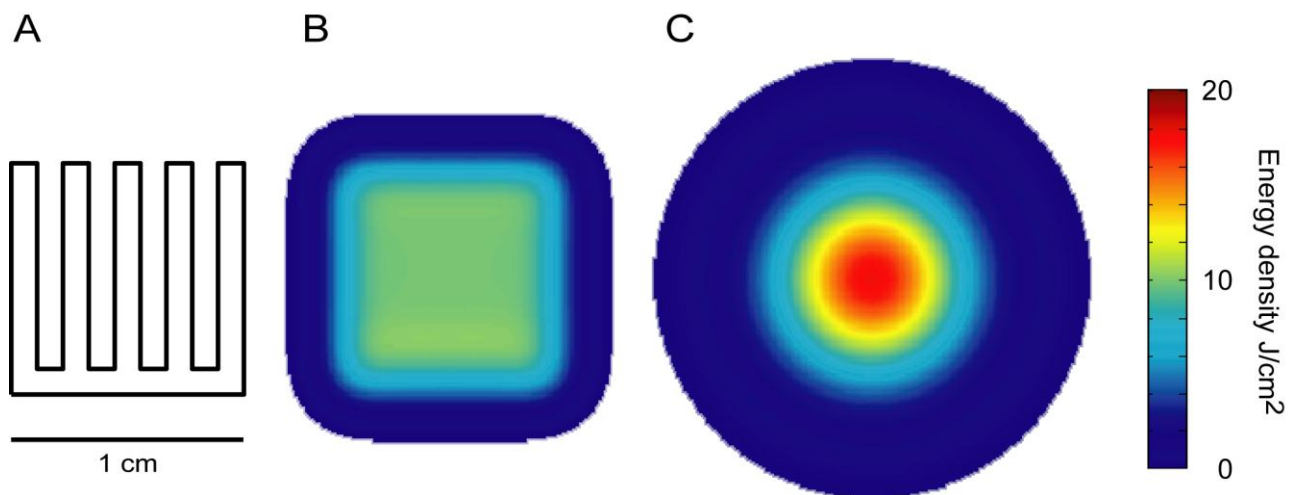
Figure 5. Energy density following laserstimualtion. Radiating a laser beam through a 'scannerhead' makes it possible to move the laser beam rapidly around in any pattern (e.g. A) during stimulation. This will enable stimulation with a 'top-hat-like' energy density profile (B). Alternatively, a stationary Gaussian energy density profile (C) can be expanded in order to stimulate a larger area, but will result in a high temperature at the center.

In study II, heat was applied to the dorsum of the foot and the front of the lower leg with a CO_2-laser and EMG was recorded from the iliopsoas, quadriceps vastus lateralis, biceps femoris, tibialis anterior, and soleus muscles. Poor correlation was found between heat intensity and the EMG responses of iliopsoas, quadriceps vastus lateralis, biceps femoris, and tibialis anterior muscles, whereas no correlation was found for soleus muscle. The correlation between the heat intensity and

the perceived pain intensity was also significantly, but poorly, correlated (linear regression, P < 0.05, R = 0.53), which resembles the result from Campbell et al. (1991). In contrast to laser stimulation of the lower limbs it is possible to elicit nociceptive protective craniofacial reflexes using brief (< 40 ms) pulses applied to a small (< 1 cm^2) perioral cutaneous area (Ellrich et al., 1997b;Cruccu and Romaniello, 1998;Romaniello et al., 2002). The jaw-opening reflex can be observed as single silent period of ongoing MAS muscle activity following Th:YAG-laser (Ellrich et al., 1997b) or CO_2-laser (Cruccu and Romaniello, 1998) stimulation resembling the long latency silent period following electrical stimulation, as both are assumed to be mediated by nociceptive Aδ- afferents. Heat evoked nociceptive reflexes seems to be more readily evoked in the craniofacial area compared to the extremities. Comparing the laser evoked cortical potential following stimulation of the foot, hand and perioral skin showed progressively smaller amplitudes at distal areas (Cruccu et al., 1999). Cruccu et al. (1999) speculated that longer afferent conductance routes may temporally disperse the afferent barrage of the spinal cord DH nociceptive neurons and thus cause weaker laser evoked cortical potentials. Furthermore, the perception threshold of laser stimulation was found to be lower for facial compared to stimulation of the extremities. This was explained by a possible denser afferent innervation. The temporal dispersion and less dense innervation of the extremities may explain the difficulties in evoking NWR in the extremities by heat stimulation. Therefore, larger area was stimulated to compensate for the lower innervation density in the lower limb compared to the facial areas in study II.

3.2 Organization of the NWR

Already the early studies of the NWR indicated that the reflex may not be a stereotype flexion reflex as originally described by Sherrington (1910). Hagbarth (1960) observed a flexion reflex when stimulating the limb electrically, but not when stimulation was applied above the extensor muscles. Furthermore, Grimby (1963) systematically studied the dependency of the site of stimulation on the sole of the foot, and observed a dorsiflexion of the ankle when stimulation the front of the foot, but a plantar flexion when stimulating the foot towards the heel. Similar results were found by Hagbarth in both humans (Hagbarth, 1960) and cats (Hagbarth, 1952). The stereotyped flexor reflex organization of NWRs was further challenged by Schouenborg and Kalliomaki (1990), who showed that muscles in the hindlimb of rats could be activated by noxious stimulation in a restricted area specific for each muscle. These specific areas were termed 'reflex receptive fields', and interestingly not only flexor muscles but also extensor muscles had such

reflex receptive fields (Schouenborg et al., 1994). The combination of muscles and their reflex receptive fields was shown to constitute a system where the reflex movement optimally removed the stimulated skin area away from the stimulation (Schouenborg and Weng, 1994). Each muscle or synergistic muscles and their reflex receptive fields were termed a module and the NWR was proposed to have a modular organization (Schouenborg et al., 1994). Such a modular organization of the NWRs has also been shown in humans (Andersen et al., 1999;Sonnenborg et al., 2000;Sonnenborg et al., 2001). The human studies were all performed by electrical stimulation and showed that the NWR is organized so that a noxious stimulation of the foot would remove the stimulated area away from harm by an appropriate movement, e.g. plantarflexion for heal stimulation and dorsiflexion for fore foot stimulation at the sole of the foot.

3.2.1 Natural stimulation e.g. mechanical and heat.

The modular organization has also been shown when reflexes were evoked by natural stimulation in animal studies. Evidence for a modular organization of reflexes has been obtained in rats for mechanical (Schouenborg and Kalliomaki, 1990) and radiant heat (Weng and Schouenborg, 1996) stimulation applied to plantar and to some extend the dorsal side of the hind paw. Furthermore radiant heat applied to the tail of the rat evoked a movement directed away from the stimulus (Cleland and Bauer, 2002). A possible modular organization of the NWR in the cat has been investigated. Heat evoked NWR could be evoked in non-flexor-muscles (Schomburg et al., 2000), and a modular organization of the NWR was shown following mechanical pinch stimuli (Levinsson et al., 1999). Study II did not show a modular organization of the heat evoked NWR in humans; however, the stimulated sites may have been located within the same reflex receptive field. Another explanation may be that a possible modular organization of the NWR is less pronounced on the dorsal side compared to the plantar side of e.g. the foot (Clarke and Harris, 2004).

The differences between plantar and dorsal side.

Two similar studies have shown a modular organization of electrically evoked responses both plantar (Andersen et al., 1999) and dorsal (Sonnenborg et al., 2001) stimulation of the foot. However, NWR evoked at the dorsal side had lower thresholds, were 'smaller', and did not show as clear modular organization as NWR evoked at the plantar side for the foot. Other human studies have shown differences in reflex activities evoked by laser stimulation of the plantar or dorsal of the hand (Romaniello et al., 2004), and withdrawal movement analysis showed that modular organization of reflexes are most readily seen when stimulation was applied to the plantar side of

the foot (Clarke and Harris, 2004). In study II, radiant heat stimulation applied to the dorsum of the foot did not show a modular organization of the NWR, and no plantar flexion as responses in the soleus muscle was sparse. However, a study investigating the organization of the heat evoked reflexes evoked from the plantar side of the foot is difficult to design as the plantar skin is glabrous and therefore contain type I AMHs and CMHs, but not type II AMHs. As described in section 1.1, type I AMHs have long response delay, and the conduction velocity of the CMHs is low. This results in a late arrival of afferent information of these nociceptors to the spinal cord that is not capable of eliciting NWR. The type II AMHs, that are only located in the hairy skin, have short response latencies and relatively fast conduction velocity, resulting in a sufficiently short response delay to evoke NWR (study II).

In a study performed to indirectly reveal the role of heat sensitive afferents in the organization of nociceptive reflexes, a conditioning radiant heat stimulation was applied and the reflexes were evoked by electrical stimulation of the tibial nerve (Ellrich et al., 2000). Independent of whether the heel, the forefoot, or the dorsum of the foot was conditioned an increase in the reflex size of both the biceps femoris and tibialis anterior muscles was observed which is not in compliance with a modular organization of the reflexes. This may indicate that a modular organization of the NWR can be demonstrated by electrical stimulation, but not heat stimulation in humans.

3.3 Convergence of muscle afferents to the reflex pathway

Several studies have shown that cutaneous and muscle afferents converge onto common interneurons in the spinal cord or medullary DH (Kniffki et al., 1981;Sessle et al., 1986;Hoheisel and Mense, 1990) (Study I). In the craniofacial area, experimental MAS muscle pain reduced the degree of silent period suppression following perioral cutaneous CO_2-laser (Romaniello et al., 2002) or electrical (Wang et al., 1999) stimulation. The convergence of muscle and e.g. cutaneous afferents onto common spinal or medullary DH may along with central sensitization form the neural basis for referred pain. Muscle pain may be associated by referred pain (Sinclair D.C. et al., 1948), where pain is perceived from the muscle that receives noxious stimulation, but also from distant parts of the body including the skin. Experimentally, intramuscular injection of hypertonic saline into the tibilais anterior muscle has been shown to produce pain in the injected muscle but also in the dorsal aspects of the ankle joint (Graven-Nielsen et al., 1997a).

3.3.1 Modulation of the NWR by tonic noxious muscle stimulation

In a pilot study performed to study the interaction between muscle afferents and AMHs, radiant heat was applied to the dorsum of the foot and frontal lower leg before, during, and after muscle pain in the tibialis anterior muscle. However, the reflexes were not significantly modulated by the muscle pain (Figure 6), although a similar study using electrical stimulation to evoke NWR did show an increase of the initial part of the reflexes (Andersen et al., 2000). In the pilot study, the NWR was evoked by heat stimulation and thus activation of the type II AMHs. These nociceptors may exhibit an adaptational behavior that may not have been completely avoided by long inter- stimuli time intervals. Therefore, the nociceptors may have adapted to the heat stimulus during the pre- muscle pain test stimuli. This may have caused the difference between the study of Andersen et al. (2000) and the pilot study. However, there were no significant differences between VAS scores before, during, and after muscle pain, although the VAS score to Argon laser stimulation has been shown to increase during muscle pain (Graven-Nielsen et al., 1997b). Furthermore, there were no differences between the NWR evoked at different sites, although one site was at a small part of the skin between the first and second metatarsal that, like the tibialis anterior muscle, is innervated by the deep peroneal nerve (Lawrence and Botte, 1995), one was at the anterior aspect of the ankle where referred pain are commonly observed (Graven-Nielsen et al., 1997a) and two others sites that were not directly associated with referred pain. However, previous studies have shown pain ratings depending on the location of the stimulation site in relation to the referred pain area (Graven-Nielsen et al., 2002;Ge et al., 2003).

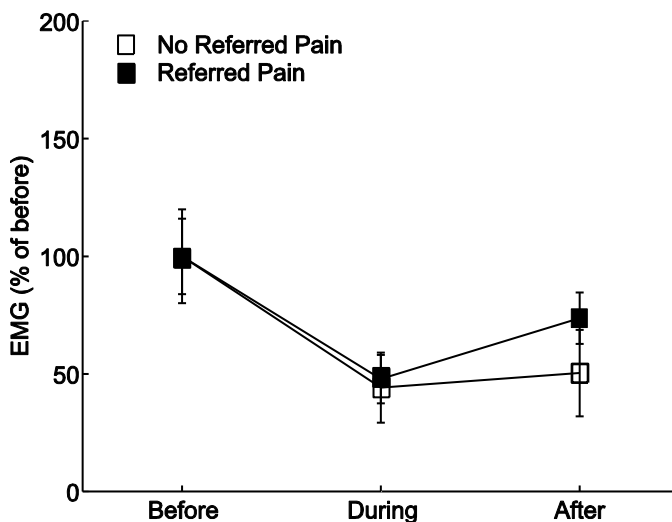

Figure 6. The effect of tonic muscle pain on CO_2-laser scanning evoked reflexes. The radiant heat evoked reflexes of two groups of subjects; one group perceiving referred pain and one group that did not perceive referred pain.

Radiant heat stimulation was applied before injection of hypertonic saline to the tibialis anterior muscle, during the perception of pain in the tibialis anterior muscle and 15 minutes after pain perception was no longer reported. The electromyography (EMG) responses were normalized to the pre-pain response and calculated as the mean over the four stimulation sites and three stimulation intensities. The group of subjects that experienced referred pain was compared to the group that did not experience referred pain, however there was no significant difference between these groups neither when the muscle pain was perceived nor after the perception of pain had vanished, nor were the any differences before during and after muscle pain (ANOVA; n.s.).

Besides the direct convergence of muscle and cutaneous afferents onto interneurons in the reflex pathway, two different mechanisms may be involved in muscle pain modulation of the reflexes; central hyperexcitability and descending inhibition. High intensity (C-fiber) stimulation has been shown to produce a prolonged increase in the excitability of flexion reflexes in spinalized animals (Wall and Woolf, 1984;Laird et al., 1995). Central hyperexcitability evoked by chemical (Hu et al., 1992) or electrical (Cook et al., 1987) noxious muscle stimulation may be associated with an expansion of cutaneous RF and formation of new deep tissue RFs in rats (Hoheisel et al., 1993). Therefore, referred pain may be a consequence of convergence of e.g. cutaneous and muscle afferents onto common interneurons and a hyperexcitability of these interneurons following the nociceptive barrage from muscle afferents (Mense, 1994;Arendt-Nielsen and Svensson, 2001). However, the appearance of new cutaneous RFs occurred after minutes in the animal studies (Steffens and Schomburg, 1993) but referred pain appears after seconds in human experiments (Graven-Nielsen et al., 1997a), which may be explained by difference between animal and human experimental models.

Descending pathways inhibit nociceptive responses after long lasting noxious muscle stimulation (Gjerstad et al., 1999). This decrease in responsiveness may be related to diffuse noxious inhibitory controls (Le Bars et al., 1979) in which noxious stimulation of distant tissue decreases the responsiveness of the nociceptive pathway (Schouenborg and Dickenson, 1985;Hu, 1990;Xian-Min and Mense, 1990;Falinower et al., 1994). In humans, similar depression of the reflex excitability by remote thermal noxious stimulation has been shown (Willer et al., 1984;Willer et al., 1989). Therefore, the tendency to reduced size of the NWR in the pilot study may reflect a counteraction of any central hyperexcitability and an activation of the descending inhibitory pathways both caused by the induced muscle pain and both acting on neurons in the spinal cord where cutaneous and muscle afferents converge. The modulation caused by muscle pain seems to depend on the

particular experimental setup and the stimulation modality applied. It is likely that the induced pain affect a dynamic balance between excitatory and inhibitory modulation of the NWR.

3.3.2 Sensory convergence of homotopic transient stimuli

Several studies have shown that radiant heat stimulation may modulate electrically evoked NWR. Noxious heat stimulation applied by xenon lamp radiation of the blackened sole of the foot has shown a facilitation of the electrically evoked NWR, and thus showing a convergence between cutaneous Aδ- and C- fibers (Andersen et al., 1994). In a conceptual similar study, Plaghki et al. (1998) applied non-noxious heat stimulation at the dorsum of the foot and elicited NWR by electrical sural nerve stimulation. The NWR was facilitated at latencies that indicated both Aδ and C fiber mediated heat sensitive afferent convergence onto the spinal cord neurons. Radiant heat applied to the sole of the foot to condition NWR evoked by electrical stimulation of the medial plantar (Ellrich et al., 1998) or tibial (Ellrich et al., 2000) nerve also showed a facilitation of the NWR. However, in the craniofacial area the electrically evoked long latency silent period was reduced by painful heat conditioning stimulation applied to the cheek (Andersen et al., 1998).

3.3.3 Modulation of the NWR by phasic conditioning stimulation

The design of study III followed the ideas of Andersen et al. (1994) and Plaghki et al. (1998), in that simultaneous arrival at the spinal cord of afferent input was achieved by varying the conditioning-test time-interval. To study the influence of muscle pain on the NWR tonic muscle stimulation may be undesirable due the possible activation of supraspinal control mechanisms and central hyperexcitability as described above. Phasic activation of muscle nociceptive muscle afferents (type III and IV) can be achieved by intramuscular electrical stimulation. Although intramuscular electrical stimulation provides controllable timing and intensity, non-nociceptive muscle afferents (type I and II) are activated alongside. Furthermore, electrical stimuli evoke muscle contraction that may evoke a mechanical afferent component. Also, repeated high-intensity intramuscular electrical stimulation may cause muscle fatigue. Still, conditioning the NWR by intramuscular electrical stimulation provides better opportunity to control the conditioning-test interval.

In study III, facilitation of the heat evoked reflexes was seen when the conditioning (intramuscular electrical stimulation) was applied 275 ms and 300 ms after the onset of the test (radiant heat) stimulation. Thus, at these intervals the heat evoked reflexes were significantly larger than the unconditioned heat evoked reflexes. The transduction delay of the type II AMHs was estimated to be 40 ms in accordance to Bromm and Treede (1984), the afferent conduction delay was estimated

to 40 ms, and a temperature that could activate the nociceptors were assumed to be achieved at the end of the 200 ms pulse. Therefore, the earliest possible afferent input was assumed at 280 ms after stimulation onset. However, the latency of the heat evoked reflexes was seen at 354 ± 9 ms. The 74 ms difference may be assigned to central delay, and efferent conduction time from the ventral horn to the semitendinosus muscle, but also an overestimate of the conduction velocity of the Type II AMHs (Burgess and Perl, 1973) or an underestimate of the transduction time as it has been estimated to 70 ms (Plaghki et al., 1998).

The facilitation found in study III was similar to that observed in studies where electrically evoked NWR were modulated by painful (Andersen et al., 1994) and non-painful (Plaghki et al., 1998) radiant heat stimulation. Surprisingly, in a study similar to study III, NWR evoked electrical stimulation of the sural nerve was depressed by a brief conditioning intramuscular electrical stimulation of the tibialis anterior when the conditioning was applied 15 ms to 1500 ms before the test stimulus (Ge et al., 2006). Conditioning stimulation applied to the contralateral tibialis anterior muscle and the contralateral trapezius muscles also attenuated the NWR, therefore the main reason for the reflex inhibition was assumed to be activation of descending inhibition (Ge et al., 2006). Furthermore, presynaptic inhibition induced by activation of group I and II muscle afferents (Rossi et al., 1999;Knikou and Conway, 2005) by conditioning the ipsilateral tibialis anterior muscle may also cause inhibition when the test stimulus was applied soon after the conditioning stimulation. The facilitation observed were at negative conditioning-test time-interval values in study III and may therefore have occurred before the inhibition reported by Ge et al. (2006). Another reason for the difference may be that heat stimulation of the dorsum of the foot evoked reflexes in 42 % of the control trials in study III, whereas electrical stimulation of the sural nerve evoked reflexes following every stimulation (Ge et al., 2006), even though the stimulation was applied at two times pain threshold in both studies. Heat stimulation applied at two times pain intensity was just around the reflex threshold, whereas electrical stimulation at two times pain threshold was well above the reflex threshold. Therefore, the conditioning may have provided sufficient additional afferent input to the reflex pathway to facilitate the heat evoked NWR (floor effect). Furthermore, the electrical stimulation may have evoked a highly synchronized afferent input from a wide range of afferent fiber, whereas heat stimulation evoked a natural barrage of only heat sensitive afferents.

The functional effect of the facilitation of the heat evoked NWR by noxious muscle stimulation, could be to protect the anterior part of the lower leg. However, the heat evoked reflex was not facilitated by ongoing muscle pain (pilot study), that presumably resembles muscle soreness more than the transient electrical stimulation (study III). This may be related to activation of the inhibitory systems e.g. diffuse noxious inhibitory control (Bars et al., 1979) evoked by tonic but not transient muscle stimulation as described in section 3.3.1.

A study investigating the central effect of visceral nociception, lower limb NWRs were not affected by painful dilation of the esophagus (Drewes et al., 2003). However, sensitization of the esophagus by acid perfusion significantly increased the NWRs. Furthermore, after the sensitization painful dilation of the esophagus decreased the NWRs showing a complex interaction between visceral and cutaneous nociception in healthy human subjects. Correspondingly, Bouhassira (1998) showed that transient rectal stimulation facilitated the NWRs of the lower limb whereas tonic rectal stimulation inhibited the NWRs in the lower limb. These findings correspond to the present findings where tonic muscle pain possibly inhibited the NWRs whereas transient muscle pain facilitated the NWRs (Study III).

Animal studies have shown that stimulation of persistent ongoing pain in deep structures was associated with activity of the ventrolateral PAG and resulted in passive emotional coping (hyporeactivity and vasodepression), whereas cutaneous stimulation was associated with activity in the lateral PAG and resulted in active emotional coping (fight-flight) (Keay and Bandler, 1993;Keay et al., 1994;Clement et al., 2000). However, it has been shown that also noxious inescapable cutaneous stimulation was associated with activity in the ventrolateral PAG, resembling the response to deep muscle pain (Keay et al., 2001). Therefore, regional activation of the PAG may be associated by the escapability of the noxious stimulation rather than the stimulation modality per se. This may reflect differences between the tonic and transient muscle pain on heat evoked NWR (Study III).

3.3.4 NWR evoked by intramuscular electrical stimulation

Studies of spinal cord injured subjects has shown that strong phasic activation of the tibialis anterior muscle afferents itself was capable of evoking withdrawal responses (Hornby et al., 2004), likewise cutaneous stimulation may also evoke reflexes in areas not observed in spinal intact subjects

(Kugelberg et al., 1960;Schmit et al., 2003;Andersen et al., 2004). In study III, significant EMG response was observed in the semitendinosus muscle in 10 % of the trials following intramuscular stimulation of the tibialis anterior muscle (Figure 7). The intramuscular electrical stimulation may directly have activated group III and/or IV muscle afferents thereby evoked the NWR as these afferents have been reported to belong to the flexor reflex afferents (Eccles and Lundberg, 1959). The mean latency of the response was 125 ms which was somewhat longer than latencies observed following cutaneous stimulation e.g. 70 ms (Andersen et al., 2000). This probably indicated that the group III muscle afferents are slower conducting than the cutaneous Aδ fibers. Alternatively, the intramuscular stimulation may have evoked the reflex indirectly as the stimulation caused contraction of the tibialis anterior muscle and therefore a perturbation of the ankle joint. In spinal cord injured subjects, both plantar- and dorsi- flexion of the ankle has been shown to evoke a multi-joint reflex resembling the flexor reflex (Schmit et al., 2000). In decerebrated cats, free nerve endings innervated by group II, III, and IV muscle afferents but neither Golgi tendon organ nor spindle afferents (Cleland and Rymer, 1990) have been proposed to be responsible for reflexes (Schmit et al., 2000). Such reflexes is often called the clasp-knife reflex (Rymer et al., 1979), and is followed by an inhibition at long stretches (Cleland et al., 1990). Whether the reflex response was evoked directly by electrical stimulation of muscle afferents or indirectly by the joint perturbation, these findings supported the notion that group II-IV muscle afferents belong to the flexor reflex afferents.
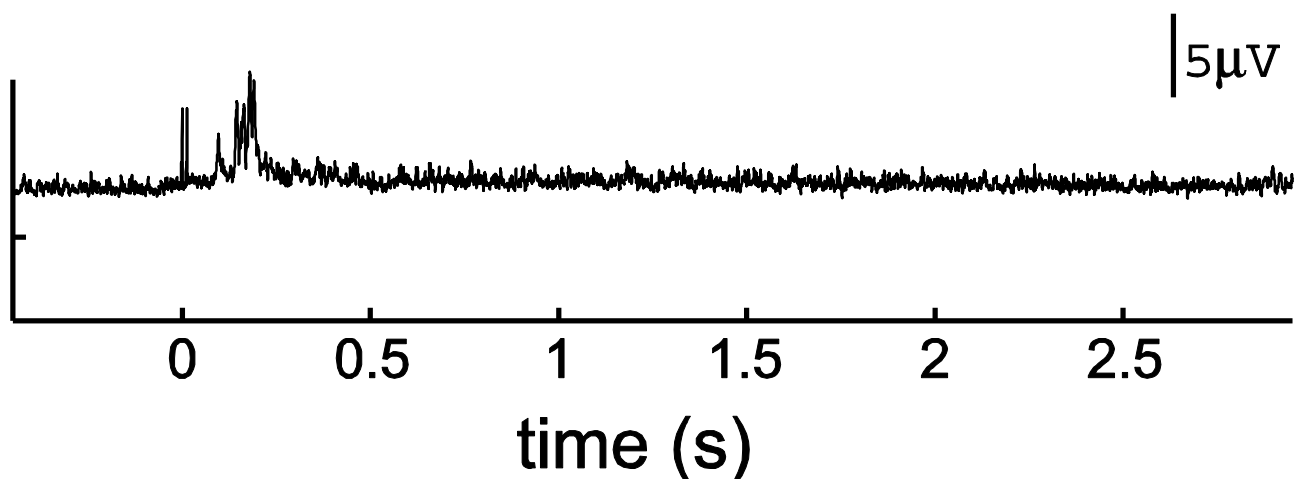

Figure 7. Electrical intramuscular stimulation of the tibialis anterior evoked withdrawal response. The grand mean of all trial rectified EMG responses recorded in the semitendinosus muscle is displayed, although only 10% of the trials showed a significant response as defined in Study III.

3.4 Short summary

The NWR has been used to study the nociceptive system at the spinal level in humans. These NWR have usually been evoked by electrical stimuli which may not resemble natural stimuli. The present thesis presented a new method where heat was uniformly applied to a large skin area by a scanning of a CO_2-laser beam. This provided a method to activate heat nociceptors sufficiently to evoke NWR and thus provides a method for investigation NWR to natural noxious stimuli. The method was used to study the correlation between nociceptive cutaneous afferents and muscle afferents. The present studies showed a facilitation of the heat evoked NWR by transient but not tonic muscle pain, indicating afferent convergence at the spinal level that may be modulated by descending control mechanisms.

4 Future perspectives

The convergence of several afferent sources in the trigeminal and cervical territories onto nociceptive C1 DH neurons was investigated in the present thesis in rats. Systematical investigations of DH neurons the remaining of the spinal cord have not been performed. Such investigations, although cumbersome, may provide information and deeper understanding of the spinal processing of nociceptive information and the complex nature of pathologic pain. However, Sessle et al. (1986) systematically investigated the sensory convergence in nociceptive and non-nociceptive DH neurons cats.

Uniformly application of heat to a large skin area by a scanning of a CO_2-laser beam proved to be a useful method to elicit NWR and to investigate sensory convergence in humans. However, only evoked reflexes in 1 out of 3 stimuli at the highest intensity that did not cause tissue damage. Further development of the system may enable even higher response rates. Stimulation by other infrared laser e.g. YAG, YAP or diode lasers will provide radiation that penetrates deeper into the skin and may raise the temperature closer to the heat sensitive nociceptors than the CO_2-laser radiation (Plaghki and Mouraux, 2003;Baumgartner et al., 2005). Furthermore, the radiation of the YAG, YAP or diode lasers may readily be mediated through optic fibers, and thus a more flexible system may be developed.

The present thesis presented a method to elicit NWR by activation of Type II AMHs. Nilsson et al. (1997) presented a method to specifically activate nociceptive afferents by intra-epidermal electrical stimulation applied through small electrodes. This method has been used to treat itch (Nilsson et al., 1997), to study the analgesic effect (Nilsson and Schouenborg, 1999), and to study long term effects

of specific nociceptive stimulation (Klein et al., 2004). The evoked cortical potentials the intra-epidermal stimulation has also been investigated e.g. (Inui et al., 2002). However, it is not know if the intra-epidermal electrical stimulation is capable of eliciting NWR.

5 Summary and conclusion

Sensory convergence of afferent input to second order neurons in the spinal and medullary DH has been shown by anatomical and electrophysiological studies. Animal electrophysiological studies investigating the input from a larger number of afferent sources have shown that nociceptive neurons on the medullary (Sessle et al., 1986) and C1 (study I) DH receive afferent input from a wide range of cutaneous, deep, dural and visceral afferent sources. The central convergence and hypersentization of the DH neurons may form the neural basis for several craniofacial pain disorders (Sessle, 2000) such as temporomandibular disorder (Dworkin et al., 1990), whiplash (Munglani, 2000), angina pectoris (Foreman, 1999;Foreman, 2000), and headache (Bogduk, 2001;Bartsch and Goadsby, 2003b). In order to study indications of sensory convergence in human subjects, the NWR have been used as a window into the nociceptive human physiology at the spinal level. A method to evoke pure nociceptive NWR has been developed by applying heat stimulation to a large area of the skin of the dorsum of the foot by CO_2-laser radiation through a scannerhead (study II). Several studies have shown a modular organization of the NWR animals and electrically evoked the NWR humans, however a similar modular organization could not be shown for the heat evoked NWR on the dorsum of the foot and anterior of the lower leg (study II). The NWR is modulated by numerous afferent sources possibly by sensory convergence of afferent activity onto second order neurons of the DH (Lundberg, 1979). In animal studies a correlation between DH neurons and reflex responses have been shown (Jankowska and Roberts, 1972a;Jankowska and Roberts, 1972b;Brink et al., 1983;Morgan, 1998;You et al., 2003). Study III showed facilatory modulation of the NWR by varying the time interval between the test stimulus and the conditioning muscle stimulus. In this way, electrical muscle conditioning stimulation has been shown to facilitate the heat evoked NWR. This indicated convergence of muscle afferents onto neurons in the NWR pathway.

6 Dansk sammenfatning (Danish Summary)

Undersøgelse af sensorisk konvergens i rygmarven

(Assessment of sensory convergence in the spinal cord)

Smerte er under normale fysiologiske omstændigheder en vigtig oplevelse, som er nødvendig for individets overlevelse. Smertesystemets perifere del består af afferente nerver, som bringer informationer om mulige trauma fra periferien til rygmarven. En vigtig funktion af somatiske smerter er afværgereflekser, hvor den sensoriske information viderebringes til det motoriske system, her aktiveres muskler, så legemsdelen fjernes fra det mulige trauma. Ligeledes bringes informationen om muligt trauma til hjernen, hvor oplevelsen af smerte kan opstå. Imidlertid kan informationen konvergere på samme neuroner i centralnervesystemet. Denne konvergens har betydning for smerteopfattelsen og kan resultere i smertespredning og meddelt smerte. Ligeledes kan konvergens resultere i en ændring i afværgereflekserne. Formålet med dette projekt har været at undersøge sensorisk konvergens i smertesystemet hos dyr og mennesker.

Afhandlingen bygger på 3 separate videnskabelige arbejder:

I   Convergence of cutaneous, visceral and muscle afferents onto nociceptive dorsal horn neurons at the first cervical level. Mørch C.D., Hu H.W., Arendt-Nielsen L., Sessle B.J.

II   Nociceptive withdrawal reflexes evoked by uniform-temperature laser heat stimulation of large skin areas in humans. Mørch C.D., Andersen O.K., Graven-Nielsen T., Arendt-Nielsen L. Accepteret af Journal of Neuroscience Methods

III   Modulation of heat evoked nociceptive withdrawal reflexes by painful intramuscular conditioning stimulation. Andersen O.K., Mørch C.D., Arendt-Nielsen L. Accepteret af Experimental Brain Research

I studie I undersøgtes identificerede nociceptive neuroner i den første cervikale del rygmarvens baghorn hos rotter. Elektrisk stimulation blev givet til hornhinden, hjernehinderne, den anden cervikale nerven, nerven som innerverer tungen (hypoglossus), kæbeledet, kæbemusklen (m. masseter) og nerven, som innerverer struben (n. superiolingualis). Ligeledes blev huden stimuleret i områderne, som innerveres af første, anden og tredje gren af den trigiminale nerve, huden bag øret (innerveret af anden og tredje cervikale nerve), huden over skulderen og huden på forpoten. Yderligere blev mikroinjektioner med glutamat givet i tungen, nakkemusklerne, området omkring hjertet og dryppet på hjernehinden. Ligeledes blev acetylsyre injiceret i bughulen, for at undersøge om de nociceptive neuroner i den første cervikale del af rygmarven modtager nociceptive viscerale informationer fra dette område.

Undersøgelserne viste, at nociceptive neuroner i den første cervikale del af rygmarvens baghorn modtager nociceptiv afferent information fra kutane og muskelskeletale organer i det trigiminale område, hjernehinde og n. superiolingualis. Undersøgelsen viste også, at elektrisk stimulation af disse organer resulterede i en dobbelt logaritmisk stimulus respons funktion, hvilket indikerer, at disse neuroner er i stand til at kode og transmittere information om smerte fra alle disse organer. Undersøgelsen viste yderligere, at de nociceptive neuroner modtager afferent information fra adskillige af disse afferente kilder. Imidlertid modtog de nociceptive neuroner i den første cervikale del af rygmarvens baghorn kun ganske sporadisk information fra afferente kilder uden for det trigeminiale område. Den substantielle konvergens af trigiminale afferente kilder kan have betydning for spredning og meddeling af smerte i forbindelse med smertelidelser i det trigiminale område, så som migræne, piskesmæld og kæbebetændelses lignende tilstande.

Undersøgelser af enkelte rygmarvsneuroner hos mennesker er imidlertid ikke en simpel opgave. Derfor blev to yderligere studier planlagt for at se på sensorisk konvergens i forbindelse med afværgereflekser. Afværgereflekser drives af afferent input gennem et antal neuroner eller netværk af neuroner i rygmarven til at udløse et muskelrespons. Idet afværgereflekserne drives af en sådan spinal refleksbue, giver de mulighed for at undersøge smertesystemet og specielt i denne sammenhæng konvergens i smertesystemet på det spinale niveau. I mange studier udløses afværgerefleksen ved elektriske stimulation på huden, hvilket direkte aktiverer nociceptive og non-nociceptive afferente fibre. Yderligere aktiverer den elektriske stimulation de afferente fibre samtidigt, hvilket givetvis medfører et unaturligt synkroniseret input til centralnervesystemet. Derfor var formålet med studie II at udvikle en metode til selektivt at stimulere nociceptive

receptorer på en måde, der ville udløse afværgereflekser. Til dette formål benyttedes en infrarød CO_2-laser til varmestimulation af oversiden af foden og forsiden af underbenet. Studiet viste, at varmestimulationen kunne udløse reflekser i iliopsoas, quadriceps vastus lateralis, biceps femoris, tibialis anterior og soleus musklerne. En lineær stimulus respons sammenhæng sås i alle disse muskler undtagen soleus musklen. Dette studie påviste en metode til at udløse rent nociceptive afværgereflekser ved hjælp af varmestimulation.

I studie III blev konvergens mellem smertereceptorer i huden og musklerne undersøgt. Til dette formål benyttedes metoden til at udløse afværgereflekser (som beskrevet i studie II) ved at stimulere oversiden af foden og måle afværgerefleksen i semitendinosus musklen. Elektrisk intramuskulær stimulation af tibialis anterior musklen benyttedes til en transient aktivering af smertereceptorer muskelen. Tidsintervallet mellem den intramuskulære stimulation og varmestimulationen blev varieret mellem -400ms (varmestimulationen givet først) til 8s (den intramuskulære givet stimulation først). Når varmestimulationen blev givet 300ms eller 275ms før den intramuskulære stimulation, øgedes størrelsen af afværgerefleksen signifikant. Det er derfor sandsynligt, at afferent information fra huden ankommer til rygmarven samtidig med afferent information fra musklen ved disse tidsintervaller. Den afferente information fra huden og musklerne konvergerer et sted i refleksbuen givetvis på nociceptive baghornsneuroner, som beskrevet i studie I. Funktionelt betyder faciliteringen af afværgerefleksen med transient muskelsmerte en øget afværgereaktion. Med metoden, som blev benyttet i studie III, er det muligt at undersøge sensorisk konvergens på rygmarvsniveau hos mennesker. Studier af sensorisk konvergens i smertesystemet kan bidrage til forståelse reflekssystemet og smertespredning og meddelt smerte i patologiske tilstande.

7 References

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