Physiological and pharmacological modulation of the human nociceptive withdrawal reflex

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Ph.D thesis,
Center for Sansory-Motor Interaction,
Aalborg University
1996

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LIST OF COMMON ABBREVIATIONS IN THE THESIS

HA	=	hyperalgesia
1°HA	=	primary hyperalgesic area
2°HA	=	secondary hyperalgesic area
NWR	=	nociceptive withdrawal reflex
ISI	=	inter stimulus interval
WDR	=	wide dynamic range (neuron)
NS	=	nociceptive specific (neuron)
LTM	=	low threshold mechanoreceptor

The present thesis is based on the papers listed below (in the text referred to by roman numerals). The original papers are attached.

I
Andersen OK, Jensen, LM, Brennum J and Arendt-Nielsen L
Modulation of the human nociceptive reflex by cyclic movements
Eur J Appl Physiol 70 (1995) 311-321

II
Andersen, OK, Jensen, LM, Brennum, J, Arendt-Nielsen, L
Evidence for central summation of C and Aδ nociceptive activity in man
Pain 59, (1994) 273-280.

III
Andersen, OK, Gracely, RH, Arendt-Nielsen, L
Facilitation of the human nociceptive reflex by stimulation of Aβ-fibres in the a secondary hyperalgesic area sustained by nociceptive input from the primary hyperalgesic area
Acta Physiol Scand 155 (1995) 87-97

IV
Andersen, OK, Felsby, S, Nicolaisen, L, Bjerring, P, Jensen, TS, Arendt-Nielsen, L
The effect of ketamine on stimulation of primary and secondary hyperalgesic areas induced by capsaicin - a double-blind, placebo-controlled, human experimental study
Pain, in press

PREFACE

The present studies have all been carried out at Center for Sensory-Motor Interaction, Aalborg University in the period from 1992 to 1995.

I wish to express my deepest and sincere gratitude to my supervisor Professor Lars Arendt-Nielsen, Ph.D., dr.med., for his never failing interest in discussing and commenting on the research projects, paper drafts, and future ideas. I also wish to thank all the co-workers for helping with the experimental studies and completing manuscripts for publication. It has been a pleasure.

Finally, I thank all my colleagues at Center for Sensory-Motor Interaction for a positive research milieu and for always being willing to help solving technical problems and proofreading various manuscripts.

The study has received financial support from The Danish National Research Foundation, Direktør Ib Henriksens Fond, Løvens Kemiske Fabriks Forskningsfond, Mogens and Else Wedell-Wedellsborgs Fond, and Therese Maria Hansen, født Beers legat.

Aalborg, April 1996

CONTENTS

1 INTRODUCTION

The founder of the International Association for the Study of Pain (IASP), Dr. John Bonica, published in 1989 a study on the incidence of patients suffering from pain in the Western World. The figures are as high as 15-20% of the population having acute pain annually, but more important 25-30% of the population has chronic pain. For the individual subject, pain affects the quality of life in general and it also means a great economic loss to the community due to expenses for treatment and lost working capacity. Thus, further research in the mechanisms and treatments of pain is highly needed. Pain research is a fairly new research field (IASP founded in the early Seventies) probably reflecting the fact that pain is associated with many different diseases and therefore research in this field demands a multi-disciplinary action.

Pain is defined by the IASP as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (Merskey 1979), and therefore pain is a purely individual and subjective experience. On the other hand nociception is the evoked activity in afferent nerve fibres and neurons in the entire neuraxis receiving potential tissue damaging information. Nociceptors are the peripheral tissue receptors signalling potential or actual tissue damage.

Pain research involves both basic animal research and clinical research. In addition, healthy subjects can participate in experimental studies to bridge the gap between basic and clinical research.

1.1 Human experimental pain research

The basic principle in human experimental pain research is to activate nociceptors by a well-defined stimulus and then record and quantify the evoked response. The response may be of verbal and/or physiological character. Quantification of verbal responses to (painful) stimuli is also denoted psychophysical research. Physiological responses may be of nociceptive character e.g. the nociceptive withdrawal reflex (NWR) but may also reflect behavioral responses to a given stimulus (e.g. changes in movement patterns with presence of pain).

In basic animal pain research, all measurements are indicators of direct nociception (e.g. single unit recordings) or behavior to a standardised stimulus but never a pain perception, while clinical pain research deals with pain as individual subjective response. However, in clinical research no patients are alike and therefore the specific source of pain is different compromising direct comparisons. The advantage of experimental human pain research is the possibility of administering quantitative and standardised somatic stimulations and at the same time assessing the response.

1.2 Nociceptive withdrawal reflexes

The nociceptive withdrawal reflex may be used as an additional physiological measure in human experimental pain research to supplement psychophysical methods.

The NWR may be evoked by stimulations of a sensory nerve at a strength sufficient to depolarise nociceptive afferents (Aδ, group III fibres, Kugelberg 1948). Nociceptive withdrawal reflexes are a subpopulation of flexion reflexes as these may be evoked by several afferents. Nociceptive afferent activity may evoke a response in both ipsilateral and contralateral muscle groups in order to avoid further tissue damage. The generation is initiated by the nociceptive input but an extensive processing takes place within the spinal cord. The neural connection from the primary sensory neurons to the motor neurons is a poly-synaptic pathway. Therefore, other afferent input, descending activity, and the excitability of the neurons in this pathway modulate the generation of the spinal nociceptive reflex, see figure 1.1.

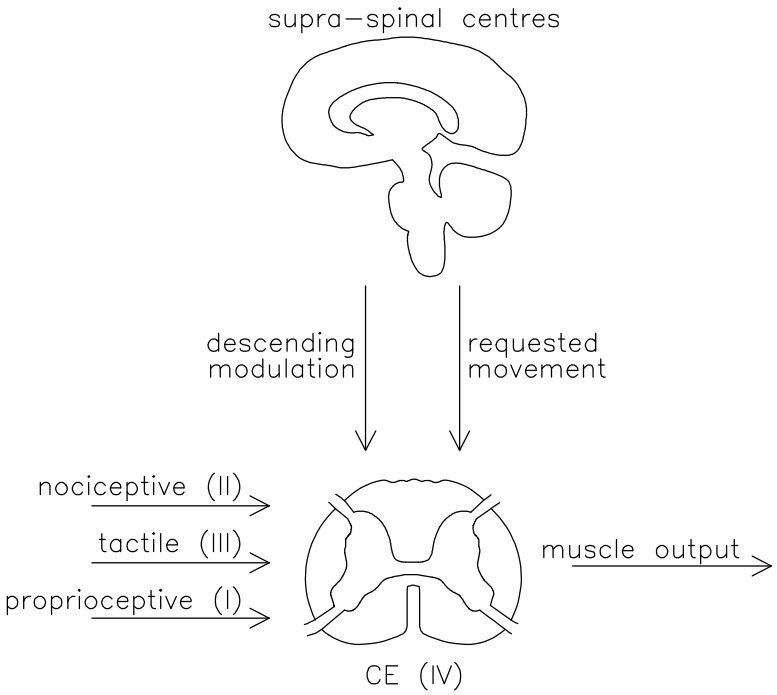

Figure 1.1. Schematic illustration of the different parameters modulating the reflex generation. Afferent input from joint, muscle, and cutaneous tactile receptors may modulate the NWR generation. Each input is studied in article I-III. Further, descending tonic and phasic activity modulate the sensitivity of spinal neurons is present. Descending motor command signals either directly or via spinal inter-neuronal structures (central pattern generators) modulate the reflex generation. Within the spinal cord, the intrinsic excitability of the neurons in the reflex pathways may also vary after e.g. robust nociceptive input studied in IV. The numbers I-IV refer to the four studies enclosed in the thesis. CE - central excitability.

1.3 Aim of Ph.D. project

The aim of this Ph.D. project is to investigate how the spinal nociceptive withdrawal reflex is modulated during various human non-invasive physiological and pharmacological experimental conditions. The purpose of this is to collect information about spinal nociceptive mechanisms under normal and hyperexcitable conditions. Further, the studies provide information about the NWR as a quantitative tool in experimental pain research. A part of the aim is to develop a method with a minimal subjective component for determination of the NWR threshold.

The experimental work has been published in four papers dealing with four different aspects of the reflex generation (see figure 1.1). The thesis consists of a presentation and a discussion of methods and main findings in the four papers. In the first paper (I), the modulation of the reflex by voluntary dynamic contractions was investigated. In the second work (II), a potential central summation of activity from cutaneous nociceptors innervated by Aδ- and C-afferents was studied. Finally, the third (III) and fourth (IV) papers involve mechanisms behind central hyperexcitability. In III, a synergistic summation of nociceptive and normally tactile activity with presence of central hyperexcitability was studied. In IV, the involvement of the NMDA receptor system (a specific neuronal receptor involved in fast excitatory neurotransmission) in the increased central excitability was investigated.

The conclusion outlines the main findings and implications of the results. The studies are referred to as I, II, III, and IV throughout the thesis.

2 METHODS USED IN THE EXPERIMENTAL STUDIES

In this chapter the method for generation and quantification of the NWR will be discussed followed by a brief description of other stimulation methods used in I-IV to modulate the NWR.

2.1 Historical findings related to the nociceptive withdrawal reflex

This preface briefly describes some historical findings regarding the characteristics of the nociceptive cutaneous withdrawal reflex, i.e. how it is generated and modulated.

Sherrington (1910) carried out an extensive study of the limb reflexes in animal preparations in the beginning of our century. He observed the characteristically ipsilateral flexion and extension in the contralateral limb as to preserve balance. Often the movement is associated with flight responses in the three unaffected limbs to escape the potential damage. This entire reflex pattern was denoted the *flexion reflex.* In addition, he also observed an oscillation between contraction of flexors and extensors in the flexion reflex later described in detail by Meinck et al. (1981). Kugelberg (1948) used electromyographic techniques to study which afferent fibre types are involved in eliciting the reflex. He was able to evoke two separate responses by either natural (pin-prick or heat) or by electrical stimulation activating Aδ- and C-fibres, respectively which also corresponded to the perceptions of first and second pain. Stimulation strengths sufficient to depolarise Aδ-fibres were most effective in eliciting the first reflex component, though large fibres may contribute. Hugon (1973) then separated the early A-fibre mediated reflex response further into a component mediated by tactile (group II) afferents with a latency of 40-60 ms after onset of a stimulation at the ankle, and a component mediated by group III afferents having latencies of 85-120 ms. He denoted these two reflex components the *RII* and *RIII* reflex, respectively. In 1960, Hagbarth systematically investigated the reflex response in a number of extensor and flexor muscles as function of stimulation site. It was observed that the general ipsilateral response to an electrical stimulation (“causing an intense burning sensation”) is a flexion of the limb as suggested by Sherrington, except when stimulating directly above extensor muscles in which case extension reflexes were evoked. In the same year, Kugelberg et al. (1960) also reported systematic observations of reflex patterns after stimulation at different locations with emphasis on the plantar response elicited by stimulation at various sites on the sole of the foot. Shahani and Young (1971) studied the influence of stimulus intensity on the latencies and the size of flexor reflexes evoked by stimulation under the sole of the foot. They observed a shortening of latency to both A-fibre mediated reflex components (RII and RIII), and increased amplitude and duration of the withdrawal reflexes (both RII and RIII) with increased stimulus intensity confirming the findings by Kugelberg et al. (1960).

In a voluntary pre-contracted muscle, inhibition of the motor activity prior to an excitatory reflex pattern is seen (Kugelberg et al. 1960; Brown and Kukulka 1993; Shahani and Young 1973). This inhibitory period is often denoted a *silent period*. After the reflex burst another silent period may occur followed by a secondary excitatory activity which may continue as a damped oscillation for up to one second (Meinck et al. 1981). Reciprocal inhibition in antagonistic muscles has also been observed in several studies (Kugelberg et al. 1960; Meinck et al. 1981; Shahani and Young 1971) and has been interpreted as a response to facilitate the withdrawal response.

2.2 Generation and recording of experimental nociceptive withdrawal reflexes

In an experimental situation, the nociceptive withdrawal reflex can be elicited by heat (Willer et al. 1979b; Campbell et al. 1991) or electrical stimulations. Electrical stimulation may either be of a pure sensory nerve e.g. the sural nerve (Willer 1977), or cutaneous stimulation (stimulation of distal cutaneous afferents) on the foot (Kugelberg et al. 1960; Willer 1977). In the present experiments (I-IV), a train of five unipolar rectangular 1 ms pulses was released with a frequency of 200 Hz (Dowman 1991 used 250 Hz; Willer 1977 used 300 Hz) and caused a sharp pin-prick-like sensation beginning around a stimulation intensity of 4-8 mA depending on the stimulation position and individual differences. Lower intensities than the above result in a tactile sensation. In I-IV, the stimulation intensity needed to evoke a NWR was *usually* above the pain threshold (cf. Campbell et al. 1991) when the subject was relaxed but aware of the coming stimuli. This is in contrast to Bromm and Treede (1980), who found the opposite relationship, and several studies observing a close relationship between the pain and reflex thresholds (Willer 1977; Chan and Dallaire 1989; Dowman 1991). Our results could reflect an unconscious descending inhibition of the motor response as the subjects were basicly aware of the coming stimuli due to the experimental set-up even when using random ISI. Alternatively, different methods of estimating the NWR threshold may also influence the intensity needed to evoke the EMG response. In a study of the effect of self-triggered stimulation versus externally triggered stimulation, Arendt-Nielsen (unpublished observations) found reduced reflexes when the subjects controlled the stimuli release. This could indicate a change in descending inhibition of the reflexes due to a shift in attention.

The evoked muscle recruitment pattern highly depends on the stimulation position. When stimulating the sural nerve (I, III,IV) in a sitting/supine position, the typical response is a flexion of the knee joint (often combined with an adduction and rotation of the limb) so recording from the biceps femoris muscle (short head) is an apparent choice for quantification of the response. In supine position (IV), extensor activity in the rectus femoris is also often seen. When stimulating the sole of the foot (II) on a sitting subject, a dorsi-flexion of the ankle by contraction of the tibial muscle is part of the stereotyped response. The latency to nociceptive withdrawal reflexes is typically 80-85 ms from stimulus onset (Kugelberg 1948; Hugon 1973).

2.3 Estimation of the NWR-threshold

Different methods may be used to determine the threshold in order of minimising inter- and intra-individual variability:

- The intensity evoking a reflex response in 50% of the stimuli alike the pain definition by IASP (Merskey 1979). Alternative percentage values have been used: 60-70% by Willer and Bathien (1977), or 70-80% by Willer and Bussel (1980).
- Bouhassira (1994) used four successive increments in the stimulus intensity and estimated the threshold as the average lowest intensity evoking a reflex in a fixed post stimulus interval from 90-180 ms.
- Random stimuli at different intensities resulting in a stimulus response function from which the intersection with the abscissa is interpreted as the threshold (Willer 1985).
- Another normalisation criterion has been used by Dowman (1991). He recorded the sural nerve compound action potential (CAP) with surface electrodes and estimated the lowest current that evoked a "just maximal CAP". One advantage of this procedure is that individual differences in descending modulation of the sensory excitability do not affect the threshold estimation as it is the case in other muscle output based threshold estimations.
- Alternatively, the inter-subject normalisation has also been achieved using the evoked pain intensity. Kiernan et al. (1995) used a level of 2-2.5 on a ten point VAS scale.

In the present studies (II-IV), a computer programme was used to estimate the reflex threshold as objectively as possible. Thus, an (arbitrarily chosen) amplitude of 20 μV in the rectified EMG response for a period of 5 ms (ten samples in all experiments) in a post-stimulus recording interval (70-200 ms) was set as EMG activity threshold. From the training prior to all the experiments, a stimulus intensity below the rough estimate of the reflex threshold was chosen and a computer programme was activated. This programme stimulated the subject approximately once every 10 s (range 8-12 s) and if the evoked EMG activity crossed the EMG activity threshold, the next stimulation was at the same intensity. Otherwise the intensity was increased by 1 mA. The NWR reflex threshold was then defined as the lowest stimulus intensity at which the EMG activity on three successive stimulations exceeded the EMG activity threshold after which the programme automatically stopped. The computer automation secured minimal influence by the experimenter on the threshold localisation. Using this fixed EMG-amplitude controlled by the computer, no subjective evaluation of the threshold was necessary so a simple on-line threshold estimation by one investigator was possible. However, this definition probably also resulted in higher thresholds than a definition of a fixed percentage of stimuli evoking EMG activity (Willer and Bathien 1977; Willer and Bussel 1980). But the present threshold estimation procedure did most likely also involve fewer stimuli.

In a study involving 14 subjects, the reflex threshold was estimated on five different days to analyse the reproducibility of thresholds estimated by the method outlined above. The mean thresholds are shown in figure 3.1. The stimulation position was on the sural nerve and reflex activity was recorded as the difference between m. biceps femoris EMG and rectus femoris EMG with the subject in supine position having the ipsilateral leg placed in a brace flexing the knee joint 30°. All electrode positions were marked to ensure equal positions between the recording days. A global average of the individual variation coefficient (standard deviation divided by the mean) for the five recording days is 0.27. An ANOVA-analysis shows no difference between the five days. The tendency to increasing threshold could be a training effect.

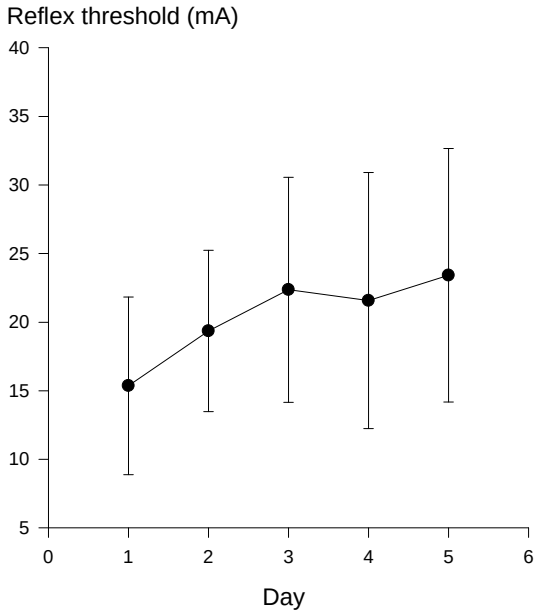

Figure 3.1. Mean reflex thresholds (±SD) for a group of 14 male subjects tested once a week using the technique described in the text. No significant difference (one-way ANOVA) was found between the days but an indication of a gradual increase in the mean reflex threshold values is seen.

2.4 Quantification of nociceptive withdrawal reflexes

Normally, quantification of a reflex response involves two different parameters; one describing the temporal aspects and one quantifying the energy in the reflex response. The typical procedure is to full-wave rectify and average a number of individual responses (five in II, 12 in I, eight in III and IV, ten in Willer et al. (1989), and 20 in Chan et al. (1985)), because of the stochastic nature of the response. Based on the averaged signal onset and offset, latencies may be scored. In the present work, this was typically achieved by visual inspection on the computer screen using a measurement cursor. Finally, the reflex duration may be calculated.

The energy in the NWR response may be quantified using different methods. In the present experiments (I-IV), a Root-Mean-Square (RMS) of the individual recordings in fixed

intervals after the first stimulus pulse was used. Various methods as areas under rectified responses, peak-peak measures, areas under rectified and low-pass filtered responses have been used by different research groups, see table 2.1.

Meinck et al. (1981) used the mean profiles of full-wave rectified EMG signals to describe silent periods and reflex bursts (see I) in recordings during static muscle contraction.

Supra-spinal startle responses (involuntary reactions) to the stimulus may also affect the EMG response leading to wrong reflex size assessment (Dowman 1992). Reaction time measurements based on contraction of the biceps muscle after sural nerve stimulation just above detection threshold have shown a mean reaction time of 177 ms (Arendt-Nielsen et al. 1994). Willer estimated the latency to supra-spinal reactions to 250 ms (Willer 1985). In the present experiments, a 'training' period initiated the experiment as it was often observed that the NWR response decreased once the subject became familiar with the stimulus intensities and experimental set-up. Often this uncertainty resulted in a large variation in the late part of the measurement window of the NWR-response which diminished with the training. This phenomenon was not studied systematically. However, it is important to be aware of possible supra-spinal generated components in the late part of the NWR EMG-response.

In II, an alternative quantification based on integrated energy (70-300 ms) was used because the duration of the NWR response varied between the conditions contaminating amplitude measures based on fixed intervals (e.g. RMS). Integrated measures of the rectified response have previously been used, see table 2.1.

The use of different quantification intervals calls for caution in direct comparison of different studies.

2.5 Experimental protocols

The protocols used in the present pain studies basically used two different paradigms. Either a baseline recording followed by some intervention (III and IV) and a post-recording or alternatively recording of the NWR under different randomised conditions (I, II). In all experiments a fixed intensity was chosen initially based on the individual reflex threshold (1.5 × NWR-threshold), and the effect of the intervention, or variation between different conditions were estimated by variation in the size of the reflex. By choosing a supra-threshold intensity of 1.5 × threshold, there is a window for changes in NWR size. Willer used 1.2 × reflex-threshold (Willer 1985; Willer et al. 1989). Alternatively, variation in the reflex threshold can be used (Willer and Bathien 1977) or a linear regression of a stimulus response function in order to obtain more complete dynamic information about the NWR modulation (Willer 1985). To achieve the stimulus response function, approximately 20-30 recordings (Willer 1985) at different intensities are necessary increasing the total number of stimuli presented to the subject during the experiment which is often not ideal. When the experiment only involves one stimulus intensity (as in the present studies), there is of course the possibility of not discovering a

differential effect in the NWR response window. In I-IV, a minimisation of the number of stimuli was found to be important of ethical and methodological reasons. Thus, repetitive stimulations may result in a habituation of the NWR response.

2.6 Habituation and facilitation of the NWR

Several authors have observed that repetitive, nociceptive electrical stimulations (inter-stimulus-interval higher than approximately 3 s) result in a gradual decrease in the NWR size (Hagbarth and Kugelberg 1958; Shahani and Young 1971; Dimitrijevic et al. 1972; Fuhrer 1976; Granat et al. 1991). This phenomenon is denoted *habituation* and reflects most likely a spinal adaptation to identical afferent input (Dimitrijevic and Nathan 1970). This decrease in responsiveness is in contrast to a reflex facilitation that occurs by repetitive stimuli at short ISI. Thus, Dimitrijevic and Nathan (1970) observed an increase in the NWR in spinal cord injured man in the course of the first three to four stimuli followed by the habituation phenomenon using an ISI of 0.3 - 1 s. The increase in NWR reflects a spinal temporal summation and has later been investigated further regarding both sensory and reflex responses (Hugon 1973; Arendt-Nielsen et al. 1994). The degree of habituation highly depends on the ISI as Fuhrer (1976) did not find any habituation using ISI of 25 s at fixed stimulus intensities in spinal cord injured subjects but found habituation at an ISI of 5 s. Random ISI diminishes the degree of habituation (Dimitrijevic et al. 1972). Further, habituation occurs more often at low stimulus intensities than at high intensities (Hagbarth and Kugelberg 1958; Dimitrijevic et al. 1972). Thus, it is very important to limit the number of weak stimuli (around reflex threshold) if this is the intensity-window of interest and further to use variable ISI especially in the determination of the NWR threshold as in the present studies.

2.7 “Pain” and reflexes

In the early seventies, studies of the nociceptive reflexes in correlation with psychophysical studies of pain sensations in humans were initiated by the work of Hugon (1973). Melzack and Wall (1965) proposed their gate control theory and Hugon saw an opportunity to study the interaction of tactile and nociceptive input on the reflex response in relation to the pain perception. Willer continued the use of the nociceptive reflex in pain research and one of the main findings was that the pain and reflex thresholds coincide (Willer 1977). Secondly, a high correlation between the pain intensity stimulus-response curve and the reflex size stimulus-response curve (Willer 1984; DeBroucker et al. 1989; Chan and Dallaire 1989; Dowman 1991) led to the suggestion of using the reflex as an “objective” measure of experimental pain (DeBroucker et al. 1989). Willer has also used the nociceptive reflex to assess the effect of e.g. various pharmacological interventions (Willer and Bathien 1977; Willer and Bussel 1980)

corresponding to the protocol for IV. See section 3.8 for a discussion of the present findings in relation to the suggestions of the NWR as an “objective” pain measure.

2.8 Additional stimulators and conditioning stimuli used in the studies

Some additional stimulators have been used to assess other aspects of the nociceptive processing and as conditioning stimuli in the studies of hyperalgesia (III/IV).

2.8.1 Thermal stimulation

Thermal stimulation is a natural modality to activate warm receptors and nociceptors in the skin. Thermal polymodal nociceptors are innervated by both Aδ- and C-afferents (Meyer et al. 1994). Activation of the thermal nociceptors may be achieved by either contact thermodes or radiant heat. The latter is preferable in pain research as it does not activate tactile receptors at the same time. In the present studies both stimulation techniques have been used. A contact thermode with a Peltier element of the dimensions 16 × 24 mm (Termotest, Somedic AB) was used in III and IV for estimating the pain threshold and as a conditioning stimulator.

An argon laser was used in IV as a radiant heat stimulators. This device emits visible green-blue light at two different wavelengths (488 and 515 nm). A single quarts fibre is used to transfer the light from the laser to the application area. To secure a standardised heat stimulation, the fibre was placed in a spacer resulting in a 3 mm diameter beam width. The output power was controlled electronically and a meter indicated the actual power (Arendt-Nielsen and Bjerring 1988).

In II, visible white light (broad spectrum) from a high energy xenon lamp was focused and guided to the skin in a liquid light guide (diameter 3 mm). The power was controlled electronically and a spacer ensured a constant distance to the skin. The output from the xenon lamp includes also light with infrared wavelengths so a lot of the energy is absorbed superficially (epidermis/dermis) in contrast to the green/blue light from the argon laser that is expected to penetrate deeper (Arendt-Nielsen and Bjerring 1988).

Xenon-generated light applied on black painted glabrous skin with a thick epidermal layer purely evoked a second pain sensation (a burning or throbbing pain starting approximately one second or more after onset of the light (Price 1988)). Most likely this reflects transmission in C-nociceptors (see Treede et al. 1995).

2.8.2 Electrical stimulation

Electrical stimulation has been used widely in experimental pain research though it is not a natural stimulation (Gracely 1994) as it by-passes the peripheral receptors and depolarises the innervating axons. In this process, thick myelinated axons are depolarised at the lowest current intensities while high current intensities in addition depolarise thinner fibres (Wall and Woolf 1984). The afferent depolarisation evoked is synchronised totally and the compound axon

potential depends on the stimulus intensity. In a sense higher stimulus intensities correspond to increased spatial stimulation as additional afferent fibres are depolarised by the increase in electric field for high stimulus intensities. Due to the unnatural afferent activation, the perception of electrical stimulation does not resemble any natural experience. Instead several subjects described it as an “electrical” sensation. Often the subjects described the pain evoked by electrical stimuli in affective terms rather than describing the sensory intensity. However, the advantage of the electrical stimulator is that it is easy to control the intensity which was the reason for using it in III and IV as activator of tactile fibres in the 2°HA. By the verbal description of the evoked perception, a non-painful stimulus intensity was chosen for conditioning in the 2°HA. Tactile stimulus intensities are associated with activity in Aβ-fibres (Gracely et al. 1993).

2.8.3 Chemical stimulation

Chemical activation of the nociceptors by capsaicin (the pungent extract of chilli-pebbers) has been used in study III and IV to induce an experimental state of clinical hyperalgesia. In both studies, capsaicin was applied topically in a moisturising cream in concentration of 0.1% and 1% in III and IV, respectively. Topical application of capsaicin results in an area with hyperalgesia to mechanical and thermal stimuli closely related to the application region (Culp et al. 1989; Kilo et al. 1994) which is denoted the primary hyperalgesic area (1°HA). Surrounding the 1°HA, an area with hyperalgesia to mechanical stimuli is induced called the secondary hyperalgesic area (2°HA) (Kilo et al. 1994; Grönross and Pertovaara 1993). Beside these changes in sensory sensitivity, a local flare reaction (Koltzenburg et al. 1992) is provoked.

The immediate pain evoked by capsaicin is highly dependent on the application method (intradermal or topical). Recordings from single sensory fibres in animals and humans have shown that Aδ-mechanoheat fibres (AMH) and polymodal C-fibres (CMH) respond to capsaicin application. Thus, both AMH and CMH afferents in monkeys respond to topical capsaicin according to Baumann et al. (1991) but Kenins (1982) only observed activation of CMH in rats. Using topical capsaicin, a high degree of spatial summation of impulses from the activated nociceptors contributes to the pain sensation as only low doses of capsaicin reach the receptors due to the epidermis acting as diffusion barrier (Baumann et al. 1991). Therefore, divergent findings as to the ability of topical capsaicin application in inducing secondary hyperalgesia have been reported. Baumann et al. (1991) suggest that only intradermal application is capable of inducing 2°HA (particularly allodynia) while others have observed 2°HA after topical capsaicin (Koltzenburg et al. 1992; Grönross and Pertovaara 1993; Grönross et al. 1994; Kilo et al. 1994). The temporal extent of allodynia has been reported to at least 30 min. (Koltzenburg et al. 1992), 30-60 min. (Kilo et al. 1994), or *within a day or two* (Grönross and Pertovaara 1993). All studies describe a large dependency on persistent afferent nociceptive inflow.

Lamotte et al. (1991) proposed a model to explain how capsaicin application induces hyperalgesia. A chemosensitive receptor innervated by C-fibres is the neural basis behind the model. This chemonociceptor is supposed to respond vigorously to capsaicin and thereby induce (and maintain) the state of central sensitisation. Using microneurography, Torebjörk and colleagues have identified a number of new receptors; one of these responding only to chemical substances as mustard oil and capsaicin (Torebjörk and Handwerker 1995).

2.8.4 Tactile stimulation

To substantiate and estimate areas of hyperalgesia to mechanical stimuli, different natural, mechanical stimulators have been used. Traditionally, the areas of brush-evoked pain (allodynia) have been assessed by cotton swaps, soft brushes or other soft material. Stiff nylon filaments have been used to estimate areas with hyperalgesia to stroking stimuli (Kilo et al. 1994; Park et al. 1995; Simone et al. 1989a). In IV, a nylon-filament was used to assess the areas of hyperalgesia to punctuate stimuli (also called pin-prick hyperalgesia). We did not map the allodynic area in III, nor in IV.

Of more differentiated mechanical stimuli, Koltzenburg et al. (1992) used both static (pressure pain threshold) and dynamic (cotton-tipped applicator stroked along the skin) stimuli. Mechanical impact stimuli by firing a small light cylinder against the skin at different velocities have been used to assess stimulus-response functions in hyperalgesic tissue (Kilo et al. 1994). In III, von-Frey hairs placed on an axis parallel to the skin and rotating with a fixed frequency of 6 Hz were used to activate tactile receptors dynamically and repetitively in the 2°HA by stroking the skin.

3 MODULATION OF NOCICEPTIVE WITHDRAWAL REFLEXES

The NWR as such is a motor output from the spinal cord. The response may involve many muscle groups within the entire body, and the withdrawal reflex does not always follow a stereotyped pattern but is determined by many factors. The response is primarily generated within the spinal cord in which information from somatic afferents, supra-spinal centres (reflex sensitivity, motor control signals etc.), and spinal intrinsic information about the current motor programme are integrated resulting in appropriate withdrawal commands. Further, sensory information is mediated to supra-spinal structures.

In the present chapter, different models describing the NWR pathway and various input to the spinal cord modulating the NWR are discussed. This also includes supra-spinal modulation of the reflex gain. Finally, the main results of the four studies will be discussed.

3.1 Flexor reflex afferents

The flexor reflex may be evoked by a number of different input to the spinal cord (not only nociceptive input) according to Eccles and Lundberg (1959b). Thus, the term *Flexor Reflex afferents* (FRA) was introduced to describe the different modalities of afferent activity that may evoke a flexion of the limb. In FRA, the following afferents are included (Schomburg 1990):

1) Group II muscle afferents
2) Joint afferents
3) Cutaneous mechano- and nociceptive afferents (Steffens and Schomburg 1993)
4) Group III and IV muscle afferents (Eccles and Lundberg 1959b; Lundberg 1979)

In the concept of FRA, the afferents converge on common interneurons in the flexor reflex pathway to α-motor neurons, although this does not exclude non-FRA *private* sensory-motor pathways (Lundberg 1979; Schomburg 1990). The findings of Hagbarth (1960) are characterised as private reflex pathways. One advantage of this multisensorial (Schomburg 1990) convergence is that FRA serve automatically as peripheral feedback for on-going motor programmes initiated by supra-spinal centres (Lundberg 1979).

Descending motor commands are projected to the same group of interneurons. Thus, these interneurons integrate both descending motor commands and the multisensorial feedback and project the output to motor neurons (Lundberg 1979; Schomburg 1990). Therefore, a movement is initiated by selection of the appropriate interneural FRA reflex pathway and is thereby inhibiting other FRA reflex pathways. Evidently, the function of decreased ‘gain’ of non-active neural pathways is to ensure low response to afferent activity from uninvolved receptors that otherwise may disturb the movement. However, nociceptive activity may still elicit the reflex.

Ascending information from the FRA reflex interneurons is mainly signalling the state of the interneurons and probably not sensory information from primary afferents (Oscarsson 1973).

Persistent short-latency input from tactile and proprioceptive activity evoked by the on-going movement serve as feedback to the current motor programme, alternatively as assistance in flexion during normal movement (Chung et al. 1983). Nociceptive input evoke very effectively the flexion reflex interrupting the on-going motor programme and thereby facilitate motor corrections when potential tissue damaging activity is signalled.

Thus, convergence to the same interneural network assures fast regulation (Behrends et al. 1983) of the on-going motor programme. This model merely describes how spinal, neuronal network integrates all available information (Baldissera et al. 1981; Jankowska and Lundberg 1981) to generate the appropriate withdrawal reflex under the present motor programme. The model does not describe how sensory information is projected to supra-spinal centres. In this concept, individual processing of motor output and ascending information is possible.

3.2 Individual receptive fields to each muscle in the NWR - Schouenborg model

Recent findings in rats by a Swedish group (Schouenborg et al. 1992; Schouenborg et al. 1995) suggest that WDR-neurons in deep lamina have receptive fields coinciding with the cutaneous area in which nociceptive reflex activity may be evoked in a particular muscle. Further, a high correlation between WDR-neuron responsiveness and spatial reflex responsiveness was observed. These putative interneurons are organised in a musculotopic way in the deep dorsal laminae (IV-VI) (Schouenborg et al. 1992). Therefore, this model suggests parallel reflex pathways to each muscle (Schouenborg et al. 1995) with an individual spatial sensitivity (Schouenborg and Weng 1994). In addition, the model outlines a detailed spinal reflex system in which reflexes in individual muscles may be evoked independently and do not necessarily involve flexion of the entire limb (Schouenborg and Weng 1994).

It has not been possible to stimulate the deep putative NWR neurons antidromically from the upper cervical cord indicating that they are pure spinal interneurons (Schouenborg et al. 1995). If this model is true discrepancies between recordings of reflex size and the associated pain intensity in humans are possible. Thus, individual regulation of reflex sensitivity may take place at the spinal level.

3.3 Supra-spinal modulation of the NWR

Descending modulation may be divided into tonic and phasic components. NWR elicited in spinal cord injured humans result in a more intense and long-lasting response (Dimitrijevic and Nathan 1968) suggesting a tonic inhibitory modulation to be present in normal subjects.

Regarding the phasic descending activity, several findings suggest that activity generated by modulation of the psychological/mental state can modulate the NWR. In a study by Bathien

and Hugelin (1969), the subjects completed tests that required their attention resulting in an inhibition of reflexes which suggested an increase in the descending inhibition. Willer et al. (1979a) found similar results when the subjects performed a calculation test, again diverting their attention away from the stimulus. Stress induced by instructing the subject that a very painful shock is coming may also result in inhibition of the NWR (Willer 1980). This mechanism is probably mediated by endogenous opiods because naloxone reversed the effect (Willer and Albe-Fessard 1980). However, stress may also facilitate the NWR (Willer et al. 1979a). Of a more methodological concern, Dowman (1992) investigated a possible contamination of the RIII-reflex by a supra-spinal startle response affecting muscle output late in the reflex response (after 150 ms). It was concluded that it is not an extensive problem but that it may occur in individuals.

These supra-spinal phasic modulating mechanisms indicate the importance of instructing subjects about what they are going to experience during the experiment in order to minimise individual supra-spinal modulation of the NWR. Further, it also stresses the importance of initial training to minimise any startle response.

Recently, two studies have investigated the influence of hypnotic suggestions on the NWR (Kiernan et al. 1995; Zachariae et al. 1996). In both studies, an effect of hypnotic suggestions of analgesia on the NWR was observed by a decrease in the reflex size suggesting direct descending mechanisms onto the spinal nociceptive system. Only one of the studies divided the subjects in accordance with hypnotic susceptibility in the design of the study. Thus, both low hypnotic susceptible and high hypnotic susceptible subjects are able to reduce the NWR size during suggestions of foot analgesia, but highly susceptible subjects showed the largest reduction compared with neutral hypnosis (Zachariae et al. 1996). Further, the neutral hypnosis recording was systematically lower than the baseline recording supporting the possibility of e.g. a startle response contaminating the NWR, which is then eliminated under neutral hypnosis.

3.4 Modulation of the NWR by current motor programme and/or proprioceptive input

Human withdrawal reflexes are highly modulated during various locomotion patterns. Probably, this modulation emanates from two different origins; central neuronal circuitry responsible for the current motor task and activity from sensory receptors.

3.4.1 Functional reflex modulation

For both non-nociceptive and nociceptive, elicited cutaneous reflexes during various cyclic stereotyped movements, a phase dependent reflex generation takes place. To a degree the reflex modulation reflects the background spontaneous EMG activity (see Tax et al. 1995), and the gain of the cutaneous withdrawal reflex arc is generally increased during active movements (Schomburg 1990). However, the consensus is that the principle for reflex generation comprises several functions. The main purpose is to withdraw the limb to compensate for an external

perturbation but the response pattern also takes preservation of balance and continuation of the movement with minimal change in cadence into consideration. Indeed, the intensity of the stimulus controls the size of the withdrawal response (Shahani and Young 1971; Forssberg 1979; Duysens et al. 1993) and therefore, a tactile stimulus serves to adjust the movement to the external perturbation (sort of a sensory feedback) while the nociceptive intensity causes a more vigorous withdrawal response to escape the pain stimulus.

The phasic modulation of the reflexes is expected to take place in the spinal cord (Crenna and Frigo 1984). Thus, convergence of descending information, various afferent input, and intrinsic information about the type and phase of the movement onto interneurons in the reflex arc modulate the withdrawal reflex. The spinal intrinsic information is expected to originate from 'central pattern generators': anatomically unspecified centres at an interneuronal level in the spinal cord generating control signals for stereotyped movements (e.g. walking) (Eccles and Lundberg 1959a; Grillner and Wallén 1985; Sillar 1991). In addition, a sensory gating of the afferent input takes place based on information from the central pattern generators (Sillar 1991).

3.4.2 NWR and pain intensity modulation during a voluntary movement (I)

While the subject was sitting in an elevated chair, a simple motor task consisting of a loaded continuous cyclic extension/flexion movement of the lower leg was used to study how proprioceptive input and/or possibly spinal pattern generators may modulate electrically evoked NWR. This exercise did not resemble any functional locomotion but the nature of the movement only involved one joint and therefore a relatively simple interpretation of the results.

Reflexes were elicited at a fixed joint angle and movement velocity, either when the knee extensor (rectus femoris) was loaded (resistance during knee extension) or alternatively when biceps femoris was loaded. Additionally, recordings during corresponding static conditions were carried out. In general, the NWR was found to vary with the direction of the movement.

The general reflex pattern consisted of a silent period followed by an excitatory burst in the active muscle, and in the antagonistic muscle a reflex burst was observed.

The largest excitatory response in rectus femoris occurred in the dynamic extension condition while the muscle was active in accordance with the findings of Crenna and Frigo (1984). They found the largest reflexes in the knee extensor muscles (vastus lateralis) during normal contraction of the muscle in the support-phase of locomotion. On the other hand, stimulation in the stance phase, in which the muscle is normally inactive, resulted in a weak reflex response. Under stationary conditions, Rossi and Decchi (1994) found a modulation of the nociceptive reflex in tibialis anterior depending on which limb supported the body while standing. Increased reflexes were observed when standing on the contralateral leg and decreased reflexes when supporting on the ipsilateral leg compared with symmetrical stance (standing on both legs). Apparently, this result is in contradiction to the findings of Crenna and Frigo (1984). Most likely this reflects a difference in balance protection as there was no chance of falling in the

experiment of Rossi et al. (1994). In agreement herewith are findings by Patla and Belanger (1987) in which cycling on an ergometer resulted in no real modulation of the non-nociceptive cutaneous reflex size. The authors suggest the lack of postural stability and potential contralateral support (through the crank) as explanation for the lack of phasic modulation (Patla and Belanger 1987). As the subjects were seated in I, the most likely reason for the large reflex in rectus femoris during dynamic extension would be to preserve the cadence of the movement in agreement with a functional cutaneous reflex modulation (Yang and Stein 1990; Berger et al. 1984; Crenna and Frigo 1984) as the posture was of no importance.

During active flexion of the knee joint in I, the active muscle (biceps femoris) was inhibited at a shorter latency than it was the case for rectus femoris during extension. However, as no real reciprocal activation occurred in rectus femoris during the silent period in biceps femoris, the net result was an increased flexion of the knee joint induced by the reflex burst succeeding the silent period in biceps femoris. Crenna and Frigo (1984) also found a phase-dependent modulation of this muscle with the largest response in the swing phase, i.e. initially in the normal activation period.

The phase-dependent results outlined above are in agreement with the findings of correspondence between muscle activity and reflex size. Comparing tibialis anterior responses for running and walking (Duysens et al. 1991), the cutaneous evoked reflex sizes (non-nociceptive intensities) are generally large during running where the background activity is also largest. However, it is not a linear relationship as relatively (to the baseline muscle tone) larger reflexes were found during running than compared to standing (Duysens et al. 1993).

Comparing the responses in the non-active muscles, the excitatory reflex burst in biceps femoris during the extension phase was larger than the burst seen in RF while flexing the leg. One possible explanation for this could be the direction of the movement relative to the stimulus position on the foot dorsum (Dimitrijevic and Nathan 1968; Shahani and Young 1971). Thus, when the knee is extended, this could be interpreted as a movement against the potential pain source and against the ‘normal’ withdrawal direction (knee flexion). In accordance herewith, the latency to the BF reflex burst during flexion was longer than to the BF burst during extension (I).

Further, reflexes elicited during the voluntary dynamic movement were generally larger than reflexes elicited in a static condition with the same load and joint angles. This is in accordance with studies which investigated the relationship between reflexes elicited during running and standing (Duysens et al. 1993) or between walking and different isometric contractions (Kanda and Sato 1983). Though, both studies did not use nociceptive stimulus intensities. Brown and Kukulka (1993) compared cycling and static contractions and observed large reflexes in the dynamic conditions, however, using non-noxious intensities.

3.4.3 Subjective pain ratings and reflex size

In I, a systematic discrepancy between changes in the reported pain intensity and the size of the nociceptive reflexes between the static and the dynamic conditions was found. Thus, the

lowest pain intensities were observed together with the largest reflexes for the dynamic conditions (see figure 7 in I). However, findings of other studies could support the decrease in perceptual sensitivity during movement. Chapman et al. (1987) have observed an increase in the perception threshold during movement, and a Finnish group has found a decrease in both thermal and pain sensitivity when doing physical dynamic exercise (Kemppainen et al. 1985) while isometric contractions did not change the pain threshold nor supra-threshold ratings (Feine et al. 1990).

Further, the results in I may contradict the influence of attention described in section 3.3, as the cyclic movement most likely directs the attention away from the coming stimulus towards maintaining the rhythm and should, therefore, result in NWR inhibition.

3.4.4 Conclusions (I)

The main findings in I are: 1) General increased reflexes during voluntary dynamic conditions compared with corresponding static conditions. 2) An opposite-related modulation of reflex size and pain intensity ratings with high NWR and low pain intensity scores during dynamic conditions. The latter indicates a possible, independent processing of the excitability in reflex and ascending sensory pathways.

Under certain conditions (e.g. during a motor task), cutaneous withdrawal reflexes may be evoked by non-nociceptive stimulus intensities and undergo severe modulation to comply with the functional context. The findings in I could indicate the cutaneous reflex modulation to occur independently of the sensory transmission.

3.5 Facilitation of the nociceptive withdrawal reflex by concurrent C-fibre input

3.5.1 Spinal afferent convergence

In light of the multisensorial convergence described in section 3.1, the involvement of different nociceptive afferents in spinal integration has been discussed. In spinal cats, integration of nociceptive (activated by radiant heat) and classic FRA (Steffens and Schomburg 1993) or of nociceptive and cutaneous mechanoreceptors (Behrends et al. 1983) result in increased post-synaptic potentials in α-motoneurons. This could indicate a central summation of activity in these afferents on the spinal level. However, excitatory convergence of nociceptive input in man resulting from different afferent populations is not fully investigated.

3.5.2 Central summation of nociceptive input (II)

Central summation is a term including both temporal and spatial summation. Several psychophysical studies of temporal and spatial summation have been conducted. In human pain studies, summation of afferent nociceptive input has been shown for repetitive heat stimuli denoted *temporal* summation (Price et al. 1977) and for increased stimulus area - *spatial*

summation (Price et al. 1989; Douglass et al. 1992). Temporal summation requires a stimulation frequency above 1/3 Hz (Price et al. 1977). However, for heat stimulations, only second pain (burning or throbbing pain (Price 1988)) corresponding to activation of afferent C-fibres exhibits the facilitatory mechanism. Aδ-activity (evoking first pain) does not show this build-up tendency to repeated heat stimuli. This is most likely due to fatigue of the type-II A-mechano-heat (AMH) nociceptors (Treede et al. 1995) resulting in a gradual suppression of the first pain intensity (Price et al. 1977). The brief thermal stimuli used in studies of temporal summation do not activate type-I AMH (Treede et al. 1995). Regarding spatial summation, increased pain ratings (intensity and unpleasantness) for increased stimulus areas (less than 3 cm^2) of contact thermodes have been observed both within (Price et al. 1989) and between dermatomes (Douglass et al. 1992).

In II, a possible central summation between Aδ- and C-fibre activity was investigated. With a 200 ms radiant heat stimulation on the sole of the foot using a xenon-lamp, burning pain (second pain) was evoked. Based on pilot studies, the pure second pain sensation was only achieved when the skin was painted black at a position with a relatively thick epidermal layer. According to Treede et al. (1995), glabrous skin is not innervated by type-II AMH fibres but only type-I AMH that usually have very high thresholds (median above 53°C) and with long response latencies. Therefore, the radiant heat most likely activated polymodal C-fibres (CMH). In II, the mean reaction time to these light stimuli was 961 ms indicating that in some of the subjects, conduction of the heat information must have taken place in faster afferents than C-fibres. Still, all subjects described the pain quality as a profound burning sensation appearing one to two seconds after light onset, which is usually associated with C-fibres (Price 1988). Next to the black skin area (i.e. presumably within the same dermatome), electrodes for electrical stimulation were mounted by which NWR were elicited in the anterior tibial muscle. Thus, afferent Aδ-activity was achieved by electrical stimuli while afferent C-fibre activity was evoked by the radiant heat, and a possible spatial summation of the afferent activity at the spinal level was investigated.

When selecting different inter-stimulus-intervals varying between 0 and 3000 ms, the central processing of these different stimulus modalities was investigated taking the afferent conduction velocity into consideration. For ISI values between 800 and 1200 ms, significantly increased NWR values and pain intensities were recorded. In addition, the reflex and associated pain intensity at 3000 ms ISI were lower than the recordings with 1000-1200 ms ISI but still above baseline. Thus, a local maximum occurred in the NWR response when elicited approximately 1 s after light onset. One possible explanation for this finding could be the prolonged heat sensation described by most of the subjects, which likely reflected a prolonged firing in dorsal horn neurons (Mendell and Wall 1965; Sivilotti et al. 1993; Dickenson and Sullivan 1987; Schouenborg and Dickenson 1988).

The most direct interpretation of the potentiation of the NWR approximately 1 s after onset of the light (i.e. colliding with the second pain sensation) is a convergence onto common interneurons within the spinal cord. Both Aδ- and C-fibres evoke prolonged "slow" discharges in dorsal horn neurons (Wagman and Price 1969; Sivilotti et al. 1993), however, C-fibre input is most efficient. Furthermore, a progressive increase in 'flexion' reflexes in the spinalised cat to repetitive C-fibre stimuli has been observed but no change in reflex size to Aδ-fibre stimulus intensity was found (Price 1972). Of course, the spinal mechanism behind the integration in II cannot be decided on the basis of a non-invasive study.

Second-order WDR neurons are likely candidates for several reasons. Only these neurons show prolonged firing to C-fibre input (Dickenson and Sullivan 1990). Indeed, only activity in WDR-neurons wind-up to repeated C-fibre input while NS neurons are unaffected by prolonged nociceptive input (Schouenborg and Dickenson 1988). WDR neurons alone can encode the intensity and spatial distribution of thermal stimuli (Coghill et al. 1993). One possible aspirant could be the putative last-order WDR interneurons in the NWR spinal pathway (located in rat lamina IV-VI) suggested by Schouenborg et al. (1992), see section 3.2. However, if this model holds, the correlation found in II between the pain intensity (mean 20% increase, approximately) and NWR size (mean 100% increase, approximately, see figure 2 in II) must, therefore, reflect two different spinal WDR neurons integrating afferent input; one projecting to the ventral horn and the other projecting to supra-spinal structures. Alternatively, independent modulation takes place in the reflex pathway and ascending pathways beyond the dorsal horn.

A mechanism behind the central summation could be an opening of the NMDA receptor system on WDR neurons by the nociceptive heat pulse resulting in increased reflex sensitivity (Dickenson and Sullivan 1987), see the next section. However, there is no indication of a general increase in excitability as all the conditions in II were randomised.

Alternative mechanisms for the NWR potentiation in the ISI-interval between 800 and 1200 ms could be a potential long loop reflex pathway involving supra-spinal centres and thereby changes in the descending inhibition of the spinal reflex pathway.

3.5.3 Conclusions (II)

The main finding of II was a potential central summation of activity in nociceptive Aδ- and C-afferents. This was observed both in the subjective pain ratings and in the NWR size.

3.6 Central hyperexcitability - reflex quantification and NMDA involvement

Mendell and Wall (1965) discovered that repetitive stimulations of afferent C-fibres caused gradual increased firing in dorsal horn neurons (wind-up) followed by an after-discharge and an increased sensitivity lasting seconds. This observation initiated research in persistent changes in central excitability. This wind-up phenomenon is likely the basis for increased central excitability (Dickenson 1995). By repeating a C-fibre electrical stimulus to rats once every

second for 20 s, an increase in central excitability is induced lasting up to 90 min. (Wall and Woolf 1984; Woolf and Wall 1986). The increased central excitability is not induced by activity in thick myelinated A-fibres (Woolf and Wall 1986). Woolf and King (1990) discovered an expansion of receptive fields, increased responsiveness, and lowering of thresholds after application of mustard oil on cats which are all indicators of increased central excitability. The same increased central excitability may be induced by natural stimuli injuring the tissue. Thermal stimuli (75°C for 60 s) to the rat paw induced a marked decrease in the threshold for mechanically evoked withdrawal reflexes (Woolf 1983) lasting for hours.

Generally, it is agreed that persistent nociceptive (C-fibre) input after tissue injury induces central hyperexcitability (Dubner 1991; McMahon et al. 1993; Yaksh 1993; Woolf 1994).

3.6.1 Hyperalgesia

After tissue injury, hyperalgesia (decreased pain threshold) to both thermal and mechanical stimuli appears at the injury (primary hyperalgesia, 1°HA). The heat hyperalgesia reflects a receptor sensitisation by release of various inflammatory agents from the nerve endings. Mechanical HA at the injury is not a result of sensitisation of normal responding peripheral receptors (Meyer et al. 1994). However, silent mechano-sensitive C-fibres (silent nociceptors i.e. not responding under normal conditions to natural stimuli) are recruited after tissue injury (Schmelz et al. 1994) causing an increase in the afferent barrage to mechanical stimuli from the injured region and this may contribute to the primary mechanical hyperalgesia. This corresponds to a receptive field expansion and thereby increased spatial summation (Meyer et al. 1994). An alternative mechanism could be a disinhibition, as a lowering of LTM responsiveness has been observed after a heat injury (Beck et al. 1974) and thereby a potential disinhibition of nociceptive input (Meyer et al. 1994).

Surrounding the injury, an area with hyperalgesia to mechanical stimuli (secondary hyperalgesia, 2°HA) and allodynia (pain mediated by tactile mechano-receptors) appears (the phenomenon of cutaneous hyperalgesia is reviewed in Treede et al. (1992)). Often the term secondary hyperalgesia is used to include both hyperalgesia and allodynia (Meyer et al. 1994; Treede and Magerl 1995; Dubner 1991). The mechanism responsible for 2°HA is believed to be a central sensitisation (Torebjörk et al. 1990; Gracely et al. 1993; Meyer et al. 1994) meaning increased excitability of dorsal horn neurons to mechanical input. However, peripheral mechanisms have been suggested (Lewis 1936) involving a peripheral axon-reflex by which antidromic activity should sensitise neighbour receptors surrounding the injury. It has not been possible to show any sensitisation of mechano-receptors (Thalhammer and LaMotte 1982; Treede and Magerl 1995). In the 2°HA, altered central changes such as facilitated processing of nociceptive input and/or altered synaptic efficacy of LTM input to nociceptive mediating neurons (Treede et al. 1992; Dubner 1991) have been suggested.

Experimentally, cutaneous hyperalgesia in humans may in a reversible manor be induced by either chemical or thermal activation of C-afferents. Capsaicin, the pungent extract of chili peppers (see section 2.8.3), activates C-fibres (Kenins 1982) with high affinity causing intense burning pain, and thereafter cutaneous hyperalgesia. However, the latter depends on the application method. The most frequently used route of application is probably intradermal injection (Simone et al. 1989b; Gracely et al. 1993; Torebjörk et al. 1992) causing a short-lasting extreme pain intensity. The pain intensity and duration are capsaicin dose-dependent and this also applies for the size of the 2°HA (Simone et al. 1989b), see table 4.1. The alternative administration method is topical application under occlusion, see the method section.

Several studies have been conducted to investigate mechanisms of hyperalgesia through the capsaicin hyperalgesia model, see table 4.1.

Sensitisation of nociceptors as described above accounts for the primary heat-HA, however, the central changes associated with the increased excitability and 2 °HA are not clear. In a study of spino-thalamic tract neurons in monkeys (Simone et al. 1991), the response of WDR-neurons correlated well with the pain intensity reported by humans to the same capsaicin injection. On the other hand, both NS and WDR neurons increased their firing to mechanical stroking of the skin suggesting that after intense C-fibre input, NS neurons act as WDR neurons and the total spinal excitability is thereby increased (Simone et al. 1991; Dubner 1991).

3.6.2 The NMDA-system and its involvement in central hyperexcitability

The induction of increased central excitability involves a cascade of modulatory events within the dorsal horn. Activation of nociceptors causes a release from presynaptic terminals of various neurotransmitters such as fast acting excitatory amino acids (EAA, aspartate and glutamate) and more slowly released neuropeptides (NP). Under normal physiological conditions, EAA activates post-synaptic AMPA and kainate receptors and only partly depolarises the post-synaptic neuron via the NMDA (N-methyl-D-aspartate) receptor. When the NMDA associated channel is opened (Mg^{2+} block is removed), Ca^{2+} enters the cell, see figure 4.1. After robust nociceptive input, the NMDA receptor plays, therefore, a major role in central sensitisation by opening for Ca^{2+}-influx resulting in an increase in intracelluar Ca^{2+} concentration which initiates central plastic changes (Coderre et al. 1993).

The NMDA-receptor is unique because it is both voltage and ligand gated (Dickenson 1994). Further, it requires presence of glycine before the binding of glutamate is capable of opening the ion-channel. Even with presence of both glycine and glutamate, the ion-channel does not open under normal synaptic conditions because it is blocked by a magnesium ion. This Mg^{2+}-ion is removed when the cell is depolarised (the voltage gate). Thus, the NMDA receptor ion channel requires other depolarising synaptic activity prior to a potential opening. This may happen via other fast transmission (e.g. EAA on the non-NMDA receptors) or slow synaptic potentials after presynaptic release of neuropeptides (Dougherty and Willis 1991).

NMDA-receptors are important for wind-up in WDR-neurons (Dickenson 1990) and increased central excitability (Woolf and Thompson 1991; Yaksh 1993). Repetitive electrical stimulations with a frequency above 0.1 Hz (Dickenson 1994) of receptive fields of dorsal horn WDR-neurons evoke wind-up which is then blocked by AP5 (a competitive NMDA antagonist) (Dickenson and Sullivan 1987; Dickenson and Sullivan 1990) or ketamine (Davies and Lodge 1987). The first neuronal response is not affected but only the firing build up to successive stimuli. Under normal physiological conditions, both A- and C-fibre stimuli are most likely mediated by non-NMDA receptors (Dickenson and Sullivan 1990) while wind-up involves the NMDA-system. Further, both the induction and maintenance of central hyperexcitability depend on the NMDA receptor system as observed in the Woolf-model of 20 s C-fibre input or in a model of chemical irritation with mustard oil (Woolf and Thompson 1991).

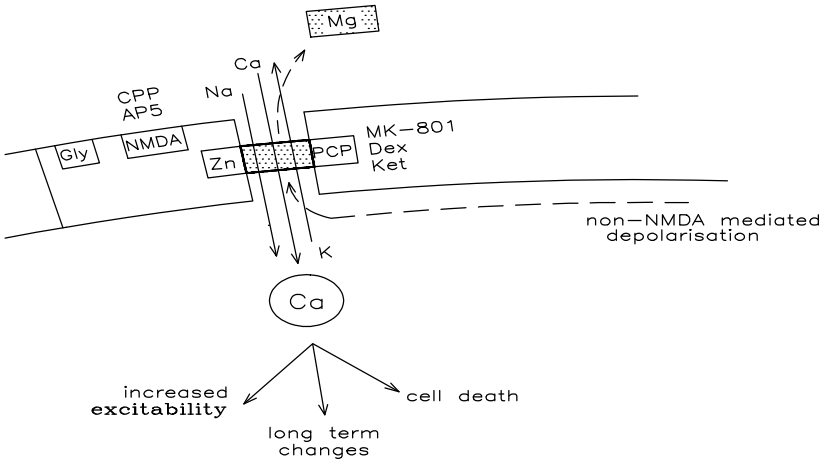

Figure 4.1 The NMDA-receptor system. The Mg^{2+} ion block of the ion channel is removed by depolarisation of the cell by other receptor systems. Abbreviations: Ket - ketamine, Dex - Dextromethorphan, Gly - glycine, and PCP - phencyclidine. CPP, AP5, and MK-801 are all antagonists to the receptor.

There is a number of different sites in the NMDA-receptor complex where pharmacological modulation is possible (Dickenson 1994). Competitive antagonists for the NMDA recognition site exist, e.g. CPP, which has been used to abolish ‘wind-up’ pain in a patient with severe neuropathic pain (Kristensen et al. 1992). A phencyclidine (PCP) receptor is found within the ion-channel and agents binding to this receptor act as non-competitive NMDA-antagonists by blocking the channel for ion transport. One of the potential drugs acting at the PCP site is ketamine (Maurset et al. 1988; Øye et al. 1992), which has been investigated under several experimental and clinical conditions (see table 4.2). In most models, ketamine has positive effect reducing pain intensity and various pain correlates. However, it is also often associated with mild or severe psychotomimetic side effects (Maurset et al. 1989; Klepstad et al. 1990; Max et al. 1995) when given intravenously. Regarding the site of action of ketamine, it has been argued that the analgesic effect after intravenous infusion may also reflect blocking of

NMDA-receptors in the brain (Øye et al. 1992). However, the blocking of wind-up in single dorsal horn neurons by ketamine (Davies and Lodge 1987) suggests a spinal action as well.

3.6.3 Cutaneous hyperalgesia and the involvement of the NMDA receptor system (III, IV)

Ketamine (an i.v. bolus of 0.2 mg/kg over 10 min. followed by a dose of 5 μg/kg/min. to maintain plasma constant plasma concentration) was used to test the involvement of the NMDA receptor system (IV) in the central processing of experimental stimuli in a model of cutanous HA. None of the subjects in IV reported any major side effects of the ketamine infusion and were therefore alert and fully capable of verbally responding to the different stimuli presented to them.

By topical application of capsaicin (concentrations of 0.1% in III and 1% in IV), cutaneous hyperalgesia was induced on the dorsal, lateral side of the foot. Independent of the doses, capsaicin consistently evoked a burning, painful sensation which lasted a few minutes after the capsaicin cream was removed from the skin after one hour application.

Heat hyperalgesia was present within the application area in both studies with heat pain thresholds lowered to around 35°C (see III/IV) probably reflecting a sensitisation of CMH receptors (Baumann et al. 1991). Hyperalgesia to mechanical stimuli in the 1°HA (Culp et al. 1989) was not quantified but a degree of tenderness (III) was present just after removal of the capsaicin. Hyperalgesia to static pressure (non-noxious) in the 1°HA mediated by C-fibres has also been observed (Koltzenburg et al. 1992). This was often observed in III/IV as constant, weak pressure to the application area, e.g. by the thermode, suddenly evoked a burning sensation.

In the part of the 2°HA in which all tests were performed, no thermal hyperalgesia was observed in any of the tested subjects. Systematical mapping of the 2°HA was only performed in IV where a von-Frey hair was used to assess the area of pin-prick HA. There was a tendency to NMDA sensitivity in the size of the 2°HA (IV, though not significant), as observed by Park et al. (1995). In a study by Arendt-Nielsen et al. (1996) using the same capsaicin dose and application method as in IV, the area with hyperalgesia to single argon laser stimuli showed a striking high correlation with the hyperalgesic area to a von-Frey hair (punctuate HA) (Arendt-Nielsen et al. 1996). This could indicate that punctuate HA reflects central sensitisation of Aδ-afferent input as the argon laser is mainly stimulating typeII AMH. This supplements the observation that hyperalgesia to punctuate (von-Frey) stimuli persisted after an ischemic nerve compression of all A-fibres indicating that punctuate HA is mediated partly by C-fibres but probably also by Aδ-fibres (Kilo et al. 1994). In contrast, Serra et al. (1993) suggest that 2°HA alone reflects sensitisation of C-afferents proximal to the receptor. Punctuate hyperalgesia outlasts the on-going pain (Kilo et al. 1994) as it was also the case in both III and IV. This is supported by findings of LaMotte et al. (1991) and Treede et al. (1993) using intradermal capsaicin injections.

Pain evoked by light brushing (allodynia) has been found after topical capsaicin application (Koltzenburg et al. 1992; Grönross and Pertovaara 1993) or intradermal application (Torebjörk et al. 1992; Treede and Cole 1993). A nerve compression which normally blocks the

sensation of touch totally abolished the allodynia suggesting that it is mediated by Aβ-afferent (Koltzenburg et al. 1992). In the same study, cooling (25°C) of the capsaicin-exposed skin abolished the on-going (spontaneous) pain, and consequently no allodynia was present. A successive re-heating (36°C) of the skin rekindled the spontaneous pain and brought back the allodynia (Koltzenburg et al. 1992). Therefore, on-going (nociceptive) afferent input seem vital for the presence of allodynia (Gracely et al. 1992). As the on-going pain disappeared within a few minutes in the present studies, this explains why the allodynia also waned (III, IV).

In III and IV, the degree of central hyperexcitability induced by the capsaicin application and the dependency of the NMDA system were assessed by modulation of the NWR. Different mechanical and thermal conditioning stimulations were applied to both the 1°HA and 2°HA in order to explore central mechanisms of HA.

Assessment of the NWR by electrical stimulations of the sural nerve was not affected (facilitated) by the presence of cutaneous hyperalgesia within the same dermatome (III). Under similar conditions, Grönross and Pertovaara (1993) observed an increase in the NWR. Yet, the increased NWR in that study was highly dependent on continuous input as cooling the application site and thereby stopping the spontaneous pain reversed the facilitated reflex. Therefore, a central summation mechanism may as well account for the increased reflexes in the light of the findings in II. In IV, there was no significant difference between placebo and ketamine regarding the reflex size with presence of cutaneous HA (in mean values, ketamine caused a decrease in reflex size in accordance with Arendt-Nielsen et al. (1995) while the placebo infusion did not change the NWR size). However, an effect of ketamine was found on the associated pain intensity. In both III and IV, no on-going pain was present when recording unconditioned reflexes.

On-going pain was re-evoked by a thermal (less than 40°C, normally non-painful) conditioning stimulus in the 1°HA followed by a reflex recording either while the spontaneous pain was still present or just after it ceased (the latter condition only in III). When the on-going pain was still present, enhanced reflexes were recorded (III), while reflexes recorded after the on-going pain ceased were not changed (III). Two possible mechanisms can explain these facilitated reflexes. Either, the C-fibre afferent barrage evoked by the thermal stimulus rekindled a state of increased central excitability (Woolf and Wall 1986; Woolf and Thompson 1991). Alternatively, it is a central summation of afferent C- and Aδ- activity as found in II. Ketamine did not reverse this reflex facilitation but caused lower pain ratings (a pain score of the ‘overall’ pain intensity) compared with placebo treatment.

Tactile conditioning stimuli by continuous high frequency (40 Hz), low intensity (not painful), electrical stimulations or continuously rotating von-Frey hairs (see chapter 2) to the 2°HA were used to investigate the interaction of afferent Aβ-activity from a 2°HA and Aδ-activity. With a lack of on-going pain, the tactile conditioning stimuli did not evoke any pain sensation and no modulation of the NWR was observed in III, indicating no or weak 2°HA.

However, as described above, a thermal conditioning stimuli to the 1°HA rekindled the 2°HA by briefly evoking on-going pain. Thus, the tactile conditioning stimuli (only electrical) evoked pain when tested just after the on-going pain waned. Correspondingly, increased NWR and pain ratings (III) were recorded in association with the painful, tactile, conditioning stimuli indicating a central excitatory convergence of tactile and nociceptive afferents with presence of 2°HA. This potential convergence is in agreement with findings in cats by Steffens and Schomburg (1993). Indeed, this positive integration may serve to facilitate the withdrawal of an injured region (Schomburg 1990). The central summation could be a result of altered central synaptic efficacy of the Aβ-afferents. This facilitation of NWR is mediated through the NMDA receptor system, as ketamine reversed this tactile mediated facilitation (IV). An effect of ketamine on spinal reflexes supports a spinal site of action.

Finally, in IV repetitive electrical stimuli (five stimuli presented at four different frequencies 0.5, 1, 2, and 3 Hz) were applied to the 2°HA to test the involvement of the NMDA receptor system in temporal summation with presence of cutaneous HA. Prior to this experiment, a facilitation of the temporal summation mechanism had been observed in a corresponding 2°HA to both electrical and argon laser stimuli (Arendt-Nielsen et al. 1996). The results presented in IV indicate a very unequivocal effect of ketamine for the repetitive stimuli while no effect was observed on single electrical stimuli to the same area. Thus, temporal summation in a 2°HA for all the applied frequencies involves the NMDA receptor system. This is in accordance with several other basic (Dickenson and Sullivan 1987; Davies and Lodge 1987; Dickenson and Sullivan 1990) and human studies (Warncke et al. 1994; Arendt-Nielsen et al. 1995). Whether the temporal summation mechanism is facilitated beyond what could be expected from the lowering of the pain threshold in the 2°HA cannot be clarified in IV due to the drug infusions.

3.6.4 Conclusions (III/IV)

In III, a potential central summation of Aβ- and Aδ- afferent activity was shown both regarding subjective pain ratings and size of the NWR. The NWR facilitation involved the NMDA receptor system. Further, with presence of on-going pain from the primary hyperalgesic area, the NWR and associated pain ratings were facilitated (III) in accordance with the observations in II. This facilitation was only reversed regarding pain intensity by the NMDA antagonist.

3.7 Motorneuron excitability

All four papers present modulation of the nociceptive reflex by various physiological and pharmacological manipulations. Potentially, this modulation may also take place at a motor neuronal level by e.g. descending activity directly on the motor neurons and not in the pain sensory pathway. In II-IV, control studies of the excitability of the motor neuron pool were

performed using the Hoffman-reflex technique. In principle this procedure should be followed in every experimental pain study using the NWR. Thus, the effect of the radiant heat stimuli on the soleus H-reflex was tested in II, the effect of capsaicin was tested in III, and in IV the effect of ketamine was tested. None of the control studies found any changes in the monosynaptic reflex suggesting none of these interventions had any effect on motoneuronal excitability. The findings in III are in accordance with the findings by Hugon (1973) who found no facilitation of the stretch reflex in the biceps muscle while the NWR (RIII) reflex was facilitated by repetitive, electrical stimuli (RIII - sensitisation). Further, application of another C-fibre irritant, mustard oil, on the skin of a rat induces a state of central hyperexcitability with increased NWR (flexor reflexes) (Woolf and Wall 1986), but increased motorneuronal excitability in the biceps muscle only occurred a few minutes after the application of the mustard oil (Cook et al. 1986).

Shahani (1970) found a short lasting modulation of the H-reflex by electrical, nociceptive conditioning stimuli of the tibial nerve at the ankle, as a facilitation occurred just after the conditioning stimulus followed by an inhibition. This modulation lasted up to 400 ms. Capaday et al. (1995) found an inhibition in the soleus H-reflex 100-120 ms after a non-nociceptive conditioning stimulus was applied to the common peroneal nerve. The radiant heat stimuli used in II correspond somewhat to the conditioning stimuli used by Shahani (1970) but no modulation of the H-reflexes was observed in II.

Retrospectively, the H-reflex tests in III should also have been carried out after the thermal and/or electrical conditioning (tactile stimulation to the 2°HA) stimuli as an increase in the NWR was only observed under these conditions. With the present findings it cannot be excluded that the increased NWR responses seen after various conditioning stimuli were partly due to increased motorneuronal excitability.

3.8 Discrepancy between modulation of the subjective pain perception and the NWR

Especially in I and IV, the correlation between the modulation of the subjective pain intensity and the size of the NWR did not comply with the findings in the literature. In I, an inverse relationship was observed indicating a parallel but individual sensory processing of the reflex pathway and the ascending sensory pathway. In IV, tendencies in mean values indicate the proportional relationship as several groups have observed under relaxed conditions (Chan and Dallaire 1989; DeBroucker et al. 1989). A methodological explanation for this could be that in I-IV a fixed stimulus intensity was used by which modulation of the response was quantified. Thus, in the study by Campbell et al. (1991), in which heat stimuli were used to elicit the NWR, they found a very poor correlation between the pain intensity ratings and reflex size at each stimulus. In the studies by Chan and Dallaire (1989) and DeBroucker et al. (DeBroucker et al. 1989), a varying stimulus intensity was used resulting in high correlation between NWR and subjective responses.

The present findings could indicate that the sensitivity of the method is not sufficient to reveal changes in spinal excitability based on one stimulus intensity only. Instead a stimulus response function is needed. On the other hand, it could also support the independent spinal processing in sensory and reflex pathways as mentioned previously. The findings by Schouenborg and colleagues of individual receptive fields for each muscle in the NWR in rats (see section 3.2), which did also not project to the cervical spinal level, could support our observations. Further, in II in which significant changes in subjective ratings and the NWR size were found, the size of the changes does not support a linear relationship. Also, different findings of the correlation between pain intensity and NWR thresholds could support individual modulation. Thus, Bromm and Treede (1980) found withdrawal reflexes for non-painful intensities. Campbell et al. (1991) needed supra-threshold intensities to elicit the NWR (as we observed) while others have observed identical pain intensity and NWR thresholds. Finally, findings of a dissociation between pain intensity and NWR size (RIII) in the contralateral limb after an anterolateral cordotomy led to suggestions of a potential descending modulation in ventral columns/ventrolateral funiculi. Or alternatively, it led to interruption of the information loop to and from the brainstem which only affected the motor response (García-Larrea et al. 1993). Thus, several lines of evidence are found for a less strict common modulation suggesting that the NWR should alone be used as an additional tool in experimental pain research.

DeBroucker (1989) suggested that the NWR could be used as an ‘objective’ physiological correlate of experimental pain intensities in humans based on the linear proportionality between subjective pain and size of the NWR (RIII). However, given the definition of pain listed in chapter 1, this really does not make any sense as pain is in nature an personal subjective experience. Further, if these two measures are always correlated there would often be no point in measuring the NWR as psychophysical assessments could instead provide the required information about e.g. analgesic efficacy. Though, the site of action would of course be less clear. Instead, the NWR should be used when information about e.g. spinal nociceptive excitability is a key question.

4 GENERAL CONCLUSIONS AND IMPLICATIONS

An automatic system for determining the NWR threshold has been developed by which a standardised procedure was used for the recordings. This system makes it possible for one investigator to carry out studies without subjectively influencing the threshold estimation. The technique of recording the NWR was used in four human experimental pain studies.

In I, increased spinal NWR excitability was observed during voluntary dynamic movements while at the same time a decrease in subjective experience of the pain was found. This calls for a more differentiated view on common spinal processing of sensory input in the generation of nociceptive withdrawal reflexes and ascending sensory information than other studies on experimental pain may suggest.

A possible spinal integration of activity in various afferents was observed in II and III. Under normal conditions, activity in C-afferents summates with activity in Aδ-afferents (II). In III, this spinal integration was extended to activity in thick myelinated afferents (Aβ) with presence of cutaneous hyperalgesia. Thus, these studies indicate that the subjective pain intensity and spinal generated NWR are both enhanced with increase in the global pain signalling inflow. In general, the results in II and III do not suggest any divergence in spinal processing of information to the ventral horn and ascending information.

In IV, ketamine (a NMDA receptor channel blocker) was used to investigate the involvement of this ion-channel in the central mechanisms involved in cutaneous hyperalgesia. The interpretation of the results in the NWR-involving tests was less apparent as the pain intensity ratings and the size of the NWR did not co-vary significantly in any of the test-conditions (though, mean values indicate an effect on both parameters). However, with presence of hyperalgesia, the potential spinal summation between Aβ- and Aδ-activity seems to involve the NMDA receptor system.

Though, there is no evidence for specific neuronal NWR pathways nor for the ascending nociceptive pathways, the most apparent model to explain the present findings is an afferent convergence onto WDR neurons (multisensorial convergence alike the FRA-system). The firing rate of these neurons decides the size of the withdrawal reflex. With normal spinal excitability, tactile activity does not in itself elicit the withdrawal reflex, perhaps except during an active movement. After tissue inflammation, the excitability of the WDR-neurons is elevated, most likely via the NMDA receptor system (IV), and the increased synaptic efficacy to Aβ-input now results in pain and large withdrawal reactions (III). Whether such a processing involves independent neuronal integration (one WDR neuron mediating sensory information and one mediating to the motorneurons) is not clear. The purpose of such an individual organisation is also not clear. However, part of our findings and the observations by the Schouenborg group may support (partly) individual spinal pathways. This divergence between pain intensity ratings and NWR size does not exclude the use of the NWR method in experimental research to elucidate *spinal* nociceptive processing.

5 DANSK SAMMENFATNING

Denne Ph.D. afhandling er baseret på 4 videnskabelige artikler, som er optrykt bagest i afhandlingen. Målet med Ph.D. studiet er at afdække spinale modulationsmekanismer under normale og hyperalgetiske forhold v.h.a. registrering af den nociceptive afværgerefleks. Derudover evalueres afværgerefleksen som et redskab i eksperimentel smerteforskning.

I forbindelse med dette Ph.D. studie er der udviklet et system til bestemmelse af afværgereflekstærsklen ved brug af feedback til den elektriske stimulator baseret på det evokerede respons. Dette system muliggør standardiseret fastlæggelse af reflekstærsklen uafhængig af forsøgslederen.

I den første artikel (I) blev betydningen af en aktiv dynamisk bevægelse på størrelsen af den nociceptive afværgerefleks undersøgt. Forsøgspersonerne udførte en cyklisk bevægelse af underbenet med en belastning på skiftevis ekstensions- og flektionsfaserne. Ved en fast ledvinkel og bevægelseshastighed stimuleredes huden på den dorsolaterale side af foden innerveret af n. suralis. Afværgereaktionen blev registreret i m. rectus femoris (RF) og m. biceps femoris (BF). Målinger under tilsvarende statiske muskelkonditioner blev tillige gennemført. Resultaterne viser en fasisk modulation af afværgeraktionerne, således at de største reflekser i RF/BF forekom, medens disse muskler var naturligt aktive i bevægelsen. D.v.s. størst refleks i RF under dynamisk ekstension og størst refleks i BF under dynamisk flektion. Reflekserne var generelt større under dynamiske forhold relativt til de statiske målinger, selvom refleksmønstrene var identiske. Forsøgspersonerne scorede desuden smerten evokeret af den elektriske stimulation. Smerteperceptionen var generelt lavere under de dynamiske forhold relativt til de statiske målinger, hvilket umiddelbart indikerer en uafhængig spinal modulation.

I artikel II blev afværgerefleksen målt fra m. tibialis anterior ved elektrisk stimulation under foden. Ved siden af den elektriske stimulationselektrode blev kraftigt lys anvendt til varmestimulation. Lyset kom fra en xenon-lampe (hvidt lys). Pilotforsøg viste, at dette lys evokerede en forsinket brændende smerteperception, når det blev påført en sortmalet hårløs hudoverflade. Det er tidligere vist, at denne karakteriske perception oprinder fra ledning i ikke-myeliniserede fibre (C-fibre). Ved systematisk at registrere afværgerefleks og smerteperception ved forskellige forsinkelser af el-stimulationen mellem 0 og 3000 ms relativ til lysstimulationen blev den spinale interaktion af disse to stimulationer undersøgt. Givet de forskellige ledningshastigheder for Aδ- og C-fibre vil synkroniseret aktivitet i disse fibre ankomme med forskellige forsinkelser til rygmarven. Både smerteperceptionen og størrelserne af afværgereflekserne viste markant kraftigere respons ved en forsinkelse på ca. ét sekund, hvilket sandsynligvis indikerer, at en summation af nociceptiv aktivitet på tværs af sensoriske modaliteter sker på rygmarven.

I tredie artikel (III) blev capsaicin (ekstrakt af chillipeber) brugt til at inducere kutan hyperalgesi. Måling af afværgerefleksens størrelse samtidig med mekaniske og termale

konditioneringsstimuli til de primære og sekundære (2°HA) hyperalgesiområder blev anvendt til at undersøge forskellige mekanismer bag 2°HA. Således var afværgerefleksen kraftigere med tilstedeværelse af spontan smerte fra området påført capsaicin, hvilket umiddelbart underbygger resultatet fra II, idet den spontane smerte sandsynligvis oprinder fra aktivitet i sensitiserede C-fibre. Svage elektriske stimuli (normalt ikke smertefulde) påført det 2°HA faciliterede afværgerefleksen samt medførte kraftigere smerteperception til reflekssstimuleringerne, hvilket kan indikere, at afferent aktivitet i Aβ- og Aδ-fibre summerer spinalt med tilstedeværelse af central hyperexcitabilitet. Denne facilitering forekom alene efter konditionering af hyperalgesien v.h.a. svag termal stimulering i primær-området, hvilket tillige indikerer, at kutan hyperalgesi afhænger af vedvarende (nociceptiv) input.

I artikel IV blev betydningen af NMDA receptoren for tilstedeværelse af central hyperexcitabilitet undersøgt ved bl.a. at gentage målingerne af de signifikante parametre i III under dobbelt-blind blokade af NMDA receptoren v.h.a. ketamin. Resultaterne viser, at potentieringen af afværgerefleksen med vedvarende taktile stimuli kunne reverseres v.h.a. ketamin og derfor (delvist) er medieret via NMDA receptoren. Ketamin havde dog ingen signifikant effekt på den tilhørende smerteperception.

Specielt i artikel I og IV var modulationen af afværgerefleksen og smerteperceptionen forskellig. I artikel I resulterede den aktive bevægelse i større reflekser, men lavere smerteintensitet relativt til målingerne under statiske forhold. Virkningen af ketamin i IV bevirkede ikke samtidige signifikante ændringer af reflekssstørrelsen og smerteintensiteten i de enkelte forsøgskonditioner. Disse afvigelser kan have metodologiske årsager, men kan også skyldes (delvis) individuel spinal modulation af sensoriske nervebaner og ventral konvergerende baner. Motorneuron eksitabiliteten blev som kontrol testet i m. soleus i forbindelse med kutan varmestimulering (xenon-lampen), capsaicin application samt administration af ketamin. Ingen af disse kontrolforsøg viste ændret motorneuron eksitabilitet.

Nærværende forsøg viser en spinal integration af afferent aktivitet, som signalerer smerte med kraftigere reflekser og smerteperception til følge. Denne integration er delvis NMDA-medieret. Discrepancen mellem smerteperception og reflekssstørrelse kan indikere en delvis uafhængig spinal modulation. Studierne viser også, at den nociceptive afværgerefleks kan anvendes i eksperimentelle studier til at undersøge spinal nociception.

6 TABLES

Table 2.1 Different quantification methods for analysing the NWR.

Group	Quantification method
I	Root-Mean-Square amplitude in a 50 ms interval after reflex onset. Subtraction of background activity.
II	Root-Mean-Square amplitude in the 50-200 ms interval
III-IV	Root-Mean-Square amplitude in the 70-200 ms interval
(Dowman 1992)	Mean amplitude of the rectified response in the 90-180 ms post-stimulus interval
(Meinck et al. 1985)	Area under the rectified reflex
(Chan and Tsang 1985)	Area under the reflex envelope (in 40-150 ms interval) of the rectified and averaged reflex measure, or a peak-peak measure.
(Chan and Dallaire 1989)	Area under the reflex envelope in 60-200 ms, though based on individual responses
(Bromm and Treede 1980)	An integrated amplitude measure (50-250 ms)
(Willer et al. 1989; Bouhassira et al. 1994)	Integrated rectified EMG (90-180 ms)

Table 4.1 Studies of hyperalgesic mechanisms using capsaicin

Intradermal capsaicin				
Research group	Capsaicin dose	2°HA punctuate/brush	Main method	Primary findings
(LaMotte et al. 1988)	100 µg	? / +	mechanical testing analgesic blockade	– allodynia and flare around cap. Allodynia persisted long after on-going pain ceased. Allodynia mediated by Aδ-fibres. No CMH/AMH receptor sensitisation to mech. st. – cooling → reduced/eliminated allodynia, re-warming brought it back. i.e. peripheral neural activity maintains allodynia – peripheral 'axon reflex' behind spread of allodynia (assessed by analgesic blocade) – CH receptors respond to capsaicin. Introduces C-chemonociceptor
(Simone et al. 1989b)	0.01 → 100 µg	? / +	pain intensity	– pain (intensity/duration) & mechanical HA: dose dependent – capsaicin evoked a flare area
(Simone et al. 1989a)	100 µg	?	single unit recording in cats	– HT/WDR responded to cap – HT/partly WDR increased response to stroking after cap – low-threshold not affected
(LaMotte et al. 1991)	100 µg	+ / +	area assessment (punctuate/stroking) analgesic blockades	– mechanical-HA temperature dependent – peripheral spread blocked by anesthetic strip → cutaneous nerves involved – existence of chemosensitive afferents postulated – evidence for central sensitisation (complete proximal nerve block → no HA) – model to explain HA
(Simone et al. 1991)	100 µg	+ / +	recording from STT in monkeys/ human psychophysical	– heat HA in 1°HA (humans). WDR sensitised to heat (monkeys) – 10/12 WDR, 4/7 HT excited by capsaicin – WDR response profile correlated with human pain profile – HT (less WDR): increased response to stroking after capsaicin – increased excitability to electrical root stimulation
(Baumann et al. 1991)	100 µg / 1% topical	?	recording from afferent fibres in monkey	– CMH/AMH responded weakly to cap, CH higher response. potential 'chemonociceptors' introduced. No receptor sensitisation – de-sensitisation around injection site – topical cap: excited CMH/AMH, sensitised CMH to heat and/or stroking
(Torebjörk et al. 1992)	100 µg	? / +	intraneural stimulation (IS) pressure cuff analgesic blockades	– C-fibres: cap pain and heat-HA, A-fibres stroking HA – proximal IS evoked pain from 2°HA at normally non-painful intensities – local anesthesia: no effect on pain from IS→central sensitisation
(LaMotte et al. 1992)	100 µg / 1% topical	+ / +	microneurography	– C-fibres: respond to cap. Profile follows pain profile – desensitisation at injection site / no receptor sensitisation – topical cap: excited CMH. sensitised to heat. 2/5 units responded to stroking evoking faint pain – cooling: decreased on-going response, abolished pain
(Serra et al. 1993)	?	+ / -	human psychophysical mechanical, heat, thermography	– no allodynia – suprathreshold mech./heat pain exaggerated. Flare and 2°HA matched → possibly C-fibre mediated
(Treede and Cole 1993)	60 µg	+ / -	subject with loss of myelinated afferents	– no allodynia. Response to punctuate stimuli enhanced after capsaicin (Aδ or C)
(Gracely et al. 1993)	?	? / +	cutaneous electrical stimulation cool stimuli	– pain at detection threshold → allodynia Aβ-mediated – cold HA in 4/7 subjects
(Serra et al. 1995)	100 µg	+ / ?	microneurography, electrical, mechanical, and heat stimulations	– lower thresholds to heat/mechanical – recruitment of 'silent' C-nociceptors

Table 4.1, continued.

Topical capsaicin				
Research group	Capsaicin dose	2°HA punctuate/brush	Main method	Primary findings
(Kenins 1982)	1%	-	single fibre recording in rats	– only CMH responded to cap
(Konietzny and Hensel 1983)	1%	?	microneurography	– some CMH increased firing to heat – lower threshold for spontaneous activity
(Culp et al. 1989)	0.006 → 6%	?	heat (thermode) mechanical (pressure algometer)	– heat and mechanical HA – dependent on cap dose – mechanical-HA temperature dependent – mediated by CMH (no effect of cuff)
(Koltzenburg et al. 1992)	1%	? / +	mechanical stimuli: pressure algometer + stroking thermal	– spontaneous pain/heat HA mediated by C-fibres – mechanical HA temperature dependent – allodynia mediated by Aβ-afferents – static pressure (1°HA) mediated by C-fibres
(Grönross and Pertovaara 1993)	1%	? / +	NWR H-reflex	– increased NWR, dependent on spontaneous activity – allodynia is dependent on skin temperature – H-reflex not affected by capsaicin
(Kilo et al. 1994)	1%	+ / +	mechanical stimuli: punctuate/ brushing/ pressure algom/mechanical impact	– punctuate & brushing HA in 2°HA – heat and pressure HA only in 1°HA – brushing HA mediated by Aβ-afferents – punctuate also mediated by C-fibres
(Arendt-Nielsen et al. 1996)	1%	+/?	argon laser (single/repetitive) electrical (single/repetitive)	– close correlation between punctuate (v-F) and laser estimated 2°HA – faciltation of the central temporal summation mechanism

Abbreviations: + present, - not present, ? not assessed

Table 4.2 Ketamine administration as a potential NMDA antagonist

Group	Model, design	Ketamine dose	Type of stimuli	Measures	Effect of ketamine
Basic research					
(Kitahata et al. 1973)	cats, ket	2.5 mg/kg i.v.	mechanical pinch	single unit, lamina I-VI	spontaneous + evoked activity suppressed in lamina I/V
(Lodge and Anis 1984)	cats, ket/alphaxalone+alphadolone /methohexitone/diisopropylphenol	2.5-10 mg/kg i.v.	electrical stimuli	monosynaptic reflex polysynaptic reflex	suppression of polysynaptic reflexes
(Collins 1986)	cats, ket	→20 mg/kg i.v.	noxious (pinch, heat) non-noxious (brush, air puff, von-Frey, brushing, rubbing, squeezing, cooling, joint rotation, muscle pressure)	single-unit recording (low threshold, WDR, and proprioceptive)	-WDR: suppression of noxious evoked activity. Non-noxious not changed -low threshold neuron: not modulated -proprioceptive neurons: varied effect
(Davies and Lodge 1987)	rats, ket/kynurenate	25mM, ionophoretic, 2-4 mg/kg i.v.	electrically evoked wind-up	WDR single-unit	abolished wind-up
(Headley et al. 1987)	rats/cats, ket/PDA	50mM, electrophoretic →20 mg/kg i.v.	noxious (electric, pinch, heat) non-noxious (tactile)	single unit. Both dorsal/ventral horn	DH: no effect VH: only effect on nociceptive stimuli
(Haley et al. 1990)	rats, ket/MK801/AP5/DGG	1-8 mg/kg (3 min) i.v.	formalin-model	WDR single-unit	inhibited 2nd formalin response, dose-dependently
(Hartell and Headley 1991)	rats, different degree of surgery, ket/alphadolone+alphaxalone /alpha-chlorase	0.5-16 mg/kg	noxious pinch	NWR	surgery enhanced NWR excitability ketamine depressed CH
(Schaible et al. 1991)	cats, inflammation ket/AP5	50mM ionophoretical, 1-8 mg/kg i.v.	kaolin/carrageenan → inflammation mechanical pressure	single-unit with afferent from knee	suppressed increased spontaneous activity + evoked response
(Ren et al. 1992)	rats, with inflammation ket/AP5/CPP/kynurenate/CNQX/DAMGO/DPDPE	161µg i.t. (cumulative dose)	carrageenan injection → inflam. noxious thermal	hindpaw withdrawal latency	attenuation of behavioral (heat) HA no motor disturbance
(Sher et al. 1992)	rats, ket/APV/ morphine/pethidine	1-5 mg/kg intra-periton	ischemic pain (tail cuff) rotarod (motor function)	tail flick latency	no post-ischemic HA, dose dependent no motor dysfunction
(Mao et al. 1993)	rats, mononeuropathy, ket/dextrorphan	12.5-100 nmol i.t.	radiant heat	foot withdrawal latency behavior (spontaneous pain)	attenuated heat HA + spontaneous pain
(Nagasaka et al. 1993)	cats, ket	0.5-10 mg/kg i.v.	bradykinin (femoral artery)	WDR single-unit	suppressed excited firing, dose dependent
(Song and Zhao 1993)	cats, ket/APV/ kynurenate	50mM electrophoretic	cutaneous nociception (electrical, thermal) muscular nociception (electrical, bradykinin inj. in gas/sol artery)	WDR single-unit lamina I, IV-VI	cutaneous nociception inhibited (>50%) less effect on muscular nociception (electrical: 25% reduction in <20% of neurons, BK 30% reduction in 2/6 neurons)
(Carter 1994)	mice, ket/dextrorphan/ various competitive antagonists	variable	200 mg/kg NMDA (lethality test), rotarod test for NMDA-antag effect on motor control	inhibiting death dose, motor disturbance dose	strong correlation between lethal inhibiting and motor dysfunction dose

Table 4.2, continued.

Group	Model, design	Ketamine dose	Type of stimuli	Measures	Effect of ketamine
Human research					
(Islas 1985)	post-operative (n=50)	4 mg epidural		pain relief category scale (0-3)	• pain relief • side effects: No
(Ravat et al. 1987)	post operative (n=10), ket/morphine	4-6 mg epidural, repeated bolus infusion if pain relieved		pain relief, category scale (0-5)	• only relieved pain in 1/10 • side effects: No
(Maurset et al. 1989)	experimental (n=6), post operative (n=6), pla/naloxone X ket/pethidine	0.3 mg/kg (2 min) i.v.	ischemic pain	VAS	• "effective analgesic" • Side effect: dizziness, floating sensation dysperception, less visual acuity
(Øye et al. 1992)	experimental (n=6), (R)-ket/(S)-ket/pla in vitro binding to human brain	(R)-ket 0.2→0.8 mg/kg (S)-ket 0.05-0.2 mg/kg (2 min) iv.	ischemic pain	VAS different side effects binding to PCP-site	• (S)-ket 4 times as potent as (R)-ket at PCP-site • "reduced pain perception". Same potency difference • side effects: auditory/visual/proprioceptive disturbances. Reduced ability to recall objects seen after infusion
(Stannard and Porter 1993)	phantom limb pain (n=3)	2 pt. X 0.2 mg/kg/h s.c. 1 pt. 0.125 mg/kg/h s.c.		qualitative pain relief	• pain relief, 1 pt stopped (due to uncomfortable feeling) • side effects: No (dose adjusted to no s.e.)
(Eide et al. 1994)	Post Herpetic Neuralgia (n=8) ket/morphine/pla	0.15 mg/kg i.v.	tactile brush, von-Frey (single/repetitive) thermal	VAS thresholds	• abolished wind-up like pain and allodynia, no threshold changes • side effects: discomfort/less visual acuity (marked in 3 subjects)
(Backonja et al. 1994)	neuropathic pain (n=6) ket/pla	0.25 mg/kg (5 min) (two pt → 16 mg/kg)	light brush pin-prick	on-going pain HA/allodynia/after sensation	• effect on evoked pain + after sensation, less on on-going pain • psychotomimetic side effects
(Tverskoy et al. 1994)	post operative, pre-emptive test (n=9), ket/fentanyl/pla	anesthesia: 2 mg/kg + 20 µg/kg/min i.v.	pressure algometry in wound	VAS: -spontaneous pain -movement assoc. pain -evoked pain	• No effect on spont. + movement pain. decreased wound HA
(Warncke et al. 1994)	experimental HA (n=12), ket/morphine/pla	0.15 mg/kg i.v.	thermal injury → HA mechanical (von-Frey/brush) thermal (heat/cold)	wind-up HA areas thresholds	• reduced 2°HA area, -8/12 no wind-up in 2°HA, -no threshold changes • side effects: ?
(Eide et al. 1995)	Post Herpetic Neuralgia (n=5), ket	0.05→0.15 mg/kg/h s.c. continuously	von-Frey filament (continuously at 3/s) brush	VAS wind-up like pain allodynic area	• less continuos pain, both wind-up like and allodynic area reduced • side effects: nausea, fatigue, dizziness (intolerable)
(Park et al. 1995)	experimental HA, ket/alfentanil/pla	0.07 mg/kg + 0.57 mg/kg/h i.v. (+ additional bolus)	capsaicin (250µg i.d.) → HA brush (gauze pad) and safety pin (pin-prick)	on-going pain allodynia and pin-prick areas	• reduced on-going pain (49±7%), reduced pin-prick/allodynic areas • side effects: sensation of mild intoxication + psychotomimetic side effects in 2 subjects

Table 4.2, continued.

(Arendt-Nielsen et al. 1995)	experimental (n=12), ket/pla	0.5 mg/kg (3 min) + 9µg/kg/min i.v.	argon laser pressure algometry electrical (single + repetitive)	psychophysical thresholds NWR	• inhibits temporal summation, hypoalgetic (high int. stimuli) • side effects: 11 had hallucinations
(Max et al. 1995)	posttraumatic pain with allodynia (n=8). ket/alfentanil/pla	0.75 mg/kg/h (total 58±5 mg over 2 hours)	brush (allodynia test) on-going pain	VAS	• relief of pain + allodynia • but associated with side effects
(Mathiesen et al. 1995)	orofacial pain. acute (n=16), chronic (n=7), (R)-ket/(S)-ket/ket (no pla)	acute: ket 0.45 mg/kg i.m. (R)-ket 0.8 mg/kg (S)-ket 1.8 mg/kg chronic: variable		VAS	• all 3 ketamine forms relieved postoperative pain. 3 chronic pt experienced pain relief while 5 (pain > 3 years) did not • side effects: similar for all types (blurred vision, altered hearing, dizziness etc.) dreams/hallucinations: only racemic ket (3/7)
(Felsby et al. 1996)	neuropathic pain (n=10), ket/Mg-chloride/placebo	0.2mg/kg (10 min) + 0.3 mg/kg/h i.v.	thermal (cold/heat) cotton swap (allodynia), von-Frey (sensibility) temporal summation (mechanical)	VAS spontaneous pain thresholds allodynic area	• reduction in spontaneous pain and allodynic area • psychotomimetic side effects in 7/10 subjects
(Nikolajsen et al. 1996)	phantom limb pain (n=11), ket/pla	0.1 mg/kg (5 min) + 7µg/kg/min i.v.	mechanical: pressure + repetitive tapping Thermal	VAS McGill Pain Questionnaire reaction time	• lower stump/phantom limb pain, increased pressure pain thresholds, less “wind-up” like pain • side effects (7/10): insobriety, discomfort

Abbreviations: pla-placebo, ket-ketamine, HA-hyperalgesia, CH-central hyperexcitability, DH-dorsal horn, VH-ventral horn

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