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Plasticity in mice nociceptive spinal circuits

– role of cell adhesion molecules

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Lund, Sweden 2005

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Original papers

The thesis is based on the work in the following papers which will be referred to by roman numerals.

- I.** Thelin J and Schouenborg J. Disturbed nociceptive processing in mice with deficient hippocampal LTP. (*Submitted to NeuroReport*)
- II.** Thelin J, Waldenstrom A, Bartsch U, Schachner M, Schouenborg J. Heat nociception is severely reduced in a mutant mouse deficient for the L1 adhesion molecule. (2003) *Brain Res* 965: 75-82.
- III.** Thelin J and Schouenborg J. Intrasegmental transmission of nociceptive input in normal and L1 deficient mice (*Manuscript*)

Abbreviations

I	Paper I
II	Paper II
III	Paper III
AEP	A-fibre Evoked field Potentials
CEP	C-fibre Evoked field Potentials
EMG	ElectroMyoGraphic
IB4	Isolectin B4
m.	musculus
NCAM	Neural Cell Adhesion Molecule
NMDA	N-methyl-D-aspartate
NWR	Nociceptive Withdrawal Reflex
LTD	Long Term-Depression
LTP	Long Term-Potential
STP	Short Term-Potential
T	Threshold for A β -fibre evoked field potential
Th	Thoracic
TRPV1	vanilloid receptor 1
TRPV2	vanilloid receptor 2
WDR	Wide Dynamic Range neurons
WGA-HRP	Wheat Germ Agglutinin-HorseRadish Peroxidase conjugate

Summary

Introduction: To understand the function of the genes and their products in the pain system, studies will have to deal with complex issues related to intercellular communication, e.g. plasticity in neuronal networks. To provide a basis for such studies, the present thesis compares basic features of the nociceptive spinal systems including the organization of nociceptive withdrawal reflexes (NWR), laminar organization of the nociceptive C-fibre input to the spinal cord and plastic mechanisms in the mouse and rat. On this basis, the role of adhesion molecules, in particular L1 adhesion molecules, in the nociceptive system is analyzed for the first time by using mutated mice.

Results: It is confirmed that sensorimotor transformations performed by the NWR circuits abide the same principles as in the rat, at least for two of the wild-type mouse strains tested. This finding indicates that mice NWR has a modular organization as previously demonstrated in the rat. Interestingly, mouse strains with a deficit in LTP mechanisms also exhibit a deficient sensorimotor transformation, suggesting that LTP mechanisms are involved in the developmental mechanisms that fine-tune the NWR. Furthermore, basic features such as nociceptive C-fibre evoked field potentials and response characteristics like short term potentiation in deep dorsal horn neurones appear to be very similar in mouse and rat. By contrast, marked differences were found in the properties of nociceptive transmission in the superficial laminae. In particular, apparently normal wild type mice seem to lack both short and long term potentiation in the first order synapses, mechanisms that are powerful in the rat. These findings suggest that the current view on the locus of the central sensitization mechanisms needs to be reconsidered.

In the second part of the thesis, the role of the cell adhesion molecule L1 in the pain system was studied in mutated mice.

Interestingly, these animals were found to be almost analgesic. This hypoalgesia is not due to a general lack of nociceptive input to the spinal cord as evidenced by a normal termination pattern of C fibres and C fibre evoked potentials in the superficial laminae in L1 deficient mice. Instead, a selective defect in the nociceptive transmission to the deeper laminae of the dorsal horn and a markedly reduced wind-up in the WDR neurones were found.

Conclusions: The present thesis demonstrates that there are important differences in plastic mechanisms in the spinal nociceptive pathways in the mouse and rat. In addition, it points to a key role of adhesion molecules in pain transmission.

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General introduction

The pain system has for a long time been supposed to be a genetically ‘hard wired’ structure. This notion is presumably due to the fact that newborn animals and humans exhibit pain related motor reactions already at birth and that there are very few noxious stimuli during early development (Fitzgerald and Jennings, 1999). Over the recent years it has become increasingly clear, however, that experience dependent mechanisms are involved in the functional sculpturing of this system during development and in setting the excitability and gain in the nociceptive pathways in adults (Schouenborg, 2003). Still, the cellular mechanisms underlying plasticity in the nociceptive system is not well known. In fact a myriad of molecular and genetic mechanisms have a putative role in this plasticity (Waxman et al., 1999; Levenson and Sweatt, 2005).

Since the complete map of the mouse genome was determined in 2002, there has been an explosion in genetically modified mice, thus providing an unprecedented arsenal of methods to study the role of different genes in nociception. Unfortunately, a clarification of the function of genes in nociception will rely on time consuming experiments in physiologically well defined neuronal networks. A complicating factor in this context is that the main network studies have been made in rats, cats and monkeys and comparatively few studies have been made in mice. Despite striking similarities in body anatomy, the mouse is not a small rat. In fact, there is a gap of 10 million years of evolution between the species and many species differences are known today. Therefore, information from the rat should not be directly translated to mouse (Wilson and Mogil, 2001). A factor further complicating the issue is that the nociceptive system is composed of many parallel subsystems with only partially understood functions. In this thesis, the

focus is on plastic properties in spinal nociceptive networks in the mouse and their relation to those in the rat.

I. Functional organization of nociceptive networks in the spinal cord

The spinal cord and the trigeminal system receive and process all nociceptive input from the body. After processing, the information is distributed to various spinal sensorimotor circuits and numerous sites in the brain. Below a short account for the spinal circuits will be given.

Anatomy

The termination of different kinds of somatosensory primary afferents in the dorsal horn is segregated such that nociceptive fibres synapse in the superficial laminae I-II and V, tactile fibres in laminae III-IV and proprioceptive fibres in laminae V-VI and the ventral horn (Light and Perl, 1979; Levinsson et al., 2002). Several types of thin unmyelinated afferents exist that are more or less specialized to a certain type of noxious stimuli and that have different properties in terms of which types of chemoreceptors are present on their membrane. A group of nociceptive afferent fibres express TRPV1 and TRPV2 receptors for capsaicin, whereas others have chemoreceptors for e.g. mustard oil (Guo et al., 1999). Presumably these subgroups mediate different types of sensations and to some extent they will also give rise to different motor reactions. For example, high threshold mechano-heat C-fibres provide a strong input to withdrawal reflex networks (see below), whereas histamine sensitive chemoreceptors mediating the itch sensation are likely to have access to scratch reflex circuits instead (Schmelz et al., 1997).

Nociceptive neurones are often classified into two major groups: Nociceptive specific neurones; that respond only to nociceptive input, and wide dynamic range neurones, which respond to both innocuous

and noxious inputs. The latter group is often referred to as wide dynamic range neurones (WDR). Many of the WDR neurones in the deep laminae of the dorsal horn appear to be involved in sensorimotor transmission (Schouenborg et al., 1995), although a role in the sensory aspects of pain cannot be ruled out.

Few studies have been made on the physiology of dorsal horn neurones in mice (Weng et al., 2001; Suzuki et al., 2003; Martin et al., 2004). Species differences between mouse and rat can be expected, as the organization of the superficial dorsal horn in some respects differs substantially in these species. For example, lamina II occupy a much larger portion of the dorsal horn in the mice than in the rat (Woodbury et al., 2000).

On the modular organization of the spinal cord

A major function of the spinal cord is to use somatosensory input for rapid corrections of ongoing movements. This requires that information about the anatomical and mechanical properties of the body is represented in the neuronal networks. Studies on sensorimotor transformation in the nociceptive withdrawal reflex (NWR) system in the rat and cat has revealed such a body representation. In these species, NWR is now known to have a modular organization, each module essentially controls a single muscle and receives a sensory input from a characteristic receptive field (Schouenborg and Kalliomaki, 1990; Levinsson et al., 1999).

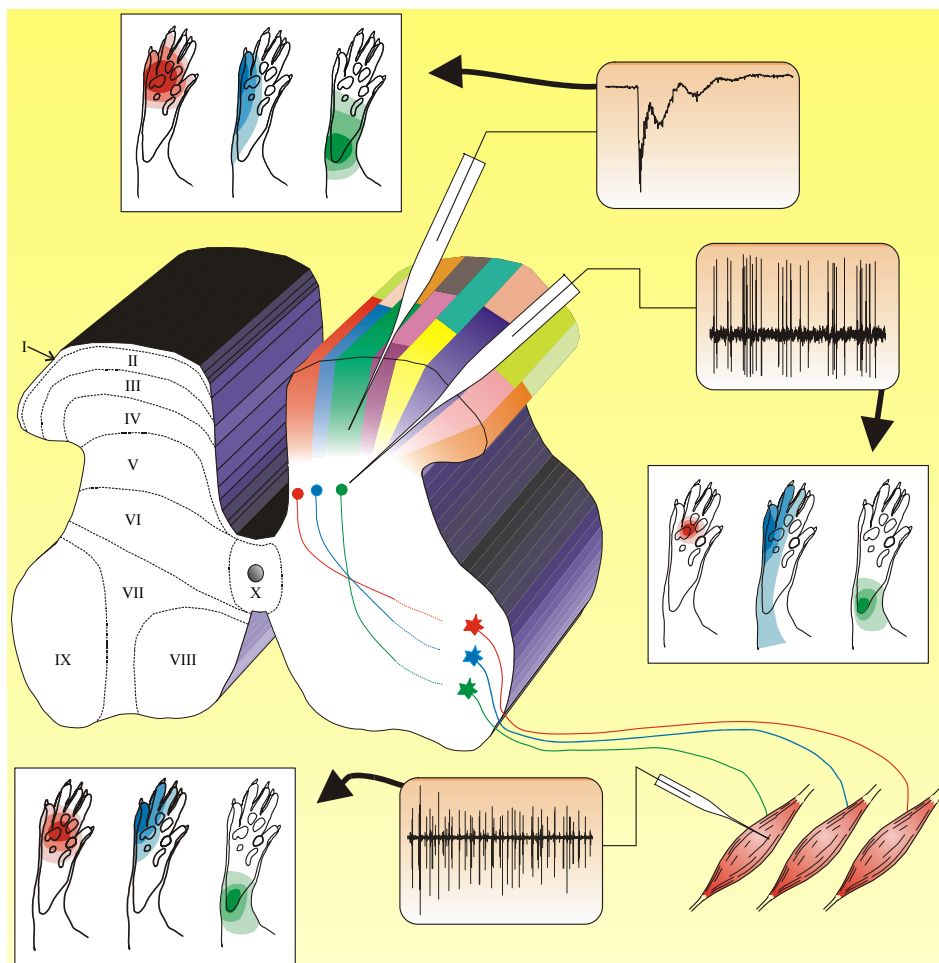


Figure 1. A schematic over three NWR modules in the spinal cord. Receptive fields recorded at different sites are illustrated by schematics of hind paws. Top, field potential, middle, extra cellular single cell recordings and bottom, EMG recording. Note the similarities between the receptive fields at the various recording sites. Rexed laminae are indicated by roman numbers.

The strength of the connections between skin receptors within the receptive field and the modules is proportional to the withdrawal efficacy of the muscle. Hence, the withdrawal movement pattern is, in a sense, imprinted on the neuronal networks of the NWR (Schouenborg and Weng, 1994).

Also the topographic organization of the primary afferent input to the dorsal horn appears to be related to the information processing in reflex modules. Rather than primarily being a representation of the body, cigar shaped zones in the dorsal horn of the lower lumbar cord receive a convergence pattern from the skin that is very similar to that of an individual module (Levinsson et al., 2002). The 'body representation' in the spinal cord thus appear to be fragmented into a number of modules, each of which process information related to a particular function. Hence, a traditionally somatotopic organization does not appear to exist in the spinal cord. An important practical consequence of this is that the topographical organization of the cutaneous input to the spinal cord can be assessed by mapping the receptive fields of individual reflex modules (see also Fig. 1).

II. Plasticity in the spinal cord

In the adult, there are numerous examples of plasticity in the nociceptive system in the spinal cord. Phenomena like wind-up and long term-potential (LTP) are believed to be part of the mechanisms behind changes in pain sensation such as, sensitisation, hyperalgesia and allodynia (Cervero and Laird, 1996; Cervero et al., 2003).

LTP/LTD

In the adult animal, nociceptive spinal connections exhibit short term-potential (STP) (termed 'wind-up', frequency potentiation or central sensitization) and long term-potential and long term-depression (LTD) depending on the intensity of the afferent input. *In vivo* LTP,

lasting several hours has been demonstrated in deep dorsal horn neurones after severe noxious stimulation (Svendsen et al., 1998) and in superficially located neurones after intense stimulation of C-fibres (100Hz) (Liu and Sandkuhler, 1997). LTP of C-fibre evoked response in the superficial laminae is assumed to be part of a pain memory (Ikeda et al., 2003).

Less intense stimulation protocols like C-fibre stimulation at 1 Hz instead cause short lasting (3-15 minutes) potentiation of C-fibre synapses in the superficial laminae and "wind-up" in dorsal horns neurones (Mendell, 1966), notably the deeply located WDR neurones. The physiological meaning of wind-up is still under debate and it now appears that wind-up is not equivalent to central sensitisation or hyperalgesia (Woolf, 1996). Wind-up does, however, share common mechanisms with these phenomena making wind-up useful as model for sensitization (Li et al., 1999). While these mechanisms are likely to contribute to the acute responses to injury, it may also be that they are involved in more long lasting changes in nociceptive pathways that occur during chronic pain condition.

Somatosensory imprinting

The reflex system in the spinal cord has, for a long time, been assumed to be innate and genetically 'hard-wired', i.e. not requiring experience. Several recent findings indicate, however, that this notion is wrong. Firstly, the withdrawal movement pattern of single muscles is 'imprinted' on the NWR during development. Secondly, the NWR can adapt to both altered peripheral innervation and altered movement patterns (Holmberg and Schouenborg, 1996; Holmberg et al., 1997). Thirdly, anaesthetics applied to the skin during a critical time window abolishes the adaptation (Waldenstrom et al., 2003b). Recently, it became clear that the adaptation of the NWR depends on tactile feedback on spontaneous movements, thus solving the puzzle of how

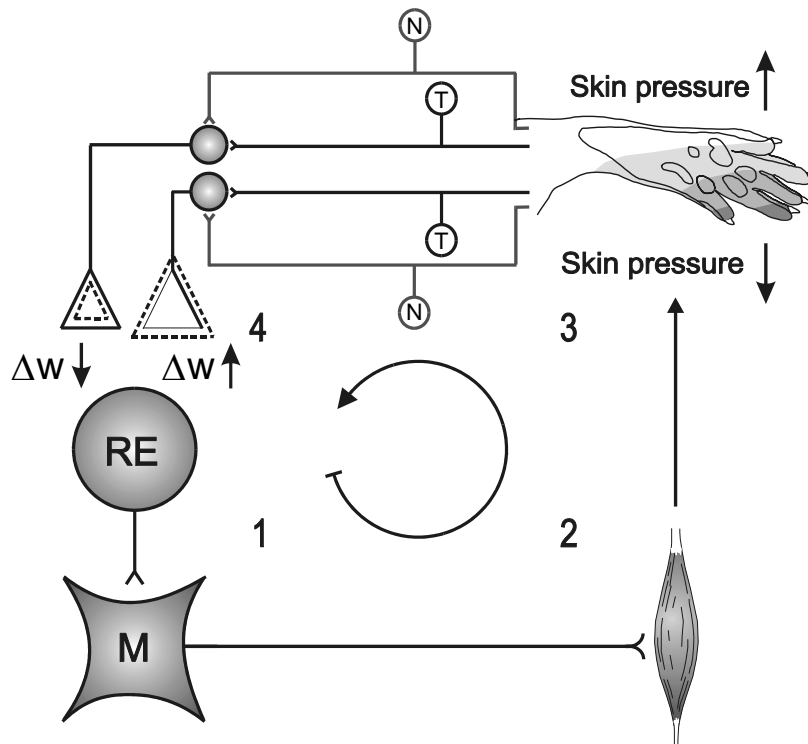


Figure 2. The proposed self-organizing network that uses tactile information related to withdrawal efficiency to adjust the strengths of nociceptive connections. One learning cycle (indicated by circular arrow) consists of the following chain of events: (1) spontaneous bursts in REs. (2) Motoneuron (M) activation leading to muscle contraction (3) Increased or decreased skin pressure. Schematic of receptive field of hind paw. (T) represent afferents from skin low-threshold mechanoreceptor and (N) symbolize nociceptive input. (4) The strengths of erroneous connections are weakened and that of appropriate ones are strengthened. ΔW symbolise change of synaptic strength. Note that although the nociceptive input is not required for the learning to take place in this model, nociceptive input, if present, would indeed cause learning effects. Modified from Waldenstrom et al, 2003b.

a pain related system can be learned despite a relative absence of noxious stimuli during development (Fig. 2) (Pettersson et al., 2003; Waldenstrom et al., 2003b).

The cellular mechanisms behind the somatosensory imprinting are not known. The functional tuning of the NWRs elicited from the tail takes place between day 12 and 21 after birth (Waldenstrom et al., 2003a). This time period coincides with several changes in the expression of glutamate receptors and ion channels. For example, the expression of NMDA receptor NR2B subunits decreases while that of NR2A subunits increases (Brown et al., 2002), thus altering the kinetic properties of the NMDA receptors (Cull-Candy et al., 2001). Moreover, calcium channels are expressed differently over time (Morisset and Nagy, 1999). Such channels may play an important role in e.g.

the induction phase of the self-organizing networks, see Fig 2. Since somatosensory imprinting results in a stable network in the adult, it may be that the consolidation phase involves structural changes. Such changes are known to occur in for example filial imprinting (Horn, 2004).

III. Role of cell adhesion molecules in nociception

The formation of memory, on a cellular level, is based on the facilitation/depression of transmission at specific synapses. Over time, memory is thought to be transformed from an initially short lived and labile phase into a longer lasting stable form, a process called consolidation. Short term memory depends on preexisting molecules at the synapses such as receptors, enzymes and transmitters (Kandel, 2001). The long term

memory, on the other hand, depends on synthesis of proteins and structural modifications at the synaptic level (Bailey et al., 1996). The presence of adhesion molecules in or near the synaptic cleft in the adult raises the possibility that they participate in maintaining synaptic changes. Traditionally, cell adhesion molecules have been recognized as important during ontogenesis of the nervous system, but also in the adult nervous system undergoing regeneration after lesions. Interestingly, recent papers report that the expression of adhesion molecules is altered in animals that have memorized objects (Skibo et al., 1998; Fox et al., 2000), and interference with adhesion molecule function modifies memory function (Wolfer et al., 1998; Stork et al., 2000). Several adhesion molecules have been shown, *in vitro* and *in vivo*, to be involved directly or indirectly in synaptic plasticity (for reviews see (Schachner, 1997; Murase and Schuman, 1999). One adhesion molecule participating in synaptic plasticity is the neural recognition molecule L1 (Luthl et al., 1994). L1 is a transmembrane glycoprotein of the immunoglobulin (Ig) superfamily (Moos et al., 1988). The protein serves important functions in the developing and adult nervous systems, and is involved in many processes such as nerve cell migration, axonal outgrowth and fasciculation, neuronal survival, axonal regeneration, and synaptic plasticity. Research on the role of cell adhesion molecules in spinal nociceptive networks is mainly focused on regeneration and development. Almost nothing is known on their role in pain systems. (Dahme et al., 1997)

Aims

The general aim of this thesis is to provide a basis for studies of gene functions in the pain system. Specifically, this thesis aims to;

- develop techniques for neural network studies in genetically modified mice
- obtain a comparison of basic features of the nociceptive spinal systems in mouse and rat, including the organization of nociceptive withdrawal reflexes, the laminar organization of the nociceptive C-fibre input and plastic mechanisms
- clarify the role of adhesion molecules, in particular the L1 adhesion molecule, in the nociceptive system, by using mutated mice

Material and Methods

Animals used

Four different strains of wild type mice NMRI, C57BL/6, 129S6/SvEvTac and DBA/2 and mouse strains deficient in the L1-adhesion molecule based on the C57BL/6 and 129S6/SvEv strains were used. Approval for the experiments was obtained in advance from the Malmö/Lund ethical committee on animal experiments.

Anaesthesia

The animals were anaesthetized with halothane/isoflurane (0.9 - 2.0%), in a mixture of 65% nitrous oxide and 35% oxygen, and were ventilated artificially via a tracheal cannula. Local infiltration of 2.0mg/ml lidocaine (Xylocaine) with 1.2µg/ml adrenaline was used to reduce nociceptive input during surgery.

Some animals were subsequently decerebrated by removing the brain rostral to the inferior colliculus and a spinal transection was then made at the thoracic (Th) segments Th 10-11 using a pair of fine scissors. The halothane/ isoflurane anaesthesia was then discontinued.

Laminectomy and nerve preparation

To record neuronal activity, a laminectomy of the vertebrae T13, L1, and L2 was made. Mechanical stabilization of the preparation was accomplished by clamping the spinal processes of the vertebrae rostral and caudal to the laminectomy. The dura was removed, and the exposed spinal cord was covered by agar (2% w/v) in saline. The right sciatic nerve was dissected free for bipolar electrical stimulation with silver electrodes and was then covered by paraffin oil.

Stimulation protocols

Noxious thermal stimulation

To determine the thermal reflex threshold for a skin area, a CO₂-laser (Directed

Energy Inc., Irvine, CA) was used. This method allows a precise temporal and spatial stimulation of cutaneous nociceptive Aδ and C afferent fibres in the epidermis. The animals were accustomed to the experimental environment for about two hours before the behavioural tests. The skin temperature was measured before the threshold tests $29.6 \pm 1.3^{\circ}\text{C}$ (measured with a Thermonitor C-1600M infrared detecting probe, Linear Laboratories, Los Altos, CA). The threshold was defined as the stimulation duration that elicited a withdrawal response in at least three out of five tests (unfocused beam, diameter 2 mm, intensity 10 W, pulse duration 8–45 ms). The duration of the laser pulses was increased in steps of 2 ms until the reflex threshold was reached. The time interval between the stimulation pulses was at least 10 seconds. To avoid overt tissue damage, the maximal pulse duration used to determine the reflex threshold was 45 ms. To elicit a robust reflex response (in studies of reflex movement direction), the duration of the laser stimulation was increased by 5 ms from threshold. Laser stimulation did not elicit vocalization or avoidance behaviour other than the withdrawal reflexes in the stimulated limb.

Mechanical tactile stimulation

The mechanical reflex thresholds of different body parts of the animals were assessed with calibrated Semmes-Weinstein nylon monofilaments (usually referred to as von Frey monofilaments) (Monofilaments set (20), North Coast Medical, Inc. Stauffer Boulevard San Jose, Ca.). The monofilaments were tested in a sequence from weak to strong until a reflex response was elicited. The reflex threshold was defined as the weakest force eliciting a response in at least three of five tests.

Nerve stimulation

The sciatic nerve was stimulated with a rectangular pulse of 1 ms duration. The stimulation strength was expressed as a

multiple of the threshold strength (T) for stimulation of the largest myelinated afferent fibres as determined by the A β -fibre evoked field potential in the dorsal horn. The A β fibre threshold was checked repeatedly during the experiments.

Data sampling

Electromyography recordings

For EMG recordings, a small opening was made in the skin overlying the muscle belly, and a reference electrode was inserted in an adjacent skin flap. Fine steel needle electrodes, insulated except for about 80 μ m at the tip were used for EMG recordings. The recording electrodes were inserted into the mid-region of each muscle belly. The identity of the muscles was determined by observing the movements evoked by cathodal pulses (100 Hz, 200 ms, 20 μ A, 20 pulses), delivered via the EMG electrodes (Schouenborg and Kalliomaki, 1990). Generally, the EMG activity in three hind limb muscles was recorded simultaneously in each experiment (m. peroneus longus, tibialis anterior and semitendinosus).

Field potential mappings

The early component of local C-fibre evoked field potentials (CEP) in substantia gelatinosa has been shown to be mainly due to currents in first order synapses (Schouenborg, 1984). Thus, to obtain information on the functional projection of primary afferents, extra cellular field potentials evoked by electrical stimulation to the sciatic nerve were studied. Glass microelectrodes filled with 3 M sodium chloride (4-8 M Ω at 1 kHz) were used for recordings of field potentials. Data were collected using computer software "Signal" and stored on hard disk (Cambridge Electronic Design Limited). The field potentials were recorded throughout the dorsal horn starting at a depth of 700 μ m (corresponding to Laminae VI in the mice).

Single cell recordings

Receptive fields of single cells in laminae V were recorded with glass microelectrodes filled with 3 M sodium chloride (7-12 M Ω at 1 kHz). The receptive fields of neurons receiving both nociceptive and tactile input (wide dynamic range (WDR) type) were established with brushing and pinching the hind paw. The number of neuronal spikes evoked by sciatic stimulation was counted.

Frequency potentiation, LTP and wind-up protocols

To elicit frequency potentiation of the CEP, 100 stimulations at 0.2-2 Hz at 100-200T were used, then 3 stimulations every 10s and 7 stimulations every 30s to study recovery. To elicit LTP of CEP, an intense stimulation was given (100Hz at 100T, 0.5ms duration, 400 stimuli given in 4 trains of 1s duration at 10s intervals) (Liu and Sandkuhler, 1997). For wind up of C-fibre evoked responses in dorsal horn neurones, 20 stimulations at 1Hz at 100T were given.

Transganglionic labelling experiments

For transganglionic labelling experiments, three L1-deficient and three control mice were used. One male of each genotype was labelled and processed simultaneously in each experiment. During anaesthesia induced by halothane, 0.5 μ l wheat germ agglutinin-horseradish peroxidase conjugate (WGA-HRP, 2%; Sigma-Aldrich Sweden AB Solkraftsvagen Stockholm Sweden) was injected into the heel, into digit 3 on one hind paw, and into one of the central pads on the contralateral hind paw of each mouse. Approximately 36 hours after the injection of WGA-HRP, the mice were deeply anaesthetized with sodium pentobarbital and killed by transcardial perfusion. After rinsing with phosphate-buffered saline, the mice spinal cords were fixed with 1.25% glutaraldehyde and 1% paraformaldehyde.

The isolated lumbar spinal cord segments were cut parasagittally at 40 μm on a freezing microtome (Leica CM 1325, Leica Microsystems, Nussloch, Germany). All sections of each animal were saved and processed and mounted on slides. All sections through laminae I–IV that showed transganglionic labelling were photographed at a magnification of 40 \times . Prints at a final magnification of about 60 \times were prepared and used to construct dorsal-view maps of the labelling. Blood vessels and other structures were used to align adjacent sections. For the reconstruction of dorsal-view maps from serial horizontal sections, the medial border of the dorsal horn was placed at a fixed distance from the midline.

Analysis

NWR movements

The reflex responses on CO₂ laser stimulation were documented with a digital video camera (50 pictures/s, JVC GR-DVL 9600) for an analysis of movement direction. The video recordings were analyzed frame by frame, and the reflex movements were characterized as either correct (away from the stimulus) or erroneous (towards the stimulus).

Topographical representation of receptive fields

To map the receptive fields of neurones we used a system termed “Receptive Field Imaging” (Pettersson et al., 2001). Key features of this computerized system are a random stimulation of 16 sites and an averaging procedure that calculates the strength of the input from each of the stimulated sites. The sampling frequency was 10kHz/channel, and a 12-bit voltage resolution of the total assigned voltage span.

For each muscle in each mouse, the response magnitude on stimulation (counted as total number of motor unit spikes during 0-900 ms after onset of laser pulse) were normalized and expressed as

percentage of the maximal response. A mean of five stimulations was then calculated for each muscle in each mouse. From these mean values, an average receptive field, divided into three areas of differing sensitivity: Maximal sensitivity (70-100% of maximum), medium sensitivity (30-70% of maximum) and low sensitivity (<30% of maximum), was constructed. The areas of differing sensitivity were delimited with the aid of computer generated isoresponse lines (Kriging algorithm and contour program, Surfer 6.01 from Golden software Inc. 809 14th street, Golden, Colorado 80402-1866).

Statistical analysis

The Spearman's correlation coefficient was used as a measure of similarity between the NWRs receptive fields. The mean correlation values from five consecutive maps in each animal and muscle was determined. Comparisons of C-fibre latency between different mouse strains and verification of foci in the NWRs receptive fields were done with one-way ANOVA and Bonferroni post-hoc test. For comparison of reflex thresholds, the median and percentile values are given, since some reflex thresholds exceeded the predetermined maximal stimulation duration (45 ms). A Mann-Whitney U-test was used for statistical evaluation.

A paired student's t-test was used when comparing wind-up between L1 deficient mice and their littermates. Significant differences were assumed at the level of $p < 0.05$.

Results and comments

The spinal nociceptive system in the mouse – comparative aspects

The starting point for this thesis was to provide a basis for further studies on genetic and molecular mechanisms underlying learning in the pain system. As the genetic arsenal of techniques has been developed on mice we first set track on comparing the spinal cord in rat and mouse with respect to sensorimotor transformation in the NWR system. It is known that the mouse and the rat are in some respects different with regard to nociceptive input to the dorsal horn of the spinal cord (Guo et al., 1999; Zwick et al., 2002). To obtain an overall view of the processing of nociceptive information in mouse spinal cord, we mapped the receptive fields of the reflex networks in different strains, A- and C-fibre evoked field potentials and wind-up properties of nociceptive dorsal horn neurones. These properties were then compared with those in the rat.

Modular organization

Comparing the spatial organization of connection strengths within receptive fields in the NMRI and C57BL/6 mouse strain (mouse strains with normal LTP) and rats, a strong correlation for all the muscles examined was found (Fig. 3), indicating that the NWRs in these two species are organized in the same way. Since the hind limb anatomy, and thereby withdrawal movement patterns on contraction in single muscles, is very similar for mouse and rat, it follows that the sensitivity distribution in individual reflex networks is also an imprint of the withdrawal movement pattern in mice. These results support the notion from studies in rats and cats, that the modular organization of the spinal cord is a general principle in mammals.

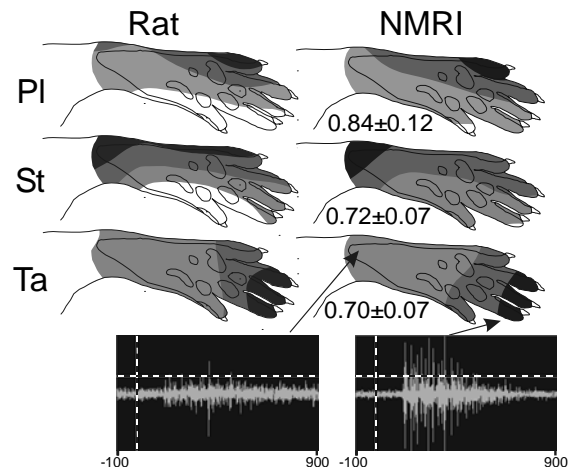


Figure 3. Comparison of the receptive fields of NWRs in rat and NMRI mouse for three hind limb muscles, peroneus longus (Pl), semitendinosus (St) and tibialis anterior (Ta), each map is an average of 5 mappings. For each muscle, responses on stimulation were normalized and expressed as percentage of the maximal response. Sensitivity differences are presented in different shades of grey, black indicates maximal sensitivity. The mean \pm SD of the Spearman correlation, rat compared to NMRI mice, for receptive fields are presented. Below, arrows indicate EMG recordings from the Ta muscle on stimulation of the focus and a peripheral site within its receptive field. The sweeps are one second long, the horizontal dashed line indicates the threshold for counting spikes and the vertical dashed line shows stimulation onset.

Field potentials

A map of A β -fibre and C-fibre evoked field potentials was characterized in mice of the outbred NMRI strain as this mice show a 'normal' NWR organization. A short latency field potential (onset 2ms) was evoked in the dorsal horn on stimulation of A β -fibres with a focus in laminae III-IV. In addition, a long latency field potential (onset 50ms \pm 10ms) could be evoked when stimulation strength was above 30T \pm 10 (Fig. 4). This potential increase with stimulation strength up to about 100T (Fig. 4A). Moreover, it has maximum amplitude at depths corresponding to laminae I-II and laminae IV-VI (Fig. 4B). Onset latency, thresholds and depth profile indicate that these

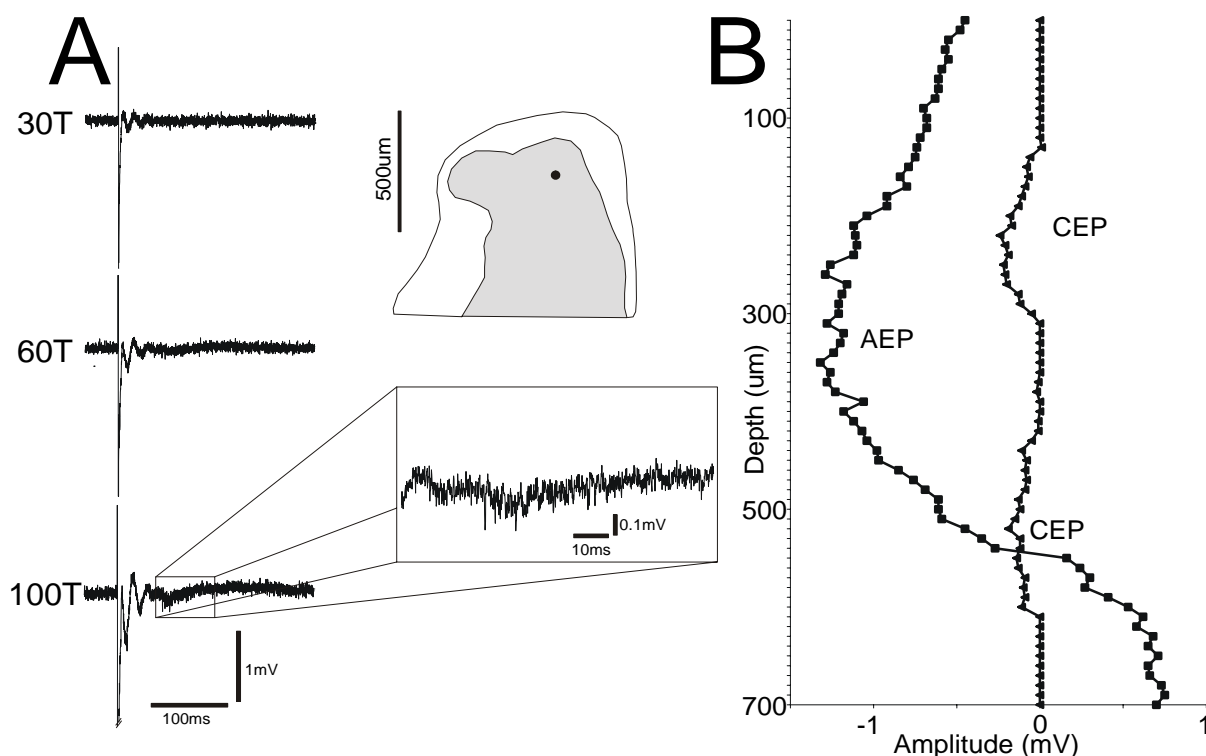


Figure 4. **A.** Example of field a potential recorded in laminae II at different stimulation strength. T is the threshold strength needed to elicit an A β fibre potential. Note the neurone discharges superimposed on the CEP. The schematic spinal cord indicates recording site.

B. Depth profile of AEP (■) and CEP (◆) in the dorsal horn in the NMRI strain.

potentials are directly equivalent to A β - and C-fibre evoked field potentials elicited in rats (Schouenborg, 1984). The short and long latency potential is henceforth denoted A β -fibre evoked field potential (AEP) and C-fibre evoked field potential (CEP).

Plastic mechanisms

Surprisingly, repetitive stimulation at 100T (0.1-2Hz, 100 stimulations) failed to potentiate the CEP in laminae I-II in mice. In the rat, such stimulation always results in a marked increase (100-200%) in amplitude of the CEP (Schouenborg, 1984). Moreover, intense C-fibre stimulation (100-200T, 100Hz, 100 stimulations), that elicits LTP in the rat (Sandkuhler et al., 1997), also failed to potentiate the CEP in NMRI mice. Control experiments were made in decerebrated and spinalized animals since it is known that isoflurane and/or descending inhibition from supraspinal structures can affect LTP and wind-up in the spinal cord (Hillman and Wall, 1969; Schouenborg

and Dickenson, 1985). Still, neither frequency potentiation nor LTP could be demonstrated suggesting fundamental differences in the superficial parts of the dorsal horn between the species.

Neural responses

WDR neurons in laminae IV-VI with an input from both A- and C-fibres were studied. Receptive field and response characteristics seemed virtually identical to those previously found in rats (Schouenborg and Sjolund, 1983; Schouenborg et al., 1995). As in rats, mice WDR neurons display a clear wind-up on repetitive stimulation at C-fibre strength (100T, 20 stimuli, 1Hz), mean increase 350% as compared to starting levels ($p < 0.01$).

Conclusion

While the overall organization of nociceptive C-fibre activated systems in the spinal cord are similar in mouse and rat, there appear to be fundamental species differences with regard to the plastic

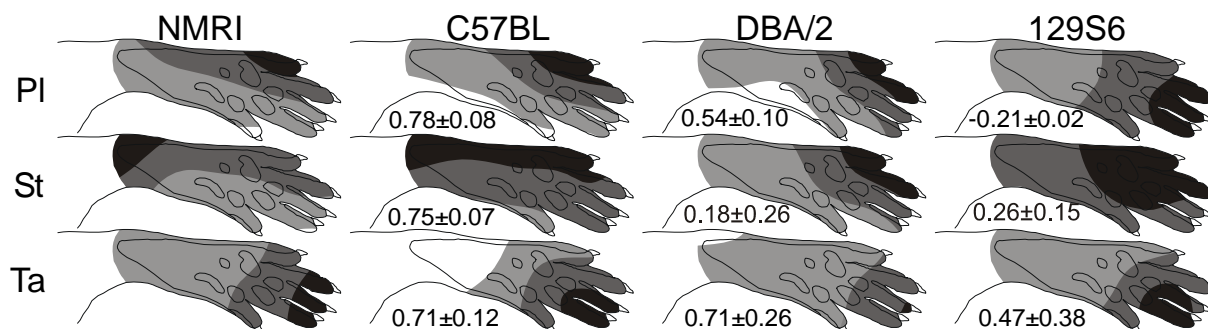


Figure 5. Comparison of NWRs receptive fields in four different strains of mice in three hind limb muscles, peroneus longus (Pl), semitendinosus (St) and tibialis anterior (Ta). Each map is a mean of five maps recorded in one mouse. The mean \pm SD of Spearman correlation, NMRI compared to C57Bl/6, DBA/2 and 129S6/SvEvTac (129S6) is indicated for each muscle and mouse strain. The mean values from five consecutive mappings in each muscle were used for correlation analysis.

mechanisms in the superficial laminae of the dorsal horn.

Differences in mouse strains somatosensory imprinting with regard to defects in LTP

To further clarify the types of learning mechanisms involved in somatosensory imprinting we also studied receptive fields in mice with a deficient LTP. We used four different “wild-type” strains of mice: two strains (NMRI and C57BL/6) with a normal LTP in hippocampus and a normal spatial learning in various mazes (Klapdor and van der staay, 1996; Vicens et al., 1999), and two strains (DBA/2 and 129S6/SvEvTac) which exhibit poor results in behavioural tasks (spatial learning) (Crawley et al., 1997; Gerlai, 2002) and impaired LTP in the hippocampus (Nguyen et al., 2000a). It is known that DBA/2 mice exhibit a normal induction but poor maintenance of LTP in

the hippocampus (Nguyen et al., 2000b). The 129S6/SvEv strain is deficient in both induction and maintenance of LTP in the hippocampus (Gerlai, 2002), probably partly due to a defect NMDA receptor (Kolesnikov et al., 1998). The spatial organization of NWR receptive fields, thresholds, reflex latency and overall reflex gain were determined in individual reflex networks (NWRs in m. peroneus longus, tibialis anterior and semitendinosus). The results show that the mouse strains studied with deficient LTP; DBA/2 and 129S6/SvEvTac mice, exhibit much more variable receptive fields than mice of the NMRI strain (Fig. 5). For example, the foci of maximal sensitivity do not always correspond to the foci of maximal withdrawal as in the NMRI mice.

It may also be of interest to note that the DBA/2 strain exhibit less disturbed NWR receptive fields than the 129S6/SvEvTac strain. It can be speculated that this difference is related to the different

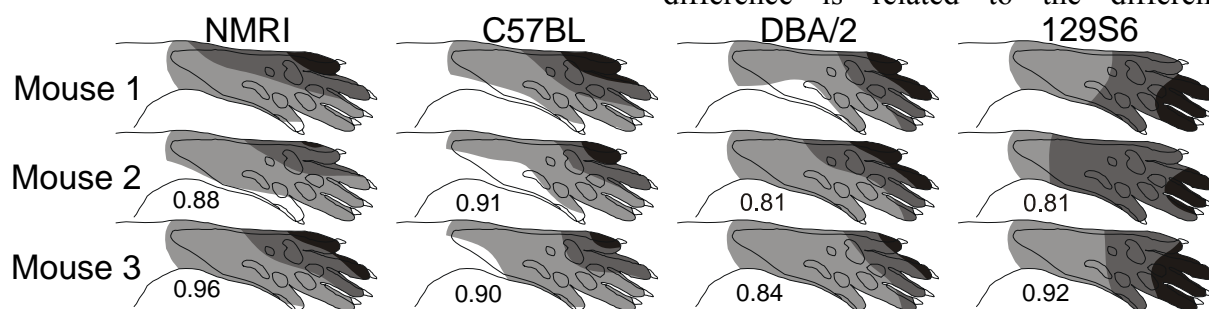


Figure 6. Comparison of receptive fields in individual mice of the same strain for m. peroneus longus. Each map is based on data from five consecutive mappings. The Spearman correlation ranged between $r=0,81-0,96$ for the strains examined.

severity of the LTP defect.

To further clarify the nature of the deficient somatosensory imprinting in LTP deficient mice, the reflex thresholds amplitude and onset latency in peroneus longus were analyzed in the four different mouse strains. We found no significant difference in NWR thresholds amplitude (one-way ANOVA) or in onset C-fibre latency between respective foci and “normal” focus on digit 5, indicating that there are no differences in the composition of the primary afferents eliciting NWR or in the general sensitivity in the networks eliciting NWR response.

From an analysis of the variability in response amplitudes we could also rule out the possibility that the deficient somatosensory imprinting is an artefact due to a greater variation in excitability in the reflex networks, which could have blurred the mapping. Moreover, the averaged receptive field in each animal had the same spatial organization for all

animals in each strain, suggesting a uniformity in the altered spatial organization of the spinal reflex networks (Fig. 6). In conclusion, there appears to be a selective defect in the somatosensory imprinting mechanism in the LTP deficient mouse strains.

Cell adhesion molecule L1 role in the nociceptive system

In view of the stability of receptive fields of NWRs, once they have been established, it is conceivable that structural mechanisms are involved in the consolidation of somatosensory imprinting. One group of molecules known to play an important role in the consolidation phase of LTP are the cell adhesion molecules (Solomon et al., 1998; Merino et al., 2000). Little is known about the role of these molecules in nociception.

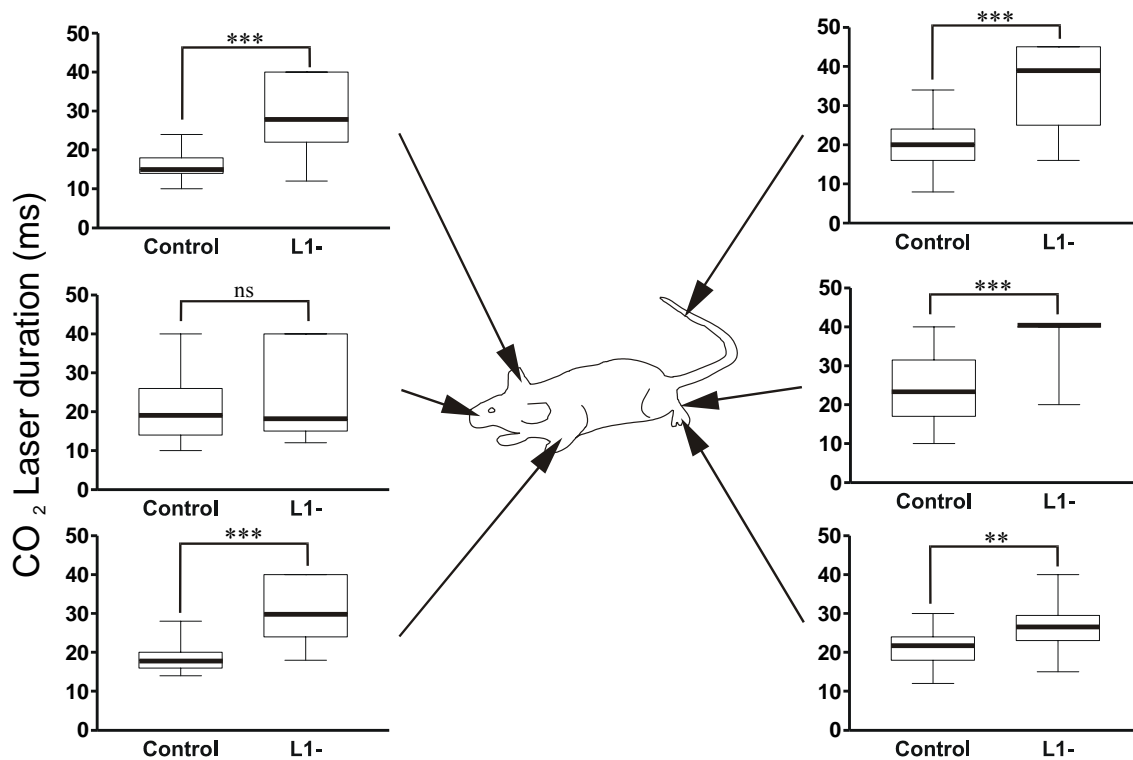


Figure 7. Nociceptive thresholds in different body regions. Median value, 25th and 75th percentiles, and range of data are shown by a thick line, box, and bars, respectively. Note the high thresholds for the L1-deficient mice on all body parts, except the nose. ** $p < 0.01$; *** $p < 0.001$

Reflex studies

In a preceding paper by (Dahme et al., 1997), defects in mechanoreception were noted in mice deficient in the cell adhesion molecule L1, but it was not clear whether these defects included the pain system. For example, the nociceptive reflex responses studied had extremely long latencies (>20s) and their thresholds were not determined.

We therefore studied NWR in such animals and characterized their thresholds and receptive fields with nociceptive CO₂ laser pulses. Moreover, possible topographical differences in nociceptive sensitivity were analyzed. It was found that the nociceptive reflex threshold in L1-deficient mice is dramatically increased in all body parts, with the exception of the nose (Fig. 7).

The mechanical thresholds tended to be increased in all body parts, except the tail but were clearly in the innocuous range (von Frey hair, mean 10mN) indicating that these reflexes are elicited by low threshold mechanoreceptors.

Notably, there was only a weak correlation between the change in nociceptive and tactile thresholds ($r = 0.39$). Skin areas with marked hypoalgesia such as the tail, exhibited normal tactile reflex thresholds and vice versa. Thus, the increased heat reflex thresholds do not appear to be due to a general insensitivity in spinal reflex pathways. To determine if L1 deficient mice also exhibit defects in somatosensory imprinting, the direction of the reflex movements elicited by nociceptive heat stimulation was investigated for the hind limb and tail. In both wild-type and L1-deficient mice, stimulation of the distal plantar digits elicited a dorsiflexion of the digits and the ankle, whereas heel stimuli evoked plantar flexion of the ankle. In both groups, the tail flick response was always directed away from the laser. Hence, it seems that the somatosensory imprinting is not abolished and that mechanisms that control the gain in the nociceptive system

are selectively affected in the L1 deficient mice.

Somatotopic organization of the spinal cord in L1-deficient mice

To clarify whether the behavioural deviations observed were due to abnormalities in the afferent termination in the spinal cord or to an altered central processing we investigated the projection pattern of thin cutaneous fibres in the spinal cord of L1-deficient mice. WGA-HRP was injected into three sites in the hind paw skin (digit 3, lateral central pad, and heel) in three L1-deficient and three control mice. Dense foci of labelling in laminae 1-2 were found in L2–L5 in animals of both genotypes. (Fig. 8).

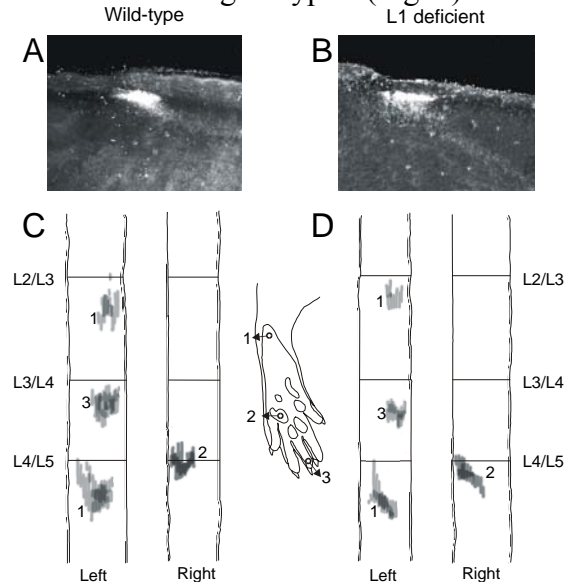


Figure 8. Left, microscopic images (60 \times) showing sagittal sections of the spinal cord with WGA-HRP staining. Right, reconstruction of the spinal cord, horizontal view. Injection sites are marked in paw schematics.

Areas of spinal cord labelling had essentially the same rostrocaudal location. As evident in Fig. 8, there was no marked difference in topography or extent of termination between L1-deficient and control mice, indicating that the thin fibre projection from the foot is not markedly affected by the L1 mutation. This conclusion is in line with the findings of (Itoh et al., 2004).

Network analysis

Having determined that the hypoalgesia in L1 deficient mice is not likely to be due to a reduction of nociceptive primary afferents we carried on with an investigation on a cellular level in the spinal cord networks. The aim of the third study in this thesis was to pin point the locus in the spinal cord of the deficiency in the nociceptive pathways in L1 deficient mice. Clarifying this may prove to be valuable for finding the critical steps in the normal nociceptive processing that ultimately lead to pain.

To this end, A β - and C-fibre evoked synaptic currents and neuronal discharges were recorded in superficial and deep dorsal horn in adult L1 deficient mice, littermates and normal mice of the NMRI strain. Frequency dependent potentiation and LTP of C-fibre evoked potentials in substantia gelatinosa and wind-up in wide dynamic range neurons in the spinal cord, respectively, were studied.

Field potentials

As shown in Fig. 9, the AEP in the dorsal horn has a normal depth profile in the L1 deficient mice.

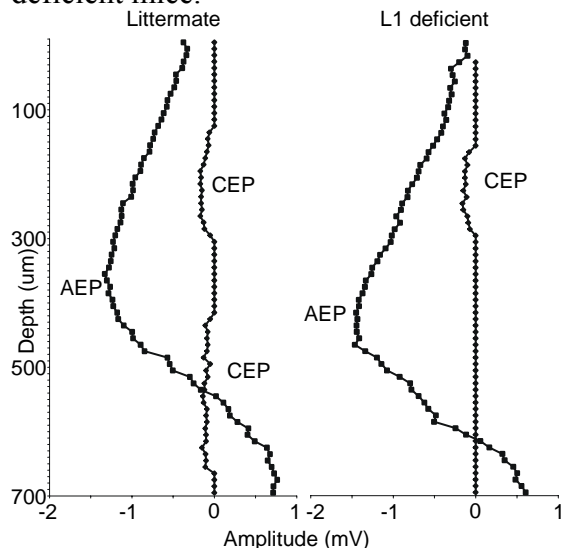


Figure 9. Depth profile of the field potentials in the dorsal horn in littermates and L1 deficient mice, from a depth of 700 μ m to the surface. Amplitude of potentials is indicated by ■ for AEP and ◆ for CEP. Note that CEP is lacking in deep dorsal horn in the L1 deficient mice

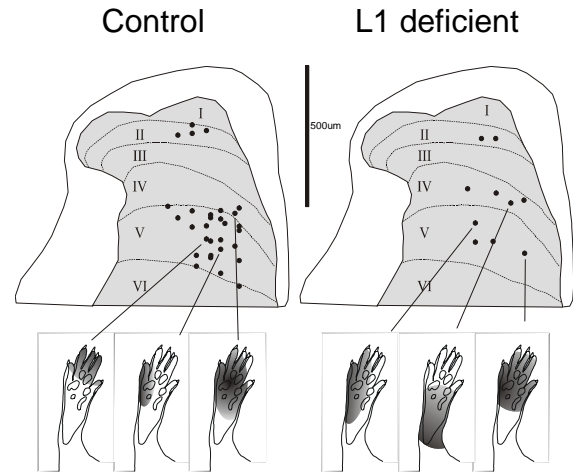


Figure 10. Recorded cells are plotted above. Below, typical receptive fields of nociceptive mechanical input to cells found in the deep part of the dorsal horn.

By contrast, CEP was only elicited in the superficial laminae in the L1 deficient mice. No trace of a CEP was found in the deep laminae. In laminae I-II at a depth of 120-220 μ m we found the same CEP with respect to latency and amplitude in the L1 deficient mice and its littermates. The L1 deficient mice exhibited the same lack of wind up of the CEP in laminae I-II as found in the control material. Furthermore, C-fibre evoked neural spikes were often superimposed on the CEP, indicating that the synapses between C-fibres and dorsal horn neurons are functional in L1 deficient mice.

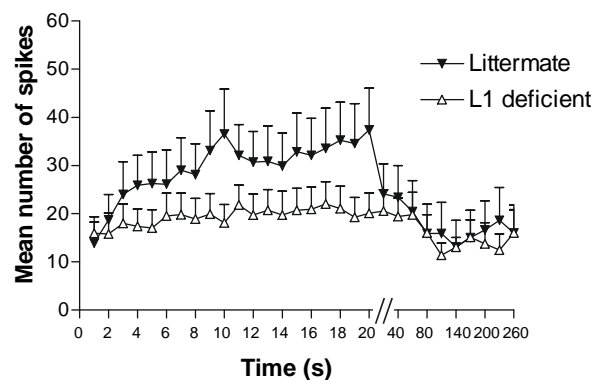


Figure 11. Mean number of neural spikes during wind-up protocol. The littermates (n=7) are indicated with ▼ and the L1 deficient animals (n=8) are indicated by Δ. Bars indicating SD values.

Neural responses in L1 deficient mice

Despite extensive searching for neurones in seven L1 deficient mice we only found 8 cells in the deep part of the dorsal horn that exhibited C-fibre elicited neural spikes as compared to 24 cells in the same number of control animals (Fig. 10). These 8 cells failed to exhibit a significant wind up (Fig. 11). Thus, L1 deficient mice appear to have a strikingly reduced number of WDR cells and a reduced wind-up as compared to controls.

Taken together, the normal termination pattern and normal CEP in the superficial laminae and lack of a CEP and WDR wind-up in the deep parts of the dorsal horn, suggest a selective defect in the nociceptive C-fibre connections with deeply located neurons in the L1 deficient mice. This defect is likely to underlie the hypoalgesia in these animals.

General discussion

Much of the current knowledge in molecular biology and genetics is based on mouse studies (Winder and Schramm, 2001; Bucan and Abel, 2002). To understand the physiological function of the genes and their products, the analysis has, however, to be extended to a cellular and network level. Future studies will for example have to deal with the complex issues of the role of different genes in cell to cell communication, e.g. plasticity in neuronal networks. As mentioned in Introduction much of the available information on physiological principles and mechanisms have been obtained through studies in the rat. It is therefore important that comparative studies are carried out on the system physiology of the mouse. The present thesis compares basic features of the nociceptive spinal systems in the mouse and rat and demonstrates that, despite expected overall similarities, such as their modular organization, there are also significant differences concerning plastic mechanisms. Moreover, this thesis addresses the role of adhesion molecules in the nociceptive system for the first time. These findings and the future perspective are discussed below.

Pain physiology in the superficial dorsal horn in mouse

Few studies have directly addressed the possible differences in the nociceptive spinal networks between the rat and mouse (Wilson and Mogil, 2001). It is known, though, that there are differences with regard to TRPV1-receptors on nociceptive afferents in the two species (Woodbury et al., 2004) and substantia gelatinosa is much larger in the mouse than in the rat (Woodbury et al., 2000). It was also recently shown that neurons in the superficial part of the mouse spinal cord exhibit somewhat different membrane and firing properties as compared to the rat

(Graham et al., 2004). Hence, there appears to be important species differences already at the very first steps in the nociceptive pathways. Moreover, the sensitivity of the opiate receptors appears to differ; the mouse exhibit a greater sensitivity towards ligands for both μ and δ -receptors (Yoburn et al., 1991), indicating differences in endogenous pain control systems as well. The functional importance of these differences is not known, however.

The work in this thesis, confirms that the withdrawal reflex system in the mice has a modular organization by demonstrating that the sensorimotor transformation performed by these circuits abide to the same principles as in the rat (Schouenborg and Kalliomaki, 1990). Since this transformation is attained through a learning mechanism involving spontaneous movements in the rat (Petersson et al, 2003) it appears likely that the developmental learning mechanisms underlying the functional tuning of the mouse and rat spinal nociceptive system are similar in principle. This does not, however, imply that the molecular mechanisms underlying the plasticity are identical. This caution appears to be particularly obvious in the case of wind-up and sensitization as we, in sharp contrast to the situation in the rat (see Schouenborg, 1984) found no evidence for frequency potentiation or LTP of C-fibre transmission in the mouse superficial dorsal horn. This is a remarkable finding in view of that a 'normal' wind-up was found in deeply located WDR neurons on repetitive stimulation of C-fibres. LTP in the first order nociceptive C-fibre synapses has been considered to be important for several clinical phenomena related to enhanced pain transmission (often referred to as "central sensitization") such as hyperalgesia and allodynia (Sandkuhler, 2000; Ji et al., 2003; Cervero et al., 2003). To our knowledge differences regarding sensitization between mouse and rat have not previously been reported. Sensitization

of mice nociceptive system appears to be as powerful as in the rat. It may thus be that the mouse exhibits a different locus and/or different mechanisms of central sensitisation compared to the rat. Alternatively, and equally possible, is that LTP in the superficial part of the dorsal horn is not necessary for the wind-up and sensitization of the deeply located WDR neurones, a possibility that would be consistent with the striking difference in time course of frequency potentiation in monosynaptic C-fibre evoked potentials in substantia gelatinosa and neuronal wind-up in the rat (Schouenborg, 1984).

Not much is known that could explain the lack of frequency potentiation and LTP in the nociceptive C-fibre input to substantia gelatinosa in the mouse. A recent report on comparative differences in the populations of C-fibres in mouse and rat may be relevant to this issue, however. From rat studies, it is known that polymodal nociceptive C-fibres are responsible for the initial component of the C-fibre evoked field potential in the substantia gelatinosa, that show frequency potentiation (Schouenborg, 1984). A large group of the polymodal nociceptive fibres consist of the IB-4 positive afferents (Bennett et al., 1998) and 65-75% of these express the capsaicin receptor TRPV1 in the rat (Guo et al., 1999). In contrast, only 2-3% of C-fibres are both TRPV1 and IB4 positive in the mouse (Woodbury et al., 2004). Interestingly, the TRPV1 receptors on the presynaptic terminals have been shown to be activated under physiological conditions. These receptors enhance the Ca^{2+} influx during arrival of action potentials and may thereby boost transmitter release (Ahern et al., 2005). Since it is likely that frequency potentiation relies, at least partly, on presynaptic mechanisms, (Schouenborg, 1984) it may be speculated that the relative lack of TRPV1 receptors in the mouse is related to the absence of frequency potentiation in the first order synapses. Whether the lack of LTP in C-fibre

synapses in substantia gelatinosa can be explained in the same way is uncertain as there are clear evidence for a postsynaptic mechanism underlying LTP in the rat (Liu and Sandkuhler, 1995; Liu and Sandkuhler, 1997).

Differences between mouse strains in learning mechanisms

Not only are there clear examples of differences in fundamental mechanisms of plasticity between mouse and rat, differences in plasticity have also been noted for a number of different wild-type mouse strains (Crawley et al., 1997). Different mouse strains exhibit differences in their capacity for learning and memory (Nguyen et al., 2000a). The present thesis shows that exploring such differences may shed light also on the plastic mechanisms underlying the developmental tuning of the pain system. It was found that the mouse strain (129S6/SvEvTac strain) exhibiting defects in both induction and consolidation of LTP tended to have a less well organised NWR system as compared to the mouse strain (DBA/2) that exhibit a normal induction phase but a defect in the consolidation of LTP (Nguyen et al., 2000b; Gerlai, 2002). These results may suggest that molecular mechanisms underlying LTP, e.g. NMDA related mechanisms, are also involved in somatosensory imprinting.

It has been reported that the 129S6/SvEvTac strain exhibit defects in the NMDA receptor (Kolesnikov et al., 1998). It is well known that NMDA receptors are important for tuning the spinal cord during development (Baba et al., 2000; Bardoni, 2001). For example, the termination pattern of tactile fibres is disturbed by NMDA antagonists if given during the first two to three weeks after birth in the rat (Beggs et al., 2002). In addition, recent findings indicate that NMDA receptors determine gain within the receptive fields of NWR in the adult (Petersson et al., 2004) and in ascending nociceptive pathways to cortex

(Kalliomaki et al., 2003). Taken together, it is likely that NMDA receptors are involved in spinal somatosensory imprinting.

Role of the cell adhesion molecules in the nociceptive system

Adhesion molecules are known to play important roles in plasticity in hippocampus (for reviews see (Schachner, 1997; Murase and Schuman, 1999). However, almost nothing is known about the physiological role of these molecules in nociception. Given that the functional adaptation of the pain system during development depends on learning mechanisms, and that these learning mechanisms result in very stable connections, a role of adhesion molecules is conceivable. In a pilot study on mice deficient in either the adhesion molecules L1, NCAM or Tenascin-C we found a disturbance in the nociceptive system only in L1 deficient mice (Thelin, Waldenström & Schouenborg, unpublished data). In the L1 deficient mice, the nociceptive threshold was strikingly increased rendering these animals almost analgesic with exception of regions innervated by the trigeminal nerves. From the analysis (II, III), it is clear that the hypoalgesia in these animals is not due to a general lack of nociceptive input to the spinal cord. Both the termination patterns of afferent fibres in the superficial laminae and C-fibre evoked potentials are normal in L1 deficient mice. Instead, the C-fibre input to the deeper laminae appears to be weak as evidenced by a lack of CEP and a markedly reduced wind-up in the WDR neurones. A reduced nociceptive input to the deeper laminae is consistent with the observed lack of withdrawal reflexes on nociceptive stimulation as these layers contain last order reflex interneurons (Schouenborg, 2003). Further analysis is required to determine if the deficient nociceptive input to the deep laminae is also associated with a lack of pain

perception, for example through the spinothalamic tract.

It may be worth noting that despite marked hypoalgesia, somatosensory imprinting is not abolished in the L1 deficient mice. This apparent paradox might be explained by the recent finding that somatosensory imprinting does not require a nociceptive input but is dependent on tactile feedback (Pettersson et al., 2003; Waldenström et al., 2003). Since tactile sensitivity is still present in the L1 deficient mice, this mechanism could operate normally.

Importantly, from both the studies on L1 deficient mice (II, III) and the one on different mouse strains (I), it appears that the gain and threshold can be selectively reduced despite a normal somatosensory imprinting and vice versa. Hence, mechanisms underlying somatosensory imprinting do not seem to be the same as those underlying the overall gain in the pain system. One testable possibility would be that the differential input strength caused by somatosensory imprinting in the sensorimotor circuits is dependent on LTP like mechanisms in the dendritic spines in the reflex encoders involving e.g. NMDA receptors, while the mechanisms setting the overall gain involve ion-channel mechanisms close to the soma of these neurons.

Future perspectives

Unravelling the synaptic memory mechanisms that determine the strength of the nociceptive transmission in the nociceptive system is a major task in pain research and will undoubtedly result in new and highly specific targets for analgesics. To bridge the knowledge in molecular biology and system physiology through comparative studies is important and necessary in this respect. The present thesis, by comparing basic features of the nociceptive system in rat and mouse, demonstrates striking differences in plastic mechanisms between these two species and also between mouse strains. Such

differences provide clues to the mechanisms underlying sensitization and somatosensory imprinting in the nociceptive pathways and can be further analyzed by e.g. gene knock-out/ knock-in mice.

The role of cell adhesion molecules in the nociceptive system is a novel area of research. Our finding that a deficient L1 adhesion molecule results in profound analgesia points towards an important role of these molecules in the development of nociceptive systems and in the gain of the threshold for pain. Moreover, these findings also suggest new targets for development of analgesic drugs in the future. Further studies in the L1 deficient mice, might also clarify the roles of superficial and deeply located nociceptive neurones in pain perception, which has been a long standing issue in pain research (Ikeda et al., 2003; Almeida et al., 2004).

Svensk sammanfattning

Medan akut smärta ofta kan bemästras på ett framgångsrikt sätt saknas ofta adekvat behandling av kroniska smärttillstånd. Smärta är ett dominerande symptom vid en mängd sjukdomar såsom till exempel, reumatoid artrit, cancer, migrän, ryggsador, fibromyalgi och tillgängliga analgetika är ofta otillräckliga. Det finns därför ett stort behov att utveckla nya och effektiva analgetika. Ett stort problem har varit bristen på adekvata djurmodeller i arbetet med att utveckla nya läkemedel. En förutsättning för en sådan utveckling är i sin tur att smärtsystemets organisation och kanske i synnerhet dess inlärningsmekanismer, är känd hos de djur som testas.

Ett sätt att angripa problemet med smärta är att undersöka de gener som är aktiva under uppbyggnaden av ryggmärgens nätverk. För några år sedan kartlades alla musens gener och parallellt med denna kartläggning har massor av olika möss som är genetiskt modifierade tagits fram. Tyvärr har musen, pga. sin lilla storlek, inte använts särskilt ofta i fysiologiska studier. De flesta studierna på smärtsystemets funktioner har i stället gjorts på andra djurarter som råtta, katt och apa. Det skiljer dock över 10 miljoner år av evolution mellan t.ex. mus och råtta och det går därför inte att direkt överföra data om t.ex. nervkretsars organisation och minnesfunktioner mellan dessa djurslag. Det finns således ett behov av en kartläggning av musens smärtsystem för att kunna undersöka genernas funktion i t.ex. nervkretsars inlärningsförmåga. En stor del av denna avhandling har därför ägnats åt att beskriva musens smärtsystem i ryggmärgen, både med avseende på dess grundstruktur och dess inlärningsförmåga.

Vår kropp är full med ”smärtreceptorer” om är specialiserade på olika typer av skadliga stimuli. Dessa receptorer förmedlar information genom perifera

nerver till ryggmärgen. I ryggmärgen sker den första sorteringen av stimuli och smärta separeras i ryggmärgen för att snabbt kunna skydda individen från skada. Ryggmärgen har nätverk av nervceller som kan utföra snabba precisa rörelser som tar bort den del av kroppen som har utsatts för smärta. Detta fenomen kallas för smärtutlöst bortdragningsreflex och styrs av ryggmärgen. Smärtsignalerna skickas vidare från ryggmärg till strukturer i hjärnan och hjärnstam. Samtidigt står nätverken i ryggmärgen under kontroll från hjärna. Ryggmärgen har således en nyckelroll i smärtsystemet och det är därför av största vikt att i detalj förstå dess organisation och funktion för att kunna utveckla nya metoder för att lindra smärta.

Denna avhandling syftar till att öka förståelsen för hur nätverken i ryggmärgen fungerar och vad som krävs för att de ska utvecklas normalt. Vi har bland annat använt den smärtutlösta bortdragningsreflexen som modell system. Smärtsystemets basorganisation hos mus undersöktes också genom att kartlägga musens signalbearbetning i ryggmärgens smärtsystem med mikroelektroder. Medan den fundamentala organisation hos bortdragningsreflexen och nervcellsegenskaper i ryggmärgens bakhorn visade sig vara densamma hos mus och råtta fann vi klara skillnader när det gäller inlärningsmekanismer. Från studier på råtta har man tidigare dragit slutsatsen att sensitisering i smärtbanorna efter skada beror på ett ”smärtminne” i första omkopplingen mellan smärtreceptorer och ryggmärg. Musen verkar helt sakna samma mekanism men uppvisar ändå sensitisering. Detta talar för att den tidigare uppfattningen om var ”smärtminnet” sitter inte är korrekt. Avhandlingen visar också att det förekommer stora skillnader mellan olika musstammar när det gäller inlärningsförmåga i smärtsystemet. Möss som är dåliga på rumsinläring på grund av bristande inlärningsmekanismer i

hippocampus, visar sig ha försämrade förmågan att lära sig att utföra en funktionell bortdragning från ett smärtsamt stimuli. Det verkar därför som om det finns gemensamma mekanismer för att lära sig att orientera i en labyrint och att lära sig en funktionell bortdragnings reflex.

Avhandlingen har också syftat till att, mot denna bakgrundsinformation om musens smärtsystem, karakterisera betydelsen av de så kallade adhesionsmolekylerna för smärtsystemets inläring. Det är känt att dessa molekyler är viktiga för anläggning av nervsystemet och även för inläring hos vuxna. De har till uppgift att leda de olika nervbanorna rätt när de växer ut samt att stabilisera synapskontakterna mellan nervceller. En av dessa molekyler heter L1 och den har bland annat visat sig vara inblandad i inlärningsmekanismer. Vi har därför undersökt en musstam med en bristfällig L1 molekyl och jämfört med ”normala” möss. Intressant nog visade sig dessa möss ha en extremt hög smärttröskel. Genom en detaljerad analys kunde vi fastslå att orsaken till smärtfriheten ligger i att en viss grupp av smärtaktiverade nervceller inte aktiveras på ett normalt sätt av smärtsignalerna. I synnerhet uppvisar dessa nervceller inte en förstärkningsmekanism som kallas ”wind-up” (ett slags korttidsminne i smärtsystemet) och som innebär att smärtsvaret ökar i storlek vid upprepad smärtstimulering. Studien tyder därför också på att just denna mekanism är av största betydelse för smärtsignaleringen i ryggmärgen.

Sammanfattningsvis så ger avhandlingen en beskrivning av basala egenskaper i musens smärtsystem och visar att det finns klara skillnader beträffande inlärningsmekanismer i detta system mellan mus och råtta som man bör ta hänsyn till när man studerar musens smärtsystem. Avhandlingen visar också på att de inlärningsmekanismer i hjärnan som har med rumsinläring och orientering att göra

verkar används i ryggmärgen för att lära in smärtekretsarnas funktion. Slutligen visar detta arbete för första gången att adhesionsmolekyler spelar en avgörande roll för att smärtnätverken ska fungera på ett korrekt sätt. Adhesionsmolekyler skulle därför kunna vara en intressant angreppspunkt för framtida smärtlindrande läkemedel.

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I started at the section for neurophysiology at the first of September in the year of our lord 2000 as an innocent student that wanted to do my master thesis in neurobiology. Before I knew years have passed and I just finished my master thesis. As a side project the book you hold in your hand came about. I have really enjoyed these past years, besides achieving knowledge in the art of electrophysiology; I have been married and got two wonderful kids. Obviously this thesis is not a one man show and there are plenty of people that helped me thru this who I would like to thank...

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