DESCENDING MODULATION OF PAIN BY MOTOR CORTEX STIMULATION IN THE RAT

EFFICACY AND MECHANISMS IN PERIPHERAL NEUROPATHY

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ACADEMIC DISSERTATION

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Helsinki 2012
Dedicated to my family
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ABSTRACT

Stimulation of the primary motor cortex (M1) alleviates neuropathic pain in humans and animals. However, the roles of various subcortical relay mechanisms in the antinociceptive effect of motor cortex stimulation are not yet fully understood.

The aim of this study was to characterize the M1 stimulation-induced antinociceptive effects, and to find out whether the descending antinociception is relayed through the noradrenergic, the serotonergic or the dopaminergic pathways in an animal model of chronic neuropathic pain. Moreover, the aim was to investigate whether the response properties of neurons in a potential relay nucleus, the locus coeruleus (LC), are changed following the development of experimental neuropathy.

The assessment of the noxious heat-evoked limb withdrawals which reflect spinal nociception, and the recordings of single LC and spinal dorsal horn units were performed in spinal nerve-ligated neuropathic and in sham-operated and/or unoperated control rats under light pentobarbital anesthesia.

Electric stimulation of M1 produced an equally strong spinal antinociception in the nerve-ligated and sham-operated animals, as revealed by noxious heat-evoked responses of spinal dorsal horn nociceptive neurons.

The M1 stimulation-induced spinal antinociceptive effect was attenuated by blocking the rostroventromedial medulla (RVM), a main source of serotonergic innervation of the spinal dorsal horn, or by blocking the spinal 5-HT1A receptor. This suggests that the RVM and the descending serotonergic pathway that acts on the spinal 5-HT1A receptor both contribute to the spinal antinociception induced by M1 stimulation in neuropathic animals.

The attenuation of the M1 stimulation-induced spinal antinociceptive effect by striatal administration of a dopamine D2 receptor antagonist on presumed pain-relay neurons of the spinal dorsal horn suggests that striatal dopamine D2 receptors contribute to the spinal antinociception induced by M1 stimulation in nerve-ligated animals. The descending dopaminergic pathway, involving the dopaminergic A11 cell group in the hypothalamus and the spinal dopamine D2 receptor, also contributes to the M1 stimulation-induced spinal antinociception in neuropathic animals. This was demonstrated by the reversal of the M1 stimulation-induced spinal antinociception following a lidocaine-induced block of the A11 cell group or a block of the spinal dopamine D2 receptor.

Characterization of the pathophysiological changes in the function of LC neurons revealed that their responses to noxious somatic stimulation were increased. This increased responsiveness is likely to promote noradrenergic feedback inhibition of neuropathic hypersensitivity while the enhanced inhibition of the LC from the amygdala is likely to suppress noradrenergic pain inhibition and promote neuropathic pain. However,
blocking the spinal $\alpha_2$-adrenoceptor failed to attenuate the M1 stimulation-induced spinal antinociception, indicating that the contribution of coeruleospinal noradrenergic pathways acting on the spinal $\alpha_2$-adrenoceptors may not be critical for the M1 stimulation-induced spinal antinociceptive effect in neuropathic animals.
LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by the Roman numerals I–IV:


III Viisanen H, Pertovaara A. Roles of the rostroventromedial medulla and the spinal 5-HT_{1A} receptor in descending antinociception induced by motor cortex stimulation in the neuropathic rat. Neurosci Lett. 2010; 133–137.

IV Viisanen H., Ansah O.B., Pertovaara A. The role of the dopamine D_{2} receptor in descending control of pain induced by motor cortex stimulation in the neuropathic rat.

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ABBREVIATIONS

5-HT  serotonin
5-HT_{1A}  subtype of the serotonin receptor
α_{1}  α_{1}-subtype of the adrenoceptor
α_{2}  α_{2}-subtype of the adrenoceptor
A1–A7  noradrenergic nuclei
A8–A14  dopaminergic nuclei
Aβ  mechanosensitive peripheral nerve fiber
Aδ  nociceptive specific peripheral nerve fiber
ACC  anterior cingulate cortex
AMPA  α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
Amy  amygdala
ANOVA  analysis of variance
Atip  atipamezole
AUC  area under the curve
C  nociceptive specific peripheral nerve fiber
C1–C2  adrenergic nuclei
c-Fos  transcription factor
CeA  central nucleus of the amygdala
COMT  catechol-O-methyltransferase
D_{1}  D_{1}-subtype of the dopamine receptor
D_{2}  D_{2}-subtype of the dopamine receptor
D_{3}  D_{3}-subtype of the dopamine receptor
DHPG  3,5-dihydroxyphenylglycine
DA  dopamine
DLPFC  dorsolateral prefrontal cortex
DStr  dorsal region of the striatum
GABA  gamma-aminobutyric acid
GABA_{A}  subgroup of the GABA receptor
Gi  nucleus gigantocellularis
Gia  gigantocellularis pars alpha
Glu  glutamate
GP  globus pallidus
GPCR  G-protein-coupled receptor
GPi  internal segment of the globus pallidus
GPe  external segment of the globus pallidus
HT  hypothalamus
i.c.  intracerebral
i.p.  intraperitoneal
i.t. intrathecal
Lid lidocaine
L2 lumbar 2
L4 lumbar 4
L5 lumbar 5
L6 lumbar 6
LC locus coeruleus
MAO monoamine oxidase
M1 primary motor cortex
MCS invasive motor cortex stimulation
mGluR1 subtype of the metabotropic glutamate receptor
mGluR3 subtype of the metabotropic glutamate receptor
mGluR5 subtype of the metabotropic glutamate receptor
Musc muscimol
NMDA N-methyl-D-aspartate
NA noradrenaline
NRM nucleus raphe magnus
NS nociceptive specific
NTS nucleus tractus solitarius
OFC orbitofrontal cortex
PAG periaqueductal gray matter
PB parabrachial nucleus
PGi paragigantocellularis
Raclo raclopride
rTMS repetitive transcranial magnetic stimulation
RVM rostroventromedial medulla
S1 primary somatosensory cortex
S2 sacral 2
SC superior colliculus
SEM standard error of mean
SN substantia nigra
SNe substantia nigra pars compacta
SNr substantia nigra pars reticulata
SNL spinal nerve ligation
Str striatum
T12 thoracic 12
VTA ventral tegmental area
WAY-100635 N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinylcyclohexanecarboxamide maleate salt
WDR wide-dynamic range
1 INTRODUCTION

The International Association for the Study of Pain (IASP) defined pain as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage” (Merskey et al. 1994). Pain is divided into short-term acute pain and long-term persistent or chronic pain. While persistent pain enhances healing processes, chronic pain appears to have no purpose. Persistent and chronic pain can be subdivided into nociceptive and neuropathic pain. The direct activation of nociceptors in the skin or soft tissue caused by tissue injury and the accompanying inflammation produce nociceptive pain, whereas direct injury to nerves in the peripheral or central nervous system produces peripheral or central neuropathic pain with a burning or electric sensation (Basbaum and Jessell 2000, Ossipov et al. 2006). Approximately 7–8% of the general population in Europe suffer from neuropathic pain. The treatment of chronic neuropathic pain is challenging and the response to most treatments is generally modest (Attal and Finnerup 2010). In 1991 Tsubokawa introduced motor cortex stimulation (MCS) as an alternative treatment for pain (Lefaucheur 2006, Tsubokawa et al. 1991). Motor cortex stimulation has also served as a treatment for chronic pain in patients who are resistant to other treatments such as drugs (Lefaucheur 2006).

The mechanisms of motor cortex stimulation in pain relief are still poorly understood although several hypotheses exist. Human studies indicate that the brain systems involved in the emotional appraisal of pain and in the descending pain modulation may have a role in the motor cortex stimulation-induced pain suppression (Garcia-Larrea and Peyron 2007, Garcia-Larrea et al. 1999, Peyron et al. 2007, Xie et al. 2009).

In 1965 Melzack and Wall proposed the gate control theory according to which painful signals are modulated in the spinal cord before they reach the supraspinal nervous systems. Supraspinal descending modulation systems, such as the noradrenergic locus coeruleus (LC), serotonergic rostroventromedial medulla (RVM) and opioidergic periaqueductal gray matter (PAG) filter and modulate nociceptive transmission in the spinal cord (Fields et al. 2006, Millan 2002). Moreover, neuroplastic changes in the descending modulation systems lead to hypersensitivity and the development and maintenance of neuropathic pain (Ossipov et al. 2006, Saadé and Jabbour 2008). Thus, a study focusing on the role of noradrenergic pain modulation mechanisms and the LC in experimental animals with spinal nerve ligation could be considered a potential source of new information about the involvement of descending noradrenergic inhibitory systems in neuropathic pain.

Motor cortex stimulation is effective also in experimental animals (Fonoff et al. 2009, Rusina et al. 2005, Senapati et al. 2005, Vaculin et al. 2008). Animal studies would, therefore, provide new insight concerning the involvement of descending modulation by noradrenergic and serotonergic systems in the primary motor cortex (M1) stimulation. Also dopaminergic systems could be involved. Especially the basal ganglia, which serve together with the motor cortex, as integrators of motor commands, are involved in the pain processes (Chudler and Dong 1995).
2 LITERATURE REVIEW

2.1 Nociceptive pathways

Thermal, mechanical or chemical stimuli, at the intensity reaching the noxious range and potentially causing tissue damage (Basbaum et al. 2009), activate distal parts of nociceptive nerve fibers, which are free nerve endings located in the skin, mucosa, membranes, deep fascias, connective tissues of visceral organs, ligaments and articular capsules, periosteum, muscles, tendons, and arterial vessels (Almeida et al. 2004).

Nociceptive nerve fibers are classified on the basis of their diameter, structure and conduction velocity as Aδ and C fibers (Almeida et al. 2004, Meyer et al. 2006, Todd et al. 2006). Aδ fibers are medium-sized, myelinated fibers and conduct at intermediate velocities, whereas C fibers are thin, unmyelinated and slowly-conducting (Millan 1999). Myelinated Aδ fibers give rise to acute, well-localized, sharp pain evoked by heat or mechanical stimuli. C fibers are polymodal fibers signaling poorly-localized slow and burning pain elicited by intense heat stimuli or sustained mechanical pressure. Nociceptors responding to cooling and silent nociceptors responding to heat but not to mechanical stimuli exist as well (Basbaum et al. 2009, Meyer et al. 2006, Todd et al. 2006). Moreover, both myelinated and unmyelinated nociceptors signal pain from chemical stimuli (Meyer et al. 2006, Todd et al. 2006). Other types of cutaneous peripheral afferent fibers are large diameter, myelinated, rapidly conducting Aβ fibers, which respond to innocuous mechanical stimulation, such as light touch. Under normal conditions, Aβ fibers do not themselves mediate pain sensation but they modulate pain perception and alleviate pain by activating inhibitory interneurons in the spinal cord (Basbaum et al. 2009, Millan 1999, Todd et al. 2006).

The cell bodies of the nociceptive nerve fibers are located in the dorsal root and trigeminal ganglia (Basbaum et al. 2009). The proximal endings of nociceptive primary afferent fibers ascend in the Lissauer Tract and project to the dorsal horn of the spinal cord where they form synapses with second-order neurons (Almeida et al. 2004). Nociceptive Aδ fibers project to the superficial lamina I and deep lamina V, while nociceptive C fibers project to the superficial laminae I and II, and collaterals of Aβ fibers to the deep laminae III, IV, and V (Basbaum et al. 2009).

In the spinal dorsal horn, neurons are classified according to their functionality. The nociceptive specific (NS) neurons in laminae I, II, V and VI respond to noxious signals from Aδ and C polymodal nociceptors. The NS neurons code the location and physical quality of the painful stimuli. A second class of neurons, the wide-dynamic range (WDR) neurons in laminae I, II, IV, V and VI respond to mechanical, thermal and chemical stimuli. The WDR neurons receive information directly from nociceptive Aδ and non-nociceptive Aβ fibers, and indirectly from nociceptive C fibers, and integrate a innocuous and noxious input. The WDR neurons code the stimulus intensity and are involved in the mechanisms...
of segmental suppression of pain (Basbaum et al. 2009). The non-nociceptive spinal dorsal horn neurons in laminae I, II and IV respond to innocuous stimuli from nociceptive Aδ and non-nociceptive Aβ fibers and are indirectly involved in segmental suppression mechanisms (Almeida et al. 2004, Basbaum et al. 2009).

Among the spinal cord interneurons, interlaminar and intrasegmental intralaminar neurons integrate the afferent stimuli within laminae and segments. Moreover, intersegmental propriospinal neurons integrate signals between several spinal levels, ipsilaterally and contralaterally (Almeida et al. 2004). Primary afferent fibers either stimulate projection neurons directly, which then relay information to the brain, or indirectly via excitatory and inhibitory interneurons that interact with projection neurons or with the terminals of primary afferent fibers (Millan 1999). Projection neurons transfer integrated nociceptive signals to supraspinal centers via at least five ascending pathways: spinothalamic, spinoreticulothalamic, spinomesencephalic, spinoparabrachio–amygdaloid and spinoparabrachio–hypothalamic tracts (Almeida et al. 2004, Basbaum et al. 2009, Millan 1999) (Fig. 1).

The spinothalamic, spinoreticular and spinomesencephalic tracts relay information from NS, WDR and non-nociceptive neurons, whereas the spinoparabrachio–amygdaloid and spinoparabrachio–hypothalamic tracts relay information from NS neurons only. The tracts ascend mainly contralaterally in the ventrolateral or dorsolateral funiculus to the higher brain areas. In addition, the spinoreticular tract ascends ipsilaterally through dorsal columns (Millan 1999). The spinothalamic tract ascends from laminae I, II, IV, V and VI to the thalamus and has collaterals to the midbrain periaqueductal gray matter (PAG). The spinoreticulothalamic tract from laminae I, V and VI ascends to the reticular formation of the medulla, lateral reticular nucleus, nucleus gigantocellularis (Gi), medial thalamus, and the dorsal raphe nuclei. The spinomesencephalic tract from laminae I, II, IV and V ascends to the midbrain PAG, superior colliculus, nucleus cuneiformis, and the parabrachial nucleus (PB). The spinoparabrachio–amygdaloid tract from laminae I and II ascends to the PB, which has further projections to the amygdala and stria terminals. The spinoparabrachio–hypothalamic tracts from laminae I and II ascend to the PB and send projections to the hypothalamus (Almeida et al. 2004, Basbaum et al. 2009, Millan 1999). All the above-mentioned tracts relay information related to the motivational-affective dimensions of pain. The spinothalamic tract projections to the ventroposterolateral and ventroposteromedial thalamus relay, instead, information related to the sensory-discriminative aspects of pain. Aside of this, the spinothalamic and spinoreticular tracts are involved in the descending modulation of pain (Millan 1999).

From the brainstem and thalamus, information ascends to cortical areas to build up the sensory-discriminative and affective-cognitive components of the pain experience (Almeida et al. 2004, Treede et al. 1999). Activation of the somatosensory cortex is involved in the sensory-discriminative component of pain, while activations of the anterior cingulate cortex (ACC) and the insular cortex are involved in the emotional aspects of pain. Moreover, prefrontal cortical areas, the basal ganglia and cerebellum are activated as well (Basbaum et al. 2009).
Figure 1  Ascending pain pathways arising from the dorsal horn: A) spinothalamic, B) spinomesencephalic, C) spinoreticular, spinoparabrachio–hypothalamic and spinoparabrachio–amygdaloid tracts. Amygdala (Amy), glutamate (Glu), hypothalamus (HT), parabrachial nucleus (PB), periaqueductal gray matter (PAG), superior colliculus (SC).
2.2 Descending modulation of pain

Before the nociceptive impulse reaches the supraspinal centers, descending modulation systems, which include the supraspinal brain structures and their descending fibers, filter and modulate the nociceptive transmission in the dorsal horn of the spinal cord (Fields et al. 2006, Millan 2002). This involves modulation of neural, behavioral and arousal responses, and also that of attention and expectation related to painful stimuli. Suppression or enhancement of nociceptive reflexes and other responses elicited by noxious stimuli tend to enhance the survival of the individual. When facing a threat, suppression of nociceptive reflexes might facilitate escape behavior, while during tissue damage and inflammation enhancement of pain could promote recuperative behavior to help healing (Fields et al. 2006).

The major pain modulatory areas in the brainstem include the PAG, the LC, and the RVM which in turn consists of the nucleus raphe magnus (NRM) and the neighboring gigantocellular nuclei. Supraspinal pain-modulation regions receive inputs through collaterals from ascending pain pathways (Almeida et al. 2004, Basbaum et al. 2009, Millan 1999) and send direct descending projections to the spinal dorsal horn. The RVM, PAG, LC and the dorsal reticular nucleus of the medulla, for instance, innervate directly the spinal cord. Moreover, other brainstem areas involved in pain modulation, such as the hypothalamus, the PB and the nucleus tractus solitarius (NTS) also project to the spinal cord. Several areas relay descending information through the RVM, which is a major relay nucleus involved in pain modulation and contains serotonergic and non-serotonergic projection neurons (Fields et al. 2006). Additionally, the noradrenergic cell groups A5, LC (A6), and A7 provide noradrenergic input to the spinal cord (Millan 2002). (Fig. 2.)

Descending pain modulation includes mechanisms of both descending inhibition and facilitation. The balance of inhibition and facilitation determines the final modulation state in the spinal cord. Descending pathways modulate nociception via various types of interaction in the spinal dorsal horn; these include presynaptic interaction with central terminals of nociceptive peripheral afferents, postsynaptic interaction directly with projection neurons, indirect postsynaptic interaction with projection neurons via inhibitory or excitatory interneurons, and interaction with terminals of other descending pathways. In inhibition, an attenuated release of pronociceptive mediators suppresses the activity of projection neurons, whereas in facilitation an enhanced release of pronociceptive mediators enhances it (Millan 2002).
Figure 2  Descending pain modulation pathways originating in the midbrain, pons, medulla and other supraspinal brain areas. Serotonergic, noradrenergic, opioidergic and dopaminergic neurons originating in the rostral ventromedial medulla (RVM), locus coeruleus (LC), periaqueductal gray matter (PAG) and the hypothalamus (HT), respectively. α2-Adrenoceptors (α2AR), dopamine (DA), dopamine D2 receptors (D2R), enkephalin, GABA, glutamate (Glu), noradrenaline (NA), opioids, serotonin (5-HT), 5-HT1A receptors (5-HT1A,R) in the synapses. Amygdala (Amy), striatum (Str), superior colliculus (SC). Ascending pain pathways (dashed lines).
2.3 Neuropathic pain

A lesion along a nociceptive pathway either in the periphery or the central nervous system can lead to peripheral or central neuropathic pain, respectively. The lesion may induce hyperalgesia, which is an increased reactivity to thermal or mechanical nociceptive stimulation, or allodynia, in which light touch or innocuous thermal stimuli may evoke a painful sensation. Moreover, neuropathic pain may involve ongoing spontaneous pain (Saadé and Jabbur 2008).

Peripheral tissue injury or damage to peripheral nerves triggers neurogenic and immunogenic mechanisms in the injured nerve fibers and also their neighboring nerve fibers (Saadé and Jabbur 2008). Injury produces discharge and abnormal firing in both the injured and intact nerve fibers, and secretion of neuropeptides and proinflammatory mediators at the site of injury. The inflammatory products and an expression of new receptors or ionic channels will then produce a sensitization of the nociceptors and activation of silent nociceptors (Ossipov et al. 2006, Saadé and Jabbur 2008). In neuropathic pain, an accumulation of sodium channels appears at the site of the nerve injury and also at the corresponding dorsal root ganglion (Ossipov et al. 2006). Additionally, an enhanced activation of immune cells induces acute and long-term functional and structural changes in the dorsal root ganglia of the injured dermatome (Saadé and Jabbur 2008).

Repetitive activity of injured nociceptive C fibers produces central hyperexcitability and an increase in immediate early-gene expression in the pain pathway. Injury-induced changes in the synthesis and secretion of neuropeptides, neurotransmitters and proinflammatory mediators will occur at various levels of the nervous system. These changes are accompanied by alterations in synaptic transmission. Among the injury-induced changes in the pain pathway is an increased release of excitatory neurotransmitters as well as such alterations in their receptors that contribute to the hyperexcitability. Additionally, the injury-induced pronociceptive changes, like alterations in expression and localization of receptors for Substance P, bradykinin, opioids, serotonin and glutamate transporters can occur (Ossipov et al. 2006, Saadé and Jabbur 2008). Following nerve injury, the activation is enhanced in pain-mediating glutamate receptors, such as the NMDA and AMPA receptors (Ossipov et al. 2006). Repetitive injury discharge in nociceptive fibers may induce wind-up in pain-relay neurons, which means that the response to each successive noxious stimulus increases, leading to enhanced pain sensation (Ossipov et al. 2006). Moreover, injury may cause an impairment of descending pain inhibitory mechanisms, which includes a reduction in both GABA release and GABA receptors, leading to disinhibition in the spinal dorsal horn. Increased spontaneous activity of dorsal horn neurons has been correlated with spontaneous pain, dysesthesia and hyperalgesia in experimental pain models (Chapman et al. 1998, Palecek et al. 1992, Pertovaara et al. 1997, Saadé and Jabbur 2008, Takaishi et al. 1996). Moreover, nerve injury may induce an abnormal sprouting of nerve fibers.
in the periphery and spinal cord. For instance, sprouting of Aβ fibers in the spinal dorsal horn has been associated with mechanical allodynia in some but not all studies (Ossipov et al. 2006, Saadé and Jabbur 2008).

Nerve injury-induced changes in the periphery and spinal cord lead to tonic and sustained bilateral neuronal activity in the brain areas known to be involved in pain perception (Saadé and Jabbur 2008). Bilateral increases in general metabolic activity occur in cortical somatosensory areas, the cingulate cortex, amygdala, thalamus, posterior thalamic nucleus, hypothalamic arcuate nucleus, central gray matter, superior colliculus, pontine reticular nuclei, PB, gigantocellular reticular nucleus, and the paragigantocellular nucleus (Mao et al. 1993). The acute as well as permanent changes involve alterations in the expression of neurotransmitters and their receptors (Saadé and Jabbur 2008). Areas that are normally not activated by noxious stimuli but show an increased abnormal activation following nerve injury include those basal forebrain areas that are considered to be part of the limbic system, and also cortical areas such as the prefrontal lobe, ACC and rostral insular cortex (Mao et al. 1993, Saadé and Jabbur 2008). Nerve injury-induced changes occur also in areas that are not considered as parts of the nociceptive system, such as the striatum (Str) (Saadé and Jabbur 2008). Moreover, increased activation after peripheral nerve injury in the PAG, LC, and the pontine and medullary reticular formation point to changes in the pain modulation system (Mao et al. 1993, Saadé and Jabbur 2008). Persistent nociception triggers both descending facilitation and inhibition systems, which may lead to an imbalance between the descending inhibitory and facilitatory control of the nociceptive input (Vanegas and Schaible 2004). Enhanced descending facilitatory action from the PAG–RVM areas may contribute to the maintenance of neuropathic manifestations (Gonçalves et al. 2007, Pertovaara et al. 2001, Pertovaara and Wei 2000, Wei et al. 2001). Loss of inhibition from several descending pathways in the spinal dorsal horn occurs as well. In spinal nerve-ligated animals, for instance, PAG stimulation-induced descending inhibition of noxious heat responses in WDR neurons were attenuated (Pertovaara et al. 1997).
2.4 Noradrenergic system

2.4.1 Noradrenaline, adrenoceptors and noradrenergic pathways

Noradrenaline is biosynthesized from tyrosine, which is first changed by tyrosine hydroxylase to dihydroxyphenylalanine (DOPA), which in turn is converted to dopamine by dopa decarboxylase. In noradrenergic cells dopamine is further converted to noradrenaline by dopamine-β-hydroxylase (Hein 2006, Pertovaara 2006). Noradrenaline is metabolized in noradrenergic cells to glycoaldehyde by monoamine oxidase (MAO) or extraneurally to normetanephrine by catechol-O-methyltransferase (COMT). Metabolites are further metabolized in sequential actions of COMT, MAO, aldehyde reductase and aldehyde dehydrogenase, and converted to vanillylmandelic acid in the liver (Cooper et al. 2003a, Eisenhofer et al. 2004).

Adrenoceptors can be divided into two different groups: α- and β-adrenoceptors. α-Adrenoceptors are further classified into several subtypes, α₁-receptors (α₁A, α₁B, α₁D) and α₂-receptors (α₂A, α₂B, α₂C). β-Adrenoceptors are classified into subtypes β₁, β₂ and β₃ (Hein 2006). Pain regulatory effects of noradrenaline are mediated via α-adrenoceptors, G-protein-coupled receptors, whereas β-adrenoceptors mediate the adrenaline-induced modulation of pain (Civantos Calzada and Aleixandre de Antinano 2001, Pertovaara 2006).

The main sources of noradrenaline are the noradrenergic brainstem nuclei A1–A7 and, peripherally, sympathetic nerves (Pertovaara 2006). The bulbospinal noradrenergic system, including A5, LC (A6) and A7, is the main source of the spinal noradrenergic innervations. It is involved in the modulation of the nociceptive transmission and pain control in the spinal cord (Jones 1991, Kwiat and Basbaum 1992, Takagi et al. 1979, Westlund et al. 1983). A5, LC and A7 all receive projections from other areas involved in pain modulation, such as the RVM (Clark and Proudfit 1991, Sim and Joseph 1992) and the PAG (Bajic and Proudfit 1999). Noradrenergic fibers and terminals descend ipsilaterally to the dorsal horn (Jones 1991, Westlund et al. 1983) and to motoneurons of the ventral horn as well as to the preganglionic autonomic neurons of the lateral cell columns (Westlund et al. 1983). Moreover, supraspinal noradrenergic areas receive direct information about pain, temperature and metabolic rate from collateral branches of ascending projections from lamina I to the PB and PAG regions. Ascending sensory pathways and descending noradrenergic pathways form a feedback loop for noradrenergic control of spinal sensory, autonomic and motor activity (Westlund and Craig 1996). Noradrenaline is also present as a neurotransmitter in peripheral sensory and sympathetic nerves (Cooper et al. 2003a, Pertovaara 2006).
2.4.2 Noradrenergic pain modulation

Noradrenaline is involved in the intrinsic control of pain in the endogenous pain inhibitory modulation system (Pertovaara 2006). Noradrenaline and noradrenergic receptors have various pain modulatory effects depending on the supraspinal site, the type of the adrenoceptor, the duration of the pain and the pathophysiological condition. The main effect of noradrenaline is antinociceptive action (Garraway and Hochman 2001a, Li et al. 2002, Takagi et al. 1979, Wei and Pertovaara 1997). The noradrenergic system produces not only antinociceptive but also pronociceptive actions. Following nerve injury, autotomy behavior and the reorganization of somatosensory pathways, particularly in the cerebral cortex, depend on central noradrenergic activity (Al-Adawi et al. 2002), which is an example of the pronocceptive role of noradrenaline.

In the spinal cord, noradrenaline modulates presynaptically the activity of central terminals of primary afferent fibers, and postsynaptically pain-relay neurons or inhibitory and excitatory interneurons (Pertovaara 2006, Yoshimura and Furue 2006). Noradrenaline depresses glutamate release from nociceptive Aδ and C fibers (Pertovaara 2006, Yoshimura and Furue 2006), and hyperpolarizes postsynaptically pain-relay neurons or excitatory interneurons of the spinal dorsal horn (Pertovaara 2006, Yoshimura and Furue 2006).

In healthy conditions, supraspinal or peripheral noradrenaline has little influence on pain, whereas under pathophysiological conditions in injured tissues it has variable effects, including the causation of irritating pain (Green et al. 1998, Malmberg et al. 2001, Pertovaara 2006). For instance, a knockout of the dopamine β-hydroxylase gene, leading to the absence of noradrenaline (Jasmin et al. 2002), has little effect on baseline nociception. Pathophysiological conditions and sustained pain influence noradrenergic feedback inhibition as well as noradrenergic top–down control of pain. Noxious peripheral stimulation produces spinal release of noradrenaline in animals with an intact spinal cord, but not in spinalized animals (Takagi et al. 1979, Tyce and Yaksh 1981); this is due to the changes in the descending noradrenergic feedback systems, such as the LC (Hodge et al. 1983, Tyce and Yaksh 1981, Wei and Pertovaara 2006a). Following nerve injury, plastic changes in the noradrenergic systems may attenuate antinociceptive influences. Moreover, an increased expression of noradrenergic receptors, sprouting of sympathetic nerve fibers, and pronociceptive changes in the ionic channel properties of nociceptors may contribute to the pronociceptive effects in the periphery (Pertovaara 2006). In contrast to this, some changes are likely to promote antinociception. An interaction with the immune system, for instance, may contribute to peripheral antinociception (Pertovaara 2006), and also an increased noradrenergic innervation of the spinal cord may promote antinociception (Ma and Eisenach 2003).
2.4.3 Locus coeruleus

The locus coeruleus (the LC or A6 cell group) in the dorsal pons has an important role in the descending noradrenergic inhibitory system and in the processing of noxious stimuli (Fig. 2). In addition to the role in pain modulation, the LC is involved in central cardiovascular control and in emotional and stress-regulating processes (Pertovaara 2006).

The LC receives afferents from the nucleus paragigantocellularis (PGi), the perifascicular area of the nucleus prepositus hypoglossi (Astier et al. 1990, Aston-Jones et al. 1991, Chiang and Aston-Jones 1993, Clark and Proudfit 1991, Ennis et al. 1992) and the nucleus raphe magnus (NRM) (Sim and Joseph 1992). The LC receives minor input from the dorsal cap of the paraventricular hypothalamus and from the spinal intermediate gray (lamina X) (Aston-Jones et al. 1991). Moreover, LC dendrites extend into the pericoerulear regions (Aston-Jones et al. 1991) receiving afferents from the PAG, the central nucleus of the amygdala (CeA), the stria terminalis, the NTS, the dorsal raphe and the spinal dorsal horn (Aston-Jones et al. 1986, Aston-Jones et al. 1991). Afferent inputs from the limbic forebrain, CeA and stria terminals coordinate emotional responses to external stressors (Van Bockstaele et al. 2001).

The LC sends ascending efferent projections to other supraspinal structures such as the cortex, thalamus, amygdala, hippocampus and hypothalamus (Foote et al. 1983). LC activity shapes, for instance, the response properties of various sensory networks en route to the cortex via bilateral projections to the thalamus (Voisin et al. 2005). The LC axons descend bilaterally through the dorsolateral funiculus to laminae I–IV in the lumbar dorsal horn of the spinal cord (Clark and Proudfit 1992). Some LC axons descend in the ipsilateral side of the spinal cord and cross the midline at spinal segmental levels (Tsuruoka et al. 2004). The LC axons descend also in the ipsilateral ventromedial funiculus to laminae VII and VIII, and to the motoneuron pool of laminae IX and X mainly within the cervical spinal cord (Clark and Proudfit 1992, Mokha 1986, Proudfit and Clark 1991).

Noradrenergic neurons in the LC respond to external, environmental stimuli and influence behavioral functions such as vigilance, alarm and anxiety reactions to novel and threatening stimuli. Autonomic or visceral functions can affect behavior and, conversely, environmental stress can affect autonomic functions through the LC (Elam et al. 1986a, 1986b). Peripheral sympathetic nerves and noradrenergic neurons of the LC respond in parallel to stress-related stimuli, resulting in noradrenaline release both peripherally and centrally (Elam et al. 1986a, 1986b, Hentall et al. 2003, Kaehler et al. 2000). Moreover, neurons in the LC are activated by both innocuous and noxious thermal (Elam et al. 1986a, Hajos and Engberg 1990, Hajos et al. 1986), mechanical, chemical (Hong et al. 1992) or electrical stimuli (Hirata and Aston-Jones 1994). Painful stimuli induce in the LC a release of excitatory amino acids (Hajos and Engberg 1990), c-Fos expression (Voisin et al. 2005) and release of noradrenaline (Kaehler et al. 2000).
Electrical stimulation of the LC produces antinociception and increases the level of noradrenaline and its metabolites in laminae I and IV–VIII of the spinal dorsal horn (Hentall et al. 2003, Janss et al. 1987, Jones and Gebhart 1986, Jones 1991, Tsuruoka et al. 2004, West et al. 1993). In laminae II–III, however, a decrease has been described in the noradrenaline content following LC stimulation (Hentall et al. 2003). The LC may modulate nociception not only through its descending but also via ascending pathways to the somatosensory thalamus (Voisin et al. 2005, Zhang et al. 1997).

Under healthy conditions, the LC has seemingly little influence on nociception, since the background activity, the heat-evoked response of dorsal horn neurons, nor the paw-withdrawal latency showed any change after lesion in the LC (Tsuruoka et al. 2003b). In sustained pain and under pathophysiological conditions, however, the LC has an important role in pain modulation. Painful stimulation induces in the LC enhanced activity (Elam et al. 1986a, 1986b, Ennis et al. 1992, Hirata and Aston-Jones 1994, 1996), increased expression of immediate-early genes (Traub et al. 1996) and release of catecholamines (Hong et al. 1992, Kaehler et al. 2000, Sajedianfard et al. 2005). Inflammation in a hindpaw, for instance, induces bilateral activation of the LC, resulting in enhanced descending inhibitory modulation in the dorsal horn (Tsuruoka et al. 2003a, 2003b). Lesion of the LC in inflamed animals leads to enhanced background activity and increased heat-evoked responses in spinal pain-relay neurons, which is accompanied by an enhanced behavioral response to noxious stimulation (Tsuruoka et al. 2003b, Tsuruoka and Willis 1996).

The LC may have as well an important role in the pain modulation of peripheral neuropathic pain. Hyperalgesia and spontaneous pain involve bilateral increases in general metabolic activity in the LC of animals with an experimental neuropathy (Mao et al. 1993). Following nerve injury, stimulation of the LC attenuates peripherally evoked responses of spinal dorsal horn neurons when the dorsal roots are intact, but not when they are injured (Hodge et al. 1983). Moreover, increased noradrenergic innervation of the spinal cord is associated with increased immunoreactivity in the LC (Ma and Eisenach 2003). These studies indicate that the antinociceptive efficacy of the LC may be changed in neuropathy.

### 2.4.4 α₂-Adrenoceptors and pain

α₂-Adrenoceptors are located in the LC, brainstem, cerebral cortex, septum, hypothalamus, hippocampus, amygdala, thalamus, basal ganglia, and the olfactory tubercle (Pertovaara 2006, Scheinin et al. 1994). In the spinal cord, α₂-adrenoceptors are located in all laminae of the spinal dorsal horn, in the lateral spinal nucleus and ventral horn, suggesting that α₂-adrenoceptors are involved both in sensory and motor processing (Pertovaara 2006, Shi et al. 1999). α₂-Adrenoceptors are also located in peripheral primary afferents and sympathetic nerves (Gold et al. 1997).
Noradrenaline and descending noradrenergic pathways in the spinal cord have, through $\alpha_2$-adrenoceptors, a suppressive effect on nociceptive transmission (Hämäläinen and Pertovaara 1995, Proudfit 1988, Yoshimura and Furue 2006, Zhang et al. 1998). The antinociception induced by electrical stimulation of the LC is relayed through the spinal $\alpha_2$-adrenoceptors (West et al. 1993). In the LC, $\alpha_2$-adrenoceptors are autoreceptors that inhibit the release of noradrenaline from the LC (Aghajanian and VanderMaelen 1982). Moreover, the LC has, through $\alpha_2$-adrenoceptors, inhibitory effects also at the level of medial thalamus (Zhang et al. 1998). In the RVM, activation of $\alpha_2$-adrenoceptors produced an inhibition of responses in spinal neurons. However, this may have been due to a spread of the $\alpha_2$-adrenoceptor agonist to the spinal dorsal horn, where the agonist could have acted directly on the spinal $\alpha_2$-adrenoceptors (Hämäläinen and Pertovaara 1995).

The $\alpha_2$-adrenoceptor-mediated inhibitory system is dormant under normal conditions. For instance, a knockout of various subtypes of $\alpha_2$-adrenoceptors has little effect on baseline nociception (Malmberg et al. 2001). Prolonged pain and nerve damage, though, induce a change in endogenous noradrenergic descending inhibition, acting via spinal $\alpha_2$-adrenoceptors (Green et al. 1998, Hämäläinen and Pertovaara 1995). Following a knockout of the $\alpha_2A$-adrenoceptor, nociceptive behavior evoked by sustained noxious stimulation is enhanced (Mansikka et al. 2004). Peripheral nerve injury may produce a tonic activation of noradrenergic feedback inhibition by acting on spinal $\alpha_2$-adrenoceptors (Hämäläinen and Pertovaara 1995, Wei and Pertovaara 2006a). In line with this, $\alpha_2$-adrenoceptor agonists increased antinociceptive efficacy in experimental peripheral neuropathy, attenuating pain responses in mechanical nociception and hyperalgesia (Pertovaara and Wei 2000, Wei and Pertovaara 1997, Wei et al. 2002, Yaksh et al. 1995). The mechanisms promoting noradrenergic antinociception in nerve-injured animals include increased noradrenergic innervation of the spinal cord by the LC (Ma and Eisenach 2003). In accordance to this, peripheral nerve injury induces changes that tend to attenuate noradrenergic feedback inhibition (Rahman et al. 2008). These changes include a tonic activation of pontine $\alpha_2$-adrenoceptors, which promotes neuropathic hypersensitivity by attenuating the descending noradrenergic inhibition (Wei and Pertovaara 2006a). Another mechanism suppressing noradrenergic inhibition in peripheral neuropathy is a decrease in the number of $\alpha_2$-adrenoceptors in the spinal cord (Stone et al. 1999). Moreover, in peripheral nerve injury noradrenaline may produce hyperexcitability in the dorsal root ganglion cells via $\alpha_2$-adrenoceptors (Leem et al. 1997, Tanimoto et al. 2011).
2.5 Serotonergic system

2.5.1 Serotonin, serotonin receptors and serotonergic pathways

Serotonin (5-hydroxytryptamine, 5-HT) is synthesized from tryptophan, which is changed by tryptophan hydroxylase into 5-hydroxytryptophan, which in turn is converted to serotonin by aromatic amino acid decarboxylase (Nichols and Nichols 2008). Serotonin is metabolized to 5-hydroxyindoleacetaldehyde by monoamine oxidase (MAO) and further converted to 5-hydroxyindoleacetic acid by aldehyde dehydrogenase or to 5-hydroxytryptophol by aldehyde reductase (Cooper et al. 2003b, Frazer and Hensler 1999). Serotonergic receptors are divided into the seven different groups: 5-HT\textsubscript{1} through 5-HT\textsubscript{7}. The receptors are further classified into several subtypes: 5-HT\textsubscript{1} receptors (A, B, D, E, F), 5-HT\textsubscript{2} receptors (A, B, C), 5-HT\textsubscript{3} receptors (A, B, C) and 5-HT\textsubscript{5} receptors (A, B). Serotonin activates G-protein-coupled receptors (GPCR) and 5-HT\textsubscript{3} receptor that is an ligand-gated ion channel (Hoyer et al. 2002).

The 5-HT cells are located in the brainstem on or near the midline. The serotonergic brainstem area is divided into a rostral group that includes nuclei: the caudal linear, median raphe, dorsal raphe and B9 nucleus, and a caudal group that includes the raphe obscurus, raphe pallidus, raphe magnus, ventral lateral medulla (lateral paragigantocellular nucleus and intermediate reticular nuclei) and area postrema (Jacobs and Azmitia 1992). Non-5-HT cells exist within serotonergic nuclei, especially in B9, ventrolateral medulla, median raphe nucleus, lateral wings of dorsal raphe nucleus, and medullary raphe nuclei. Fibers descending from raphe nuclei to the spinal cord, however, are mainly, but not exclusively, serotonergic (Jacobs and Azmitia 1992, Kwiat and Basbaum 1992, Mason 1997, 2001).

Serotonergic areas in the dorsal raphe send projections to the cortex, thalamus and striatal regions, whereas the median raphe nucleus projects to the limbic system, e.g. the hippocampus (Jacobs and Azmitia 1992). The RVM is the classic serotonergic area (Mason 1997, Potrebic et al. 1995), which sends descending projections to the spinal dorsal horn (Jacobs and Azmitia 1992, Kwiat and Basbaum 1992). The input to the raphe nuclei is mainly coming from the raphe nuclei themselves. Serotonergic nuclei receive afferents also from other brainstem nuclei, hypothalamus, prefrontal cortex, limbic forebrain (Jacobs and Azmitia 1992) and spinal cord (Braz et al. 2009).

Serotonergic innervation of the spinal cord is derived only from supraspinal sources. Serotonergic pathways descend to all spinal laminae and all segmental levels of the spinal cord that act on projection neurons, preganglionic sympathetic and parasympathetic neurons, and somatic motoneurons. In the spinal dorsal horn, serotonergic fibers innervate laminae I, II\textsubscript{o}, III and IV (Marlier et al. 1991a, Millan 2002). Furthermore, supraspinal 5-HT neurons receive direct sensory input from the spinal cord through the spinoreticular pathway (Braz et al. 2009, Menetrey et al. 1980). Serotonin also acts as a neurotransmitter in peripheral sensory and sympathetic nerves (Nichols and Nichols 2008, Pierce et al. 1996).
2.5.2 Serotonergic pain modulation

Brainstem serotonergic neurons show both antinociceptive and pronociceptive actions (Braz et al. 2009, Mason 2001) depending on the 5-HT receptor subtype and the anatomical location of the activated 5-HT receptor (Hoyer et al. 2002, Millan 2002). In the spinal dorsal horn, serotonin may facilitate or inhibit spinal dorsal horn neurons by postsynaptically depolarizing or hyperpolarizing the neuron, respectively. The direction of this serotonergic effect depends on the spinal 5-HT receptor type activated (Abe et al. 2009, Braz et al. 2009, Garraway and Hochman 2001a, 2001b, Mason 2001, Yoshimura and Furue 2006). Moreover, serotonin may presynaptically increase the release of inhibitory transmitter from interneurons, which may depress the glutamate release from the primary afferents and thereby attenuate the transmission of pain-related signals (Abe et al. 2009, Yoshimura and Furue 2006).

During sustained pain and in pathophysiological conditions, such as inflammation or peripheral nerve injury, changes will occur in the descending 5-HT system. The development of chronic pain after spinal nerve lesion has been associated with a potentiation of the descending facilitatory 5-HT system (Suzuki et al. 2004) and an attenuation of the descending inhibitory 5-HT system (Liu et al. 2010). Both of these changes may contribute to the development of central sensitization and in that way to nerve injury-induced neuropathic pain. The changes involve, for instance, decreased levels of 5-HT in the NRM (Souvoravong et al. 2004) and a decreased inhibitory effect of 5-HT on the evoked-responses of spinal dorsal horn neurons (Liu et al. 2010). Moreover, in inflammation and nerve injury peripheral 5-HT contributes to sensitization and hyperalgesia (Sommer 2004).

2.5.3 Rostral ventromedial medulla

The RVM in the brainstem consists of the NRM and the adjacent reticular formation, including the nucleus gigantocellularis pars alpha (Giα) and paragigantocellularis ventralis (PGi) (Pertovaara and Almeida 2006). The RVM is a major final common pathway for the top–down modulation of the spinal processing of sensory inputs. Thus, the RVM is involved in the control of the relay of pain-related sensory information between the spinal cord and brain. Additionally, the RVM is involved in thermoregulation, vasomotor control, sleep-wake cycle, motor control, and the control of sexual functions (Mason 2001).

The RVM receives afferents from the hypothalamus, medial preoptic region, amygdala, PAG, and PB (Jacobs and Azmitia 1992, Mason 2001, Sandkühler and Gebhart 1984). Moreover, several catecholaminergic (A5, LC, A7) and cholinergic cell groups, several brainstem reticular nuclei, and the NTS all project to the RVM (Braz et al. 2009, Jacobs and Azmitia 1992). The NRM, the Giα or the PGi neurons project to the lateral hypothalamus, parafascicular nucleus, PAG and NTS (Sim and Joseph 1992). Moreover, the RVM has ipsilateral projections to the LC, PB, A7, and A5 (Chiang and Aston-Jones 1993, Clark
and Proudfit 1991, Ennis et al. 1992, Sim and Joseph 1992). The RVM may contribute to the modulation of spinal nociception through its reciprocal connections with the midbrain PAG, and also via its efferent projections to the noradrenergic LC, A7 and A5, each of which sends direct pontospinal projections to the spinal dorsal horn (Sim and Joseph 1992). Furthermore, medullary 5-HT neurons receive direct information about pain from the spinoreticular pathway that arises from the deep spinal cord laminae V–VIII, and may trigger descending serotonergic antinociceptive control (Braz et al. 2009, Menetrey et al. 1980). (Fig. 2.)

The RVM sends bilateral descending projections, via the dorsolateral funiculus, to innervate the spinal dorsal horn laminae I, II and V (Mokha 1986), the intermediolateral cell column, laminae VII and VIII of the intermediate and ventral horns, and the central canal region (Fields et al. 1995, Mason 2001). Additionally, the RVM has been described to send descending projections through the ventral and ventrolateral funiculi (Zhuo and Gebhart 1997). The descending facilitatory influences of the RVM travel in the ventral and ventrolateral funiculi, whereas the inhibitory influences travel in the dorsolateral funiculi (Zhuo and Gebhart 1997). The RVM fibers form monosynaptic connections with dorsal horn cells, whereas their connections with preganglionic autonomic neurons or premotoneuronal and other interneurons can be monosynaptic or disynaptic (Mason 2001).

The RVM contributes to the modulation and integration of nociceptive and affective information through its descending projections to the spinal cord and via ascending projections to the forebrain (Mico et al. 2006). Moreover, the RVM receives somatic and visceral inputs from various areas of the body (Braz et al. 2009). There are three classes of nociceptive modulatory neurons in the RVM that are defined by their responses associated with nocifensive reflexes: on-cells, off-cells, and neutral cells (Fields et al. 1983, Mason 2001). Noxious stimuli such as strong thermal or mechanical stimuli increase on-cell activity and decrease off-cell activity (Leung and Mason 1998). Activation of on-cells facilitates spinal nociceptive transmission and reflexes (Heinricher et al. 1989, Morgan and Fields 1994), whereas off-cells inhibit them (Heinricher et al. 1989). Off-cell discharge is not, however, sufficient to completely suppress nociceptive withdrawal reflexes because it may result from rather than cause changes in nociception. In addition, changes in on-cell discharge alone fail to alter spinal reflexes if there is no contribution from off-cells (Mason 2001).

The responses of the RVM on-, off- and neutral cells to noxious stimulation depend on the stimulation site, i.e. RVM neurons may respond differently to noxious stimulation depending on the site of the stimulation on the body surface. For example, the response pattern evoked by noxious mechanical or noxious thermal stimulation of the limbs may differ from the response to noxious heating of the tail. In addition, it is proposed that the neutral cells are probably subtypes of the on- and off-cells (Ellrich et al. 2001, Leung and Mason 1998, Schnell et al. 2002). A subgroup of neutral cells, though, consists of serotonergic neurons, while both on- and off-cells are nonserotonergic (Mason 1997,
Although most of the neutral cells fail to respond to noxious stimuli, some of the serotonergic cells are weakly excited or inhibited by both noxious mechanical and heat stimuli (Leung and Mason 1998, Mason 1997). Interestingly, 5-HT within the RVM may contribute to nociceptive modulation by directly acting on the cell bodies of the RVM neurons (Potrebic et al. 1995). It predominantly facilitates but in some cases inhibits the ongoing or evoked activity of RVM neurons, independent of the RVM neuron’s subtype (Roychowdhury and Heinricher 1997). Administration of a 5-HT1 receptor agonist to the medulla only inhibited RVM neurons of any subtype (Roychowdhury and Heinricher 1997). The net output from descending projections of pronociceptive RVM on-cells and antinociceptive RVM off-cells will determine whether the activity of nociceptive neurons in the spinal cord is increased or decreased (Mason 2001). Under normal conditions, electrical and chemical stimulation of the RVM may produce either facilitation or inhibition of cutaneous thermally or mechanically-evoked nociceptive responses of spinal dorsal horn neurons, and of nociceptive reflexes, depending on the strength of RVM stimulation (Jones and Gebhart 1987, Zhuo and Gebhart 1992, 1997 and 2002). Additionally, lesion or inactivation of the RVM may attenuate both the RVM-induced suppression and also facilitation of nociceptive transmission in the spinal dorsal horn (Mason 2001). It has been proposed that the primary effect of 5-HT in the RVM is an inhibition of spinal nociceptive responses (Mason 2001). This suggestion is supported by studies indicating that blocking the NRM reduces the NRM stimulation-induced inhibition of the nociceptive reflex (Jones and Gebhart 1987) and also the serotonin levels in the spinal cord (Mason 2001).

Although under normal circumstances the RVM suppresses nociception, in some pathophysiological conditions, such as neuropathic pain, the facilitatory influence may predominate and lead to an enhancement of neuropathic pain symptoms and hyperexcitability via an enhanced evoked transmitter release in the spinal dorsal horn (Burgess et al. 2002, Carlson et al. 2007, Gardell et al. 2003, Pertovaara 1998, Vera-Portocarrero et al. 2006). During peripheral neuropathy, stimulation of the NRM attenuated peripherally evoked responses of spinal dorsal horn neurons when the dorsal roots were intact, but not when they were injured (Hodge et al. 1983). Neuropathy-induced changes in the RVM also include pronociceptive changes in the on- and off-cell activities (Carlson et al. 2007, Gonçalves et al. 2007, Kincaid et al. 2006, Neubert et al. 2004). Sensitization of stimulus-evoked responses of on-cells may contribute to allodynia and hyperalgesia (Carlson et al. 2007, Gonçalves et al. 2007, Neubert et al. 2004). Blocking the RVM activity, and thereby the facilitatory activity of on-cells, suppressed evoked-responses in spinal dorsal horn neurons following both nerve injury (Bee and Dickenson 2007) and inflammation (Pertovaara 1998). Despite the finding that neutral cells remained unresponsive to cutaneous stimulation after nerve injury (Carlson et al. 2007), the serotonergic neutral neurons may have a role in neuropathy by contributing to mechanical hypersensitivity via a change in their axonal targets in the spinal cord (Pertovaara et al. 2001).
2.5.4 Serotonin 5-HT\textsubscript{1A} receptors and pain

5-HT\textsubscript{1A} receptors are located in limbic areas (lateral septum, CA1 area of Ammon’s horn, dentate gyrus in hippocampus, and frontal and entorhinal cortices), anterior raphe nuclei, interpeduncular nucleus, neocortex, some thalamic and hypothalamic nuclei, nucleus of solitary tract, dorsal tegmentum, nucleus of spinal tract of trigeminal nerve, and the superficial layers of the dorsal horn in the spinal cord. 5-HT\textsubscript{1A} receptors are located both in the perikarya and dendrites of neurons (Kia et al. 1996, Marlier et al. 1991b). In healthy conditions, 5-HT\textsubscript{1A} receptors are not expressed in the periphery (Pierce et al. 1996). However, 5-HT induces hyperalgesia via 5-HT\textsubscript{1A} receptors (Taiwo and Levine 1992) whose peripheral expression is enhanced following inflammation (Liu et al. 2005).

Activation of 5-HT\textsubscript{1} receptors inhibits ongoing or evoked activity of RVM neurons (Roychowdhury and Heinricher 1997). 5-HT\textsubscript{1A} receptors in the RVM (Azmitia et al. 1996) are somatodendritic autoreceptors that inhibit the release of serotonin in terminal areas, a mechanism by which activation of 5-HT\textsubscript{1A} receptors on cell bodies within the medulla may enhance pain-related signals in the spinal dorsal horn (Mico et al. 2006). In neuropathic animals, activation of medullary 5-HT\textsubscript{1A} receptors suppresses tonically descending inhibition, as was indicated by the finding that a 5-HT\textsubscript{1A} receptor antagonist in the RVM disinhibited descending pain regulatory pathways, thus producing an attenuation of neuropathic hypersensitivity (Wei and Pertovaara 2006b).

Under healthy conditions, activation of spinal 5-HT\textsubscript{1A} receptors promotes antinociception postsynaptically (Abe et al. 2009, Colpaert 2006) by hyperpolarizing the substantia gelatinosa neurons (Yoshimura and Furue 2006). This leads to an inhibition of evoked responses in the spinal WDR neurons (Gjerstad et al. 1996). Under neuropathic conditions, activation of spinal 5-HT\textsubscript{1A} receptors effectively suppresses mechanical hypersensitivity (Wei and Pertovaara 2006b). In contrast to this, it was recently reported that the inhibitory effect of spinal 5-HT\textsubscript{1A} receptors on the responses of dorsal horn nociceptive neurons, in fact, decreased following peripheral nerve injury (Liu et al. 2010).
2.6 Dopaminergic system

2.6.1 Dopamine, dopamine receptors and dopaminergic pathways

Dopamine is synthesized from tyrosine first by tyrosine hydroxylase to dihydroxyphenylalanine (DOPA), which in turn is converted to dopamine by dopa decarboxylase (Vallone et al. 2000). Dopamine is metabolized to 3,4-dihydroxyphenylacetaldehyde by monoamine oxidase (MAO) or to 3-methoxytyramine by catechol-O-methyltransferase (COMT). Metabolites are further metabolized in sequential actions of COMT, MAO, aldehyde reductase and aldehyde dehydrogenase, and converted to 3,4-dihydroxyphenylacetic acid and homovanillic acid (Cooper et al. 2003a, Eisenhofer et al. 2004). Dopamine activates the G-protein-coupled receptors (GPCR), which are divided into two different groups: D1 and D2 receptors. The D1-like subfamily includes D1 and D5 receptors, while the D2-like subfamily includes D2, D3 and D4 receptors (Jackson and Westlind-Danielsson 1994, Vallone et al. 2000).

The dopaminergic cells are located in the substantia nigra compacta (A9), the retrorubral field (A8), and the ventral tegmental area (VTA). The A8–A9 areas innervate the dorsal striatum (DStr), forming the nigrostriatal pathway, whereas the VTA area innervates different regions of the frontal cortex, forming the mesocortical pathway. In addition, the VTA innervates the ventral striatum, the olfactory tubercle and parts of the limbic system, forming the mesolimbic pathway (Saper et al. 2000). Dopaminergic cells are also located in the olfactory system and retina (Saper et al. 2000).

Additional groups of dopaminergic cells are located in the hypothalamus. Hypothalamic dopaminergic neurons in the periventricular (A14) and arcuate nuclei (A12) of the hypothalamus (Qu et al. 2006) form the tuberoinfundibular pathway that innervates the median eminence of the hypothalamus and also the hypophyseal portal system (Van den Pol 1986). Dopaminergic neurons in the posterior hypothalamic A11 nucleus and dorsomedial hypothalamus/zona incerta area (A13 nucleus) (Qu et al. 2006) innervate the brainstem and spinal cord (Skagerberg and Lindvall 1985, Van den Pol 1986).

Dopaminergic neurons from the diencephalic A11 cell group descend several segments to the entire length of the spinal cord (Qu et al. 2006, Skagerberg and Lindvall 1985) where they innervate the dorsal horn (superficial and laminae III–IV), intermediolateral cell column, peri-ependymal region, and the ventral horn (Ridet et al. 1992, Weil-Fugazza and Godefroy 1993). In the dorsal horn, the intermediate gray matter and lamina X of the spinal cord, descending dopaminergic pathways are involved in pain modulation and the control of autonomic functions, whereas in the ventral horn they influence motor functions (Weil-Fugazza and Godefroy 1993). In addition, in periphery dopamine is present in primary sensory and sympathetic neurons (Cooper et al. 2003a, Weil-Fugazza et al. 1993).
2.6.2 Dopaminergic pain modulation

The dopaminergic system and dopamine play a critical role in natural analgesia and are involved in pain modulation in several areas of the central nervous system, such as the basal ganglia, insula, ACC, thalamus, PAG, and the spinal cord (Wood 2008). The ascending nigrostriatal pathway and the descending fibers from the A11 to the dorsal horn are involved in dopaminergic pain modulation (Chudler and Dong 1995, Pelissier et al. 2006, Skagerberg and Lindwall 1985). For instance, intraplantar injection of carrageenan, a compound inducing inflammatory pain, increased significantly dopamine and its final metabolite homovanillic acid levels in the Str, PAG, and the dorsal horn of the spinal cord (Gao et al. 2001b).

Dopamine has both a pronociceptive and an antinociceptive role in pain modulation depending on the receptor subtype activated and the anatomical location of the activated receptor (Gao et al. 2001b, Liu et al. 1992, Paalzow and Paalzow 1983, Pelissier et al. 2006, Taniquchi et al. 2011). In line with this, intrathecal administration of the dopamine agonist apomorphine induced either hyperalgesia or antinociception depending on the dose applied (Barasi and Duggal 1985, Jensen and Yaksh 1984, Paalzow and Paalzow 1983). In the spinal dorsal horn, dopamine-induced antinociception may be induced either by a presynaptic or postsynaptic inhibition of synaptic transmission in the substantia gelatinosa (i.e. the superficial dorsal horn) and the deep dorsal horn (Garraway and Hochman 2001a, Tamae et al. 2005, Taniquchi et al. 2011). In the periphery, dopamine induces hypernociception (Cunha et al. 2008, Villarreal et al. 2009). In line with this finding, carrageenan-induced inflammatory pain was prevented by a peripheral dopamine antagonist (Villarreal et al. 2009).

In pathophysiological conditions, such as inflammation or peripheral neuropathy, various changes may take place in the function of the descending dopaminergic system. Following inflammation, activation of central dopaminergic receptors attenuates inflammatory hyperalgesia, which indicates a role in the control of inflammatory pain (Gao et al. 2000, Gao et al. 2001a, 2001b). Moreover, neurochemical assessments indicated that inflammation induced bidirectional changes in the synthesis and metabolism of dopamine: an increase in the spinal dorsal horn laminae III–V and a decrease in the more superficial laminae and in lamina X (Weil-Fugazza and Godefroy 1993). The dopamine system may also contribute to the control of neuropathic pain and hypersensitivity (Ansah et al. 2007, Hagelberg et al. 2004, Pertovaara and Wei 2008), as will be discussed further in the following sections.
2.6.3 Basal ganglia

The basal ganglia consist of the striatum (Str) (caudate nucleus, putamen and ventral striatum), the globus pallidus (GP) (internal segment, GPi, and external segment, GPe), the substantia nigra (SN) (substantia nigra pars compacta, SNc, and substantia nigra pars reticulate, SNr), and the subthalamic nucleus. The caudate nucleus and putamen are also called the neostriatum, and in rodents the caudate-putamen. The Str receives afferents from the cerebral cortex and the intralaminar nuclei of the thalamus. The Str projects to the SN and GP. The SNc projects back to the Str, and the SNr to the thalamus. The GPe projects to the subthalamic nucleus and GPi, while the GPi projects to the thalamus. The Str, GP and SN may receive nociceptive information from several sources such as the cerebral cortex, thalamus and amygdala (Chudler and Dong 1995).

In addition to regulating movement, attention, rewarding and learning, the basal ganglia process and regulate pain-related responses in humans (Chudler and Dong 1995, Hagelberg et al. 2004, Neugebauer 2006) and animals (Ansah et al. 2007, Pertovaara and Wei 2008). In harmony with this, painful stimulation increases regional cerebral blood flow within the putamen and GP of human subjects (Chudler and Dong 1995, Hagelberg et al. 2004), and electrical stimulation of the Str attenuates responses related to pain in rats and nonhuman primates (Belforte and Pazo 2005, Lineberry and Vierck 1975, Saunier-Rebori and Pazo 2006).

Basal ganglia neurons are classified on the basis of their response properties to innocuous and noxious stimulation as low-threshold mechanoreceptive, wide-dynamic range, nociceptive-specific and inhibited neurons (Chudler et al. 1993). Multisensory input from several sensory modalities, both innocuous and noxious, is converging within the Str for the coordination of behavioral responses (Chudler and Dong 1995). Some neurons within the basal ganglia (SNc, striatal and pallidal neurons) are multisensory, responding to both innocuous and noxious stimulation (Bernard et al. 1992, Chudler et al. 1993, Chudler and Dong 1995). A high proportion of the striatal and pallidal neurons, which respond to somatosensory stimulation, are activated differentially or exclusively by noxious stimuli (Chudler 1998). Noxious mechanical, electrical and thermal stimuli may excite or inhibit the activity of striatal and pallidal neurons (Brown 1992, Chudler 1998, Chudler et al. 1993, Chudler and Dong 1995, Richards and Taylor 1982). Those striatal and pallidal neurons that respond to low-threshold somatosensory stimulation are located throughout the Str and GP, have large cutaneous receptive fields covering most of the body, and fail to show a somatotopic arrangement (Chudler and Dong 1995). Nociceptive neurons are mostly located along the Str-GP border, and neurons of similar functional classification are often clustered (Chudler et al. 1993). Stimulation of the hindlimb, trunk or forelimb will activate the sensorimotor striatum according to the known anatomic patterns of corticostriate terminals (Brown 1992): the hindlimb, scrotum and tail areas in the most caudal regions of the Str, while the forelimb, head and neck areas are represented in its anterior regions (Brown 1992, Chudler and Dong 1995, Richards and Taylor 1982).
The Str and GP neurons contribute to the behavioral responses that attempt to minimize bodily harm (Chudler 1998). The basal ganglia select or modify the movements responding to particular environmental demands. It is presumed that the role of the basal ganglia in the sensory-discriminative dimension of pain is to influence the direction or speed of coordinated escape behavior from the source of pain, thus to prevent further pain and injury. In the motivational-affective dimension of pain, the basal ganglia influence avoidance, attack and distress modes of behavior. In the cognitive dimension of pain, the basal ganglia are presumed to be involved in the willful control of the sensory-discriminative and motivational-affective dimensions of pain. The association of the basal ganglia with learning and memory could assign meaning to noxious events, while their association with attentional mechanisms could contribute to the orientation of the animal in relation to an environmental threat (Chudler and Dong 1995).

Dopamine is a key neuromodulator in the basal ganglia’s function and also essential for normal motor activity. Within the basal ganglia, the Str is the main functional target of the dopaminergic innervation, although dopaminergic nerve fibers are known to innervate also GP, subthalamic nucleus and SN (Smith and Villalba 2008). Dopamine has, depending on the dose administered and the receptor type activated, a pronociceptive or antinociceptive role in the Str (Lin et al. 1981, Paalzow and Paalzow 1983, Pelissier et al. 2006). Intraplantar injection of carrageenan, which produces inflammatory pain, increased significantly dopamine and homovanillic acid levels in the Str (Gao et al. 2001b). The nigrostriatal dopaminergic pathway is considered to induce predominantly antinociception (Chudler and Dong 1995, Gao et al. 2001b). In accordance with this, a decrease in presynaptic dopaminergic function in the putamen has been associated with the prolonged pain of the burning mouth syndrome (Jääskeläinen et al. 2001), and striatal lesions in rats increased nociceptive responses to chemical, thermal and mechanical stimulation (Chudler and Lu 2008, Saadé et al. 1997, Takeda et al. 2005).

Following experimental nerve injury, the observed thermal hyperalgesia, mechanical allodynia and spontaneous pain behavior have been associated with a bilateral increase in general metabolic activity and function in the Str (Anshah et al. 2007, Chudler and Dong 1995). Neuropathy-induced changes in the Str include tonic activation of striatal NMDA receptors (Pertovaara and Wei 2008) and an enhanced antihypersensitivity effect of striatal dopamine D2 receptors (Anshah et al. 2007, Lin et al. 1981, Magnusson and Fisher 2000, Pertovaara and Wei 2008).

Sensory processing within the basal ganglia affects motor control by a selection from the sensory information arriving from various cortical areas, such as the primary somatosensory cortex (S1), secondary somatosensory cortex, area 7b, and the cingulate cortex. Via projections from the cingulate cortex, amygdala and prefrontal cortex, the basal ganglia receive information concerning the affective dimension of pain. Moreover, the basal ganglia receive inputs from the dorsal raphe nucleus (Chudler and Dong 1995). In addition
to its efferent cortical projections to premotor areas, the basal ganglia send descending 
projections to the superior colliculus, and from there further to the NRM and eventually 
to the spinal cord (Ansah et al. 2007, Basso et al. 1996, Basso and Evinger 1996), this 
way providing a pathway for descending pain modulation (Fig. 2).

2.6.4 Dopamine D₂ receptors and pain

The antinociceptive effect of dopamine is mediated by the dopamine D₂ receptors (Van 
Dijken 1996, Wood 2008). These are located in several areas throughout the central nervous 
system, along the dopaminergic innervation in the Str, nucleus accumbens, subthalamic 
nucleus etc. In the Str, dopamine D₂ receptors are located on the medium-sized spiny 
neurons, and some on interneurons (Bouthenet et al. 1987, Yung et al. 1995).

A dopamine D₂ receptor can be an autoreceptor localized on dopaminergic cells or a 
heteroreceptor localized on non-dopaminergic ones (Reisine et al. 1979). Activation of 
the dopamine D₂ autoreceptors on axon terminals suppresses dopamine release from the 
dopaminergic cells innervating the Str, providing an autoinhibitory mechanism (Benoit-
dopamine modulates the signal transmission in the Str through its action on presynaptic 
and postsynaptic dopamine D₂ receptors on non-dopaminergic neurons (Flores-Barrera 

In addition to those in the basal ganglia, dopamine D₂ receptors are located in the cerebral 
cortex, cerebellum, hippocampal formation, in several septal, thalamic and hypothalamic 
nuclei, and in large tectal and numerous brainstem areas. For instance, in the hypothalamus, 
dopamine D₂ receptors are found in the A11 area (Bouthenet et al. 1987). Dopamine D₂ 
receptors are located also in the PAG (Wood 2008). Further sites are found in the spinal 
cord, i.e. in the parasympathetic area of the sacral cord and two sexually dimorphic 
motor nuclei of the lumbosacral cord, in the spinal nucleus of the bulbocavernousus, 
intermediolateral cell column, area around central canal, laminae I, III and IV of dorsal 
horn, lateral spinal nucleus, and in laminae VII and VIII of the ventral horn (Van Dijken 
1996). In the spinal cord, dopamine has via dopamine D₂ receptors both presynaptic 
and postsynaptic inhibitory effects on synaptic transmission (Taniguchi et al. 2011). 
Dopamine D₂ receptors are located also in the peripheral nervous system in peripheral 
and sympathetic nerves (Xie et al. 1998).

Under healthy conditions, activation of dopamine D₂ receptors in various brain areas 
attenuates pain-related behavior (Munro 2007, Paalzow and Paalzow 1983, Pelissier et 
al. 2006, Wood 2008, Zarrindast et al. 1999). Dopamine D₂ receptor activation produced 
antinociceptive effects, for instance, in the Str and SN (Bouthenet et al. 1987, Lin et al. 1981, 
Magnusson and Fisher 2000), and in the spinal cord (Barasi and Duggal 1985, Belforte and 
et al. 2006, Saunier-Rebori and Pazo 2006). Dopamine D₂ receptor antagonists, in contrast,
enhanced pain-related responses under healthy conditions (Lin et al. 1981, Magnusson and Fisher 2000, Pelissier et al. 2006). Additionally, a low dopamine D₂/D₃ receptor binding potential in the putamen, which presumably reflects a high activation level of striatal dopamine D₂/D₃ receptors by endogenous release of dopamine, was associated with a high pain threshold in healthy subjects (Hagelberg et al. 2002, Martikainen et al. 2005).

In the spinal cord, dopamine exerts its antinociceptive effects through dopamine D₂ receptors; this was indicated by the finding that activation of these receptors by intrathecal administration of a dopamine D₂ receptor agonist decreased pain-related responses in healthy animals (Barasi and Duggal 1985, Jensen and Yaksh 1984, Liu et al. 1992, Paalzow and Paalzow 1983, Pelissier et al. 2006). Electrophysiological studies indicate that the spinal antinociceptive action of dopamine D₂ receptors can be explained by hyperpolarization of substantia gelatinosa neurons in the spinal dorsal horn (Tamae et al. 2005).

A dopamine D₂ receptor agonist attenuated mechanical allodynia and thermal hyperalgesia in experimental neuropathy (Ansah et al. 2007), and also mechanical hyperalgesia in a carrageenan-induced inflammatory pain condition (Gao et al. 2000, Gao et al. 2001a, 2001b). In human patients with a burning mouth syndrome, a decrease in the striatal presynaptic dopaminergic function has been associated with prolonged pain (Jääskeläinen et al. 2001). Under pathophysiological conditions, also hypothalamic dopamine D₂ receptors may contribute to pain suppression, since activation of dopamine D₂ receptors in or adjacent to the hypothalamic dopaminergic A11 cell group was found to suppress hypersensitivity in experimental animals with a peripheral neuropathy (Wei et al. 2009).

2.6.5 A11

The descending dopaminergic pathway arises mainly from the hypothalamic A11 nucleus (Skagerberg and Lindwall 1985) (Fig. 2), which provides the major source of dopamine in the spinal cord (Barraud et al. 2010, Skagerberg and Lindwall 1985). The A11 and the dopaminergic diencephalospinal pathway are crucial for sensorimotor integration and pain control at the spinal cord level (Barraud et al. 2010). Dopaminergic neurons in the A11 nucleus send ipsilateral projections to the spinal cord, and a minority of crossed projections to the contralateral side. Dopamine neurons have long axons that give off collateral branches at various levels of the spinal cord (Hökfelt et al. 1979, Qu et al. 2006, Skagerberg and Lindvall 1985).

Electrical stimulation of A11 in healthy controls and also in neuropathic animals produces antinociception through an action on the spinal dopamine D₂ receptors (Fleetwood-Walker et al. 1988, Taniguchi et al. 2011, Wei et al. 2009), as shown by an inhibition of the nociceptive responses of spinal dorsal horn WDR neurons in healthy control animals (Fleetwood-Walker et al. 1988) and suppression of a noxious heat-evoked limb withdrawal reflex in nerve-injured animals (Wei et al. 2009).
2.7 Amygdala, emotions and pain

Neurons in the lateral capsular part of the central nucleus of the amygdala (CeA) form the “nociceptive amygdala”. It receives nociceptive information from the spinal dorsal horn through the spinoparabrachial–amygdala tract, which has a relay at the nucleus parabrachialis (Basbaum et al. 2009, Ikeda et al. 2007, Neugebauer 2004, 2006) (Fig. 1). The output nuclei in the amygdala project to the substantia innominata dorsalis, the bed nucleus of the stria terminalis, the posterior hypothalamus, and the interstitial nucleus of the posterior limb of the anterior commissure. Moreover, the amygdala projects to the DStr, nucleus accumbens, olfactory tubercle, nucleus of olfactory tract, and the rostral pole of the cingulate/frontal cortex. The amygdala also projects to dopaminergic, noradrenergic and adrenergic cell groups of the brainstem: the dopaminergic cell groups A8–A10, adrenergic cell groups C1–C2, and noradrenergic cell groups A2, A5, LC and A7 (Van Bockstaele et al. 2001, Wallace et al. 1992).

Noxious thermal and mechanical stimuli can excite or inhibit nociceptive neurons in the CeA, depending on the neuron. The receptive fields of nociceptive neurons in the amygdala are large. Some neurons respond similarly to stimuli from all parts of the body, and some with stronger responses when stimulations is applied to a restricted body part (Bernard et al. 1992). The amygdala may influence the descending modulation of pain through several structures such as the PAG, RVM and LC (Ansah et al. 2009, da Costa Gomez and Behbehani 1995, Van Bockstaele et al. 1996, 1998) (Fig. 2). Stimulation of the CeA may produce inhibitory or excitatory responses in the neurons of the PAG, an area involved in descending pain modulation (da Costa Gomez and Behbehani 1995).

The amygdala has an important role in anxiety, aversion and fear (Bourgeais et al. 2001). It processes and regulates information that is relevant for the aversive properties of the pain experience (Basbaum et al. 2009). In animals and humans, emotional states modulate pain reactivity. Fear may inhibit pain, whereas anxiety may enhance it (Rhudy and Meagher 2000, 2003). Prolonged pain, such as inflammation, induces neuroplastic changes in the amygdala (Han et al. 2004, 2006, Han and Neugebauer 2004). These changes probably contribute to the maintenance of the sensory and emotional components of inflammatory and possibly neuropathic pain.
2.8 Motor cortex stimulation

2.8.1 Primary motor cortex

The primary motor cortex (M1), which is also known as Brodman’s area 4 in man, is in humans located in the precentral gyrus of the brain. In the rat, M1 contains two subregions that rostrally include a typical M1 cortex and caudally an area overlapping with the primary somatosensory cortex (S1) (Chapin and Woodward 1986). M1 receives afferents from the somatosensory cortex (Farkas et al. 1999), basal ganglia (Ebrahimi et al. 1992, Hoover and Strick 1999, Miyachi et al. 2006), thalamus (Cicirata et al. 1986) and cerebellum (Hoover and Strick 1999).

M1 projects directly to other parts of the motor cortex, to the basal ganglia, thalamus, tectum, red nucleus, pontine nuclei, inferior olive, external cuneate nucleus, and the cerebellum. Unlike projections to the spinal cord, projections of M1 to other brain areas are mainly ipsilateral, except for the cerebellum where the projections are mainly contralateral. In addition, M1 has indirect projections to the globus pallidus, to deep cerebellar nuclei, cerebellar cortex, and the external cuneate nucleus (Wan et al. 1992).

The majority of the fibers projecting from M1 to supraspinal structures fail to reach the spinal cord (Canedo 1997). Corticospinal axons from the pyramidal cells of the sensorimotor cortex layer V, however, project to the spinal cord via direct pathways (particularly in primates) or indirect pathways (non-primates as well as primates). The corticospinal tract consists of a main tract and several minor pathways. As a first step, axons project via the internal capsule and cerebral peduncle to the ventral aspect of the brainstem and send collaterals to supraspinal structures such as the red nucleus, pontine nuclei, olivary complex (Canedo 1997, Brösamle and Schwab 2000), reticular formation, medial pontine nucleus, and NRM (Antal 1984, Catsman-Berrevoets and Kuypers 1981, Keizer and Kuypers 1984, 1989, Keizer et al. 1987, Newman et al. 1989). Supraspinal structures, such as the reticular formation, send their descending projections to the spinal cord, forming bilateral indirect corticoreticulospinal pathways (Jacobs and Azmitia 1992, Keizer and Kuypers 1984, Kwiat and Basbaum 1992, Umeda et al. 2010).

Most of the corticospinal fibers descend further in the spinal cord, turning dorsally and crossing the midline in the pyramidal decussation, while some of the fibers descend in the ipsilateral dorsomedial funiculus or ipsilaterally in the ventral side of the cord (Brösamle and Schwab 2000), forming uncrossed ipsilateral corticospinal projections to the spinal cord (Joosten et al. 1992, Newman et al. 1989). In the rat, the corticospinal neurons from M1 descend to all levels of the spinal cord including the lumbar region (Miller 1987). In the spinal cord, the descending pathways from M1 innervate the dorsal horn laminae I–V, the internal basilar nucleus, laminae V–VII, and motoneurons of the ventral horn (Antal 1984,
Casale et al. 1988). Axons terminating in the spinal dorsal horn laminae I and II also terminate in or are collaterals of axons innervating the deeper dorsal horn (Casale et al. 1988). Ipsilaterally, ventral corticospinal fibers descend via the ventral funiculus close to the midline, contacting presynaptically neurons in the intermediate laminae of the spinal cord at lumbar spinal levels (Brösamle and Schwab 2000). Integration between the corticospinal systems from each side occurs at the spinal cord level via interneurons (Umeda et al. 2010), and at the supraspinal level between the hemispheres through the corpus callosum and pyramidal tract collaterals (Brus-Ramer et al. 2009).

### 2.8.2 Motor cortex and pain

The main role of the motor cortex is to control movement and posture (Canedo 1997). The M1 is activated bilaterally even during unilateral movements (Brus-Ramer et al. 2009, Chapin and Woodward 1986). In the M1/S1 area, some neurons respond both to movements and cutaneous stimulation. Also in the typical M1 area some neurons are activated by cutaneous stimulation (Chapin and Woodward 1986), including noxious stimulation. Noxious cold or heat stimuli, for instance, may cause significant activation of the M1 in humans (Casey et al. 1996, 2001). Additionally, noxious intramuscular stimulation increased regional cerebral blood flow in the M1/S1 area (Svensson et al. 1997). M1 receives pain-related information from several structures such as the thalamus (Cicirata et al. 1996) and the somatosensory cortex (Farkas et al. 1999). The motor cortex selects a unique motor command by filtering sensory information and influencing the activities of the descending systems that are related to the control of distal and proximal muscles and pain modulation (Canedo 1997, Millan 2002). Moreover, direct projections from M1 to the dorsal laminae of the spinal cord and the dorsal column nuclei may be involved in the modulation of the sensory transmission in those descending systems (Antal 1984, Canedo 1997).

Peripheral nerve injury or nerve lesion induces a reorganization of somatosensory pathways (Al-Adawi et al. 2002, Sanes et al. 1990, Toldi et al. 1996) and also that of the motor cortex (Sanes et al. 1990). In rats, intraoral noxious stimulation resulted in prolonged neuroplastic changes decreasing the excitability of the M1 face area (Adachi et al. 2008), and a nerve injury in the hind limb resulted in neural plasticity within the hind paw area of M1 (Ooi et al. 2006). Plasticity of M1 may involve changes in the expression of neurotransmitter receptors (Corlew et al. 2008, Neto et al. 2001), such as an up-regulation of the mGluR₃ mRNA expression in monoarthritic rats (Neto et al. 2001). Moreover, plastic reorganization of the somatotopic order of M1 may lead to some disinhibition of this structure (Farkas et al. 2000, Schwenkreis et al. 2010). Furthermore, human studies demonstrated that the M1 was inhibited when tonic cutaneous or muscle pain was experimentally induced in healthy subjects (Farina et al. 2001, Le Pera et al. 2001).
2.8.3 Motor cortex stimulation in humans and animals

Two decades ago, Tsubokawa and colleagues introduced motor cortex stimulation (MCS) as a treatment of post-stroke thalamic pain (Lazorthes et al. 2007, Peyron et al. 2007, Tsubokawa et al. 1991, Xie et al. 2009). In patients with deafferentation pain, motor cortex stimulation produced a reduction of pain or even a complete absence of pain (Tsubokawa et al. 1991). Since then, invasive stimulation of M1 has served as a clinical treatment for chronic pain, such as post-stroke and thalamic pain (Lazorthes et al. 2007). Particularly, MCS has been effective in the alleviation of nerve injury-induced neuropathic pain (Fontaine et al. 2009, Hosomi et al. 2008, Lazorthes et al. 2007, Nguyen et al. 1999, Xie et al. 2009). Stimulation of M1 by noninvasive transcranial electric or magnetic pulses attenuated effectively chronic pain (Fontaine et al. 2009, Lefaucheur 2006) and increased the threshold for experimental pain stimuli in human patients and healthy subjects, although not under all conditions and in all individuals (Johnson et al. 2006, Lefaucheur et al. 2008, Nahmias et al. 2009, Summers et al. 2004, Valmunen et al. 2009). MCS and non-invasive repetitive transcranial magnetic stimulation (rTMS) of M1 has been used for treating patients who are resistant to other therapeutic approaches (Lefaucheur 2006, Tsubokawa et al. 1991). MCS may produce pain relief even in a half or more of the pain patients (Fontaine et al. 2009). Additionally, M1 stimulation has been used as a clinical treatment of movement disorders, such as Parkinson’s disease (Arle and Shils 2008, Lefaucheur 2006, 2009) and essential tremor (Arle and Shils 2008).

In humans, stimulation of the motor cortex has produced changes in the threshold for both cold and heat pain, decreasing the threshold temperature for cold pain detection and increasing the threshold temperature for heat pain detection (Johnson et al. 2006, Lefaucheur et al. 2008). The effect induced by M1 stimulation is influenced by the origin and site of the pain and the stimulus condition. For instance, its analgesic effect is weak in patients with a brainstem stroke, regardless of the site of the pain, whereas the analgesic effect is good in patients with facial pain (Xie et al. 2009). Also the intensity of stimulation influences the efficacy. When using rTMS for M1 stimulation, for example, a high-frequency (5 or 20 Hz) stimulus can reduce pain more efficiently than low-frequency stimulation (at 1 Hz) (Lazorthes et al. 2007, Summers et al. 2004, Xie et al. 2009). Additionally, the cortical stimulation site is important for the analgesic efficacy especially with rTMS, because the most effective cortical stimulation target may not be the M1 area corresponding to the painful zone but rather an adjacent region (Lazorthes et al. 2007, Lefaucheur 2006, Xie et al. 2009). Some studies postulate that in order to have permanent therapeutic effects with M1 stimulation, chronic stimulation using surgically implanted electrodes rather than rTMS is necessary (Lefaucheur 2006, 2009).

Stimulation of M1 exerts in experimental animals similar pain attenuating effects as in humans suffering from neuropathic pain. Electric stimulation of M1 modulates pain-related responses in healthy experimental animals, as shown by suppression of the
pain-related limb withdrawal response (Fonoff et al. 2009), attenuation of the responses in nociceptive spinal dorsal horn neurons (Rojas-Piloni et al. 2010, Senapati et al. 2005), and by a reduction in the sensory abnormalities induced by nerve injury (Pagano et al. 2011, Rusina et al. 2005, Vaculin et al. 2008). In animals with neuropathic pain, short-term and long-term stimulation of M1 produced pain relief (Pagano et al. 2011, Vaculin et al. 2008), reversing the mechanical hyperalgesia and allodynia induced by peripheral neuropathy (Pagano et al. 2011).

2.8.4 Mechanisms of motor cortex stimulation

The supraspinal and spinal mechanisms whereby M1 stimulation attenuates neuropathic pain are poorly understood. Studies in humans show that the MCS produces changes in regional cerebral blood flow in several brain areas. The MCS triggered rapid and phasic activation of the lateral thalamus (Garcia-Larrea and Peyron 2007, Garcia-Larrea et al. 1999), which led to activation of the ACC and dorsolateral prefrontal cortex (Garcia-Larrea et al. 1999, Peyron et al. 2007). During a post-stimulation period, longer time-course activation occurred in various cortical and subcortical regions, including the anterior cingulate/orbitofrontal cortices, putamen, thalamus, posterior cingulate, prefrontal areas, and in the brainstem the mesencephalon, PAG and pons. The MCS-induced cerebral blood flow changed in the supraspinal areas in proportion to the pain relief (Garcia-Larrea and Peyron 2007, Garcia-Larrea et al. 1999, Peyron et al. 2007, Xie et al. 2009).

It has been hypothesized that stimulation of M1 produces analgesia through at least two mechanisms (Garcia-Larrea and Peyron 2007). First, the MCS may influence the emotional appraisal of pain by activating the perigenual cingulate and orbitofrontal areas. Second, the MCS may modulate descending inhibition through the upper brainstem structures by activating the midbrain PAG, which leads to descending inhibition in the spinal cord (Garcia-Larrea and Peyron 2007, Garcia-Larrea et al. 1999, Peyron et al. 2007, Xie et al. 2009). Human studies have shown correlations between the analgesic effect and the activity of the pregenual ACC, PAG, basal ganglia, and the lower pons (Peyron et al. 2007). In line with this, it has been shown that the analgesic effect of MCS in patients with neuropathic pain correlated with the release of endogenous opioids in the anterior middle cingulate cortex and PAG (Maarrawi et al. 2007).

Studies in experimental animal support the hypothesis that M1 stimulation attenuates pain by action on the supraspinal structures involved in the emotional appraisal of pain as well as the hypothesis that descending pathways acting on signal transmission at the spinal cord level contribute to the M1 stimulation-induced analgesic effect. Namely, M1 stimulation increased Fos immunoreactivity in the PAG, ACC and amygdala, which are involved in the induction of descending inhibition of pain. Moreover, M1 stimulation
decreased Fos immunoreactivity in the ventral posterior lateral and medial nuclei of the thalamus, i.e. in structures that are involved in the mediation of pain (Pagano et al. 2011). The involvement of the PAG and endogenous opioids in descending pain modulation systems was further supported by an animal study (Xie et al. 2009) showing that a blockade of opioid receptors by naloxone abolished the increase in the nociceptive threshold which had been induced by epidural electrical stimulation of M1 (Fonoff et al. 2009). Moreover, a recent study suggested that corticospinal pathways might also directly mediate the M1 stimulation-induced spinal antinociception (Rojas-Piloni et al. 2010).

Also other hypotheses and suggestions have been introduced in attempts to explain the attenuation of pain by M1 stimulation. It has been proposed that the efferent signals induced by M1 stimulation in the motor cortex may diminish pain-related responses in the somatosensory cortex (Jiang et al. 1990). Even GABAergic activity in the cortex has been implicated in this phenomenon (Xie et al. 2009), and it has been suggested that M1 stimulation may act through cortical GABAergic neurotransmission, thus modulating intracortical inhibitory processes (Di Lazzaro et al. 1998, Lefaucheur et al. 2006, Xie et al. 2009).
3 RATIONALE OF THE STUDY


The M1 stimulation-induced pain relief, particularly in humans, may involve the supraspinal mechanisms that influence the perception and/or emotional appraisal of pain (Garcia-Larrea and Peyron 2007, Maarrawi et al. 2007, Peyron et al. 2007). However, in experimental animals, the spinal antinociceptive effects of M1 stimulation (Senapati et al. 2005) indicate that descending pain modulatory pathways may have a role in the M1 stimulation-induced pain suppression. This hypothesis is supported by studies showing that the M1 stimulation-induced spinal antinociceptive effect was associated with an increased activation of neurons in a number of brainstem structures that are involved in descending pain control, such as the PAG (Garcia-Larrea and Peyron 2007, Peyron et al. 2007). This hypothesis is further supported by the finding that, in animals, blocking the effect of opioid stimulation (Fonoff et al. 2009) reverses the spinal antinociceptive effect. The results to date, however, still leave open the questions 1) which of the major descending pain-regulatory pathways (Millan 2002, Pertovaara and Almeida 2006) are involved in M1 stimulation-induced antinociception and 2) whether the efficacy of noradrenergic pain modulation is changed under pathophysiological conditions, such as in peripheral nerve injury-induced neuropathy.


In addition, also the striatum (Str) and hypothalamic A11 cell group provide potential relays for an antinociceptive dopaminergic circuitry activated by M1 stimulation (IV). The Str receives projections from the M1 (Brown and Sharp 1995, McGeorge and Faull 1989), and M1 stimulation induces a release of dopamine in the Str (Kanno et al. 2004, Nieoullon et al. 1978, Strafella et al. 2003). Activation of dopamine D2 receptors in the DStr attenuates pain (Ansah et al. 2007, Lin et al. 1981, Magnusson and Fisher 2000, Saunier-Rebori and Pazo 2006) and may mediate the M1 stimulation-induced spinal antinociception. Moreover, since the A11 cell group projects to the spinal cord (Hökfelt et al. 1979, Qu et al. 2006, Skagerberg and Lindvall 2005) and produces antinociception due to its action on the spinal dopamine D2 receptors (Fleetwood-Walker et al. 1988, Taniguchi et al. 2011, Wei et al. 2009), this dopaminergic cell group may mediate the M1 stimulation-induced antinociception.

In the spinal cord, serotonin acts on various 5-HT receptors, dopamine on various dopamine receptors, and noradrenaline on numerous types of adrenoceptors. The modulatory effects on pain may vary from pronociception to antinociception depending on the receptor subtype (Millan 2002). The 5-HT1A receptors, dopamine D2 receptors and α2-adrenoceptors are considered to be among the spinal receptor types that predominantly promote antinociception (Abe et al. 2009, Barasi and Duggal 1985, Colpaert 2006, Hämäläinen and Pertovaara 1995, Jensen and Yaksh 1984, Liu et al. 1992, Pelissier et al. 2006, Proudfit 1988, Yoshimura and Furue 2006). It is not yet known whether these receptors contribute to the M1 stimulation-induced spinal antinociception but there were enough reasons to study those receptor subtypes.

The noradrenergic pontospinal pathways arising from the LC (II), the serotonergic pathway arising from the RVM (III) and the dopaminergic pathways arising from the DStr and the hypothalamic A11 nuclei (IV) are in any case potential relays for the M1 stimulation-induced antinociception.
4 AIMS OF THE STUDY

The aim of this study was to characterize M1 stimulation-induced antinociceptive effects, and to find out whether the descending antinociceptive influence is relayed through the noradrenergic, serotonergic or dopaminergic pathways in an animal model of chronic neuropathic pain. The specific objectives were:

1. To study whether experimental neuropathy (I) results from a change in the discharge properties of neurons in the LC, a potential major relay center for M1 stimulation-induced descending antinociception, or from a change in coeruleospinal antinociception per se.

2. To assess whether stimulation of the primary motor cortex (M1) attenuates pain-related spinal withdrawal responses of neuropathic and healthy control rats, and whether the descending antinociceptive effect is relayed through the LC (II).

3. To determine whether the RVM or the spinal 5-HT1A receptor contributes to the antinociception induced by stimulation of M1 in neuropathic animals (III).

4. To find out whether striatal or spinal dopamine D2 receptors play a significant role in relaying the descending antinociceptive effect from M1 in experimental neuropathy. Moreover, to determine whether the dopaminergic hypothalamic A11 nucleus contributes to the relay of the antinociceptive effect of M1 stimulation in neuropathic animals (IV).
5 MATERIALS AND METHODS

5.1 Animals

The experiments were performed in adult, male Hannover-Wistar rats (Harlan, Horst, Netherlands; weight: 200–300 g). The methods had been approved by the Ethics Committee of the Regional Government of Southern Finland and the experiments were performed according to the guidelines of the European Communities Council Directive of 24 November 1986 (86/609/EEC). All efforts were made to limit distress and to use only the number of animals necessary to produce reliable scientific data. The rats were housed in a 12-h light-dark cycle with free access to food and water. At the end of the study, the animals were sacrificed immediately after the experiments.

5.2 Techniques for producing neuropathy

The unilateral ligation of two spinal nerves (L5 and L6) was performed under sodium pentobarbital anesthesia (50 mg/kg i.p.) as described in detail earlier (Kim and Chung 1992). Briefly, left paraspinal muscles were separated from the spinous processes at the L4–S2 levels. The L6 transverse process was partly removed to identify visually the L4–L6 spinal nerves. The left L5 and L6 spinal nerves were isolated and tightly ligated with 6-0 silk thread. After nerve ligation, the wound was sutured and the rats were allowed to recover. Development of nerve injury-induced mechanical hypersensitivity was assessed in awake animals 10–14 days following the operation (Fig. 3). Of the spinal nerve-ligated rats, only those with a marked hypersensitivity to mechanical stimulation with monofilaments (hind limb withdrawal thresholds on the operated side <2 g) and with no motor impairment were selected for the neuropathic study group. The controls consisted of a group of unoperated animals (I) and a group of sham-operated animals (I–II) that had undergone the same surgical procedures as the spinal nerve-ligated animals (including partial removal of the L6 transverse process), except that their spinal nerves were not ligated. The sham-operated or unoperated animals, unlike the spinal nerve-ligated ones, were not hypersensitive to mechanical stimulation.
5.3 Anesthesia and stereotaxic surgery

The anesthesia was induced by administering 50 mg/kg of sodium pentobarbital i.p. The anesthesia was continued by administering sodium pentobarbital at the dose of 15–20 mg/kg/h. The level of anesthesia was frequently monitored by assessing the size of the pupils, general muscle tone and reflex responses to noxious pinching. Supplemental doses of sodium pentobarbital were given as required. During surgery and electrophysiological experiments, the rats were spontaneously breathing. The peripheral blood circulation was checked by examining the color of the ears and extremities.

During the nerve ligation surgery and an intrathecal (i.t.) catheter insertion, rat was lying on the table without fixation. After surgery the rat was returned to the home cage for recovery. Development of neuropathy and correct location of i.t. catheter were verified in an awake animal.

Electrophysiological recordings or assessment of the heat-evoked withdrawal response were performed 2–4 weeks after nerve ligation (Fig. 3). For electrophysiological recordings, assessment of the heat-evoked withdrawal response or intracerebral drug injections, the animal was anesthetized (see above) and placed in a standard stereotaxic apparatus. The animal’s head was fixed with ear bars and teeth and nose clamps. The skin over the skull was pulled aside and the membranes over the skull were removed. After the skull was exposed, a hole was drilled with a dental drill for placement of a recording electrode, a guide cannula (26-gauge), a concentric bipolar electrode or a 26-gauge electrode-cannula guide (see details below) according to the atlas of Paxinos and Watson (1998) (Table 1). The body temperature was maintained within a physiological range by means of a warming blanket. At the time of testing the withdrawal response, the anesthesia was kept at a level at which no spontaneous movements of extremities were observed while a noxious test stimulus applied to the hind paw produced a brief withdrawal response. After completing the study, the animals were given a lethal dose of pentobarbital and the brains were removed for histological assessment of the sites of recording, stimulation and injections.

5.4 Preparation for intrathecal drug injections

A group of animals (II–IV) had an intrathecal (i.t.) catheter fitted for drug delivery to the spinal cord level. The catheter (Intramedic PE-10, Becton, Dickinson and Company, Sparks, MD, USA) was inserted into the lumbar level of the spinal cord (Storkson et al. 1996). The i.t. catheter was inserted under sodium pentobarbital anesthesia (50 mg/kg i.p.) during the same procedure as the nerve injury or sham surgery at least 2–3 weeks before the actual drug testing (Fig. 3). Following recovery from anesthesia, the correct placing of the catheter was verified by administering lidocaine (4 %, 7–10 µl followed by
<table>
<thead>
<tr>
<th>Area</th>
<th>Coordinates (AP; ML; DV)</th>
<th>Study</th>
<th>Animals (n)</th>
<th>Drug (dose)</th>
<th>Electrical Stim (µA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amy</td>
<td>6.44; 4.2; 7.5</td>
<td>I</td>
<td>Unoper (6), Sham (7), NP (7)</td>
<td>Glu (2.5 and 25 nmol)</td>
<td>M1 (7.3–10.7; 1–3; 0.7–2.2) or LC (-1.30–(-0.68); 1.3; 6.8–7.6)</td>
</tr>
<tr>
<td>A11</td>
<td>6.0; 0.6; 7.5–8.0</td>
<td>IV</td>
<td>NP (5)</td>
<td>Lid (4%/0.5 µl)</td>
<td>30 (M1)</td>
</tr>
<tr>
<td>LC</td>
<td>-1.30–(-0.68); 1.3; 6.8–7.6</td>
<td>I</td>
<td>Unoper (9), NP (9)</td>
<td>30, 70, 100 (LC)</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>II</td>
<td>Sham (7), NP (7)</td>
<td>Lid (4%/0.5 µl)</td>
<td>30 (M1)</td>
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<tr>
<td></td>
<td></td>
<td>II</td>
<td>Sham (6), NP (10)</td>
<td>30, 50, 70, 100 (M1)</td>
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<tr>
<td>M1</td>
<td>7.3–10.7; 1–3; 0.7–2.2</td>
<td>II</td>
<td>Sham (9), NP (21)</td>
<td>Glu (2.5 and 25 nmol)</td>
<td>30 (M1)</td>
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<tr>
<td></td>
<td></td>
<td>II</td>
<td>Sham (9), NP (7)</td>
<td>DHPG (10 nmol)</td>
<td>30 (M1)</td>
</tr>
<tr>
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<td></td>
<td>IV</td>
<td>NP (18)</td>
<td></td>
<td>30 (M1)</td>
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<tr>
<td>RVM</td>
<td>-2.6–(-1.5); 0; 9.0–11.0</td>
<td>III</td>
<td>NP (8)</td>
<td>Musc (50 ng)</td>
<td>50 (M1)</td>
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<td>Spinal</td>
<td></td>
<td>II</td>
<td>Sham [8], NP [8]</td>
<td>Atip (5 µg)</td>
<td>30 (M1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>III</td>
<td>NP [8]</td>
<td>WAY (3 µg)</td>
<td>50 (M1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IV</td>
<td>NP [7]</td>
<td>Raclo (1 µg)</td>
<td>30 (M1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IV</td>
<td>NP (rec)</td>
<td>Raclo (1 µg)</td>
<td>30 (M1)</td>
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<tr>
<td>DStr</td>
<td>8.7–9.7; 3.4–4.2; 4.0–6.8</td>
<td>IV</td>
<td>NP (rec)</td>
<td>Raclo (1 µg), L471,626 (5 µg)</td>
<td>30 (M1)</td>
</tr>
</tbody>
</table>

Table 1  Experimental parameters in the study

Unilateral stereotaxic coordinates of amygdala (Amy), hypothalamic A11 (A11), locus coeruleus (LC), primary motor cortex (M1) and dorsal striatum (DStr). Stereotaxic coordinates of rostroventromedial medulla (RVM). Number of animals (n); healthy control (Unoper), sham control (Sham) and nerve injured (NP) animals. Doses of drugs administered: glutamate (Glu), lidocaine (Lid), DHPG, muscimol (Musc), atipamezole (Atip), WAY-100635 (WAY), raclopride (Raclo), L471,626. Intensity of electrical stimulations in M1 or LC.
10 µl of saline for flushing) with a 50-µl Hamilton syringe (Hamilton Company, Bonaduz, Switzerland). Only those rats were further studied that had no motor impairment before the lidocaine injection but showed a bilateral paralysis of the hind limbs following i.t. administration of lidocaine.

5.5 Preparation for intracerebral drug injections and electric stimulations

For chemical or electric stimulation, a small hole was drilled into the skull, sufficiently big for a 26-gauge electrode-cannula guide (C315G-MS303/2/SPC, PlasticsOne, Roanoke, VA, USA) (II–III), a 26-gauge guide cannula (C315G, PlasticsOne) (I–V) or a concentric bipolar electrode (Rhodes NE-100, David Kopf Instruments, Tujunga, CA, USA) (I–II, IV). The guide cannula was inserted at an angle anterior to the intended injection site in the Str because of the limited access to M1 (IV). The desired injection and stimulation sites are presented in Table 1 (Paxinos and Watson 1998).

5.6 Microinjections

Drugs or saline were microinjected through a 33-gauge stainless steel injection cannula (C315I, PlasticsOne) that was inserted so that it protruded 1 mm below the tip of the 26-gauge guide cannula (C315G, PlasticsOne) (I–IV) or to the level of the a 26-gauge electrode-cannula guide (C315G-MS303/2/SPC, PlasticsOne) (II). The microinjections were performed using a 10-µl Hamilton syringe (Hamilton Company) connected to the injection cannula by polyethylene tubing (Intramedic PE-10, Becton, Dickinson and Company). The volumes of the injections were 0.5 µl (I, II, IV) and 1 µl (III). The efficacy of the injection was monitored by watching the movement of a small air bubble through the tubing. The injection lasted 30 s and the injection cannula was left in place for at least an additional 30 s, and in most cases until the next injection was performed.

I.t. drug injections (II–IV) were performed using a 50-µl Hamilton syringe. The volume of i.t. drug injections was 5 µl, which was followed by a flush with 15 µl of saline. In electrophysiological spinal dorsal horn recordings (IV), drug or saline were injected using a 50-µl Hamilton syringe and applied directly to the surface of the spinal cord, in a volume of 50 µl.
5.7 Behavioral test stimuli

The effect of innocuous mechanical stimulation was assessed by repeated stroking stimulation of the plantar skin of the hindpaw with a brush (IV). The effect of noxious mechanical stimulation was assessed by applying a hemostatic clamp to the tail. The clamp produced pain when applied to the finger of the experimenter (I–II).

A noxious heat stimulus was applied with a feedback-controlled Peltier device (82.8 mm², LTS-3 Stimulator, Thermal Devices Inc., Golden Valley, MN, USA) to the plantar skin of the hind paw. The baseline temperature of the thermode was 35 °C, and during stimulation its temperature was increased to 54 °C (I–IV) or 52 °C (IV) at a rate of 10 °C/s and decreased to the baseline temperature of 35 °C at a rate of 4 °C/s.

Visceral stimulation (I) was determined using colorectal distension at a noxious intensity (80 mm Hg) by inflating with air a 7–8-cm flexible latex balloon inserted transanally into the descending colon and rectum. The pressure in the balloon was controlled by an electronic device (Anderson et al. 1987).

The signals from the thermostimulator and the visceral stimulator were amplified and sampled with a computer via a CED Micro 1401 interface and Spike 2 software (Cambridge Electronic Design, Cambridge, UK).

5.8 Assessment of spinal nociceptive reflex

The heat-evoked hind limb withdrawal response was used to assess spinal nociception. After induction of anesthesia with sodium pentobarbital (40–50 mg/kg i.p.), the assessment of the limb withdrawal did not start until the level of anesthesia was so low that the animal gave a withdrawal response to a noxious pinch of the skin, but did not have spontaneous movements. After that, a low level of anesthesia was maintained by administering sodium pentobarbital at the dose of 15–20 mg/kg/h. For inducing the hind limb withdrawal reflex, noxious heat (54 °C) was applied for 10 s with a Peltier device (see above) to the plantar skin of the hind paw. To determine the latency (I–III) or magnitude (IV) of the heat-induced limb reflex, a piezoceramic movement detector (Siemens Elema Ab, Solna, Sweden) was taped onto the skin over a flexor muscle in the hind limb. The signal from the thermostimulator and the movement detector were amplified and sampled with a computer via a CED Micro 1401 interface and Spike 2 software (Cambridge Electronic Design). Latency was measured from the start of the heat stimulus to the first movement of the hind limb (I–III). Magnitude of limb movement was determined as the area under the curve (AUC) from the start of the heat stimulus to time point 5 s (IV).
5.9 Unilateral electrical stimulations

The electric stimulation of M1 was performed using an electrode-cannula guide (C315G-MS303/2/SPC, PlasticsOne) (II–III) or a concentric bipolar electrode (Rhodes NE-100, David Kopf Instruments) (II, IV). The concentric bipolar electrode was used for the electric stimulation of the LC (I). The tip of the stimulus electrode was placed in the stimulation area indicated by Table 1. Electric stimuli were generated by a constant current stimulator (PSIU6 and Grass S88, Grass Instruments, Quincy, MA, USA). In each experimental condition, only one type of electrode was used. Electric stimulation was delivered at the frequency of 100 Hz (I) and 300 Hz (II–V) (duration of each stimulus pulse: 0.1 ms). Electric stimulation at varying intensities was assessed in lightly anesthetized animals. The intensity of electric stimulation was chosen for each experiment as described in Table 1.

5.10 Spinal antinociception induced by central electrical or chemical stimulation: course of the studies

5.10.1 Assessment of antinociception induced by electrical stimulation of LC (I) and M1 (II)

Spinal antinociceptive effects induced by electric stimulation of the LC (I) and M1 (II) at varying intensities were assessed in lightly anesthetized animals. For further details on stimulus production, see the section on electrical stimulations (above). Different intensities of electric stimulation were applied first in increasing and then decreasing order (Table 1). The mean of the values obtained at each stimulus intensity was used in further calculations; this was done to minimize the effect of a potential change in the level of anesthesia during the experiment. The electric stimulation started 5 s before the heat stimulation of the hind paw and continued until the end of heat stimulation. Duration of the peak temperature was 10 s. Duration of the electric stimulation was 15 s. The interval between each testing was 2 min.

The heat-evoked withdrawal latency was determined in the hind limb ipsilateral to the LC (I) or M1 (II) stimulation, and also ipsilateral to the nerve injury (I, II) or sham operation (II). Additionally, in a group of nerve-injured (I, II) and healthy control animals (I), the heat-evoked withdrawal latency was determined in the unoperated hind limb contralateral to the LC or M1 stimulation. For further details of the method of reflex measurements see the section on the assessment of spinal nociceptive reflexes (above).
5.10.2 Assessment of antinociception induced by chemical stimulation of M1 (II)

The spinal antinociceptive effect induced by glutamate in M1 was assessed in a group of lightly anesthetized neuropathic animals which had an electrode-cannula guide implanted for cortical microinjections. Saline or glutamate at doses of 2.5 or 25.0 nmol (in 0.5 µl) were microinjected into M1 in an ascending order 2 min prior to the heat stimulation of the neuropathic hind paw. The interval between injections was 5–7 min. The mean of the withdrawal latencies evoked by each dose of glutamate or saline for each animal was used in further calculations.

5.10.3 Attempted reversals of the spinal antinociceptive effect of M1 stimulation (II–IV)

The role of descending noradrenergic pathways in M1 stimulation-induced antinociception (II) was assessed by administering i.t. atipamezole, a selective α2-adrenoceptor antagonist, to the lumbar spinal cord of neuropathic and sham-operated control animals. In a separate experiment, the LC was blocked by a microinjection of lidocaine in order to assess its contribution to the M1 stimulation-induced spinal antinociception in the animals used.

The role of descending serotonergic pathways in M1 stimulation-induced antinociception (III) was assessed by administering i.t. WAY-100635, a selective 5-HT1A receptor antagonist, to the lumbar spinal cord of neuropathic animals. To find about a possible role of the RVM, a block of the RVM was induced by microinjecting a GABA_A receptor agonist muscimol into it (III).

The role of descending dopaminergic pathways in M1 stimulation-induced antinociception (IV) was determined by administering i.t. raclopride, a selective D2 dopaminergic antagonist, to the lumbar spinal cord of neuropathic animals. A possible role of the hypothalamic A11 cell group was assessed by blocking it with a microinjection of lidocaine.

Physiological saline was used for control injections. Five (Lid, Musc, WAY), eight (Atip), or ten (Raclo) min after its administration, the latency (II–III) or magnitude (IV) of the heat-induced hind limb reflex was determined in the nerve-injured limb. The doses of drugs and the intensity of electric stimulation were chosen for each experiment as described in Table 1. M1 stimulations were performed in ascending order of intensity in studies II and IV. In study III, M1 stimulations were performed in ascending followed by descending order of intensity. The mean of the repeated results at each stimulus condition was used in the statistical analysis. The results obtained by saline administration within the same study were in the final analysis pooled into one saline control group.
5.11 Electrophysiological recordings

During single unit recordings of LC (I, II) and spinal dorsal horn (IV) neurons, the animal was placed in a standard stereotaxic frame according to the atlas of Paxinos and Watson (1998). Single neuron activity was recorded extracellularly with lacquer-coated tungsten electrodes (impedance 5–7 MΩ at 1 kHz). The signal was amplified and filtered using standard techniques. Data sampling was performed with a computer connected to a CED Micro 1401 interface and using Spike 2 software (Cambridge Electronic Design, Cambridge, UK).

When determining the discharge frequency during heat stimulation (I, IV) or colorectal distension (I), a 10-s time period from the start of the stimulus rise was taken into account: the response during the first 5 s of stimulation was called the early response, and the response during seconds 5–10 of stimulation the late response. Early and late responses were assessed separately. Since the heat-evoked responses of six neurons in the unoperated group were assessed using a heat stimulus of a 5-s duration, only the early heat-evoked response was determined for these neurons (I). When determining the discharge frequencies during heat stimulation in study II, a 3-s period beginning 0.5 s before the paw withdrawal was taken into account. When determining the discharge frequency during a noxious tail pinch (I), a 5-s time period from the start of the pinch was taken into account. Additionally, an after-discharge during a period of 10 s from the end of the pinch was assessed separately.

During the further analysis of the noxious stimulus-evoked responses, the baseline discharge frequency recorded before the noxious stimulation (I, II), drug application (I, II) or electrical M1 stimulation (IV) was subtracted from the discharge frequencies determined during and after stimulation; i.e. positive values represent excitatory responses evoked by peripheral stimulation, and negative values represent inhibitory responses.

5.12 Electrophysiological recordings: course of the studies

5.12.1 Recording of neuronal activity in LC (I, II)

The skull was exposed and a hole drilled for the placement of a recording electrode in the LC (I, II), a guide cannula (26-gauge) in the amygdala (I) or M1 (II), a concentric bipolar electrode in M1 (II), or a 26-gauge electrode-cannula guide in M1 (II). For the LC recordings, the microelectrode was lowered into place according to the stereotaxic coordinates (See Table 1).
5.12.1.1 Characterization of response properties of LC (I, II)

When neuronal firing was observed, the spontaneous activity was first assessed for 2 min. Then the effect of noxious mechanical stimulation was assessed by applying a hemostatic clamp to the tail for 5 s (I, II). After the response to the tail pinch was tested, a noxious heat stimulus of 54 °C for was applied for 10 s with a feedback-controlled Peltier device to the plantar skin of the hind paw ipsilateral to the recording electrode in the LC and ipsilateral to the nerve ligation or sham operation (I). After determining the response to noxious heating of the ipsilateral limb, heat was applied to the hind paw contralateral to the LC recording (I). After assessing the heat-evoked responses of the LC neuron, the response to a 10-s visceral stimulation (colorectal distension) was determined (I). The testing procedure, including the order in which different submodalities of nociception were tested, was the same in all experimental groups (I).

5.12.1.2 Influence of central glutamatergic stimulation on ongoing neuronal activity in LC (I–II)

For the purpose of searching and characterizing response properties of LC (I, II) neurons, only a hemostatic clamp to the tail (I) was applied. The LC neurons that gave an excitatory response to noxious pinch were considered likely to be noradrenergic neurons involved in the feedback inhibition of pain (Hirata and Aston-Jones 1994). After the characterization of a LC neuron in a lightly anesthetized animal, recordings of LC neurons and drug injections into the amygdala (I) or M1 (II) were performed ipsilateral to the spinal nerve ligation (I–II) or sham operation (I–II). The effect of M1 or amygdala stimulation on the ongoing discharge rate of the LC was assessed by microinjecting saline or drugs into the amygdala (I) or M1 (II) (Table 1). The discharge rate of the LC neuron was recorded before, during and up to 3 min (I, II) or 32 min (II) following each injection. In the final analysis only those discharge rates were considered that had been measured during the second (II) or third minute (I) following glutamate or saline injection. In study II, the discharge rate from the end of a DHPG injection up to the 11th min after injection was taken into account in the data analysis. The doses of the drugs are given in Table 1.

In the calculations, the post-injection discharge rate was first compared with the corresponding pre-injection rate by subtracting the pre-injection rate from the post-injection rate (I, II). In study II, the difference between the post-saline and pre-saline activity was subtracted from the difference between the post-drug and pre-drug activity. A positive value represents a true drug-induced increase in the ongoing discharge rate, and a negative one a drug-induced decrease.
5.12.1.3 Influence of electrical stimulation of M1 on ongoing activity and heat-evoked responses of LC neurons (II)

The effects of electric stimulation of M1 on the ongoing activity of the LC and the heat-evoked responses of LC neurons (II) were studied under light pentobarbital anesthesia. After searching for and characterizing the response-properties of a LC neuron to tail pinch, its spontaneous activity and heat-evoked response were studied without and with electric M1 stimulation (Table 1). Electric stimulation was applied to the M1 ipsilateral to LC and/or nerve injury for 15 s either starting 5 s prior to 54 °C heat stimulation of the plantar skin in the injured paw, or starting without accompanying heat stimulation; this procedure ensured the assessment of the influence of M1 stimulation per se on the neuronal discharge rate. The intensity of the electric stimulation is given in Table 1.

5.12.2 Recording of neuronal activity in the spinal cord (IV)

Following the induction of anesthesia, a laminectomy was performed at the level of the vertebrae T12–L2, the dura removed, a pool of skin formed and filled with either warm mineral oil or with physiological saline when studying the effects induced by spinally administered drugs. Two spinal clamps, one rostral and one distal to the laminectomy, were used to stabilize the preparation.

The skull was exposed and a hole drilled for the placement of a guide cannula (26-gauge) in the Str and a concentric bipolar electrode in M1. Before the recordings and stimulations, the tip of the guide cannula was positioned 1 mm above the desired injection site in the Str. Desired injection and electrical stimulation sites are shown in Table 1.

5.12.2.1 Characterization of response properties of spinal dorsal horn neurons

During the search for spinal neurons, the plantar skin of the hindpaw was repeatedly stimulated with a brush at an innocuous intensity. After finding a neuron that responded to the stimulation, noxious heat of 52 °C was applied for 10 s by a feedback-controlled Peltier thermode. The neurons were classified as wide-dynamic range (WDR) neurons if they gave responses to both brush stimulation and noxious heat. The neurons classified as nociceptive-specific (NS) responded to noxious heat stimulation but failed to respond to innocuous brush stimulation. Low-threshold mechanoreceptive neurons were not further considered in this study. Only the neurons that were considered to be in the spinal dorsal horn were included (recording depth <1000 µm from the cord surface; Willis and Coggeshall 1991).
5.12.2.2 Influence of electrical stimulation of M1 on heat-evoked responses of spinal dorsal horn neurons and attempted reversal of the spinal inhibition induced by M1 stimulation

After the characterization of a spinal dorsal horn neuron, the role of striatal or spinal dopamine D<sub>2</sub> receptors in the antinociceptive effect was assessed by administering raclopride, a selective dopamine D<sub>2</sub> receptor antagonist, or physiological saline into the Str or onto the spinal cord. In control experiments, L-741,626, a dopamine D<sub>2</sub> receptor antagonist with a structure different from that of raclopride, was administered into the Str. The effects induced by striatal and spinal drug deliveries were studied in separate groups of animals. The response of the recorded spinal dorsal horn neuron was determined 10–15 min after the administration of saline or raclopride, first without and then 2 min later with M1 stimulation. The doses of the drugs and the intensity of electric stimulation are given in Table 1.

5.13 Drugs

3,5-dihydroxyphenylglycine (DHPG), a group I metabotropic glutamate receptor agonist, was purchased from Tocris Bioscience (Bristol, UK). An α<sub>2</sub>-adrenoceptor antagonist, atipamezole, and lidocaine were purchased from Orion Pharma (Turku, Finland). Glutamate, muscimol hydrobromide (a GABA<sub>A</sub> receptor agonist), raclopride (a dopamine D<sub>2</sub> receptor antagonist) and WAY-100635 or N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinylcyclohexanecarboxamide maleate salt (a selective 5-HT<sub>1A</sub> receptor antagonist) were purchased from Sigma-Aldrich (St. Louis, MO, USA). L-741,626 (a dopamine D<sub>2</sub> receptor antagonist with a structure different from that of raclopride) was purchased from Tocris Bioscience (Bristol, UK). Physiological saline was used as a control.

5.14 Statistical analysis

The data are presented as mean ±SEM. Statistical analysis was performed with Prism 4 and 5 for Windows software (GraphPad Software Inc., La Jolla, CA, USA) (I–IV) when one- or two-way analysis of variance (1-w- or 2-w-ANOVA) was conducted (I–IV). Statistical analysis was performed with STATISTICA (StatSof Inc., Tulsa, OK, USA) for Windows software when repeated-measures (rmANOVA) were conducted (II). Post hoc testing was performed by Tukey’s test (I–IV) or a t-test with a Bonferroni correction for multiple comparisons (II–IV). Comparisons between two groups were performed by t-test. P<0.05 was considered to represent a significant difference.
**Figure 3** Timeline of the experiments. Day 0 represents the day when the nerve ligation was performed. Amygdala (Amy), glutamate (Glu), hypothalamic dopaminergic nuclei (A11), locus coertuleus (LC), primary motor cortex (M1).
6 RESULTS

All spinal nerve-ligated rats included in the studies developed mechanical hypersensitivity, as indicated by the monofilament-induced limb withdrawal threshold that was always $<2$ g ipsilateral to the nerve injury. Only those results were included which the post-mortem histological analysis confirmed to have been obtained by stimulation or injection within the intended target area; i.e. results obtained by stimulations in sites within the M1 cortex (II–IV) or the LC (I), by intracerebral injections within the LC (II), amygdala (I), M1 (II), RVM (III), DStr (IV), or the hypothalamic A11 nucleus (IV), and by single cell recordings of neurons inside the LC (I, II).

6.1 Spinal antinociception induced by stimulation of M1 (Study II)

Electrical stimulation of M1 produced significant antinociception either in the ipsilateral nerve-injured or sham-operated hind limb. The magnitude of thermal antinociception varied with the intensity of M1 stimulation: the stimulus intensities from 0 to 50 μA increased the magnitude of antinociception while a further increase of stimulus intensity up to 140 μA decreased it (Fig. 4a, Table 2; See 70 and 120 μA in Study II, Fig. 2a). The effect of electric stimulation of the ipsilateral M1 on the magnitude of the thermal antinociception was not significantly different between the sham-operated and nerve-injured animals. Moreover, in nerve-injured animals, electric stimulation of M1 ipsilateral to the injury produced antinociception also in the contralateral healthy hind limb (Fig. 4a). However, the magnitude of thermal antinociception was smaller in the healthy limb contralateral to M1 stimulation than in the ipsilateral nerve-injured limb.

Chemical stimulation of M1 by glutamate failed, independent of dose (2.5 or 25 nmol), to induce a significant prolongation of the heat-evoked hind limb withdrawal latency in the injured hind limb ipsilateral to M1 stimulation (Table 2; See Study II, Fig. 2b).
6.2 Spontaneous discharge rates and heat-evoked responses of spinal dorsal horn neurons following electric stimulation of M1 (Study IV)

When analyzing noxious heat-evoked responses of spinal dorsal horn neurons, the early (0–5 s) and late (5–10 s) responses were assessed separately. In spinal dorsal horn WDR and NS neurons, the magnitude of the late heat-evoked response was stronger than that of the early one. Electric stimulation of M1 (30 µA) produced a significant suppression of the heat-evoked response in WDR and NS neurons (Fig. 4b, Table 2), independent of the response latency. Electric stimulation of M1 at the intensity of 30 µA failed to influence the spontaneous discharge rates of spinal WDR or NS neurons (See Study IV, Fig. 4b).

![Figure 4](image_url)

**Figure 4** (A) Influence of M1 cortex stimulation on noxious heat-evoked hind limb withdrawal latency at varying intensities in neuropathic (Neurop) and sham control (Sham) animals and, (B) baseline responses in spinal dorsal horn wide-dynamic range (WDR) and nociceptive-specific (NS) neurons in neuropathic animals. In A, Ipsi = M1 stimulation ipsilateral to the studied limb and nerve injury or sham operation, Contra = M1 stimulation contralateral to the studied healthy limb. In B, heat stimulation was applied ipsilateral to the studied limb and nerve injury. In B, the Y-axis represents the heat-evoked increase in the discharge rate. Early (0–5s) and late (5–10 s) responses were analyzed separately. §/* P <0.05, ** P<0.01 (t-test with a Bonferroni correction; reference: the corresponding latency without electric stimulation 0 µA). + P<0.05, ++ P<0.01, +++ P<0.005 (t-test with a Bonferroni correction for multiple comparisons; the reference was the corresponding early response). Error bars represent SEM.
6.3 Role of descending noradrenergic pathways in M1 stimulation-induced antinociception

6.3.1 Influence of peripheral nerve injury on spontaneous activity and response properties of locus coeruleus neurons (Study I)

The spontaneous discharge rates of LC neurons among neuropathic and sham-operated control animals failed to differ significantly (Fig. 5a, Table 2). The responses of LC neurons evoked during noxious tail pinch (5 s) were significantly increased in the neuropathic group (Fig. 5b, Table 2). The after-discharge (during 10 s after the end of pinch), which was separately assessed, failed to differ significantly between the experimental groups (See Study I, Fig. 4b). The responses of LC neurons evoked by noxious heat applied to the hind paw showed significant differences between the experimental groups (Fig. 5c, Table 2).

![Figure 5](image-url) Response properties of LC neurons in neuropathic (Neurop) and sham control (Sham) animals. (A) Spontaneous discharge rates. (B) Responses to noxious tail pinch. (C) Responses to noxious heating of the hind paw. Ipsilateral, heat stimulation applied to the operated limb, ipsilateral to the LC studied; Contralateral, heat stimulation applied to the unoperated limb, contralateral to the LC studied. In B and C, the Y-axis represents the stimulus-evoked increases in the discharge rate. * P<0.05, ** P<0.01 (Tukey’s test). Error bars represent SEM.
Independent of the ipsilateral or contralateral test side, both the early response (during the first 5 s of thermal stimulation) and the late response (during the following 5 s of thermal stimulation) were enhanced in neuropathy. Moreover, heat produced the strongest responses in the neuropathic group, independent of the response latency (early versus late response). The responses of LC neurons to colorectal distension at a noxious intensity were weak and failed to differ among the experimental groups (See Study I, Fig. 4d), also independent of response latency (early versus late response). See unoperated control animals in Study I: Fig 4a–d.

6.3.2 Central drive from the amygdala to the LC (Study I)

Microinjection of glutamate into the central nucleus of the amygdala produced within two minutes a dose-related decrease in the discharge rate of LC neurons in the neuropathic group but not in the control group (Fig. 6c, Table 2; See 2.5 nmol in Study I, Figs. 5, 6).

6.3.3 Spinal antinociception induced by electrical stimulation of the LC (Study I)

Electric stimulation of the LC ipsilateral to the studied hind limb increased with increasing stimulation intensity the latency of the heat-evoked hind limb withdrawal and thus promoted thermal antinociception (Table 2; See Study I, Fig. 7a). This thermal antinociceptive effect was significantly stronger in the unoperated controls than in nerve-ligated animals. The effect of contralateral LC stimulation failed to produce a significant effect on the heat-evoked withdrawal latency in either of the experimental groups (See Study I, Fig. 7b).

6.3.4 Discharge rates and heat-evoked responses of LC neurons following stimulation of M1 (Study II)

Administration of glutamate into M1 produced in nerve-injured animals a slight increase in the ongoing discharge rate of LC neurons, which was significant at a dose of 25 nmol but not at a dose of 2.5 nmol. Glutamate administration into M1 failed to influence the discharge rates of LC neurons in sham-operated animals, independent of the dose (Fig. 6a, Table 2; 2.5 nmol in Study II, Figs. 3, 4a). Cortically administered DHPG, a group I metabotropic glutamate receptor agonist, produced a slight increase in the discharge rates of LC neurons, which was significant in the nerve-injured group and short of significance in the sham-operated group (Fig. 6a, Table 2). Electric stimulation of M1 at an intensity of 30 μA produced a significant increase in the discharge rate of LC neurons, irrespective whether the M1 stimulation was accompanied by heat stimulation or not (Fig. 6b, Table 2).
Figure 6  (A) Mean changes in the discharge rates of LC neurons 1–2 min following injection of 25.0 nmol of glutamate (Glu) or during the first 10 min following injection of 10 nmol of DHPG into M1. (B) Mean changes in the discharge rates and heat-evoked responses of LC neurons by ipsilateral M1 stimulation in neuropathic animals. (C) Mean changes in the baseline discharge rates of LC neurons 2–3 min following injection of 25.0 nmol Glu into the amygdala. In A and C, Sham control (Sham) and neuropathic (Neurop) animals. In A and C, 0 Hz in the Y-axis represents the effect of saline injection into M1 or amygdala, and values >0 Hz indicate that glutamate or DHPG increased the discharge rates of neurons while values <0 Hz indicate that glutamate decreased their discharge rates. For comparison with Study II, data from Study I were recalculated in C. In A and C, + P<0.05, ++ P<0.01, +++ P<0.005 (paired t-test, reference: the corresponding saline group). In B, # P<0.05 (Tukey’s test, reference: the corresponding value without M1 stimulation) and * P<0.05 (Tukey’s test, reference: the corresponding value without heat stimulation). Error bars represent SEM.
6.3.5 Spinal antinociception induced by M1 stimulation following a block of the LC or of spinal α2-adrenoceptors (Study II)

Blocking the LC by lidocaine, or the spinal α2-adrenoceptors by atipamezole (an α2-adrenoceptor antagonist) failed to produce a significant attenuation of the M1 stimulation-induced spinal antinociception in neuropathic animals (Fig. 7a, Table 2; See Study II: Table 1).

6.4 Role of descending serotonergic pathways in M1 stimulation-induced antinociception

6.4.1 Spinal antinociception induced by M1 stimulation following a block of the RVM or of spinal 5-HT1A receptors (Study III)

Blocking the RVM by muscimol, a GABA<sub>A</sub> receptor agonist, significantly attenuated the spinal antinociception induced by electric stimulation of M1 (Fig. 7b, Table 2). Additionally, the blocking of the spinal 5-HT<sub>1A</sub> receptors by WAY-100635 (a 5-HT<sub>1A</sub> receptor antagonist) resulted in the same effect (Fig. 7b, Table 2). Moreover, muscimol in the RVM, but not WAY-100635 in the spinal cord, promoted spinal nociception in the baseline condition (without M1 stimulation) (Fig. 7b).

6.5 Role of descending dopaminergic pathways in M1 stimulation-induced antinociception

6.5.1 Role of striatal and spinal dopamine D<sub>2</sub> receptors in M1 stimulation-induced inhibition of heat-evoked responses of spinal WDR and NS neurons (Study IV)

Blocking the striatal or spinal dopamine D<sub>2</sub> receptors by raclopride failed to influence the spontaneous discharge rates of spinal dorsal horn WDR neurons, whereas the same raclopride treatment had a significant effect on the spontaneous discharge rates of spinal dorsal horn NS neurons (See Study IV, Fig. 4a) in nerve-injured animals. The mean spontaneous discharge rate of NS neurons was decreased following striatal treatment with raclopride. Electric stimulation of M1 (30 μA) failed to induce changes in the spontaneous firing-rates of WDR or NS neurons, independent of striatal or spinal coadministrations of raclopride (See Study IV, Fig. 4b).
Figure 7  (A) An attempt to attenuate the spinal antinociceptive effect of M1 stimulation by intrathecal administration of atipamezole (Atip; an α₂-adrenoceptor antagonist: 5 µg) or by administration of lidocaine (Lid: 4 %, 0.5 µl) into the LC, (B) An attempt to attenuate the spinal antinociceptive effect of M1 stimulation by intrathecal administration of WAY-100635 (WAY; a 5-HT₁A antagonist: 3 µg) or by administration of muscimol (Musc: 50 ng) into the RVM, (C) An attempt to attenuate the spinal antinociceptive effect of M1 stimulation by intrathecal administration of raclopride (Raclo; a dopamine D₂ receptor antagonist: 1 µg) or by administration of lidocaine (Lid: 4 %, 0.5 µl) into the hypothalamic A11. For comparison with Study III and IV, the data from Study II were recalculated in A. * P<0.05, ** P<0.01, *** P<0.005 (Tukeys´s test; reference was the corresponding saline group). * P<0.05, ** P<0.01, *** P<0.005 (t-test; reference was the corresponding drug-treated group without electric stimulation of M1). Error bars represent SEM. All results obtained in animals with a peripheral neuropathy.
When assessing the effects of raclopride treatments and electric M1 stimulation on heat-evoked responses, the early and late responses were pooled into one mean heat response (0–10 s). Striatal but not spinal administration of raclopride enhanced the heat-evoked responses of WDR neurons as demonstrated by the responses determined without M1 stimulation (Fig. 8a, Table 2). In addition, M1 stimulation failed to produce a significant suppression of heat-evoked responses of WDR neurons following a striatal or spinal block of dopamine D2 receptors by raclopride (Fig. 8a, Table 2). Moreover, in a separate group of nerve-ligated animals, M1 stimulation failed to produce a significant suppression of heat-evoked response of WDR neurons following striatal administration of L-741,626 (a highly selective dopamine D2 receptor antagonist with a structure different from that of raclopride) (See Study IV, Results).

A striatal raclopride-induced block of dopamine D2 receptors enhanced the M1 stimulation-induced inhibition of the heat response in NS neurons, whereas a corresponding spinal block of dopamine D2 receptors resulted, in contrast, in an enhancement of the heat-evoked responses in spinal dorsal horn NS neurons by M1 stimulation (Fig. 8b, Table 2).

### 6.5.2 Spinal antinociception induced by M1 stimulation following a block of spinal dopamine D2 receptors or of A11 (Study IV)

Electrical stimulation of the M1 of neuropathic animals produced spinal antinociception in the saline-treated group, whereas it was ineffective in the groups in which the spinal dopamine D2 receptors were blocked by intrathecally administered raclopride or the ipsilateral A11 was blocked by microinjection of lidocaine (Fig. 7c, Table 2).
A. WDR

![Graph A: Influence of M1 stimulation on the heat-evoked responses of spinal dorsal horn wide-dynamic range (WDR) neurons.](image1)

B. NS

![Graph B: Influence of M1 stimulation on the heat-evoked responses of nociceptive-specific (NS) neurons.](image2)

**Figure 8** Influence of M1 stimulation on the heat-evoked responses of (A) spinal dorsal horn wide-dynamic range (WDR) and (B) nociceptive-specific (NS) neurons following administration of saline (Sal) or raclopride (a dopamine D2 receptor antagonist: 1 µg) into the dorsal striatum (Raclo ic) or onto the spinal cord (Raclo it) in neuropathic animals. Early (0–5 s) and late (5–10 s) responses were analyzed separately. In the Y axis, 0 Hz represents the spontaneous discharge rate in each drug treatment condition before cortical and peripheral stimulations. Y-axis values >0 Hz represent heat-induced increases in the discharge rate. The data from Study IV were recalculated in A and B. 

# P<0.05 (paired t-test with a Bonferroni correction; reference: the corresponding value without M1 stimulation). 

** P<0.01 (t-test with a Bonferroni correction; reference: the corresponding value without M1 stimulation). 

+ P<0.05, ++ P<0.01, +++ P<0.001 (t-test with a Bonferroni correction; reference: the corresponding saline group). Error bars represent SEM.
Table 2  Main results in neuropathic animals

<table>
<thead>
<tr>
<th>Area</th>
<th>Study</th>
<th>Drug</th>
<th>Electrical stimulation</th>
<th>Pain</th>
<th>Influence on spontaneous activity</th>
<th>Influence on evoked-responses</th>
<th>M1-induced antinociception</th>
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<tr>
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<tr>
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<td>LC ↔</td>
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Main effects of electrical stimulations and drugs used on pain behavior, spontaneous and peripheral stimulus-evoked neuronal activity, or M1 stimulation-induced spinal antinociception in neuropathic animals. Amygdala (Amy), hypothalamic A11 cell group (A11), locus coeruleus (LC), primary motor cortex (M1), influence of peripheral nerve injury on response properties of LC neurons (NP), rostroventromedial medulla (RVM), and dorsal striatum (DStr), wide-dynamic range spinal neuron (WDR), nociceptive-specific spinal neuron (NS), colorectal distension (crd). Arrows pointing down ↓ = attenuate/suppress, arrows pointing down and in parentheses (↓) = may attenuate, arrows pointing up ↑ = enhance, horizontal arrows ↔ = no effect, empty = not studied.
7 DISCUSSION

7.1 Methodological considerations

The present series of studies was performed *in vivo* (in the living animal) because nociception and pain processes are based on complex neuronal networks including both peripheral and central nervous systems. The number of animals used was a minimum needed to produce reliable scientific data. After completing a study, the animals were given a lethal dose of pentobarbital.

As sex hormones may influence pain processes and descending modulation of pain, the processing of pain might differ between females and males (Aloisi and Bonifazi 2006). Additionally, the cyclic variation of the sex hormone level needs to be taken into account when studying females. To avoid the complicating factor constituted by sex hormones, only males were used as experimental animals. This limitation needs to be taken into account when interpreting the present results on the M1 stimulation-induced regulation of neuropathic pain.

The currently used animal model of neuropathic pain induces mechanical allodynia- and hyperalgesia-like symptoms, similar to those in human patients (Ossipov et al. 2006, Saadé and Jabbur 2008). Since nerve injury does not always produce allodynia, it was verified in the present study by behavioral testing that only those spinal nerve-ligated rats that had a marked mechanical hypersensitivity and no motor impairment were accepted in the neuropathic study groups. Nerve injury may change the distribution of an animal’s weight over its limbs, which may be a significant confounding factor when assessing hypersensitivity in some experimental pain models. However, monofilament stimulation has been shown to be a valid method for the assessment of mechanical hypersensitivity in the currently used spinal nerve injury model of neuropathy (Kauppila et al. 1998b).

In this series of studies, several types of stimuli were used to assess pain behavior. Among the test stimuli used there were, in addition to light touch, also noxious mechanical, visceral and thermal stimuli. Of these, the noxious ones induce pain in healthy volunteers as well as in pain patients, while touch evokes pain (tactile allodynia) only in patients suffering from nerve injury-induced pain (Ossipov et al. 2006, Saadé and Jabbur 2008). In the present investigation, noxious stimuli were given at intervals that were appropriate for avoiding sensitization. Moreover, as the assessment of spontaneous pain sensation is a challenge in experimental animals, the spontaneous activity of nociceptive neurons were also studied in an attempt to find a potential correlate for spontaneous pain.

The piezoceramic device used for measuring the hind limb withdrawal responses is a noninvasive, quantitative method for evaluating the magnitude of various components of nocifensive withdrawal reflexes (Hämäläinen et al. 1996). An increase in the amplitude
of the withdrawal response (IV) or a decrease in the response latency (I–III) were both considered to represent enhanced pain sensation, although one needs to be cautious when interpreting spinal reflex responses in terms of pain perception.

Anesthesia is meant to influence nociceptive responses. For instance, pentobarbital anesthesia inhibits dopaminergic neural activity in the Str (Adachi et al. 2006, Ford and Marden 1986). However, anesthesia was not likely to be responsible for the differences found between the experimental groups in the present series of studies because it was induced and maintained in an identical manner in all groups. Moreover, repeated measurements, saline control conditions, and the same order of administering the drugs or delivering the electric stimulations in the different experimental groups reduced the potentially confounding effects of anesthesia on the results. Although sodium pentobarbital is a respiratory and cardiovascular depressant possibly producing hypoxemia, hypercapnia, and prolonged hypotension (Hedenqvist and Hellebrekers 2003), it is an moderate analgesic and sedative when vital functions are constantly monitored. In the present study, the size of the pupils, the general muscle tone, noxious stimulation-induced reflex responses, spontaneous breathing, peripheral blood circulation and the body temperature were monitored during the experiments to ensure an adequate depth of anesthesia. A single bolus of sodium pentobarbital is enough to produce 30–60 min surgical anesthesia (Field et al. 1993), which is enough for performing unilateral nerve ligation and i.t. catheterization. When performing electrophysiological recordings and measuring withdrawal reflexes, the dose of pentobarbital was reduced to a level that allowed assessing brief reflex responses but that was sufficient to suppress spontaneous pain. Once a stable level of anesthesia was achieved additional doses were given when indicated by the constant monitoring of the anesthesia. Pentobarbital has been commonly used in previous electrophysiological assessments of the pain system, allowing comparisons of the present results with the previous literature. It should be noted, however, that any type of general anesthesia might influence neuronal responses, which is a potentially complicating factor when comparing neuronal results obtained under general anesthesia with behavioral results without anesthesia.

Unilateral electric stimulation of the cortex may produce adverse effects and impair motor performance. In the present series of studies, intensity and frequency of the electric stimuli were kept at levels where abnormal behaviors were not observed. An earlier study in experimental animals showed that epidural motor cortex stimulation at an intensity sufficient to produce antinociception failed to induce, in the course of the stimulation sessions, motor or other behavioral impairment as measured in the open-field test (Fonoff et al. 2009). Additionally, in humans MCS is considered a safe treatment for chronic neuropathic pain (Fontaine et al. 2009, Nguyen et al. 1999). Two types of electrodes were used: a concentric bipolar electrode and an electrode consisting of two closely spaced wires with an accompanying cannula guide. Electric stimulation of M1 with both types of electrodes produced spinal antinociception (II–IV). In each experiment, only one type of electrode was used; thus any differences found between the experimental conditions cannot be explained by a difference in the cortical stimulus electrodes.
The intracerebral drug applications and electric stimulations of the primary motor cortex (M1) were performed ipsilateral to the spinal nerve ligation (II–IV) or sham operation (II), with one exception: the electric M1 stimulation was applied contralateral (II) to the limb studied in one group of nerve-injured animals. Senapati et al. (2005) used identical parameters for the electric M1 stimulation. They found that the antinociceptive effect in the rat spinal dorsal horn was of the same magnitude ipsilateral as contralateral to the M1 stimulation (Senapati et al. 2005). The focus of the present series of studies was, however, on effects ipsilateral to the M1 stimulation and the nerve injury (II–IV) or sham surgery (II). This was done to reduce potential motor artifacts that might interfere with the withdrawal responses. Although some corticospinal axons descend ipsilaterally to the spinal cord in the rat (Joosten et al. 1992), a possible confounding effect due to activation of limb muscles by M1 stimulation was expected to be insignificant when testing sensory responses in the side ipsilateral to the M1 stimulation.

The drug applications to the hypothalamic A11 cell group (IV) and LC (II), and the recordings of LC neurons (I–II) were performed ipsilaterally to the nerve injury and the studied spinal response, since spinal projections of these structures descend mainly ipsilaterally (Hökfelt et al. 1979, Mokha et al. 1986, Qu et al. 2006, Skagerberg and Lindvall 1985, Tsuuruoka et al. 2004). Moreover, previous results have indicated that the projections of M1 to the brainstem are strongest ipsilaterally (Keizer et al. 1987). Additionally, results in the rat have indicated that stimulation of the A11 cell group (Wei et al. 2009) or LC (I) produce descending antinociception that is stronger ipsilaterally than contralaterally to the stimulation. Similarly, the chemical manipulations of the Str were performed ipsilateral to M1 stimulation and nerve injury (IV) because the main projections from the M1 to the Str are ipsilateral (Ebrahimi et al. 1992, McGeorge and Faull 1987, 1989, Wan et al. 1992). Moreover, chemical stimulation of the Str (Ansah et al. 2007, Pertovaara and Wei 2008) produced descending antinociception that was in fact stronger ipsilaterally than contralaterally.

### 7.2 Spinal antinociception induced by stimulation of M1

The results of the thesis indicate that electric stimulation of M1 produces spinal antinociception in nerve-injured (II–IV) as well as in sham-operated control animals (II). The pain-attenuating role of M1 was demonstrated by the results of study IV: in experimental neuropathy electric M1 stimulation was shown to attenuate heat-evoked responses of nociceptive spinal dorsal horn neurons. Additionally, electric M1 stimulation was also found to attenuate heat-evoked responses of nociceptive spinal dorsal horn neurons in control animals (unpublished data). The present findings are in line with previous experimental results showing that electric stimulation of M1 suppresses pain-related behavior under neuropathic (Fonoff et al. 2009, Lucas et al. 2011, Pagano et al. 2011, Rusina et al. 2005, Vaculin et al. 2008) as well as control conditions (Rojas-Piloni
et al. 2010, Senapati et al. 2005). Furthermore, previous studies have shown in line with the present results, an attenuation of responses in nociceptive spinal dorsal horn neurons (Rojas-Pili
ti et al. 2010, Senapati et al. 2005) and a decrease in noxious stimulation-induced Fos immunoreactivity in the spinal dorsal horn (Pagano et al. 2011) by M1 stimulation. Collectively, these studies indicate that M1 stimulation may suppress pain-related spinal reflex responses due to an action on spinal sensory rather than motor neurons.

M1 stimulation failed to influence the spontaneous discharge rates of nociceptive spinal dorsal horn WDR and NS neurons in study IV, supporting earlier results (Rojas-Piloni et al. 2010, Senapati et al. 2005). This suggests that the M1 stimulation-induced spinal antinociception was predominantly due to actions presynaptic to the studied spinal neurons, possibly reflecting descending effects on interneurons mediating nociceptive inputs to the studied neurons or a direct modulation of transmitter release from peripheral primary afferent fiber terminals (Canedo 1997, Umeda et al. 2010). Accordingly, several studies have indicated that the pain modulation pathways descending to the spinal dorsal horn modulate the function of spinal dorsal horn neurons by means of a presynaptic as well as postsynaptic action (Fleetwood-Walker et al. 1988, Garraway and Hochman 2001a, Pertovaara et al. 1997, Wei et al. 2001). It should also be noted that descending serotonergic (III) and dopaminergic (IV) pathways, which were shown to be involved in the spinal antinociceptive effect induced by M1 stimulation, are known to modulate spinal nociception, at least partly, by presynaptic action (Millan 2002).

Although the present results support earlier findings as to demonstrating top–down antinociception by M1 stimulation, there are also some differences. In studies II–IV, antinociception was primarily studied and found ipsilateral to the cortical stimulation site. Some of the earlier data in experimental animals indicate that M1 stimulation induces antinociception bilaterally (Lucas et al. 2011, Senapati et al. 2005) while some other studies claim that M1 stimulation induces antinociception mainly in the contralateral side (Fonoff et al. 2009, Pagano et al. 2011, Rojas-Piloni et al. 2010, Rusina et al. 2005, Vaculin et al. 2008). Moreover, earlier findings suggest that M1 stimulation may suppress pain behavior more effectively in nerve-ligated than control animals (Vaculin et al. 2008). The results of the present thesis indicate that M1 stimulation produces spinal antinociception in both nerve-ligated and sham-operated control animals (II). These differences are possibly explained by the various types of experimental parameters. The parameters for the stimulation of M1 in this thesis (0.1 ms pulses at 300 Hz for 10–15 s) (II–IV) were adopted from a previous study by Senapati and colleagues (2005) suggesting that the M1 stimulation-induced spinal antinociception is effective both ipsilaterally and contralaterally. In studies reporting mainly contralateral antinociceptive effects, in contrast, longer pulse durations (0.2 ms), lower stimulus frequencies (25–60 Hz), and longer stimulation periods (15 min) were used for cortical stimulation (Rusina et al. 2005, Vaculin et al. 2008). The diverse stimulus parameters used for M1 stimulation may activate, at least partly, different pain modulatory mechanisms, thus possibly producing the different outcome.
Interestingly, a recent study in spinal cord-injured animals indicated that M1 stimulation induces long-term antinociception bilaterally with ipsilateral predominance; and when the stimulus frequency was high enough, the contralateral effect was attenuated (Lucas et al. 2011). Similarly, in study II an increase in the intensity of M1 stimulation resulted in an attenuation of its contralateral effect. These results indicate that different cortical stimulation frequencies and intensities may influence M1 neurons diversely and produce varying antinociceptive effects. Paradoxically, intense electrical stimulation may also have an inhibitory influence on the neurons close to the stimulation electrode, while neurons far from the electrode are activated (Ranck 1975). On the basis of previous studies, it may, however, be estimated that electric stimulation at the intensities used in the present series of studies had a direct effect on neurons in an area with a diameter of about 1 mm (Ranck 1975). Moreover, the results of study II suggest that repetitive electric stimulation of M1 is a more effective way for activating descending pathways originating in the M1 than a cortical administration of a single bolus of glutamate or DHPG. This result may reflect a difference in the antinociceptive effect induced by intense or weak stimulation of the M1. Furthermore, differences between the experimental animal models (spinal nerve ligation, spinal injury, chronic constriction injury of a peripheral nerve, or dorsal root rhizotomy) and the anesthetic conditions (light pentobarbital anesthesia, ketamine/xylazine anesthesia, or awake) may contribute to differences in the results obtained in the studies on neuropathic animals (Lucas et al. 2011, Rusina et al. 2005, Vaculin et al. 2008).
7.3 Descending noradrenergic pathways in M1 stimulation-induced antinociception

7.3.1 Influence of peripheral nerve injury on spontaneous activity and response properties of LC neurons (Study I)

The results of study I showed that the spontaneous activity of LC neurons failed to change significantly after peripheral nerve injury. Spinal nerve injury produces a tonic increase in the activity of peripheral nociceptive nerve fibers, an increased spontaneous activity of nociceptive spinal dorsal horn neurons and a tonic increase in the ascending nociceptive activity, all of which promote neuropathic pain (Ossipov et al. 2006, Saadé and Jabbur 2008). This increase in ascending nociceptive activity, via action on various brainstem nuclei including the noradrenergic LC, is likely to activate feedback inhibition (Menetrey et al. 1980, Westlund and Craig 1996). Furthermore, earlier and recent studies indicate that the baseline general metabolic activity and gene transcription in LC neurons are increased in animals with experimental neuropathy (Brightwell and Taylor 2009, Mao et al. 1993). Therefore, the baseline activity of LC neurons was expected to increase in neuropathic animals. The results in study I, however, showed only a slight increase in the spontaneous discharge rate of LC neurons in nerve-ligated animals. This could be explained by an increased $\alpha_2$-adrenergic autoinhibition of LC neurons in spinal nerve-ligated animals as observed by Wei and Pertovaara (2006a). Moreover, discordant previous and present results may be explained by the differences in the experimental conditions, such as the experimental animal model (chronic constriction injury of a peripheral nerve vs. spinal nerve injury), methods of anesthesia, or metabolic versus electrical activity.

The results of study I showed that the responses of LC neurons to noxious somatic mechanical or thermal stimuli were significantly enhanced in nerve-ligated animals, while the effect of peripheral neuropathy on the response evoked by noxious visceral stimulation was short of significance. The activation of LC neurons in control animals induced by noxious somatic and visceral stimulation is in line with the results of some earlier studies (Elam et al. 1986a, 1986b, Ennis et al. 1992, Hajós et al. 1986, Hirata and Aston-Jones 1994, 1996). The viscerally evoked LC responses were weaker than the responses evoked by somatic mechanical or thermal stimulation, independent of the experimental group. In neuropathic animals, the responses of LC neurons to noxious somatic stimulation were enhanced when applied both to the injured dermatome and outside of it. This finding supports the previous evidence (Ossipov et al. 2006, Saadé and Jabbur 2008) indicating that central mechanisms contribute to hypersensitivity in peripheral neuropathy. It remains to be studied whether the increased responses of LC neurons to noxious somatic stimuli reflect the nerve injury-induced changes in the evoked responses to noxious stimuli ascending from the spinal dorsal horn (Ossipov et al. 2006,
Saadé and Jabbur 2008), or an increased synaptic gain within the LC, or both. Clinical studies (Scadding and Koltzenburg 2006) and behavioral findings in experimental animals (Pertovaara 2000) have shown that a hypersensitivity to cooling and mechanical stimulation is a frequent and prominent symptom after traumatic nerve injuries, whereas hyperalgesia to heat stimulation occurs only occasionally (Scadding and Koltzenburg 2006). These clinical results are in line with those of study I which showed that peripheral nerve injury produces a weaker hypersensitivity to heat than noxious mechanical stimulation.

An earlier study demonstrated an increased noradrenergic immunoreactivity in the LC of spinal nerve-injured animals (Ma and Eisenach 2003). Spinal nerve injury increased noradrenergic immunoreactivity (Ma and Eisenach 2003) and the release of noradrenaline also at the spinal cord level (Hayashida et al. 2008, 2010). Additionally, peripheral nerve injury produced a bilateral increase in the stimulus-evoked expression of gene transcription factors related to neuronal activity in the LC (Brightwell and Taylor 2009). These results, together with my results, suggest that the efficacy of noradrenergic feedback inhibition of pain is enhanced in neuropathy. An upregulation of the noradrenergic coeruleospinal pain inhibitory system may explain why the antinociceptive efficacy of spinally administered $\alpha_2$-adrenoceptor agonists is increased in peripheral neuropathy (Pertovaara and Wei 2000, Wei and Pertovaara 1997, Wei et al. 2002, Yaksh et al. 1995).

The finding that the responses of LC neurons to noxious mechanical and thermal stimulation were increased in nerve-ligated animals fails to support the hypothesis that the noradrenergic coeruleospinal pain inhibitory modulation is saturated in neuropathy, thus reducing the antinociceptive effect of LC stimulation. Recent studies rather indicate that the descending noradrenergic pathways may have a more complex role in peripheral neuropathy because, besides the antinociceptive actions of the noradrenergic system, the noradrenergic LC may also produce pronociceptive actions (Al-Adawi et al. 2002, Brightwell and Taylor 2009, Li et al. 2002, Wei and Pertovaara 2006a). The LC could have a facilitatory role in neuropathy, since a selective destruction of the LC neurons or a blocking of the LC by lidocaine (Brightwell and Taylor 2009) reduced or reversed the behavioral signs of neuropathic pain, respectively. It should be noted that lidocaine blocks the function of the LC completely, and possibly of the adjacent neurons as well (Sandkühler and Gebhart 1984). Thus, a non-selective lidocaine block may have a different effect than a selective block of specific neurotransmitter receptors within the LC. Tonically activated $\alpha_2$-autoreceptors in nerve injury, for instance, promote neuropathic hypersensitivity by attenuating descending inhibition (Wei and Pertovaara 2006a), while glutamate in the LC has an enhanced descending inhibitory effect in neuropathy (Hayashida et al. 2010).

The results of study I indicated that LC stimulation produced a significant spinal antinociceptive effect only in the ipsilateral hind limb. This is supported by earlier studies showing that the noradrenergic coeruleospinal pathways innervate mainly the ipsilateral spinal dorsal horn (Clark and Proudfit 1992, Mokha et al. 1986, Tsuruoka et
An inflammation in the hindpaw, for instance, induces a bilateral activation of the LC. This results in an enhancement of the descending inhibitory modulation in the ipsilateral dorsal horn (Tsuruoka et al. 2003a, 2003b). The result of an earlier study (Hodge et al. 1983), as well as those of mine, show that the LC stimulation-induced spinal antinociception was attenuated in neuropathy. These findings suggest that inflammation and neuropathy influence the function of descending noradrenergic coeruleospinal pathways in different ways.

7.3.2 Central drive from amygdala to LC (Study I)


Earlier studies in healthy and inflamed animals indicate that the amygdala has both pain-inhibiting and pain-enhancing functions (Neugebauer et al. 2004, Neugebauer 2006). Emotional states modulate pain reactivity. Fear inhibits pain whereas anxiety enhances it (Rhudy and Meagher 2000, 2003). In an experimental animal model of neuropathy, chronic pain was associated with a development of affective disorders and depressive-like behavior (Gonçalves et al. 2008, Ikeda et al. 2007).

In study I, glutamate applied into the CeA inhibited the spontaneous discharge rate of LC neurons in nerve-ligated but not in control animals. The suppression of activity in the LC neurons may have attenuated the noradrenergic feedback inhibition of pain in neuropathic animals, which in turn may have contributed to neuropathic pain and hypersensitivity. This suggestion is supported by studies showing that prolonged pain, such as inflammation (Han et al. 2004, 2006, Han and Neugebauer 2004) and peripheral neuropathy (Ansah et al. 2010, Bourbia et al. 2010, Gonçalves et al. 2008), induce neuroplastic changes that include structural and functional changes of the amygdala. These changes probably contribute to the persistence of sensory and emotional components of neuropathic or inflammatory pain. A recent study indicated that the cell numbers and the volumes of CeA and basolateral amygdalar nuclei were increased in animals with a peripheral nerve injury (Gonçalves et al. 2008). Moreover, excitatory synaptic transmission between the PB and amygdala neurons was potentiated, as shown by the enhanced postsynaptic currents of CeA neurons (Ikeda et al. 2007). The results of study I are also supported by the finding that two types of glutamatergic receptors (GluRs), group I metabotropic GluRs and NMDARs, facilitated emotional-like pain behavior in animals with a peripheral neuropathy (Ansah et al. 2009, 2010). Moreover, an increased free endogenous corticotropin-releasing factor
in the CeA was associated with cutaneous hypersensitivity and decreased emotional pain-like behavior of neuropathic animals (Bourbia et al. 2010). Increased inhibitory influence from the amygdala on LC neurons may indicate that fear or some other emotion activating the amygdala results in a reduced hypalgesic or even a pain-enhancing effect in patients with peripheral neuropathy.

7.3.3 Role of LC in descending antinociception induced by M1 stimulation

The LC provides noradrenergic innervation to the spinal cord (Kwiat and Basbaum 1992) where the descending noradrenergic inhibition of pain-related responses is predominantly mediated by $\alpha_2$-adrenoceptors (Pertovaara 2006). A contribution of spinal noradrenergic receptors to the M1 stimulation-induced antinociceptive effect could be expected on the basis of anatomic evidence showing direct M1 projections to the pontine region (Keizer et al. 1987). Alternatively or additionally, M1 stimulation could activate the LC through other subcortical structures receiving M1 projections, such as the midbrain (Catsman-Berrevoets and Kuypers 1981) and the medial bulboresicular formation (Keizer and Kuypers 1984, 1989), both of which have connections to pontine noradrenergic nuclei (Bajic and Proudfit 1999, Sim and Joseph 1992). For instance, the RVM provides excitatory afferents to the LC (Astier et al. 1990, Aston-Jones et al. 1991, Chiang and Aston-Jones 1993, Ennis et al. 1992). The finding that the RVM had a role in the M1 stimulation-induced antinociception (study III) supports the hypothesis that the RVM might relay the effect of M1 stimulation to the LC. Interestingly, a recent study in animals indicated that M1 stimulation enhanced c-Fos expression in the amydala (Pagano et al. 2011), while study I showed that in neuropathy the amygdala exerted an inhibitory action on the discharge rates of LC neurons. Based on these findings, it may be proposed that the amygdala is one of the potential relay centers in the M1 stimulation-induced circuitry influencing the LC. Even if M1 stimulation promotes activation of the LC through one circuitry, the M1 stimulation-induced inhibitory effect through the amygdala of neuropathic animals might counteract the excitatory effect, which could explain the weak effect of M1 stimulation on the LC activity in the neuropathic animals.

The results of study II indicate that stimulation of M1 increased slightly the neuronal discharge rates in the ipsilateral LC neurons, particularly in nerve-ligated animals. Since this is expected to reflect enhancement of descending noradrenergic pain inhibition, my finding suggests that descending noradrenergic inhibitory pathways contribute to the M1 stimulation-induced spinal antinociception. However, blocking of the LC by lidocaine or intrathecal administration of an $\alpha_2$-adrenoceptor antagonist failed to produce a significant attenuation of the descending antinociception in nerve-ligated or control animals. This finding suggests that the descending noradrenergic system originating in the LC may not have a critical role in the M1 stimulation-induced spinal antinociception. In line with this, although M1 stimulation increased the discharge rates of LC neurons, the increase
in the activity was evidently too weak to produce a significant antinociceptive effect. The results of a recent study indicate that a lidocaine block of the LC in neuropathic animals may promote antinociception rather than suppress any antinociceptive effects of the LC (Brightwell and Taylor 2009). Lidocaine blocks the sodium channels and may influence not only the noradrenergic function but also the function of other cells and receptors in the LC and adjacent structures (Aston-Jones et al. 1991, Hayashida et al. 2008, 2010, Koga et al. 2005). Furthermore, blocking of the LC may influence the function of $\alpha_2$-adrenergic autoreceptors whose tonic activation in nerve-injured animals may promote hypersensitivity (Wei and Pertovaara 2006a). Thus, blocking of the LC and $\alpha_2$-adrenergic autoreceptors is expected to enhance the descending noradrenergic inhibition in neuropathy, which, regarding blocking LC, was actually demonstrated by Brightwell and Taylor (2009). Moreover, M1 stimulation may relay through the LC to act on the spinal $\alpha_1$-adrenoceptors. The role of $\alpha_1$-adrenoceptors, which have both antinociceptive and pronociceptive roles in descending pain modulation (Millan 2002, Pertovaara 2006), was not studied in this thesis.

7.4 Descending serotonergic pathways in M1 stimulation-induced antinociception

7.4.1 Role of RVM and spinal 5-HT$_{1A}$ receptors in descending antinociception induced by M1 stimulation

The results of study III suggest that the RVM, a major pain regulatory area in the brainstem, and its descending serotonergic pathways, have a role in relaying the antinociceptive effect from the M1 to spinal cord in nerve-ligated animals. This suggestion was indicated by the finding that a block of the RVM or a block of the spinal 5-HT$_{1A}$ receptors attenuated the M1 stimulation-induced spinal antinociception in nerve-ligated animals.

Earlier results indicate that neuropathy sensitizes the RVM neurons and leads to hypersensitivity through actions that are mediated by descending pathways (Carlson et al. 2007, Gonçalves et al. 2007, Kincaid et al. 2006, Neubert et al. 2004). In the RVM, on-cells are considered to have a pronociceptive and off-cells an antinociceptive role in descending pain modulation (Fields et al. 1983, Heinricher et al. 1989, Leung and Mason 1998, Mason 2001). Among the third group of RVM neurons, neutral cells, a subgroup has been shown to be serotonergic (Mason 1997, 2001, Potrebic et al. 1994, 1995). Moreover, descending serotonergic pathways descending from the RVM are known to be involved in the regulation of spinal nociception (Millan 2002, Pertovaara and Almeida 2006). The function of the neutral cells, and especially serotonergic neutral cells is still unknown in neuropathy. Although neutral cells remained unresponsive to cutaneous stimulation after
nerve injury (Carlson et al. 2007), serotonergic neutral neurons might still have a role in neuropathy-induced mechanical hypersensitivity due to an injury-induced change in the axonal targets of serotonergic pathways in the spinal cord (Pertovaara et al. 2001). Descending serotonergic axons act on various types of spinal 5-HT receptors including the 5-HT$_{1A}$ receptors, whose activation has been shown to be effective in suppressing pain-related responses in healthy (Colpaert 2006, Mico et al. 2006) and neuropathic animals (Wei and Pertovaara 2006b).

The results of study III are in agreement with the above-cited earlier findings as i.t. administration of a selective 5-HT$_{1A}$ receptor antagonist attenuated the M1 stimulation-induced spinal antinociception in neuropathic animals. My finding suggests that the spinally projecting serotonergic RVM neurons, by acting on spinal 5-HT$_{1A}$ receptors, contribute to the M1 stimulation-induced antinociceptive effect. The blocking of spinal 5-HT$_{1A}$ receptors, however, failed to block totally the M1 stimulation-induced antinociception. The activation of spinal 5-HT$_{1A}$ receptors is known to inhibit nociceptive responses in the spinal dorsal horn under healthy conditions (Gjerstad et al. 1990), while under neuropathy the inhibitory effect of 5-HT$_{1A}$ receptors was decreased (Liu et al. 2010), which might have led to attenuation of the antinociceptive effect of M1 stimulation in the present study.

Earlier studies indicate that activation of GABA$_A$ receptors in the RVM has a pronociceptive action on spinal heat nociception in healthy control animals (Gilbert and Franklin 2001, 2002, Heinricher and Kaplan 1991). The pronociceptive action of GABA$_A$ receptors was explained by inhibition of antinociceptive RVM off-cells (Heinricher et al. 1991, Heinricher and Tortorici 1994). In study III, the GABA$_A$ receptor agonist-induced block of the RVM (or of RVM off-cells) enhanced slightly the spinal withdrawal response to noxious heat in neuropathic animals. This finding is in line with the earlier results indicating that the brainstem–spinal pathways suppress heat nociception in a tonic fashion under neuropathic and control conditions (Kauppila et al. 1998a). There is still research to be done to establish the roles of the various RVM neuron types (on-, off- and neutral cells) and 5-HT receptor subtypes in the M1 stimulation-induced antinociception.

M1 stimulation may influence the RVM directly (Keizer and Kuypers 1984, 1989) or also indirectly. For instance, the RVM receives, via the superior colliculus, projections from the basal ganglia (Ansah et al. 2007, Basso et al. 1996, Basso and Evinger 1996). Study IV showed that the DStr and striatal dopamine D$_2$ receptors have a role in the M1 stimulation-induced antinociception and it might be these receptors that relay the M1 stimulation-induced antinociception, presumably via the superior colliculus and RVM (Basso et al. 1996, Basso and Evinger 1996), to the spinal cord. Moreover, the amygdala, which is known to be activated by M1 stimulation (Garcia-Larrea and Peyron 2007, Pagano et al. 2011), may influence RVM neurons (Ansah et al. 2009) and thus contribute to the M1 stimulation-induced antinociception.
7.5 Descending dopaminergic pathways in M1 stimulation-induced antinociception

7.5.1 Role of striatal and spinal dopamine D2 receptors in spinal antinociception induced by M1 stimulation

Several studies indicate that the basal ganglia process and regulate responses related to pain in humans (Chudler and Dong 1995, Hagelberg et al. 2004, Neugebauer 2006) and animals (Ansah et al. 2007, Pertovaara and Wei 2008, Saadé et al. 1997, Takeda et al. 2005). A recent human brain imaging study showed that the putamen is not only involved in the processing of motor components of pain but also in the sensory components of pain-related processes (Starr et al. 2011). Dopamine acting on dopamine D2 receptors has a role in pain control in the DStr, where a dopamine D2 receptor agonist suppressed responses associated with pain (Ansah et al. 2007, Lin et al. 1981, Magnusson and Fisher 2000, Saunier-Rebori and Pazo 2006).

In study IV, blocking the dopamine D2 receptors in the DStr facilitated noxious heat-evoked baseline responses of spinal WDR neurons, while the corresponding baseline responses of spinal NS neurons were not significantly changed in nerve-ligated animals. Moreover, blocking striatal dopamine D2 receptors decreased the spontaneous discharge rate of NS but not that of WDR neurons.

The Str receives projections from M1 (McGeorge and Faull 1987, 1989), and stimulation of the M1 cortex has been found to cause a striatal release of dopamine (Kanno et al. 2005, Nieoullon et al. 1978, Strafella et al. 2003) and c-Fos expression (Fu and Beckstead 1992, Liste et al. 1995, Sgambato et al. 1997) in various species. In study IV, the blocking of striatal dopamine D2 receptors attenuated the M1 stimulation-induced inhibitory effect on WDR neurons but enhanced it on NS neurons. These results of study IV indicate that supraspinal, presumably striatal dopamine D2 receptors, are involved in the control of spinal nociception, which varies with the type of the sensory neurons involved in the spinal dorsal horn. The facilitation of the heat-evoked baseline response and the reduction in the M1 stimulation-induced inhibition of WDR neurons following a striatal block of dopamine D2 receptors suggest that they are involved both in tonic and M1 stimulation-induced phasic control of noxious inputs to WDR neurons. In the present study striatal dopamine D2 receptors suppressed noxious inputs to spinal dorsal horn WDR neurons, a finding that is supported by previous studies indicating that these receptors suppress pain-related behavioral responses (Ansah et al. 2007, Lin et al. 1981, Magnusson and Fisher 2000).
The spinal neuronal results of study IV and previous behavioral findings (Ansah et al. 2007, Lin et al. 1981, Magnusson and Fisher 2000) support the proposal that the spinal WDR neurons carry signals that are coding the sensory response to noxious stimulation from the periphery (Coghill et al. 1993). The enhanced nociception in the putative spinal pain-relay neurons following a block of the striatal dopamine D2 receptors supports the hypothesis that they contribute to the regulation of baseline pain sensitivity (Hagelberg et al. 2004).

In contrast to the results on WDR neurons, a block of striatal dopamine D2 receptors failed to influence the heat-evoked baseline responses of spinal dorsal horn NS neurons, while the inhibition of NS neurons by M1 stimulation was promoted. The dissociative effect of striatal dopamine D2 receptors on spinal dorsal horn NS and WDR neurons may reflect different functional roles of these spinal sensory neurons in pain processing. While the spinal WDR neurons encode nociceptive signals (Coghill et al. 1993), the NS neurons may have a predominant role in other functions, such as feedback inhibition of pain or control of autonomic responses (Willis and Coggeshall 1991).

The putamen relays pain information to the thalamus, and provides feedback to cortical areas (Starr et al. 2011). Motor cortex stimulation has been shown to induce activation of the thalamus in humans (Garcia-Larrea and Peyron 2007) and animals (Pagano et al. 2011). Thus, M1 stimulation may influence the cortex–basal ganglia–thalamus–cortex pain circuitry. Moreover, the results of studies III and IV, together with earlier findings (Ansah et al. 2007, Basso et al. 1996, Basso and Evinger 1996), indicate that the basal ganglia may relay the M1 stimulation-induced antinociceptive effect, presumably via the superior colliculus, to the RVM and further to the spinal cord.

Dopamine in the spinal cord has an antinociceptive effect due to its action on dopamine D2 receptors that are located presynaptically and postsynaptically to the spinal dorsal horn neurons (Fleetwood-Walker et al. 1988, Garraway and Hochman 2001a, Tamae et al. 2005, Taniguchi et al. 2011). Study IV shows that following a block of spinal dopamine D2 receptors, M1 stimulation failed to attenuate the heat-evoked responses of either WDR or NS spinal dorsal horn neurons. These results in nerve-ligated animals indicate that dopamine acting on spinal dopamine D2 receptors contributed to the M1 stimulation-induced spinal antinociception. This is supported by the finding of study IV that M1 stimulation failed to attenuate the heat-evoked withdrawal reflex following a block of spinal dopamine D2 receptors. Interestingly, the block of spinal dopamine D2 receptors was accompanied by M1 stimulation-induced facilitation of the heat-evoked response in spinal NS neurons. The functional explanation for this finding is still open.
7.5.2 Role of dopaminergic hypothalamic A11 cell group in spinal antinociception induced by M1 stimulation

The dopaminergic pathways innervating the spinal cord originate mainly from the hypothalamic A11 cell group (Hökfelt et al. 1979, Qu et al. 2006, Skagerberg and Lindvall 1985), and electric stimulation of the same area produces antinociception by acting on spinal dopamine D2 receptors (Fleetwood-Walker et al. 1988, Taniguchi et al. 2011, Wei et al. 2009). Hence, it is reasonable to propose that the M1 stimulation-induced spinal antinociception observed in the present thesis was, at least partly, mediated by the descending dopaminergic projections of the A11 cell group. Study IV supports the view that the A11 cell group has a role in the M1 stimulation-induced antinociception because M1 stimulation failed to attenuate the heat-evoked withdrawal reflex following a lidocaine-induced block of the A11. The A11 provides also a potential relay route to the noradrenergic LC via the nucleus prepositus hypoglossi (Aston-Jones et al. 1991, Iwasaki et al. 1999). However, study II failed to find a contribution of the LC to the M1 stimulation-induced antinociception.

7.5.3 Role of dopamine D3 receptors in spinal antinociception induced by M1 stimulation

The dopamine D2 receptor subfamily includes also D3 and D4 receptors. Raclopride binds preferably to both dopamine D2 and D3 receptors but it has also a low affinity to D4 receptors (Seeman and Van Tol 1994). Dopamine D3 receptors in the spinal dorsal horn (Levant and McCarson 2001) and in the caudate-putamen (Hillefors et al. 1999, Suzuki et al. 1998) play a role in pain control, as indicated by the finding that a knockout of dopamine D3 receptors produced hypoalgesia to both heat and mechanical stimulation (Zhu et al. 2010). Therefore, dopamine D3 receptor might contribute to the findings in study IV. Interestingly, a knockout of the dopamine D2 receptor produced a partially different effect: a weak thermal hypoalgesia accompanied by a weak mechanical hypersensitivity (Mansikka et al. 2005). Moreover, intraperitoneally applied dopamine D3 and D2 antagonists had a pain enhancing effect on the hot plate test in the rat (Casarrubea et al. 2006). The effect of raclopride was replicated by striatal application of L-741,626 (a highly selective D2 receptor antagonist supporting the proposal that the D2 receptors indeed play a role in the M1 stimulation-induced antinociception. In line with this, it has been shown in humans that the analgesic effect induced by transcranial sensorimotor cortex stimulation has a significant correlation with the C957T polymorphism of the dopamine D2 receptor gene (Jääskeläinen et al. 2008). These findings favor the suggestion that the dopamine D2 receptor contributes to the M1 stimulation-induced pain suppression but the involvement of dopamine D3 receptors cannot be excluded.
7.6 Other pathways potentially relaying antinociception induced by M1 stimulation

Studies III and IV suggest that the descending serotonergic and dopaminergic pathways have a significant role in the M1 stimulation-induced antinociception, unlike descending noradrenergic pathways (II). It may be proposed that M1 stimulation produces spinal antinociception via a circuitry involving RVM and spinal 5-HT$_{1A}$ receptors, striatal and spinal dopamine D$_2$ receptors, and probably also the hypothalamic dopaminergic A11 cell group. None of the tested manipulations alone produced a complete reversal of the M1 stimulation-induced spinal antinociception, suggesting that the antinociceptive effect is relayed via multiple pathways.

M1 stimulation might also activate descending systems that were not considered in the present study but are certainly involved in pain regulation (Millan 2002, Pertovaara and Almeida 2006). These might contribute to the M1 stimulation-induced descending antinociception. In line with this, the M1 has efferent projections to multiple sites in the cortex and brainstem (Canedo 1997, Keizer and Kuypers 1984, 1989). Moreover, the RVM is the final common pathway for a number of descending pain regulatory pathways originating from multiple brain areas (Fields et al. 2006, Millan 2002, Pertovaara and Almeida 2006). Therefore, the contribution of the RVM to the M1 stimulation-induced antinociception may be explained by either subcortical connections of the M1 or by cortico-cortical loops descending finally through the RVM or by both.

It is noteworthy that the PAG, a structure involved in descending opioidergic inhibition of pain is among the subcortical pain-regulatory structures projecting to the RVM (Bajic and Proudfit 1999, Sandkühler and Gebhart 1984). Human brain imaging studies and a recent experimental animal study have indeed demonstrated that the PAG is activated by M1 stimulation (see below). Studies in humans have demonstrated that M1 stimulation produces increased blood flow in the PAG (Peyron et al. 2007), and also a decrease in its opioid receptor availability reflecting an increased release of endogenous opioids (Maarrawi et al. 2007). Similarly, in experimental animals M1 stimulation increased c-Fos expression (Pagano et al. 2011) and a release of endogenous opioids in the PAG (Maarrawi et al. 2007). Moreover, an opioid receptor antagonist, naloxone, attenuated the M1 stimulation-induced antinociception in an animal study (Fonoff et al. 2009), and decreased the analgesic effect of cortical rTMS in healthy volunteers (de Andrade et al. 2011). These findings suggest that the PAG provides a potential opioidergic link for the activation of serotonergic raphe–spinal pathways by M1 stimulation.

At the cortical level, the M1 stimulation-induced descending antinociceptive effect may relay through the prefrontal, anterior cingulated and orbitofrontal areas, as indicated by the M1 stimulation-induced increase of cerebral blood flow or by a change in the neurotransmitter receptor binding in human subjects (Garcia-Larrea et al. 1999, Peyron et
al. 2007, Strafella et al. 2003). It remains to be studied whether these structures, through their connections to various nuclei converging in the RVM, DStr, A11 cell group or LC (Millan 2002, Pertovaara and Almeida 2006, Vanegas and Schaible 2004), would contribute to the M1 stimulation-induced descending antinociception. For instance, the prefrontal cortex has excitatory connections to the LC (Jodo et al. 1998), which might contribute to the activation of the noradrenergic system by M1 stimulation. This mechanism is, however, not likely to be involved in neuropathic animals according to study II. Moreover, additional possible relay links from the Str to the spinal cord might include the superior colliculus (Basso et al. 1996), which may also have a role in the M1 stimulation-induced antinociceptive circuitry. Additionally, M1 stimulation is not only relayed via indirect pathways but also via a direct projection to the spinal cord (Rojas-Piloni et al. 2010).

The present series of studies focused on the descending control of spinal nociception by M1 stimulation. It should be noted that in human clinical studies pain alleviation may be based not only on spinal but also on supraspinal actions that influence the emotional appraisal of pain (Garcia-Larrea and Peyron 2007, Ohara et al. 2005). For example, the striatal dopamine D2 receptors and the RVM may act via the amygdala non-sensory factors that influence the emotional appraisal of pain (Chudler and Dong 1995, Garcia-Larrea and Peyron 2007, Nahmias et al. 2009, Pertovaara et al. 2004). In fact, a recent animal study (Pagano et al. 2011) showed that M1 stimulation increased c-Fos expression in both the amygdala and PAG.

7.7 Implications, unsolved questions and future prospects

The use of motor cortex stimulation as a neurosurgical technique for pain control is steadily increasing. Clinical studies show that at least half of the patients with chronic neuropathic pain untreatable by other therapeutic approaches may benefit from this method (Nguyen et al. 1999). Activation of several brain structures lasting for hours or days after discontinuation of the treatment has been associated with the clinical effects of motor cortex stimulation (Garcia-Larrea and Peyron 2007). A better understanding of the mechanisms involved the M1 stimulation-induced antinociception could improve the clinical treatment of persistent pain. In this thesis, the mechanisms of the M1 stimulation-induced spinal antinociception were studied in peripheral neuropathy. The results indicate that the stimulation of M1 attenuates pain-related responses in neuropathic animals through activation of several descending pain inhibitory systems. However, a parallel supraspinal action that could also attenuate sensory and emotional components of pain cannot be excluded since it is difficult to estimate these effects in experimental animals.

My thesis indicated possible roles for the RVM (III), Str and hypothalamic A11 cell group (IV) in the M1 stimulation-induced antinociception. However, the mechanisms still remained partially open. Therefore, the future research topics should include the influence of M1 stimulation on the response properties of RVM on-, off- and neutral cells,
and of striatal nociceptive cells and A11 cells. Further studies are also needed to clarify the roles of the serotonergic receptor subtypes within the RVM, of the dopaminergic receptor subtypes and of the neuronal circuitry mediating the pain-regulatory effect from the M1 to the A11 cell group.

This thesis is focused on short-duration influences of M1 stimulation on pain. However, since clinical treatment with motor cortex stimulation has been observed to produce long-lasting effects, the future experimental studies should also focus on prolonged effects of M1 stimulation. Furthermore, it is of importance to determine how changes in the temporal parameters of M1 stimulation, such as frequency and duration, influence the antinociceptive efficacy. For instance, more studies are needed to find out whether noradrenergic pathways, which had a minor, if any, role in the current study applying short train of high-frequency M1 stimulation, might increase their influence with M1 stimulation of longer duration or lower frequency. The neurons and receptors within the M1 that are critical for inducing pain relief, need to be characterized. For example, it would be tempting to know whether and how various subtypes of glutamate and nonglutamate receptors in the M1 contribute to pain modulation. Moreover, based on human results on transcranial magnetic stimulation of M1, it has been proposed that local corticocortical inhibitory circuits might be important (Di Lazzaro et al. 1998). This should be tested in experimental animals with a peripheral neuropathy.

In the present series of studies, the focus was on antinociceptive effects on the side ipsilateral to the cortical stimulation site. Although descending pain modulation systems descend mainly ipsilaterally, contralateral M1 stimulation may influence the descending modulation via interaction between two primary motor cortices, via pyramidal tract collaterals (Brus-Ramer et al. 2009, Gerloff et al. 1998) or via interneurons at the spinal cord level (Umeda et al. 2010). Further studies are needed to determine the importance of these mechanisms in contralateral or bilateral effects induced by M1 stimulation.

Motor cortex stimulation has been used for treating symptoms of several motor disorders, including Parkinson’s disease, in which dysfunction of the nigrostriatal dopaminergic system leads to increased pain sensitivity on top of motor impairment (Djaldetti et al. 2004, Juri et al. 2010, Lefaucheur 2009). Since the dopaminergic system is involved in pain modulation (Ansah et al. 2007, Chudler and Dong 1995, Hagelberg et al. 2004, Juri et al. 2010) and motor control, it remains to be studied whether the mechanisms of the M1 stimulation-induced therapeutic effects in motor disorders are the same as in neuropathic pain.

The clinical motor cortex stimulation may produce pain relief due to an action on non-sensory aspects of pain, e.g. by interfering with emotional components of pain (Xie et al. 2009). Therefore, further experimental animal studies should be conducted to find out whether the limbic system in general, and the amygdala in particular, have a role in the M1 stimulation-induced antinociception in experimental neuropathy.
8 SUMMARY AND CONCLUSIONS

The conclusions of this thesis can be summarized as follows:

1. Stimulation of M1 has a spinal antinociceptive effect in nerve-injured and control animals (II). Stimulation of M1 induces top–down modulation of noxious heat-evoked responses in the spinal dorsal horn WDR and NS neurons of nerve-injured animals (IV).

2. Peripheral neuropathy results in bidirectional changes in the function of the pain regulatory system originating in the noradrenergic LC. The increased responses of LC neurons to noxious somatic stimuli are likely to promote the efficacy of the noradrenergic feedback inhibition of pain (I).

3. The increased inhibition of LC neurons from the amygdala is likely to increase neuropathic pain due to suppression of coeruleospinal pathways. This provides a potential pain-enhancing mechanism for emotions processed by the amygdala in peripheral neuropathy (I).

4. M1 stimulation activates LC neurons, but LC or its descending noradrenergic pathways have no major role in the spinal antinociceptive effect induced by stimulation of M1 (II) because local anesthesia of LC and a block of spinal α2-adrenoceptors failed to reverse the M1 stimulation-induced spinal antinociception.

5. RVM and the descending serotonergic pathway acting on spinal 5-HT1A receptors contribute to the spinal antinociception induced by M1 stimulation in neuropathic animals (III) because a block of the RVM and spinal 5-HT1A receptors attenuated the effect.

6. Supraspinal, presumably striatal and spinal dopamine D2 receptors are involved in mediating descending antinociceptive actions from M1 to the spinal cord because a block of striatal and spinal dopamine D2 receptors attenuated the action. The efficacy of dopamine D2 receptors had partly different effects that varied with the type of affected spinal neuron. This probably reflects the different roles that the spinal WDR and NS neurons have in pain processing (IV).

7. Blocking the dopaminergic A11 cell group in the hypothalamus attenuated the M1 stimulation-induced spinal antinociception, suggesting that this region is among the relay centers mediating the descending antinociceptive effect in this model (IV).

The main results of the mechanisms of primary motor cortex stimulation are summarized in Figure 9.
Figure 9 Possible mechanisms of antinociception induced by primary motor cortex (M1) stimulation. Excitatory projections (dark arrows), inhibitory projections (light arrows) and possible inhibitory input (dashed arrow). Striatum (Str), thalamus, descending pain modulation areas; dopaminergic hypothalamic cell group (A11), locus coeruleus (LC), periaqueductal gray matter (PAG) and rostoventromedial medulla (RVM), areas related to emotional component of pain; amygdala (Amy), anterior cingulate cortex (ACC), dorsolateral prefrontal cortex (DLPFC), orbitofrontal cortex (OFC). Dopamine D$_2$ receptors (D$_2$R), dopamine (DA), noradrenaline (NA), 5-HT$_{1A}$ receptors (5-HT$_{1A}$R). Gray circles represent structures studied in the thesis.
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10 REFERENCES


