Department of Physiology
Faculty of Medicine
University of Helsinki
Helsinki

PLASTICITY IN DESCENDING MODULATION OF PAIN: THERAPEUTIC EFFECTS OF NON-PAINFUL HEATING-NEEDLE STIMULATION AND ITS POTENTIAL MECHANISMS

Jing Lei

ACADEMIC DISSERTATION

To be presented, with the permission of the Faculty of Medicine of the University of Helsinki, for public examination in Lecture Hall 1, Biomedicum 1, Haartmaninkatu 8, on 20 March 2020, at 12 noon

Helsinki 2020

Supervisors
Professor Antti Pertovaara, M.D., Ph.D.
Department of Physiology
Faculty of Medicine
University of Helsinki
Helsinki, Finland

Professor Hao-Jun You, M.D., Ph.D.
Center for Translational Medicine Research on Sensory-Motor Diseases
Yan'an University
Yan'an, China

Pre-examiners
Professor Rashid Giniatullin, M.D., Ph.D.
A.I. Virtanen Institute for Molecular Sciences
University of Eastern Finland
Kuopio, Finland

Docent Jouni Sirviö, Ph.D.
University of Eastern Finland & Sauloner Ltd.
Finland

Opponent
Docent Xiao-Jun Xu, M.D., Ph.D.
Department of Physiology and Pharmacology
Karolinska Institutet
Stockholm, Sweden

Custos
Professor Antti Pertovaara, M.D., Ph.D.
Department of Physiology
Faculty of Medicine
University of Helsinki
Helsinki, Finland

ISBN 978-951-51-5892-5 (paperback)
ISBN 978-951-51-5893-2 (PDF)
Printed by Unigrafia
Helsinki 2020

The Faculty of Medicine uses the Urkund system (plagiarism recognition) to examine all doctoral dissertations

ABSTRACT

The role and significance of descending regulation of pain has received much attention due to its implications in the development and maintenance of pathological pain that can be associated with central sensitization. In the present series of studies, contributions of descending pain facilitatory and inhibitory pathways to behavioral pain responses were assessed under physiological conditions in humans and rats, under a simulated weightlessness condition in rats, and in an experimental model of Parkinson's disease (PD) in rats. In all experimental conditions, a long-term activation of descending pain modulatory pathways was induced by noxious conditioning stimulation to allow determining whether the studied experimental procedures cause decreases or increases in the activity of descending pathways. For noxious conditioning, acute muscle nociception was induced by intramuscular injection of 5.8% hypertonic saline. Secondary mechanical hyperalgesia (hyperalgesia outside of the injected muscle region) was used as an index of the magnitude of descending facilitation, and secondary heat hypoalgesia as an index of the magnitude of descending inhibition. To explore whether heating-needle stimulation can promote analgesia due to modulation of descending pain controls, the effect of heating-needle stimulation on descending facilitation and inhibition was also determined in different experimental conditions of the present study. To study whether thalamic dopamine is involved in descending modulation of pain, the effects induced by microinjections of dopamine or dopamine D2 receptor antagonist into the thalamic mediodorsal (MD) / ventromedial (VM) nuclei on descending pain modulation were evaluated. Furthermore, expression of Fos, a marker of neuronal activation, was determined in the spinal cord dorsal horn to reveal a spinal neuronal correlate for descending pain modulation.

In the current series of studies, simulated weightlessness and a partial depletion of dopamine in the striatum enhanced endogenous descending facilitation and depressed descending inhibition. In the spinal dorsal horn, changes in Fos expression in the superficial layers were associated with changes in descending facilitation and those in the middle/deep layers with changes in descending inhibition. Intramuscular (i.m.) heating-needle stimulation at an innocuous temperature of 43°C triggered descending inhibition without concomitant activation of descending facilitation both in physiological conditions and in experimental model of PD. In the PD model, dopamine D2 receptors in the thalamic VM nucleus were involved in mediating the enhancement of descending pain inhibition induced by heating-needle stimulation applied at the temperature of 43°C. In humans, i.m. heating-needle stimulation applied at the non-painful temperature of 43°C enhanced selectively descending inhibition, and it may be explained by a difference in the density of capsaicin- and heat-sensitive fibers innervating these heating-needle stimulation targets. Injection of capsaicin that selectively activates C-fibers produced stronger pain in acupoints than in non-acupoints.

The results indicate that pronociceptive changes in descending pain modulatory circuits contribute to pain facilitation under simulated weightlessness and in the model of PD. I.m. heating-needle stimulation at an innocuous temperature of 43°C provides a treatment method that promotes antinociception in these pathophysiological as well as physiological conditions through a selective recruitment of descending pain inhibitory circuits. Dopamine D2 receptors in the thalamic VM nucleus are mediating the attenuation of pain by heating-needle stimulation in the model of PD.

ABSTRAKTI

Tässä työsarjassa tutkittiin aivoista selkäytimeen laskevan kivunmuuntelujärjestelmän toimintaa ja merkitystä fysiologisissa ja eräissä patofysiologissa tilanteissa ihmisillä sekä koe-eläimillä (rotilla). Koe-eläimillä aikaansaatiin patofysiologinen tilanne joko painotonta olosuhdetta simuloivalla tilalla tai kemiallisesti aikaansaadulla kokeellisella Parkinsonin taudilla. Kaikissa olosuhteissa niin ihmisellä kuin koe-eläimillä aktivoitiin pitkäkestoisesti laskevat kivunmuuntelujärjestelmät antamalla toispuoleisesti raajan lihakseen hypertonista keittosuolaa. Laskevan kipua fasilitoivan järjestelmän aktivaation mittarina määritettiin mekaanisen kipukynnyksen alentuminen (mekaaninen hyperalgesia). Laskevan kipua inhiboivan järjestelmän aktivaation mittarina määritettiin kuumakipukynnyksen nousu (kuumahypoalgesia). Kynnysmääritykset tehtiin ihoalueella, joka oli etäällä hypertonisella keittosuolalla käsitellystä lihaksesta, minkä vuoksi kynnysmuutokset kuvastavat keskushermostollisiin mekanismeihin perustuvaa sekundaarista hyperalgesiaa tai hypoalgesiaa. Työsarjassa selvitettiin myös kuumaneulastimulaation terapeuttista vaikutusta ja vaikutusmekanismia kivulle herkistymiseen. Mediaalisen talamuksen dopamiinihermotuksen merkitystä laskevan kivunmuuntelujärjestelmän toiminnan häiriölle tutkittiin annostelemalla mediaalisen talamuksen tumakkeisiin dopamiinia tai dopamiini D2 reseptorin antagonistia. Mediaalisessa talamuksessa tutkimuksen kohteena olivat mediodorsaalinen (MD) tumake ja ventromediaalinen (VM) tumake. Lisäksi tutkittiin selkäytimen takasarven eri solukerrosten osuutta laskevassa kivun muuntelussa käyttäen hermosolujen toiminnan mittarina c-FOS-merkkiaineen ilmentymistä.

Painotonta olosuhdetta simuloiva tila sekä kokeellinen Parkinsonin tauti vahvistivat kivun laskevaa fasilitaatiota ja vaimensivat kivun laskevaa inhibitiota. Selkäytimen takasarvessa laskeva fasilitaatio korreloi hermosoluaktiviteetin muutoksiin selkäytimen takasarven pinnallisissa solukerroksissa, kun taas laskeva inhibitio korreloi muutoksiin takasarven syvempien solukerrosten hermosoluaktiviteetissa. Kivuton kuumaneulastimulaatio 43°C lämpötilassa olevilla neuloilla vahvisti selektiivisesti kivun laskevaa inhibitiota koe-eläimillä sekä fysiologissa oloissa että kokeellisessa Parkinsonin tautimallissa. Mediaalisen talamuksen VM-tumakkeen dopamiini 2-reseptori oli välittämässä kuumaneulastimulaation kipua inhiboivaa vaikutusta Parkinsonin tautimallissa. Myös ihmiskokeissa kivuton kuumaneulastimulaatio 43°C lämpötilassa olevilla neuloilla vahvisti selektiivisesti kivun laskevaa inhibitiota. Ihmiskoetulokset osoittivat, että ääreishermoista C-syyt ovat keskeisiä kuumaneulastimulaation vaikutuksen välittäjiä, koska kuumaneulastimulaation terapeuttinen vaikutus oli voimakkain, kun stimuloitiin ihoaluetta, jossa C-syitä selektiivisesti aktivoiva kemiallinen aine kapsaisiini aiheutti voimakkaimman poltteen.

Työsarjan tulokset osoittavat, että kipua lisäävät muutokset laskevissa kivun muuntelujärjestelmissä ovat myötävaikuttamassa kipuherkkyyden lisääntymiseen painotonta tilaa simuloivissa olosuhteissa ja kokeellisessa Parkinsonin taudissa. Kivuttoman kuumaneulastimulaation avulla on mahdollista vahvistaa selektiivisesti laskevaa kivun inhibitiota ja siten vähentää kivulle herkistymistä niin fysiologisissa kuin patofysiologisissa tilanteissa. Mediaalisen talamuksen VM-tumakkeen dopamiini D2-reseptori välittää kuumaneulastimulaation kivulle herkistymistä vähentävän vaikutuksen kokeellisessa Parkinsonin taudissa.

ACKNOWLEDGEMENTS

I would like to express my sincerest gratitude to Professor Antti Pertovaara, who provided and gave me this valuable opportunity to study in the University of Helsinki, Finland. I am grateful to his highly-effective helpfulness and friendly advice.

I am also indebted to Professor Hao-Jun You, who has been guidance, fruitful discussion and support in all aspects of this project.

I wish to thank the pre-examiners: Professor Rashid Giniatullin and Docent Jouni Sirviö, for their constructive comments to improve the thesis.

My sincere appreciation also goes to Dr. Gang Ye, Ms. Shu-Qing Shao, and other personnel in China for their clinical work and proper feedbacks in this project.

Finally, I am grateful to all my colleagues, in particular Dr. Hong Wei and Dr. ZuYue Chen, at the Department of Physiology, University of Helsinki for their friendship and help during my stay in Helsinki.

The present study presented in this thesis has been supported financially by the National Natural Science Foundation of China (81772451, 81860410), the Academy of Finland (315043), and the Sigrid Jusélius Foundation, Helsinki, Finland.

CONTENTS

Abstract....3

Abstract in Finnish....5

Acknowledgements....7

Contents....8

List of original publications....11

Abbreviations....12

1 Introduction....13

2 Review of literature....15

2.1 Ascending pain pathways....15

2.2 Endogenous descending pain modulation pathway....16

2.3 Roles of the thalamus in pain perception and pain modulation....17

2.4 Neurotransmitters/receptors involved in endogenous descending modulation on pain....19

2.4.1 Opioid peptides/receptors....19

2.4.2 Dopamine and dopamine receptors....21

2.4.3 Other neurotransmitters....22

2.5 Pain in Parkinson's disease....22

2.6 Animal models of pain in Parkinson's disease....24

2.6.1 Pharmacological PD models....24

2.6.2 Pesticide rotenone-induced PD model....24

2.6.3 Genetic PD models....25

2.6.4 Neurotoxin-induced PD models....25

2.7 Experimental muscle pain....26

2.8 Traditional acupuncture and heating-needle stimulation (H.N.S.).....28
2.8.1 Acupuncture.....28
2.8.2 Heating-needle stimulation.....29
3 Aims.....31
4 Methods.....32
4.1 Animals.....32
4.2 Subjects.....32
4.3 Drugs.....32
4.4 Experimental design.....33
4.5 Contents of the studies.....34
4.6 Surgeries.....34
4.6.1 Installation of intracerebral cannula.....34
4.6.2 Neurotomy of tail nerve.....34
4.7 Hindlimb unloading (HU).....35
4.8 Muscle nociception induced by intramuscular injection of algogen.....35
4.9 Intramuscular heating-needle stimulation.....36
4.10 Behavioral tests in animal experiment.....37
4.10.1 Measurement of mechanical sensitivity.....37
4.10.2 Measurement of heat sensitivity.....37
4.10.3 Assessment of motor function.....37
4.10.4 Apomorphine-induced rotation behavior.....38
4.11 Measurement of pain sensitivity in human study (Study II).....38
4.11.1 Measurement of pressure pain threshold.....38
4.11.2 Measurement of heat pain threshold.....38
4.11.3 Visual Analog Scale (VAS).....38

4.11.4 Pain distribution 38
4.12 Immunohistochemical detection of Fos expression 39
4.13 Statistics 39
5 Results 41
5.1 Results in animal studies 41
5.1.1 Changes in descending control of pain under special and pathological conditions 41
5.1.2 Effects of heating-needle stimulation on descending control of pain 42
5.2 Results in human study 43
5.2.1 Differences in effects of heating-needle stimulation at acupoints and non-acupoints on descending modulation of pain 43
5.2.2 Effects of heating-needle stimulation at acupoint on experimental muscle pain 44
6 Discussion 46
6.1 6-OHDA-induced PD 46
6.2 Withdrawal reflex 47
6.3 Emotional influence on pain 49
6.4 Neurotransmitters involved in PD 50
6.5 Diffuse noxious inhibitory controls (DNIC) 50
6.6 Potential clinical implications 51
7 Conclusions 52
8 Future prospects 53
References 55

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications:

I Jing Lei, Antti Pertovaara, Hao-Jun You. Effects of simulated weightlessness on intramuscular hypertonic saline induced muscle nociception and spinal Fos expression in rats. Brain Res. 2015;1594: 204-214.

II Jing Lei, Gang Ye, Jiang-Tao Wu, Antti Pertovaara, Hao-Jun You. Role of capsaicin- and heat-sensitive afferents in stimulation of acupoint-induced pain and analgesia in humans. Neuroscience 2017;358: 325-335.

III Jing Lei, Gang Ye, Antti Pertovaara, Hao-Jun You. Effects of heating-needle stimulation in restoration of weakened descending inhibition of nociception in a rat model of Parkinson's disease. (Submitted)

The publications are referred to in the text by their roman numerals.

ABBREVIATIONS

ACC	Anterior Cingulated Cortex
ATP	Adenosine 5'-triphosphate
CFA	Complete Freund's adjuvant
GS	Gastrocnemius
H.N.S.	Heating-needle stimulation
HPT	Heat pain threshold
HT	Hypertonic
HU	Hindlimb unloading
i.c.	Intracerebral
i.m.	Intramuscular
LC	Locus coeruleus
MD	Mediodorsal nucleus
NOP	nociceptin/orphanin-FQ
PAG	Periaqueductal gray
PBS	Phosphate-buffered saline
PD	Parkinson's Disease
PPT	Pressure pain threshold
PWMT	Paw withdrawal mechanical threshold
PWTL	Paw withdrawal thermal latency
RVM	Rostral Ventromedial Medulla
VAS	Visual Analog Scale
VM	Ventromedial nucleus

1 INTRODUCTION

Nociception is the response of sensory nervous system to harmful or potentially harmful stimuli. Physiological and behavioral responses triggered by nociception cause a subjective distressing feeling, pain. Usually, induction of pain is accompanied by activation of pain modulation (Yam et al., 2018).

It is well known that endogenous descending modulation of pain consists of descending facilitation and inhibition. Changes in descending pain modulation play important roles in chronic pain and central sensitization, such as induction and maintenance of allodynia or hyperalgesia (Fields, 1992; Millan, 2002). Consequently, pathological pain could be alleviated when descending inhibition were enhanced and descending facilitation suppressed by various pharmacological and non-pharmacological methods. It has been reported that descending modulation of pain is not active under physiological conditions, while it can be activated by a sufficient amount of inputs from peripheral C-fiber afferents. Most importantly, the triggering threshold of descending inhibition is significantly higher than that of descending facilitation (You et al., 2010, 2013; Lei et al., 2011, 2013).

Astronauts often suffer from skeletal muscle pain both during spaceflight and after returning to the earth. Importantly, muscle pain in astronauts is more serious than that in ordinary muscle pain patients (Buchanan & Convertino, 1989; Riley et al., 1995; Ali et al., 2009). However, whether there are changes in descending modulation of pain under weightlessness is less known. Parkinson's disease (PD) is a common neurodegenerative disorder associated with a dopaminergic deficiency and is characterized mainly by motor symptoms. However, pain is an early symptom during the development of PD, and pain has a high prevalence in PD patients (Beiske et al., 2009; Buhmann et al., 2017). Dopaminergic deficiency is characteristic for PD and the dopaminergic system regulates pain and nociception due to action on a number of central structures involved in descending control of pain (Chudler & Dong, 1995; Wood, 2008). Consequently, it remains to be studied and revealed whether the development of PD and thereby dopaminergic deficiency is accompanied by a dysfunction in descending modulation of pain.

Traditional Chinese acupuncture has been used to relieve pain for thousands of years. Previous studies showed that needle stimulation at acupoints along different meridians could exert long-term analgesia (Chiang et al., 1973; Han et al., 2003; You et al., 2006). Moreover, there are results suggesting that C-afferents innervating the acupoint area are involved in the acupuncture-induced analgesia (Zhu et al., 1990; Radhakrishnan & Sluka, 2005). To date, it is still not clear how acupuncture recruits circuits involved in descending modulation of pain.

Earlier experimental studies indicate that two specific thalamic nuclei, the mediodorsal (MD) nucleus and the ventromedial (VM) nucleus, act as a 'Thalamic nociceptive discriminator' and exhibit distinct roles in evoking activities of descending facilitatory and inhibitory controls upon noxious mechanically and heat evoked nociception (Lei & You, 2013; You et al., 2013, 2016). There are both nociceptive and non-nociceptive primary afferent C-fibers (Besson & Chaouch, 1987). It has been thus hypothesized that descending inhibitory controls could be triggered by non-painful heat stimulation of non-nociceptive C-afferents, without accompanying activation of descending facilitatory controls (You et al., 2013). Verification of this hypothesis still needs experimental evidence.

Taken together, the purpose of the current series of studies was to explore descending modulation of pain in humans under physiological conditions (Study II), in rats under simulated weightlessness condition (Study I), and in rats under a pathological state (with an experimental model of PD) (Study III). Additionally, the present studies assessed whether intramuscular (i.m.) heating-needle stimulation, a modification of Chinese traditional medicine treatment methods, applied at a non-painful (43°C) temperature has analgesic effects that are due to action on descending modulation of pain under physiological conditions in healthy human subjects (Study II) and in an experimental rat model of PD (Study III). Furthermore, the potential contribution of the thalamic dopamine D2 receptors (Study III) to the i.m. heating-needle stimulation induced changes in descending pain modulation was explored.

2 REVIEW OF LITERATURE

Pain is defined by the International Association for the Study of Pain (IASP 2011) as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage. Normally, physiological pain is experienced as an acute event and serves protective function. Pain as a sensory experience has two main components that have partly different underlying neurobiological mechanisms: i) Sensory-discriminative component that tells about the location, quality and quantity of painful stimulation, ii) Affective-emotional component that tells about unpleasantness and suffering. Pathological pain is pain expressed in pathophysiological conditions such as neuropathy, diabetes, inflammation and cancer. Among features characterizing pathological pain are hyperalgesia (increased sensitivity to painful stimuli) and allodynia (pain sensation evoked by innocuous stimuli) (Kuner, 2010).

2.1 ASCENDING PAIN PATHWAYS

The ascending pain pathway carries nociceptive signals from peripheral tissues to the brain where pain is perceived. Peripheral nociceptors are responsible for the detection of noxious stimuli. Peripheral nociceptors are free nerve endings that express various ion channel receptors that transduce noxious stimuli into nociceptive neuronal signals. Among nociceptive transducers are a family of transient receptor potential channels (Jardin et al., 2017). From the periphery, nociceptive signals are carried by primary afferent Aδ and C fibers that are distributed in different tissues throughout the body. Aδ fibers are myelinated afferents with a medium diameter. They are responsible for the transmission of well-localized 'fast' pain. C fibers are thin unmyelinated afferents that are responsible for transmission of poorly localized 'slow' pain (Treede et al., 1998; Yam et al., 2018). Aδ and C fibers transmit information to nociceptive specific neurons in lamina I and II of the spinal cord dorsal horn. In the spinal dorsal horn, nociceptive-specific projection neurons are located mainly in lamina I and scattered through lamina III-VI (You et al., 2008). Another common type of nociceptive projection cell in the lamina V of the spinal dorsal horn is wide-dynamic range neuron that receives convergent inputs from nociceptive and non-nociceptive primary afferent nerve fibers (You et al., 2003). The axons of the spinal projection cells cross the midline and ascend in the spinal white matter contralaterally as the spinothalamic tract and the spinoreticular tract that innervate the thalamus and other brainstem structures. The third-order nociceptive neurons in the thalamus send ascending projections to the primary somatosensory (Bell, 2018) and other cortical areas (Harris et al., 2019). Ascending pain pathways are illustrated in Figure 1a.

2.2 ENDOGENEOUS DESCENDING PAIN MODULATION PATHWAY

Nociceptive information ascending from the periphery to the cerebral cortex is controlled at various levels of the neuraxis by descending modulatory systems originating in multiple areas of the central nervous system (Millan, 2002; Pertovaara & Almeida, 2006). Endogenous descending modulation of pain consists of descending facilitation and descending inhibition. Because of the crucial role of descending modulation of pain in central sensitization and in induction and maintennance of pathological pain, better understanding of the underlying mechanisms and pathophysiology of descending modulation of pain will be of importance for the development of more effective pain treatments.

Endogenous descending pain modulation derives from different areas in the central nervous system, including the cerebral cortex, thalamus and brain stem. The study of the descending modulation of pain started after it was for the first time reported that sustained analgesia could be induced by electrical stimulation of the midbrain periaqueductal grey matter (PAG) (Reynolds, 1969). After that it was found that stimulation of also other sites in the brainstem, such as the nucleus raphe magnus (NRM) or adjacent reticular formation, has analgesic effects (Basbaum & Fields, 1978). It has been generally accepted that the PAG sends projections to noradrenergic pontine nuclei and the rostroventromedial medulla (RVM) that through pathways descending in the spinal dorsolateral funiculus induce inhibitory modulation of nociception at the level of the spinal cord. Additionally, descending projections from the PAG that relay in the RVM induce facilitatory effect on spinal nociception via pathways descending in the spinal ventrolatral funiculus (Bajic & Proudfit 1999; Odeh & Antal, 2001). Thalamus may activate the brainstem nuclei involved in descending pain modulation either through direct descending projections (Marini & Tredici, 1995) or through ascending projections to cortical areas that further project to the PAG and other brainstem nuclei involved in descending pain regulation (for further details, see section 2.3 and section on “Withdrawal reflex” in the Discussion). Results of a recent lesion study showed that the dorsal funiculus is also involved in descending facilitation of pain behavior (Lei & You, 2013). The pathways involved in descending modulation of pain are shown in Figure 1b.

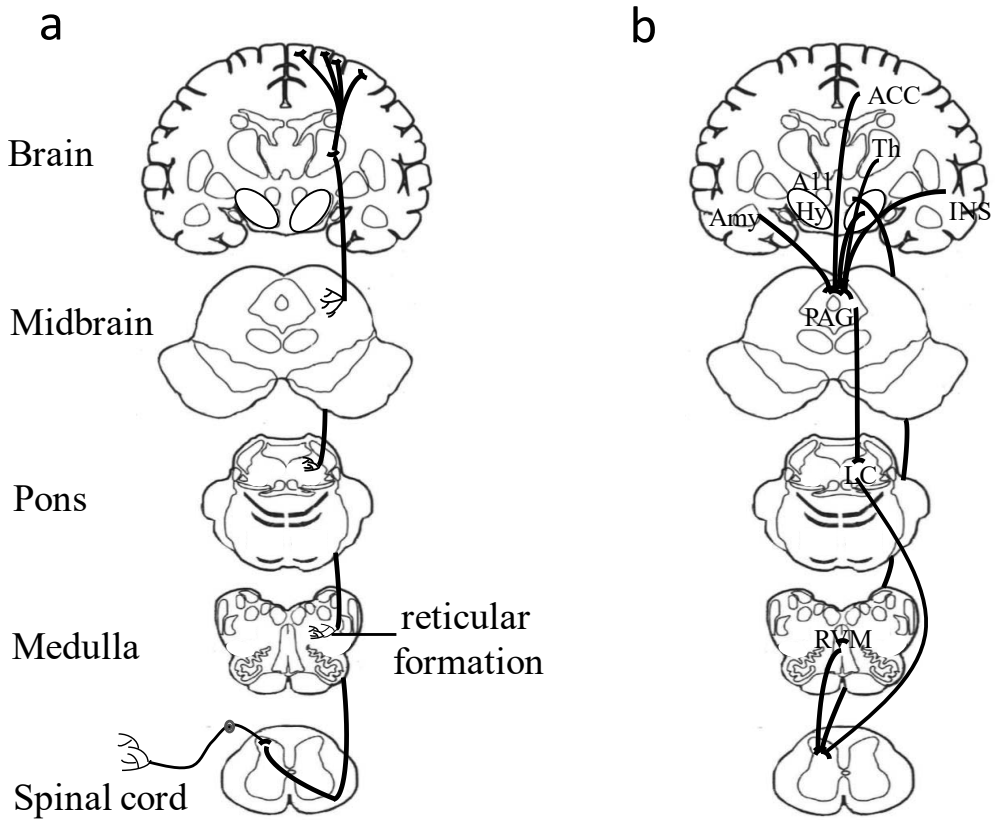

Figure 1 Ascending pain pathways and descending modulatory pain pathways

a: ascending pain pathways, b: descending modulatory pain pathways, ACC: anterior cingulate cortex, A11: hypothalamic nuclei, Amy: amygdala, INS: insular cortex, Hy: hypothalamus, LC: locus coeruleus, RVM: rostral ventromedial medulla, PAG: periaqueductal grey, Th: thalamus (modified from Cury et al., 2016)

2.3 ROLES OF THE THALAMUS IN PAIN PERCEPTION AND PAIN MODULATION

In animals with a developed cerebral cortex, thalamus is the most important relay station for sensory information arriving in the cortex. Various sensory pathways (except for those involved in olfaction) have a relay neuron in the thalamus, after which the sensory signal is carried to different areas in the cerebral cortex. The thalamus is located in the diencephalon part of the brain, below the cortex and above the midbrain. According to the anatomy and electrophysiological evidences, the somatosensory-related thalamic structures are divided into lateral and medial subdivisions. The lateral thalamic pain pathway includes the ventral posterolateral

nuclei (VPL) and posterior nuclei, and it is responsible for the sensory-discriminative dimensions of pain. The medial pathway consists of intralaminar nuclei, medial dorsal (MD) nucleus and other midline nuclei, such as ventromedial nucleus (VM). The medial pathway receives projections from the dorsal spinothalamic tract (Zhang et al., 1990), and is involved in the affective aspect of pain associated with emotional responses (Melzack & Casey, 1968). Additionally, thalamus receives descending inputs from the cortex (Yuan et al., 1985) and is connected with the hypothalamus and the striatum.

Electrophysiological studies have shown that neurons in the VPL nucleus respond to noxious and innoxious stimuli. Neurons in VPL have fixed receptive fields and respond accurately to stimuli of different intensities (Berkley et al., 1993; Harding-Forrester & Feldman, 2018). In addition, noxious stimuli could activate other thalamic nuclei, such as intralaminar nuclei, inferior central nucleus, posterior nuclei, and VM nucleus (Peschanski et al., 1981; Miletic & Coffield, 1989; Apkarian & Shi, 1994; Bester et al., 1999; Monconduit et al., 2003). However, noxious stimuli could depress the activity of the thalamic reticular nucleus, which is thought to play an important role in the regulation of sensory information processing, as thalamic reticular nucleus sends projections to the VPL and ventral basal ganglia (Alitto & Usrey, 2003). In line with experimental animal results, human brain imaging studies showed activation of the thalamus following noxious stimulation in healthy subjects (Casey et al., 1994; Derbyshire & Jones, 1998). Numerous studies have shown that the thalamus plays crucial roles not only in transmission of painful signals but also in pain facilitation. Increased peripheral responses and decreased thresholds of neurons in the VPL and ventral posteromedial (VPM) nucleus were observed following peripheral nerve injury or inflammation in rats (Guilbaud et al., 1980; 1986). Deep brain stimulation at VPL/VPM exerted pain relief in patients with chronic pain (Boccard et al., 2015). In neuropathic rats, increased cerebral blood flow was found in thalamic VPL, VM and posterior nuclei (Paulson et al., 2000). In human brain imaging studies, enhanced activities in the medial thalamus were found with innocuous thermal stimulation in subjects with heat allodynia (Lorenz & Casey, 2005).

In more recent studies performed with conscious rats, pain regulatory pathways with relays in the thalamus were connected to the early-developed, long-lasting secondary mechanical hyperalgesia and the late-developed, long term secondary heat hypoalgesia triggered by hypertonic saline-induced muscle pain (You et al., 2010). Neither mechanical hyperalgesia nor heat hypoalgesia was induced by i.m. injection of hypertonic saline in rats with a lesion of PAG and RVM. These findings indicate that the PAG-RVM-spinal cord pathway played a role in the generation of the mechanical hyperalgesia and hypoalgesia induced by i.m. injection of hypertonic saline (You et al., 2010; Lei et al., 2014). After exploring the potential pain regulatory role of thalamic nuclei one by one, the concept of the 'thalamic nociceptive discriminator' was introduced. According to it, thalamic mediodorsal

(MD) and ventromedial (VM) nuclei act as 'thalamic nociceptive discriminators' to discriminate inputs from nociceptive Aδ and C-fiber afferents. In line with this concept, the thalamic MD nucleus was shown to be involved in a circuitry that promotes descending facilitation of responses to noxious mechanical stimulation, whereas the thalamic VM nucleus was shown to play a pivotal role in a circuitry promoting descending inhibition of responses to noxious heat stimulation (You et al., 2013) (Figure 2). Further experiments showed that the triggering threshold of the descending facilitatory circuitry involving the thalamic MD nucleus is significantly lower than that of the descending inhibitory circuitry involving the VM nucleus. In addition, sex-related differences in the triggering thresholds of these descending facilitatory and inhibitory circuitries were found. Females had a lower triggering threshold of descending facilitation and higher triggering threshold of descending inhibition than males (Lei et al., 2011). Interestingly, no descending modulatory effect exerted by the thalamic nucleus Submedius (Sm) was observed in behavioral explorations (You et al., 2013), although an earlier neuroanatomical study suggested that based on its projections, the thalamic Sm nucleus might exert a role in endogenous analgesia (Coffield et al., 1992).

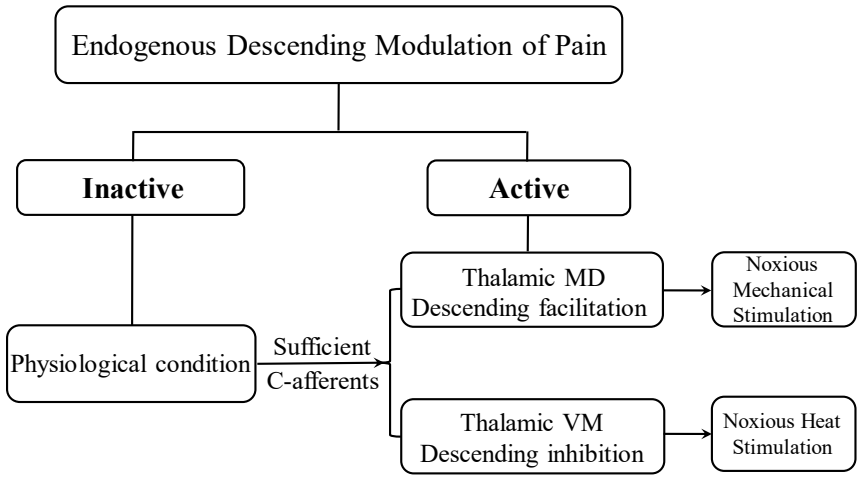

Figure 2 Roles of thalamic MD/VM nuclei in endogenous descending modulation of pain
MD: mediodorsal nucleus, VM: ventromedial nucleus

2.4 NEUROTRANSMITTORS/RECEPTORS INVOLVED IN ENDOGENOUS DESCENDING MODULATION ON PAIN

2.4.1 OPIOID PEPTIDES/RECEPTORS

Opioids are among the most important endogenous substances involved in regulation of nociceptive signals. Opioid receptors consist of μ-, δ-, κ-opioid receptors and nociceptin/orphanin FQ receptors. The first three are classical opioid receptors. Opioid receptors are widely distributed in the peripheral and

central nervous system. It has been shown that the μ-opioid receptor is enriched in different brain areas such as the cerebral cortex, striatum, thalamus and brain stem. The δ-opioid receptor is enriched in the cerebral cortex, hipocampus, caudate putamen and nucleus accumbens. The κ-opioid receptor is expressed in the cerebral cortex, hippocampus, hypothalamus, thalamus, caudate putamen and nucleus accumbens (George et al., 1994). All these opioid receptors are expressed in the superficial laminae of the spinal cord dorsal horn (Gouardères et al., 1985). Several opioid-receptor subtypes have been found. The μ-opioid receptor has μl and μ2 subtypes, the κ-opioid receptor has 3 subtypes as κ1, κ2, and κ3. The δ-opioid receptor consists of δ1 and δ2 subtypes. Opioid receptors are all G-protein-coupled receptors and highly homologous. Activation of opioid receptors inhibits the activity of adenylyl cyclase, activates potassium channels and closes calcium channels (Cunningham et al., 2019). It is worth noting that different types of opioid receptors can be found in a single neuron of the spinal cord dorsal horn or at other sites of the nervous system. In general, acute activation of opioid receptors exerts analgesic effects at peripheral and spinal cord levels. However, effects of opioid receptors, particularly that of the μ-opioid receptor, can be complicated in the supraspinal central nervous system (Ossipov et al., 2004; Schrepf et al., 2016). Following repeated or chronic administration, opioids can induce pain and hyperalgesia (Mayer et al., 1995; Celerier et al., 2000; Higgins et al., 2019) due to a switch of opioid receptor coupling from an inhibitory to a stimulatory G-protein; i.e., from Gi/o to Gs (Chakarabarti et al., 2005; Roeckel et al., 2016).

Endogenous opioid peptides are natural opioid-like substances in mammals and play important roles in pain modulation. They include dynorphin, endorphin, nociceptin/orphanin-FQ and endomorphin. The main family members of endorphin are β-endorphin, α-endorphin, δ-endorphin and γ-endorphin. Dynorphins consists of dynorphin A and dynorphin B. Enkephalin activates mainly δ-opioid receptors. Enkephalin-containing cells are widely distributed in the central nervous system, such as the amygdala, globus pallidus, striatum, thalamus, hypothalamus, brainstem and laminae I, II, V of the spinal dorsal horn (Naik et al., 1981). Enkephalin exerts antinociceptive effects at the spinal cord level as well as supraspinally (Fields & Basbaum, 1978; Kieffer 1995). β-Endorphin has a high affinity for μ- and δ-opioid receptors. β-Endorphin-containing neurons are located mainly in the arcuate nucleus of the hypothalamus, which sends projections to the midbrain PAG and pontine locus coeruleus that mediate the descending antinociceptive effect (Bloom et al., 1978; Behbehani & Fields, 1979; Basbaum & Fields, 1984). In addition, it has been shown that β-endorphin plays roles in pain inhibition in the nucleus of the solitary tract and spinal cord dorsal horn (Tsou et al., 1986; Aicher & Randich, 1990; Van der Kraan et al., 1999). Dynorphin activates primarily κ-opioid receptors, but it also has a certain affinity for μ- and δ-opioid receptors. Dynorphin exerts its antinociceptive effect by activating κ-opioid receptors in the spinal cord (Millan 1986; Ossipov et al., 1996; Hiramatsu et al.,

2001). It has been reported that dynorphin may have also pronociceptive effects in the spinal cord that are mediated by mechanisms other than opioid receptors (Dubner & Max, 1992; Laughlin et al., 1997). Endomorphin has a high affinity for μ-opioid receptors, and nociceptin/orphanin-FQ has a low affinity for the classic opioid receptors, but it has a strong affinity for nociceptin/orphanin-FQ receptors (NOP receptors), which are not sensitive to naloxone, an opioid receptor antagonist (McDonald & Lambert, 2015). NOP receptors are distributed in both ascending and descending pain pathways, including the dorsal root anglia, spinal cord and the midbrain PAG (Neal et al., 1999; Schröder et al, 2014). Activation of NOP receptors produces antinociception at peripheral and spinal cord levels (Obara et al., 2005; Rizzi et al., 2017). Species differences in the antinociceptive effect induced by activation of supraspinal NOP receptors have been described. Activation of supraspinal NOP receptors induced antinociception in monkeys (Ding et al., 2015), but hyperalgesia in rats (Pan et al., 2000; Tariq et al., 2013).

Recent study revealed that δ-opioid receptors within the thalamic VM nucleus are involved in the innocuous (43°C) heating-needle stimulation induced endogenous descending inhibition (You et al., 2014). Moreover, μ-opioid receptors in thalamic MD and VM nuclei were involved in the noxious (46°C) heating-needle stimulation induced descending facilitation and inhibition, respectively (You et al., 2014).

2.4.2 DOPAMINE AND DOPAMINE RECEPTORS

Dopamine (DA) is a monoaminergic neurotransmitter that is involved in multiple functions, including processing of pain-related signals (Girault & Greengard, 2004). A large body of evidence has shown that two of the dopaminergic cell groups in the brain exert significant roles in pain control in both humans and animals. DA neurons in the substantia nigra compacta (also known as A9 cell group) send projections to the dorsal striatum. DA neurons from midbrain ventral tegmental area (VTA) (also known as A10 cell group) send projections to the ventral striatum, the frontal cortex and parts of the limbic system. In addition, dopaminergic cells in the hypothalamic A11 cell group innervate the spinal cord (Koblinger et al., 2018) providing anatomical substrate for descending dopaminergic control of spinal nociception.

Dopamine receptors belong to a group of G-protein coupled receptors. Dopamine receptors can be classified into D1 and D2 groups according to their biochemical and pharmacological properties. The D1 receptor group consists of D1 and D5 receptor subgroups; the D2 receptor group includes D2, D3 and D4 receptors subgroups (Vallone et al., 2000). D1 receptors are distributed mainly in the cerebral cortex, caudate putamen, olfactory tubercle, nucleus accumbens, amygdala, island of Calleja, substantia nigra pars reticulata and subthalamic nucleus. D2 receptors are expressed in many brain areas, such as the cerebral cortex, caudate putamen, olfactory tubercle, nucleus accumbens, amygdala,

substantia nigra pars compacta and ventral tegmental area (Jackson & Westlind-Danielsson, 1994). In most cases, the density of D1 receptors is higher than that of D2 receptors in all brain areas (Boyson et al., 1986; Mansour et al., 1990). D3 receptors are expressed in the islands of Calleja, nucleus of the vertical limb of the diagonal band, the bed nucleus of the stria terminalis and thalamic nuclei (Bouthenet et al., 1991). D4 receptors are mainly expressed in the frontal cortex, olfactory bulb, amygdala, mesencephalon and medulla oblongata (Van Tol et al., 1992). D5 receptors have been detected mainly in the frontal cortex, hippocampal formation and mammillary nucleus (Tiberi el al., 1991). At the spinal cord level, dopaminergic fibers are distributed predominently in laminae I-VII and lamina X of the dorsal horn (Skagerberg & Lindvall, 1985; Ridet et al., 1992). D1 receptors are expressed in dorsal root ganglia and all areas of the spinal cord, whereas D2 receptors are distributed in dorsal root ganglia, superficial layers and lamina X of dorsal horn. Spinal distribution pattern of D3 receptors is similar to that of D2 receptors, but the density of D3 receptors is lower than that of D2 receptors (Dubois et al., 1986; Van Dijken et al., 1996; Levant & McCarson, 2001). Moreover, D4 receptors have been detected in the human spinal cord (Matsumoto et al., 1996).

It has been reported that D1 receptor agonists may induce pain facilitation (Aira et al., 2016). In general, D2 or D2/D3 receptors in the brain are involved in pain inhibition according to both human and animal studies (Martikainen et al., 2018). In the spinal cord, activation of the dopamine D2 receptor has also antinociceptive effects as shown by studies in which D2 receptor agonists were administered intrathecally (Jensen & Yaksh, 1984; Gao et al., 2001; Mercado-Reyes et al., 2019). In line with this, stimulation of the spinally projecting dopaminergic A11 cell group in the hypothalamus has decreased responses of nocicetive neurons in superficial and deep layers of the spinal cord dorsal horn and suppressed pain-related behavior evoked by noxious stimuli in a way that was reversed by dopamine D2 antagonistor (Carr 1984; Wei et al., 2009; Moradi et al., 2015).

2.4.3 OTHER NEUROTRANSMITTERS

Also various other neurotransmitters play important roles in descending modulation of pain. In particular, there is large amount of evidence on the contribution of serotonin and noradrenaline to descending pain controls (Pertovaara, 2006; White et al., 2018), but since these are outside the focus of this thesis, they are not described in more detail here.

2.5 PAIN IN PARKINSON'S DISEASE

Parkinson's disease (PD) is a common neurodegenerative disorder characterized by preferential loss of dopaminergic neurons, aggregation of α-synuclein as Lewy bodies (Williams-Gray & Worth, 2016), and motor symptoms that include resting tremor, bradykinesia, rigidity and postural abnormalities (Obeso et al., 2004;

Moustafa et al., 2016). Motor symptoms have been associated with a loss of dopamine neurons in nigrostriatal pathway. More recently, non-motor manifestations of PD, such as pain, depression, sleep disturbance, and autonomic disorders, have received more attention (Chen et al., 2013; Schapira et al., 2017; Rukavina et al., 2019). The prevalence of pain varies between 30 % and 95 %, and pain is an early pre-motor symptom during the development of PD (Beiske et al., 2009; Buhmann et al., 2017). Dystonic and musculoskeletal pains are the most frequently occurring types of pain in PD patients (Beiske et al., 2009). Earlier studies showed that the pain thresholds to thermal, cold, mechanical or electrical stimuli were lower in PD patients than in healthy controls (Djaldetti et al., 2004; Brefel-Courbonet al., 2005; Zambito et al., 2011; Uddin & MacDermid, 2016). However, no significant differences in pain thresholds and pain tolerance were reported in early-stage PD patients with spontaneous/ongoing pain compared with PD patients without spontaneous/ongoing pain (Mylius et al., 2009). Apart from degeneration of cerebral structures, there are studies revealing a significant loss of epidermal nerve fibers, Meissner corpuscles and an increased deposition of α-synuclein in cutaneous sympathetic fibers in PD patients (Nolano et al., 2008; Gibbons et al., 2016). It has been suggested that neurodegeneration in nociceptors may be associated with peripheral neuropathic pain in PD patients (Blanchet & Brefel-Courbon, 2018). Lewy bodies were also observed in the spinal cord, dorsal root ganglia, and sympathetic ganglia in PD patients (Sumikura et al., 2015). It has been shown that chronic PD patients may have serious atrophy of several cortical areas that have projections to descending pain control circuits (the dorsolateral prefrontal cortex, orbitofrontal cortex, and posterior cingulate cortex), which may affect the function of the descending pain modulatory system and also influence emotional aspects of pain (Polli et al., 2016). Abnormal activation of central pain producing areas such as ipsilateral prefrontal cortex, ipsilateral insula and contralateral anterior cingulate were found in PD patiens (Brefel-Courbon et al., 2005). It has been widely recognized that dopamine-dependent pathways play crucial roles in pathological processing of nociceptive inputs in PD. However, the response of PD patients' pain to levodopa treatment has been variable. A neuroimaging study showed that the abnormal response of central pain-related areas to nociceptive stimulation returned to a normal range after levodopa administration (Brefel-Courbon et al., 2005). In line with this, a significant increase in the nociceptive flexion reflex threshold was found following administration of dopaminergic medication in pain-free PD patients but not in healthy subjects (Gerdelat-Mas et al., 2007). In contrast, another study stated that no effect on abnormal processing of nociceptive inputs was observed in pain-free PD patients after administration of dopaminergic medication (Tinazzi et al., 2010). It has been suggested that the deficit in non-dopaminergic neurotransmitters including serotonergic, cholinergic, and noradrenergic systems may be also important in the modulation of pain in PD (Sauerbier et al., 2016).

2.6 ANIMAL MODELS OF PAIN IN PARKINSON'S DISEASE

Various animal models of PD including pharmacological models, neurotoxin-induced models, pesticide-induced models, genetic models and MPTP primate model have been widely used to understand the mechanisms underlying the disease and to develop treatments of PD. Most of the animal models of pain in PD have been conducted in rodents.

2.6.1 PHARMACOLOGICAL PD MODELS

Pharmacological PD models were induced by administration with pharmacological agents, reserpine and haloperidol.

Reserpine works by inhibiting the vesicular monoamine transporter and leading to loss of storage capacity and depletion of central and peripheral monoamines including dopamine, noradrenaline and 5-HT, but without accompanying neurodegeneration. Carlsson and coworker were first ones to demonstrate that L-DOPA, a compound that was later used for PD treatment in the clinic, could reverse the effects of reserpine pretreatment in mice (Carlsson et al., 1957). In spite of this, the reserpine-induced animal model was not regarded as a successful model of PD as it was not selective for dopamine. However, after it was found that noradrenergic and serotonergic systems are also affected in PD (Jellinger, 1991), the reserpine model is recognized as a relatively good model to mimic biochemical changes in PD. After reserpine treatment, there is up to 85% loss of dopamine in the substantia nigra pars compacta and more than 95% dopamine depletion in the striatum within 2 hours of reserpine injection. Moreover, dopamine content in the substantia nigra pars compacta returns to about 30% of that before treatment within 24 hours after the reserpine administration. Dopamine depletion in the striatum has been about 95% after reserpine treatment and it has lasted for at least 24 hours (Heeringa & Abercrombie, 1995). Behavioral studies indicate that reserpine treatment can cause a decrease of mechanical and thermal nociceptive thresholds (Taguchi et al., 2015). Reserpine model reflects major components of the biochemistry of PD, although reserpine does not cause dopaminergic cell degeneration in the substantia nigra. Descending pathways involved in pain control have not be screened using the reserpine model of PD.

Another pharmacological rodent model of PD is that induced by haloperidol treatment. Haloperidol is predominantly an antagonist of the dopamine D2 receptor and to a lesser degree also that of the dopamine D1 receptor. Blocking of striatal dopamine transmission with haloperidol results in abnormal downstream firing within the basal ganglia circuits. However, because of the analgesic effect of haloperidol, haloperidol model is not suitable for studying pain in PD (Salpeter et al., 2015).

2.6.2 PESTICIDE ROTENONE-INDUCED PD MODEL

Rotenone is a pesticide that has been used to induce experimental model of PD. Rotenone crosses the blood-brain barrier following systemic injections and inhibits mitochondrial complex I, which induces oxidative stress (Cicchetti et al., 2009; Abelsalam & Safar, 2015). Numerous rodent studies have shown that rotenone model mimics pathology of PD. Oxidative damage induced by protein carbonyl formation is found in many brain areas of rats treated with rotenone, a finding mimicking that reported in the brain of PD patients (Alam et al., 1997). Systemically administered rotenone induces extensive microglial activation in the substantia nigra pars compacta and striatum, which is similar to the inflammatory features observed in PD (Murakami et al., 2015). Rotenone inhibits proteasomal activity, which is implicated in PD (Wang et al., 2006). Moreover, α-synuclein and Lewy body-like cytoplasmic inclusions were found within the substantia nigra pars compacta following rotenone treatment (Johnson & Bobrovskaya, 2015).

Rotenone shows a high degree of systemic toxicity that influences e.g. the cardiovascular system and produces high mortality rates (up to 30% of animals). Only about 50% of the rotenone-treated animals show neurodegeneration (Betarbet et al., 2000). In assessments of pain behavior, rotenone did not change mechanically evoked withdrawal thresholds (Xiao & Bennett, 2012). All these factors limit the use of the rotenone model for the study of pain in PD.

2.6.3 GENETIC PD MODELS

Many transgenic models of PD, in which the gene products related to neuronal cell death in PD that include α-synuclein, PINK1, DJ-1, LRRK2, parkin, UCH-L1 and glucocerebrosidase, have been developed (Yang et al., 2009; Gubellini & Kachidian, 2015). However, very few of these models have been used in pain-related studies. Moreover, only minor changes in nociceptive responses have been observed in these models. For example, no changes in thermal thresholds were found in animals with an overexpression of SNCA gene in mice (Torres et al., 2010), and no reduction in mechanical or chemical thresholds was observed in the LRRK2 transgenic mouse model (Bichler et al., 2013). In contrast to clinical observation, nociceptive hyposensitivity was found in PINK1 transgenic animals (Gierthmühlen et al., 2009). These findings suggest that none of the gene abnormalities in the transgenic PD models developed so far is related to the development of pain in PD.

2.6.4 NEUROTOXIN-INDUCED PD MODELS

Among the most commonly used experimental models of PD is that induced by 6-OHDA treatment.

6-OHDA is a neurotoxin, which produces degeneration of dopaminergic neurons in the nigro-striatal tract and induces a PD-like pathophysiological condition (Ungerstedt, 1968; Quiroga-Varela et al., 2017). In general, when inducing experimental model of PD with 6-OHDA, it is injected into one of three locations of the nigro-striatal tract: i) the substantia nigra pars compacta, where

dopaminergic cell bodies are located; ii) the median forebrain bundle, through which the dopaminergic nigro-striatal tract ascends; iii) the terminal region, the striatum. Unilateral administration of 6-OHDA has been used most commonly, since it prevents the high mortality rate associated with bilateral full lesion models. In addition, PD model induced by unilateral injection of 6-OHDA provides the advantage that the behavior at the impaired side can be compared with that in the contralateral side. Nigrostriatal 6-OHDA-lesions cause motor impairment in the limbs contralateral to the lesion side. 6-OHDA model has been used for studying pain in PD. Numerous studies have reported hypersensitivity to thermal, mechanical, chemical or cold stimuli in 6-OHDA-lesioned rats (Gomez-Paz et al., 2018; Domenici et al., 2019). In most cases, the changes in thresholds are bilateral, although 6-OHDA was administered unilaterally. Changes in nociception have been observed as early as one week after the 6-OHDA lesion and the changes have persisted for at least 12 weeks. It has been suggested that 6-OHDA lesions induce stable alterations and persistent plasticity. Unilateral injection of 6-OHDA into the medial forebrain bundle or substantia nigra of rats shows an immediate and almost full destruction of dopamine neurons within the substantia nigra pars compacta and the ventral tegmental area. Such a treatment produces a model that mimics the end-stage of PD (Faull & Laverty, 1969; Grandi et al., 2018). A more slowly developing partial lesion of the nigrostriatal pathway has been achieved by administering 6-OHDA into the striatum, which represents an early-stage PD model (Boix et al., 2018). After striatal 6-OHDA treatment, the damage involves both the dopaminergic nerve terminals innervating the striatum and retrogradely dopaminergic cell bodies (Sauer & Oertel, 1994; Blandini et al., 2007). At doses higher than 6 μg, 6-OHDA produces a significant dopamine depletion, and at doses 6-14 μg, 6-OHDA has elicited at least 40-60 % depletion of dopamine that is accompanied by D-amphetamine/apomorphine-induced rotation behavior (Ishida et al., 2005; Miyanishia et al, 2019). Unilateral striatal lesion with 6 μg of 6-OHDA mimics clinically observed early deficits in isometric force control that affects the daily life of early-stage PD patients (Bethel-Brown et al., 2011). Assessments of pain behavior in 6-OHDA-treated rats showed decreased nociceptive withdrawal thresholds induced by mechanical or thermal stimuli (Zengin-Toktas et al., 2013; Domenici et al., 2019).

Numerous studies suggest that dopaminergic, serotonergic, and opioid pathways are involved in modulating nociceptive thresholds in the 6-OHDA lesion rat model which reflect findings in the clinic (Brefel-Courbon et al., 2013; Uchida et al., 2019). Taken together, the 6-OHDA model provides an ideal platform to explore the pathophysiology of pain symptoms and test potential pain therapy in PD.

2.7 EXPERIMENTAL MUSCLE PAIN

Intramuscular injection with hypertonic (HT) saline has been widely used to mimic

clinical muscle pain in humans since it was first introduced by Kellgren (Kellgren, 1938). Injection with HT saline into limb muscles, neck muscles, shoulder muscles, lower back or jaw muscles induces transient pain at the injected area, and the pain quality and intensity are similar to that in clinical myalgia. HT saline activates group III and IV nociceptive muscle afferents, corresponding to nociceptive Aδ and C afferents in cutaneous nerves, respectively (Iggo, 1961). In the spinal dorsal horn of lightly anesthetized rats, wide-dynamic range neurons, which are presumably pain-relay neurons, were activated by HT saline injections and this was associated with nocifensive behavior (Ro & Capra, 1999; Ro et al., 2003). The time course of neuronal and behavioral responses induced by HT saline injection in animals was similar to that of local pain ratings induced by HT saline in humans. In addition, HT saline injections have elicited pain sensations that are reported at areas remote to local pain areas; i.e., subjects have reported referred pain following injection of HT saline. Referred pain has been recognized as a characteristic property of clinical muscle pain. In human studies, referred pain has been induced by intramuscular injections of HT saline and other algogenic substances. For example, injection of HT saline into the temporalis muscle produced referred pain in teeth (Jensen & Norup, 1992). Injection of HT saline into the tibialis anterior muscle produced referred pain on the dorsal surface of the ankle (Lei et al., 2008). In experimental animals, bilateral hyperalgesia has been observed after unilateral muscle injection of carrageenan or HT saline (Radhakrishnan et al., 2003; You et al., 2016).

Human studies have shown that it is possible to induce also long-lasting pain by intramuscular infusion of HT saline (Zhang et al., 1993; Lei et al., 2008). Intramuscular injections of other agents including acidic saline, capsaicin, carrageenan, mustard oil, and complete Freund's adjuvant (CFA) have also produced long-lasting muscle pain and robust inflammatory responses. In humans, intramuscular injection of capsaicin (a TRPV1 channel agonist) induced longer-lasting local muscle pain and more extensive referred pain than intradermal administration of capsaicin (Witting et al., 2000). Injection of capsaicin into rat hind paw muscles induced long-lasting (up to 4 weeks) bilateral mechanical allodynia and unilateral heat hypoalgesia (Sluka, 2002). Repeated injection of acidic saline into the gastronemius muscle in rats produced mechanical and heat hyperalgesia that lasted for several weeks (Sluka et al., 2003). Immunohistochemical studies showed that injection of mustard oil (a TRPA1 channel agonist) or CFA into the masseter muscle induced nocifensive behavior that was associated with an increase of Fos expression (a marker of neuronal activation) in the trigeminal subnucleus caudalis, a medullary relay for nociceptive signals originating in the orofacial area (Imbe et al., 1999; Ro et al., 2004). Carrageenan, which activates group III and IV nociceptive muscle afferents and produces myositis (Berberich et al., 1988), has produced bilateral mechanical hyperalgesia when administered unilaterally into the GS muscle (Radhakrishnan et al., 2003). Among many other endogenous algogens that have been used in attempts to induce muscle pain in human and animal studies are substance P,

bradykinin, serotonin, ATP and glutamate (Capra & Ro, 2004).

Sensitization of peripheral nociceptors at the site of injury causes primary hyperalgesia at the injury site that is predominantly due to peripheral mechanisms, and secondary hyperalgesia adjacent to the injury site that is due to central mechanisms (Hardy et al., 1950; Meyer et al., 1988; Simone et al., 1991). Concerning afferents innervating the muscles, of which are 2/3 are unmyelinated (Mense, 1993), it has been demonstrated that conditioning stimulation of muscle C-fibers, unlike that of cutaneous C-fibers, produced prolonged increase in the excitability of motoneurones (Wall & Woolf, 1984). This is in line with the long-lasting secondary mechanical hyperalgesia induced by i.m. injection of different algogenic agents. For example, unilateral intramuscular injection of 5.8% hypertonic (HT) saline into the GS muscle induced a bilateral decrease of the mechanically evoked hind limb withdrawal threshold (secondary mechanical hyperalgesia) that was observed within 30 min after the injection and that lasted for 5 days (You et al., 2010). This was accompanied by a bilateral increase of heat-evoked hind limb withdrawal latency (secondary heat hypoalgesia) that was observed from the first day after the injection and that lasted for one week. Further experiments showed that the secondary mechanical hyperalgesia and the secondary heat hypoalgesia induced by experimental muscle nociception were regulated by descending facilitatory and descending inhibitory circuits, respectively (You et al., 2010). Thereby, sustained muscle pain induced by i.m. injection of algogenic compounds, such as HT saline, provides a method to activate in a tonic fashion otherwise inactive descending pain controls and to study separately descending facilitation and descending inhibition by determining secondary mechanical hyperalgesia and secondary heat hypoalgesia, respectively.

2.8 TRADITIONAL ACUPUNCTURE AND HEATING-NEEDLE STIMULATION

2.8.1 ACUPUNCTURE

Acupuncture is an important pain treatment method in traditional Chinese medicine. For acupuncture treatment, fine needles are inserted into acupoints either in the skin or in the muscle, and manual techniques including lifting, inserting and twisting of the needle are applied during treatment to activate the meridians, a concept in traditional Chinese medicine about a path through which the life-energy known as "Qi" flows. According to traditional Chinese medicine, regulation of Qi by acupuncture needles is required for therapy, since stagnation of Qi will cause pain and other diseases. Nowadays, electric stimulation with acupuncture needles is commonly used in therapy, which is called electroacupuncture. Human and animal studies have shown that analgesic effect can be achieved by electroacupuncture as well as traditional acupuncture (Cheng, 2014; Choi et al., 2015). Studies addressing neurobiological mechanisms underlying acupuncture-induced analgesia indicate that electroacupuncture may

alleviate pain by activating the descending pain inhibitory system. For example, it has been demonstrated that electroacupuncture could activate the supraspinal 5-HT-containing RVM and norepinephrine-containing LC to produce its analgesic effects. (Hu et al., 2016; Seo et al., 2016). 5-HT1 and 5-HT3 receptors have been shown to be among neurotransmitter receptors involved in mediating the electroacupuncture-induced alleviation of chronic pain (Seo et al., 2016). In addition, electroacupuncture-induced analgesia has been shown to involve recruitment also other supraspinal pain-regulating sites such as the anterior cingulate cortex, amygdala, hypothalamus, and PAG, and in addition to serotonin and noradrenaline, many other neurotransmitters such as endogenous opioids and γ-aminobutyric acid (GABA) (for review see Lv et al., 2019).

2.8.2 HEATING-NEEDLE STIMULATION

Moxibustion is another traditional Chinese pain therapy that has been used to treat pain-related diseases for thousands of years. The methods for performing moxibustion were recorded as early as about 3000 years ago. According to traditional Chinese medicine, it is necessary to apply moxibustion when neither medicaments nor acupuncture works. More than 500 years ago, the silver needle therapy was used for the treatment of shoulder pain, knee pain and many other osteoarticular dysfunctions, with a good therapeutic effect. The traditional silver needle was made of 85% silver and of copper and chromium alloy. In general, the needle body has a varying length (6-15cm), while the needle tail is 5cm long and the diameter of the needle is 1.0-1.1mm. During moxibustion treatment, a stick of moxa (a soft woolly mass prepared from the ground young leaves of a Eurasian Artemisia) is fixed at the needle tail and ignited. After recognizing that among disadvantages of silver needle therapy are air pollution from smoke and skin burns caused by burning of moxa, the temperature-controlled silver needle was first introduced in 2002. The size and materials of the temperature-controlled needle are similar to that of the traditional silver needle. The tail of the temperature-controlled needle is connected to a tubular-cased heating element that is temperature-controlled by a computer. It is smokeless and with no burn hazards. A disadvantage of the first-generation temperature-controlled silver needle apparatus is that the temperature decreases gradually from the tail to the top of the needle. In 2012, an inner-heating therapeutic apparatus was developed (see Figure 6), in which the heating element is located inside the needle, due to which there is no difference in the temperature along the needle body. It is still not known whether heating-needle stimulation at acupoints and non-acupoints induces different analgesic effects, whether heating-needle stimulation is effective against pain induced by Parkinson's disease, or which neurotransmitter receptors are involved in mediating the heating-needle stimulation induced attenuation of pain.

To determine an optimal needle temperature for heating-needle stimulation, a pilot study was performed in healthy rats. In the pilot study, single/double

heating-needle was inserted into the ipsilateral gastrocnemius muscle. The temperature of heating-needle stimulation was first set at 40°C and the treatment lasted for different periods (15, 30, and 45 minutes). Paw withdrawal reflex threshold elicited by noxious mechanical and heat stimulation was determined bilaterally 30 minutes, 1-4h and 1-7 days after completing the heating-needle stimulation Intramuscular insertion of a single/double needle without heating for 45 minutes was set as a control. In the following experiments, temperature of heating-needle stimulation was increased by 1°C from session to session, and the effect of heating-needle stimulation on endogeneous descending modulation of pain was explored at a range of stimulus temperatures from 40°C to 46°C. The results showed that insertion of needles without heating or heating-needle stimulation at temperatures varying from 40°C to 42°C for 45 min had no significant influence on paw withdrawal reflex elicited by noxious mechanical or heat stimulation. However, descending inhibition was triggered by the i.m. heating-needle stimulation at a temperature of 43°C for 30-45 min. The results of the pilot study (You et al., 2014) were used when choosing stimulus parameters for studies II and III. The effect of 44-46°C heating-needle stimulation (single needle) on descending modulation of pain in the pilot study is shown in Table 1.

Table 1 Effects of 44-46 °C H.N.S. on descending modulation of pain

	D. Facilitation			D. Inhibition		
	15min	30min	45min	15min	30min	45min
44 °C	0	0	0	0	↑	↑
45 °C	0	0	0	0	↑	↑
46 °C	↑	↑	↑	0	↑	↑

H.N.S.: heating-needle stimulation, D.: descending, 0: no effect, ↑: increase

3 AIMS

The current series of studies was performed to explore plasticity of descending modulation of pain in humans and rats under different experimental conditions and to explore whether i.m. heating-needle stimulation provides a method to attenuate pain due to action on descending pain modulatory circuitries. In all studies, sustained muscle pain induced by i.m. 5.8% saline was used as a noxious conditioning stimulus to activate descending pain modulatory circuitries and to allow using secondary mechanical hyperalgesia as an index of descending pain facilitation and secondary heat hypoalgesia as an index of descending pain inhibition. Specific aims of the study were as follows:

(1) To explore changes in descending modulation of pain in rats with simulated weightlessness. Moreover, spinal neuronal correlate of descending facilitation and inhibition was explored using immunohistochemical methods.

(2) To explore effects of non-painful (43°C) heating-needle stimulation at acupoints and non-acupoints on descending modulation of pain in healthy subjects.

(3) To explore whether an experimental model of PD causes pathophysiological pain-promoting changes in descending modulation of pain and whether non-painful (43°C) heating-needle stimulation can reverse these pathophysiological changes. Moreover, potential role of the dopamine D2 receptor in the thalamic MD/VM nuclei in the heating-needle stimulation-induced modulation of pain behavior in experimental PD was explored.

4 METHODS

4.1 ANIMALS

Male Sprague-Dawley rats at 10 weeks age were provided by Animal Center of the College of Medicine, Xi'an JiaoTong University. Rats were housed with 2 rats a group in plastic boxes under a 12:12 h light dark cycle, room temperature was at 22-26°C, food and water were available ad libitum. All experiments were approved by the Animal Care and Use Committee of Xi'an JiaoTong University. The experiments were performed according to the guidelines of European Communities Council Directive of 22nd September 2010 (2010/63/EU). Before the experiments, the rats were acclimatized to the laboratory and testing boxes for at least 1 hour per day during five consecutive days. At the end of the experiment, the rats were sacrificed with an overdose of sodium pentobarbital. All efforts were made to minimize the number of animals and the suffering of the animals.

4.2 SUBJECTS

Fifty healthy male volunteers with age range 18-43 years and mean age 23.4 years were involved in the study. None of them had signs or symptoms of peripheral or central nervous system disorders. None had either chronic pain or any medical treatment. They were informed about the experimental procedures and all signed a consent form that was in accordance with the Declaration of Helsinki. The experiments were approved by the Ethics Committee of Xi'an Jiaotong University. The experiments were performed in a quiet enviroment at a temperature of 22-23°C.

4.3 DRUGS

6-OHDA (6-hydroxydopamine), dopamine, raclopride (dopamine D2 receptor antagonist), apomorphine, sodium pentobarbital, desipramine, capsaicin (TRPV1 channel agonist) and Tween 80 were purchased from Sigma-Aldrich Chemie Gmbh (Germany). Isoflurane was purchased from Yapei Pharmaceuticals Inc. (Shanghai, China). Benzylpenicillin sodium was purchased from Harbin Pharmaceutical Group Holding Co. Ltd. (Harbin, China). Ascorbic acid, 10% saline and 0.9% saline were purchased from Shijiazhuang No.4 Pharmaceutical Industry Limited Company (Shijiazhuang, China).

In immunohistochemical experiments, rabbit polyclonal antibody against c-fos was purchased from ABCAM (Cambridge, UK), sheep-anti-rabbit IgG and ABC complex were purchased from BOSTER (Wuhan, China).

4.4 EXPERIMENTAL DESIGN

The series of experiments were performed to investigate the plasticity of descending modulation of pain as a potential cause of chronic pain under various conditions that included simulated weightlessness and experimental PD as well as physiological conditions. In particular, the effect of heating-needle stimulation applied at an innocuous temperature on descending modulation of pain was studied in healthy subjects and in rats with an experimental model of PD (Figure 3). Descending modulatory circuitries are to a large extent inactive under baseline conditions, due to which the study of descending modulation of pain is difficult, unless descending pain modulatory circuitries are activated by a noxious conditioning stimulus, such as sustained muscle nociception induced by HT saline. Therefore, in all experiments of the present study descending pain modulatory circuitries were activated by i.m. injection of HT saline.

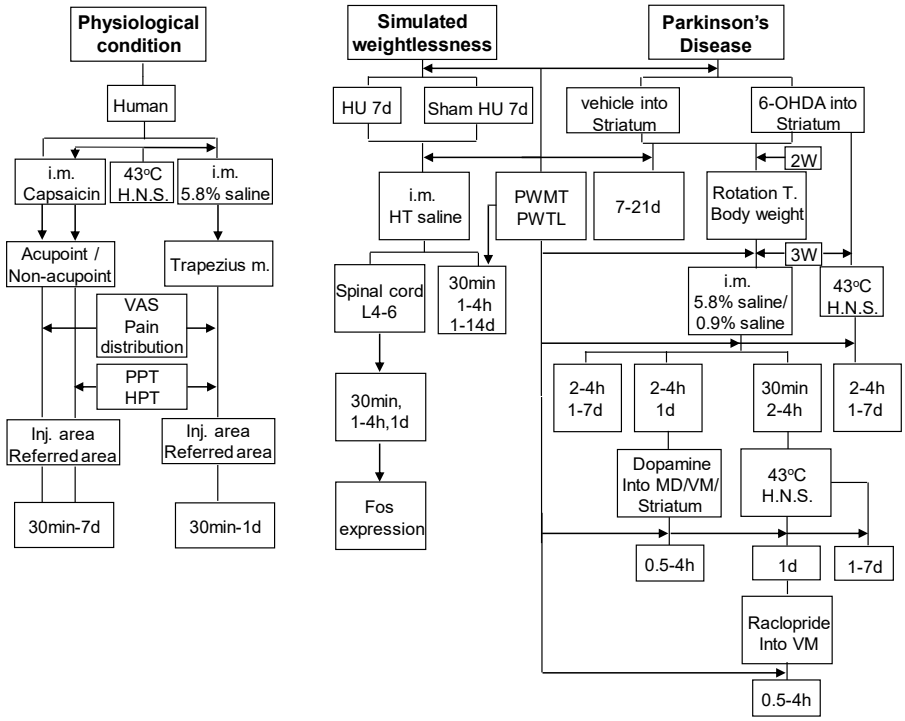

Figure 3 Experimental design

i.m.: intramuscular, VAS: Visual Analogue Score, PPT: Pressure pain threshold, HPT: Heat pain threshold, inj.: injection, H.N.S.: Heating-needle stimulation, PWMT: Paw withdrawal mechanical threshold, PWTL: Paw withdrawal thermal latency, MD: Mediodorsal nucleus, VM: Ventromedial nucleus, HU: Hindlimb unloading, Rotation T.: Rotation test, raclopride: dopamine D2 receptor antagonist.

4.5 CONTENTS OF THE STUDIES

The contents of the current series of studies are shown in Table 2.

Table 2 Contents of the studies

	Experiments	Study design	Outcome measures	Interventions	
				Pharmac.	Non-pharmac.
Study I (animal)	Muscle pain in HU rats (n=8)	HT saline	Heat Von Frey		
	Fos expression in spinal cord during muscle pain in HU rats (n=6)	HT saline			
Study II (human) (n=10)	Pain induced at acupoint and non-acupoint area	capsaicin	Heat Pressure		
	Effect of H.N.S. at acupoint and non-acupoint area				H.N.S.
	H.N.S. at acupoint induced analgesia in muscle pain	HT saline			H.N.S.
Study III (animal) (n=10)	PD model	6-OHDA	Apomorphine rotation		
	Muscle pain in PD rats	HT saline			
	Descending modulation of pain in PD rats		Heat Von Frey	Dopamine	
	Effects of H.N.S. in muscle pain in PD rats	HT saline		Raclopride	H.N.S.

H.N.S.: heating-needle stimulation, HT: hypertonic, HU: hindlimb unloading, PD: Parkinson's disease
Pharmac.: Pharmaceutical, n: number of animals/subjects in each experimental group

4.6 SURGERIES

4.6.1 INSTALLATION OF INTRACEREBRAL CANNULA

After anesthesia, the head of the animal was fixed by stereotaxic apparatus, and the skin over the skull was cut with a surgical knife. A hole was drilled in the skull to insert a guide cannula. The guide cannula with outer diameter of 0.35 mm and inner diameter of 0.25 mm (RWD Life Science Co., Shenzhen, China) was inserted to allow microinjections into the thalamic mediodorsal (MD), ventromedial (VM) nuclei or striatum according to the following coordinates: MD nucleus: anteroposterior –(2.3-2.8) mm from bregma, lateral 0.75 mm from midline, dorsoventral 5.2-5.4 mm from the cranium; VM nucleus: anteroposterior –(2.3-2.8) mm, lateral 1.2-1.5 mm and dorsoventral 7.1-7.2 mm; striatum: anteroposterior 1.0 mm, lateral 3.0 mm and dorsoventral 5.0 mm from the cranium (Paxinos and Watson, 1998). After the insertion of the guide cannula, the cannula was fixed in the skull by dental cement and the wound was sutured (Study III).

4.6.2 NEUROTOMY OF TAIL NERVE

After the induction of anesthesia with pentobarbital, sacral nerves 1 and 2 were

exposed and transected with scissors and surgical knife under a microscope (Nikon SMZ-645, Japan). After the nerve transection, the wound was sutured in layers (Study I).

4.7 HINDLIMB UNLOADING (HU)

The rat tail was washed, air-dried, after which benzoin and tincture of rosin were gently applied on the tail. The tail was wrapped with bandage, and a 1cm-wide piece of tape was attached along side of the tail bilaterally and to form a loop near the end of the tail. Then the rat was suspended in a head-tilt position with an angle of 30° from the horizontal plane in individual plexiglass box (25×25×50cm). The tape-loop was attached to a pulley system allowing the animal a free movement in the cage by using its forelimbs, but its hindlimbs could not touch any surface of the cage (Study I) (Figure 4).

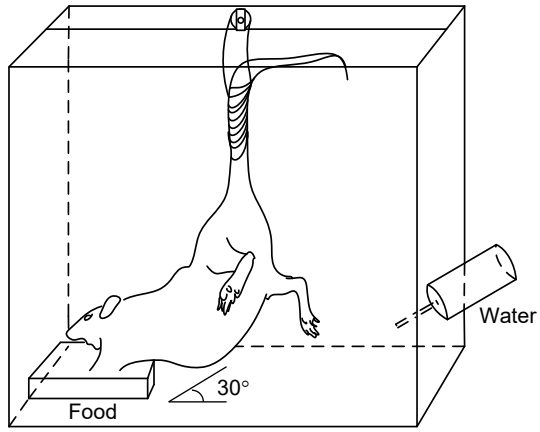

Figure 4 Hindlimb unloading (HU) by tail suspension

4.8 MUSCLE NOCICEPTION INDUCED BY INTRAMUSCULAR INJECTION OF ALGOGEN

In animal experiments, muscle pain was induced by intramuscular injection with 5.8% HT saline. The injection site was in the middle of the gastrocnemius muscle, and the depth of the injection was about 0.5 cm. The injection was performed manually and lasted more than 30 seconds. The volume of HT saline was 0.2ml (Study I, III).

In the human study, muscle pain was induced by intramuscular injection of 5.8% HT saline into the trapezius muscle or that of capsaicin into acupoints and non-acupoint sites. The injection site of HT saline was 2cm lateral to the halfway point between the spinous process of the 7th cervical vertebra and the lateral edge

of the acromion. The volume of HT saline was 1ml. The injection was performed with a computer-controlled syringe pump (RWD Co., Shenzhen, China) over 1 min. Capsaicin 100 μg/50 μl was injected intramuscularly at ST36 (Zusanli)/ ST37 (Shangjuxu) acupoints and non-acupoint sites unilaterally. ST36 is located four-finger breadths below the lower margin of the patella and one-finger breadth laterally from the anterior crest of the tibia. ST37 is located four-finger breadths below the ST36, and one finger-breadth (middle finger) from the anterior crest of the tibia (Figure 5). One non-acupoint site was located at a middle site between the ST36 and ST37 acupoints. The other non-acupoint site was located in the midway between ST35 (Dubi: below the patella in a depression lateral to the patellar ligament with knee flexed) and ST36. The depth of injections was 2 cm (Study II).

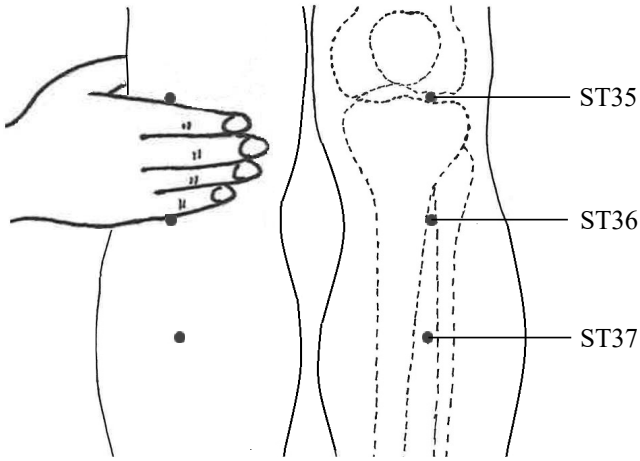

Figure 5 Anatomical diagram of acupoint ST35, ST36 and ST37
ST: stomach meridian

4.9 INTRAMUSCULAR HEATING-NEEDLE STIMULATION

The heating-needle is a stainless-steel needle filled with a heating element and a temperature probe, and is feedback-controlled by a computer (Figure 6). The accuracy of the stimulation temperature is +/- 0.25 °C. In animal studies, the inserting site was at the middle part of the gastrocnemius (GS) muscle; and the depth of the insertion was about 0.5 cm. In the human study, the inserting site was at acupoint ST36/ST37. The temperature of heating-needle stimulation was 43°C (innocuous range) (Study II, III).

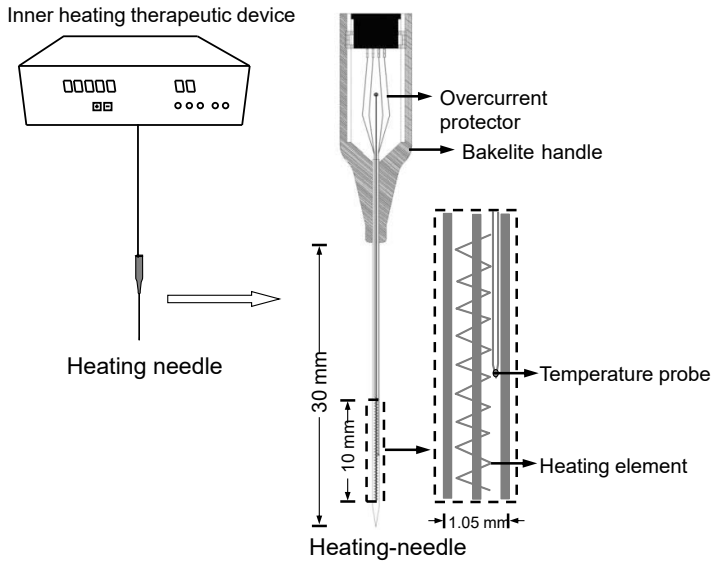

Figure 6 Schematic diagram of the heating-needle temperature control system

4.10 BEHAVIORAL TESTS IN ANIMAL EXPERIMENT

4.10.1 MEASUREMENT OF MECHANICAL SENSITIVITY

Rats were placed in individual plexiglass boxes with mesh floors and transparent covers. The paw withdrawal mechanical threshold was detected by hand-held electronic von Frey device (2290 Electrovonfrey®, IITC, Woodland Hills, CA, USA) with a rigid filament. The mechanical threshold was identified as the filament was applied to the heel of the hind paw and a paw-withdrawal response was elicited (Study I, III).

4.10.2 MEASUREMENT OF HEAT SENSITIVITY

The paw withdrawal thermal latency (PWTL) was determined by a 390G plantar stimulator Analgesia Meter (IITC, Woodland Hills, CA). Rats were placed individually in a Plexiglas cubicle which was placed onto a constant temperature-controlled glass. The temperature of the glass was maintaind at 32°C. The heat stimulation was applied on the heel of the hind paw. The time from the onset of heat stimulation to withdrawal response of the hind paw was regarded as PWTL. Baseline PWTL was set to about 10-11 seconds by adjusting the intensity of heat stimulation. Cut-off time was set at 20 seconds to avoid tissue injury (Study I, III).

4.10.3 ASSESSMENT OF MOTOR FUNCTION

Rats were placed on a Rota-Rod treadmill (Model 755, IITC, Woodland Hills, CA, USA) rotating at a gradually increasing speed from 5 to 30 rpm during 30 seconds and then maintained at 30 rpm for 120 seconds. Motor performance was defined as

the time rats were able to stay on the treadmill (Study III).

4.10.4 APOMORPHINE-INDUCED ROTATION BEHAVIOR

To confirm a PD-like motor disorder, 2 weeks after unilateral microinjection of 6-OHDA into the striatum, apomorphine (0.5 mg/kg) was injected subcutaneously to induced body rotations. The observation of body rotations started after the administration of apomorphine and lasted for 30 minutes. The rate of body rotations was quantified as the number of 180° turns contralateral to the side of the chemical injury of the striatum (Study III).

4.11 MEASUREMENT OF PAIN SENSITIVITY IN HUMAN STUDY (STUDY II)

4.11.1 MEASUREMENT OF PRESSURE PAIN THRESHOLD

Pressure pain threshold (PPT) was assessed by means of a hand-held electronic algometer (Somedic AB, Stockholm, Sweden) with a rounded rubber probe with a surface area of 1cm^2. The probe was pressed on the test site perpendicularly and the pressure was increased at a constant rate of 30 kPa/s. When the subjects felt the first pain that was considered PPT, they were requested to press a button that froze the pressure reading in the digital display of the algometer.

4.11.2 MEASUREMENT OF HEAT PAIN THRESHOLD

Heat pain threshold (HPT) was assessed by a modified heat stimulator (TaiMeng Co., Ltd, ChengDu, China). The heat stimulator was applied 2 cm away from the test area. Subjects were asked to press a button to stop the ascending stimulus temperature when they felt the first sensation of pain.

4.11.3 VISUAL ANALOG SCALE (VAS)

After the i.m. injections at acupoints or non-acupoints, pain intensity was assessed by subjects every minute for 5 min using a 0-100mm visual analog scale (VAS). The subjects were instructed that 0 mm means no pain and 100 mm means intolerable pain. The assessment was followed every 5 min for 55 min. After the i.m. injection of HT saline into the trapezius muscle, the pain intensity was scored by the subjects continuously on a 0-10 cm electronic VAS, in which 0 cm meant no pain and 10 cm meant intolerable pain. The data was saved on the computer every 5 seconds.

4.11.4 PAIN DISTRIBUTION

After i.m. injections at acupoints, non-acupoints or trapezius muscle, pain

distribution at the local pain area and the referred pain area was drawn by the subject using an anatomical map. Local pain was defined as pain around the injection site, and referred pain was defined as pain outside of the local pain area.

4.12 IMMUNOHISTOCHEMICAL DETECTION OF FOS EXPRESSION

Animals were deeply anesthetized and perfused intracardially with first 200ml of 0.1M phosphate-buffered saline (PBS), then 500ml of 4% paraformaldehyde in 0.1M phosphate buffer. The lumbar spinal cord was then removed and fixed in 4% paraformaldehyde for 3h then soaked in 10% sucrose in PBS for 1 hour and 30% sucrose in PBS at 4°C overnight. The tissues were cut into coronal section slices that had the thickness of 15 μm with a freezing microtome (CM1900, Leica, Germany). The avidin–biotin–peroxidase complex (ABC) method was used to detect Fos expression. After rinsed in 0.1M PBS, the sections were incubated with 0.3% hydrogen peroxide in 0.1M PBS for 30min. After washed thoroughly, the sections were blocked in 0.1 M PBS with 0.2% Triton-X-100 with 10% goat serum for 30 min; then incubated with a rabbit polyclonal antibody against c-fos at 4°C overnight. After rinsing in 0.1 M PBS, the sections were incubated with sheep-anti-Rabbit IgG for 1 h at room temperature, then with the ABC complex. After washing in 0.1M PBS, the reaction product was visualized with 0.05% diaminobenzidine solution with 0.006% hydrogen peroxide for 2 min and then rinsed in 0.1M PBS. Following the immunostaining, mounting on APES-coated glass slides and air-dried overnight. Slices were dehydrated and cleared in alcohol series and xylene, respectively. After covering the slides, they were observed under a light microscope (Study I).

4.13 STATISTICS

All results were expressed as mean ± SEM. The data were analyzed using SigmaStatTM (Systat Software Inc., California, USA). $P < 0.05$ was considered statistically significant.

In behavioral assessments of Study I, differences among experimental groups were analyzed using two-way analysis of variance (ANOVA) followed by post hoc Bonferroni's test for multiple comparisons. The mean numbers of labeled cells among experimental groups in immunohistochemical experiments were analyzed using one-way ANOVA followed by post hoc Bonferroni's test (Study I).

In the human study (Study II), VAS scores and pain distribution data were analyzed by one-way ANOVA. PPT and HPT were compared by two-way ANOVA, followed by Student-Newman-Keuls test for post hoc testing.

In Study III, t-test was used to analyze differences between two groups (i.e.; difference in apomorphine-induced rotations of PD rats following heating-needle stimulation). One-way ANOVA was used to compare the effects of striatal treatment with 6-OHDA/vehicle on Rota-rod results, level of dopamine in the striatum, apomorphine-induced rotations among various treatment groups, and body weight. Two-way repeated-measures analysis of variance (two-way RM ANOVA) with a post-hoc Bonferroni t-test was used for analyses of the differences in the withdrawal reflex among different groups (Study III).

5. RESULTS

5.1 RESULTS IN ANIMAL STUDIES

5.1.1 CHANGES IN DESCENDING CONTROL OF PAIN UNDER SPECIAL AND PATHOLOGICAL CONDITIONS

To assess changes in descending modulation of pain during simulated weightlessness, noxious mechanical and heat stimulation-evoked paw withdrawal reflexes were assessed with or without simulated weightlessness in animals, in which descending modulatory pathways were activated with i.m. injection of HT saline. Hindlimb unloading (HU) for 7 days was used to simulate weightlessness. Following unilateral i.m. injection of HT saline, a bilateral decrease of the paw withdrawal mechanical threshold (PWMT) was found (secondary mechanical hyperalgesia) with and without simulated weightlessness. Thirty minutes - one day following i.m. injection of HT saline, PWMT was significantly lower in rats with simulated weightlessness than rats not exposed to HU. Three days after the i.m. injection of HT, the PWMT in rats with simulated weightlessness returned to the same level as in rats without simulated weightlessness. One day after i.m. injection of HT saline, heat-induced paw withdrawal responses were significantly different in rats with as in rats without simulated weightlessness. In rats without simulated weightlessness, bilateral prolonged paw withdrawal thermal latency (PWTL) (secondary heat hypoalgesia) was observed 1 day after the i.m. injection and it lasted for 7 days. In rats with simulated weightlessness, secondary heat hypoalgesia induced by i.m. injection of HT saline was delayed and observed not earlier than 3 days after the i.m. injection, after which heat hypoalgesia declined progressively to baseline level by the 5th day following the i.m. injection.

In parallel to the assessment of pain behavior, Fos expression in lumbar segments 4-6 of the spinal cord was explored at different time points following i.m. injection of HT saline. The number of Fos-like immunoreactive (Fos-LI) cells was bilaterally increased in rats exposed to simulated weightlessness. The increases in Fos-LI cells were located only in the laminae I-II of the spinal dorsal horn. Denervation of the tail did not influence the effect of simulated weightlessness on Fos expression in the spinal dorsal horn. Following i.m. injection with HT saline, Fos expression was increased in all layers of the spinal dorsal horn within 30 min and the increase peaked at 1 hour, after which Fos expression gradually declined to the background level 4-8 hours after the i.m. injection of HT saline. In superficial layers (laminae I-II), the HT saline-induced increase in Fos expression was higher in rats with simulated weightlessness than control rats. In contrast, the increase of Fos expression in middle (III-IV) and deep (V-VI) layers of the spinal cord was significantly lower in rats with simulated weightlessness than

controls. Combined with results from parallel behavioral tests, immunohistochemical findings on Fos expression suggest that superficial layers (I-II) of the spinal dorsal horn play a role in descending facilitation and middle/deep (III-IV) layers in descending inhibition of pain (Table 2).

Early-stage PD was induced in rats by unilateral striatal lesion with neurotoxin 6-OHDA. Three weeks after striatal administration of 6-OHDA, no significant differences in PWMT and PWTL were found among PD rats, rats with a striatal sham lesion, and naïve rats. Injection of physiological (0.9%) saline at a volume of 0.2 ml into the GS muscle induced secondary mechanical hyperalgesia in PD rats, but not in sham-lesioned rats or naïve rats, which finding suggests that descending facilitatory modulation of pain was increased in the PD group. Injection of HT saline into the GS muscle, in contrast, induced secondary heat hypoalgesia in sham-lesioned and naïve rats, but not in PD rats suggesting a decrease of descending inhibition in the PD group. The increased descending facilitation and decreased descending inhibition in the PD group could not be reversed by striatal microinjection of dopamine. However, dopamine administration into the thalamic MD nucleus reduced descending facilitation and dopamine administration into the thalamic VM nucleus enhanced descending inhibition in PD rats indicating that dopamine controls descending modulation of pain, at least partly, due to action on thalamic MD and VM nuclei (Table 3).

Table 3 Changes in descending control of pain under special and pathological conditions

	Simulated weightlessness (I)		PD model (III)	
	D.F.	D.I.	D.F.	D.I.
Changes in descending control of pain	↑	↓	↑	↓
Anatomical sites involved	SDH I-II layer	SDH III-VI layer	Thalamic MD n.	Thalamic VM n.

D.F.: descending facilitation, D.I.: descending inhibition, SDH: spinal dorsal horn, PD: Parkinson's disease, I: study I, III: study III, ↑: increased, ↓: decreased

5.1.2 EFFECTS OF HEATING-NEEDLE STIMULATION ON DESCENDING CONTROL OF PAIN

In PD rats, i.m. heating-needle stimulation for 30 min at the temperature of 43°C had no effects on secondary mechanical hyperalgesia induced by i.m. injection of HT saline, whereas the HT saline-induced secondary heat hypoalgesia was significantly enhanced by heating-needle stimulation at 43°C. The 43°C heating-needle stimulation induced enhancement of secondary heat hypoalgesia in HT-treated animals was dose-dependently decreased by microinjection of

raclopride (dopamine D2 receptor antagonist) into the thalamic VM nucleus. In addition, 43°C heating-needle stimulation also reversed the increase in the number of the apomorphine-induced body rotations in PD rats. The results showed that in an experimental rat model of PD descending inhibition is decreased, but 43°C heating-needle stimulation restores descending inhibition in PD animals through a mechanism that involves the dopamine D2 receptor in the thalamic VM nucleus (Table 4).

Table 4 Effects of heating-needle stimulation on descending control of pain

	PD model (III)		
	43°C	MD n.	VM n.
D. Facilitation	0	0	0
D. Inhibition	↑	0	↑ (D2R)

PD: Parkinson's disease, III: study III, D.: descending, 0: no effect, ↑: increased, D2R: dopamine D2 receptor

5.2 RESULTS IN HUMAN STUDY

5.2.1 DIFFERENCES IN EFFECTS OF HEATING-NEEDLE STIMULATION AT ACUPOINTS AND NON-ACUPOINTS ON DESCENDING MODULATION OF PAIN

When investigating descending control of pain in healthy human subjects, capsaicin (TRPV1 channel agonist; 100μg/50μl) was injected at acupoints ST36, ST37 and two non-acupoints to activate descending pain control circuitries. One non-acupoint was located between ST35 and ST36, and the other one was located between ST36 and ST37. Stronger pain intensity and larger pain distribution were observed following capsaicin treatment at acupoints than non-acupoints. Capsaicin treatment at acupoint ST36 induced higher pain intensity and larger pain distribution than that at acupoint ST37.

After heating-needle stimulation for 15-45min at an innocuous temperature of 43°C ipsilaterally at acupoint ST36, pressure pain threshold (PPT) and heat pain threshold (HPT) was determined at the dorsal surface of the ankle. No significant change in the PPTs was observed following heating-needle stimulation for

15-45min. However, a significantly increased HPT was found following 30-45min of heating-needle stimulation at the temperature of 43°C. This finding suggested that heating-needle stimulation for 30-45min at a temperature of 43°C applied at acupoint ST36 enhanced descending inhibition of pain, but exerted no effect on descending facilitation of pain. (Table 5) (Figure 3 in publication II).

Table 5 Effect of 43°C H.N.S. at acupoint ST36 on descending control of pain (II)

	15min	30min	45min
PPT	0	0	0
HPT	0	↑	↑
D.F.	0	0	0
D.I.	0	↑	↑

H.N.S.: heating-needle stimulation, PPT: pressure pain threshold, II: study II, HPT: heat pain threshold, D.F.: descending facilitation, D.I.: descending inhibition, 0: no effect, ↑: increase, ↓: decrease

5.2.2 EFFECTS OF HEATING-NEEDLE STIMULATION AT ACUPOINT ON EXPERIMENTAL MUSCLE PAIN

Injection of HT (5.8%) saline at a volume of 1 ml into the trapezius muscle of healthy human subjects caused local muscle pain at the injection area and referred pain at posterolateral area of the neck and/or the temporomandibular region. Heating-needle stimulation applied at acupoint ST36 for 30-45min at an innocuous temperature of 43°C prior to i.m. injection of HT saline decreased the HT saline-induced VAS scores of pain (VAS peak, VAS area and VAS duration), areas of pain distributions at the injection and referred sites, and reduced the HPT at the injection area (primary heat hyperalgesia), whereas HPT in the referred pain area was increased (secondary heat hypoalgesia) (Table 6) (Table 1, Figure 5 and Figure 6 in publication II).

Table 6 Effect of 43°C H.N.S. at acupoint ST36 on experimental muscle pain (II)

	43°C H.N.S.+ i.m. inj.		
	15min	30min	45min
M. hyperalgesia (injection area)	0	0	0
M. hyperalgesia (referred area)	0	0	0
H. hyperalgesia (injection area)	0	↓	↓
H. hypoalgesia (referred area)	0	↑	↑
VAS	0	↓	↓
Pain distribution (injection area)	0	↓	↓
Pain distribution (referred area)	0	↓	↓

M.: mechanical, H: heat, VAS: Visual Analog Scale, i.m. inj.: intramuscular injection H.N.S.: heating-needle stimulation, 0: no effect, ↑: increase, ↓: decrease, II: study II

6 DISCUSSION

The main findings of the current series of studies are as follows:

(1) Decreased descending inhibition and enhanced descending facilitation was found in rats with a partial depletion of striatal dopamine (Study III) and in rats with simulated weightlessness (Study I).
(2) Non-painful intramuscular heating-needle stimulation at a temperature of 43°C alleviated pain by enhancing descending inhibition (Study II, III). This enhancement of descending inhibition was mediated by dopamine D2 receptors of the thalamic VM nucleus in PD model animals (Study III).
(3) Immunohistochemical results indicated that in the spinal dorsal horn, descending facilitatory effect influences neuronal activity predominantly in superficial layers and descending inhibition in middle/deep layers (Study I).

Together, the findings indicate that a change in descending controls may contribute to pain in various pathophysiological conditions, such as weightlessness or PD, and 43°C heating-needle stimulation provides a potential therapy for these pain conditions by activating the thalamic VM nucleus that triggers promotion of descending inhibition.

6.1 6-OHDA-INDUCED PD

PD is known as a human neurodegenerative disorder that is characterized mainly by motor symptoms (Obeso et al., 2004). However, the prevalence of pain varies between 30 % and 95 %, and pain is an early and even a pre-motor symptom during the development of PD (Buhmann et al., 2017). The main pathological feature of PD is progressive and massive degeneration of dopaminergic neurons in the substantia nigra. Chemical lesion of the dopaminergic system by administration of the compound 6-OHDA has been used to produce an experimental PD model in animals (Mokry, 1995; Jackson-Lewis et al., 2012). In the current study III, 2 weeks after the striatal lesion with 6 μg of 6-OHDA, robust apomorphine-induced rotation was found, which is consistent with earlier findings (Kaakkola & Teräväinen, 1990). In addition, no significant abnormalities in the Rota-rod locomotion test and body weight were observed in 6-OHDA lesioned rats. It has been reported that the movement deficit in forelimb stepping and skilled paw use is most significant when 6-OHDA is injected into three or four different sites, but not a single site (Kirik et al., 1998). The findings in PD rats of the current study are in line with that in early-stage PD patients (Domenici et al., 2019). In the current study, no significant effect of 6-OHDA lesion on withdrawal thresholds evoked by mechanical or heat stimulation was observed, whereas earlier studies with 6-OHDA-treated rats showed decreased nociceptive thresholds to mechanical or thermal stimulation (Zengin-Toktas et al., 2013; Campos et al., 2019). A novel

finding in the present study is that descending facilitation is enhanced and descending inhibition decreased in PD rats exposed to the i.m. 5.8 % saline. It may be proposed that the pronociceptive change in descending controls is among mechanisms contributing to pain in PD patients. There is evidence suggesting that the dopaminergic system is involved in processing and control of pain sensation in PD, as nociceptive thresholds were restored following systemic administration of dopamine D2 receptor agonist in the 6-OHDA rat model (Dieb et al., 2016). Our current results revealed that dopamine D2 receptors in the thalamic MD and VM nuclei play important roles in descending modulation of pain in PD. In the current study, dopamine level in the striatum was decreased following 6-OHDA lesion. However, dopamine level in the thalamus was not measured. On the other hand, results in the current study showed that microinjection of dopamine into thalamic MD/VM nuclei reversed the changes in descending modulation of pain in 6-OHDA lesioned rats. This finding indicates that dopamine level in the thalamus decreased in PD model animals of the present study. Earlier results indicate that microinjection of drugs into the brain at a 3.0 µl volume spreads to about a 2.9 mm diameter sphere (Myers, 1966) suggesting that the decrease in dopamine level in the thalamus may not be caused by spillover of 6-OHDA from the striatal injection site to the thalamus.

However, it is worth noting the limitations of the 6-OHDA model. First, PD is an age-related disease. In current experiments, all the rats were at young age, and the ability of plasticity in central nervous system is much better at young age than in old animals. Secondly, the process of PD in patients is slow and may last for decades. In the present study (Study III), the progress of the 6-OHDA lesion-induced PD was too fast to induce compensation in the central nervous system, due to which the currently used rat model may not fully reflect the pathological changes of PD in the clinic. Furthermore, no hallmarks of PD, i.e. Lewy bodies, have been detected in the current 6-OHDA model.

6.2 WITHDRAWAL REFLEX

In the present study, limb withdrawal reflex evoked by mechanical or thermal stimulation was used to assess pain in experimental animals (Study I, III). The withdrawal reflex (nociceptive flexion reflex or flexor reflex) is a spinal reflex to withdraw the limb from injurious agents (Sherrington, 1910). Noxious stimulation applied to the paw induces neuronal activity in peripheral A-δ and C fibers that travels to the dorsal horn, in which it synapses with interneurons that further project to motor neurons in the ventral horn of the spinal cord. Motor neurons send polysynaptically excitatory and inhibitory signals to the flexors and the extensors, respectively, to induce the flexion reflex. Interneurons mediating the polysynaptic flexor reflex pathways are subject to descending control from supraspinal regions (Lundberg, 1969; Schomburg et al., 1998). Among supraspinal structures involved in the modulation of the withdrawal reflex are the cerebral

cortex, cerebellum, basal ganglia and various brainstem nuclei (Schomburg, 1990). In primates, neurons in the primary motor cortex send projections directly to motor neurons and interneurons in the ventral horn of the spinal cord, whereas in other species such as rodents the motor commands descending from the cortex have a relay in brainstem nuclei (Lemon, 2008). The VPL thalamus relays non-nociceptive and nociceptive somatosensory information to the primary somatosensory cortex (S1), which is of importance for the perception of touch and pain. However, S1 has also direct projections to the spinal dorsal horn (Casale et al., 1988; Liu et al., 2018). The direct corticospinal projections originating in the S1 may modulate spinal nociception (Senapati et al., 2005; Olivares-Moreno et al., 2017; Favorov et al., 2019). Moreover, ascending somatosensory signals can arrive in the motor cortex through multiple pathways that include cortico-cortical connections from the S1 to motor cortex, a subcortical loop from S1 via the striatum and motor thalamus to motor cortex, and also from the VPL thalamus directly to the motor cortex (Horne & Tracey, 1979; Guo et al., 2018). In the present study, the focus was on modulation of descending pain controls by two intralaminar nuclei of the thalamus, MD and VM. Thalamic MD nucleus projects to most of the frontal cortex and the amygdala (Krettek & Price, 1977), and thalamic VM nucleus projects to rostral parts of the medial agranular cortex (Haque et al., 2010). In line with these anatomical tract-tracing results, functional results indicate that a cortical projection from the thalamic MD nucleus activates the cingulate cortex and that from the thalamic VM nucleus the insular cortex (Xiao et al., 2015). These cortical projection areas have efferent projections to midbrain and pontine areas that are involved in descending regulation of nociception (Hardy, 1986; Reep et al., 1987), which provides a plausible explanation for the modulation of descending pain controls by MD/VM thalamic administration of drugs in the present study (Study III).

In a previous study, paw withdrawal reflex induced by mechanical and heat stimulation was chosen to evaluate experimental muscle pain (You et al., 2010). Secondary mechanical hyperalgesia and heat hypoalgesia were observed following i.m. 5.8% HT saline into the GS muscle, which is in line with the present findings (Study I, III). Lesioning of the RVM with kainic acid significantly depressed the secondary mechanical hyperalgesia and heat hypoalgesia indicating involvement of descending facilitatory and inhibitory controls, respectively, in the previous (You et al., 2010). In the earlier study, it was suggested that supraspinal structures have the ability to discriminate noxious mechanical stimulation-induced inputs from noxious heat-evoked inputs that promote descending facilitation and inhibition, respectively (You et al., 2010). Neurophysiological studies of primary afferent nerve fibers have shown that noxious mechanical stimuli preferentially activate nociceptive Aδ-fibers and noxious heat stimuli C-fibers (Magerl et al., 2001). Electrolytic lesion of the thalamic MD and VM nuclei depressed secondary mechanical hyperalgesia and heat hypoalgesia induced by i.m. injection of HT saline, respectively (You et al., 2013). The results of this earlier lesion study are in

line with the present finding showing that dopamine microinjection into the thalamic MD nucleus attenuated descending facilitation and that into the thalamic VM nucleus enhanced descending inhibition in PD animal as revealed by changes in secondary mechanical hyperalgesia and secondary heat hypoalgesia, respectively (Study III). These findings of the present study support the proposal that thalamic MD and VM nuclei discriminate between inputs from nociceptive Aδ- and C-fiber afferents, and that the thalamic MD nucleus is involved in a circuitry promoting descending facilitation of nociception, whereas the thalamic VM nucleus plays a role in a circuitry promoting descending inhibition of nociception. The finding that i.m. HT saline had opposite effects on mechanically and heat-evoked limb withdrawal responses, although the motor component of the withdrawal response was identical, indicates that the facilitatory and inhibitory effects in the present study (Study I, III) are due to actions on spinal sensory rather than motor neurons.

6.3 EMOTIONAL INFLUENCE ON PAIN

It is important to consider the impact of emotion on pain. Accumulating evidence indicates that emotional state has a powerful influence on pain perception; e.g., a positive emotional state depresses pain (Koechlin et al., 2018). Among many areas of the central nervous system that have been reported to be involved in emotional control of pain are the anterior cingulate cortex, amygdala, midbrain PAG and RVM (LeDoux, 2000). In the current study, simulated weightlessness was induced by means of HU (Study I). Earlier rat studies indicate that HU causes only slight (Knox et al., 2004) or no reduction in weight gain (Wronski & Morey-Holton, 1987). In line with this, no significant differences in body weight gain were found between the HU and control groups (Study I). In addition, no significant activation of the corticoadrenal stress axis has been found in this model (Knox et al., 2004) indicating that the HU model provides a possibility to study physiological effects of weightlessness in an ethically acceptable fashion, with a minimal confounding factor induced by stress or emotions. It has been reported that, anxiety and depression can occur in PD patients even before the appearance of the motor symptoms (Faivre et al., 2019). In experimental animal model of PD induced by 6-OHDA used in the present study (Study III), however, anxiety has not been a prominent feature (Branchi et al., 2008; Matheus et al., 2016), although depressive-like behavior has been reported in many previous studies (Shimura et al., 2002; Matheus et al., 2016; Ilkiw et al., 2018). It should be noted that the magnitude of the abnormality varies with the 6-OHDA lesion site. In the current study (Study III), 6-OHDA-lesion was limited to the striatum. An earlier study reported that 6-OHDA-lesions limited to the striatum fail to cause depressive-like behavior (Branchi et al., 2008).

6.4 NEUROTRANSMITTERS INVOLVED IN PD

PD is a neurodegenerative disease associated typically with loss of dopamine neurons in nigrostriatal pathway. Accumulating evidence indicates that the decrease in other neurotransmitters including serotonin (5-HT) and noradrenaline (NA) also plays a role in the pathophysiology of PD (Politis & Niccolini, 2015; Sommerauer et al., 2018). It has been shown that noradrenergic cells in the locus coeruleus (LC) also degenerate in PD (Greenfield & Bosanquet, 1953; German et al., 1992; Bertrand et al., 1997). LC modulates a variety of central functions by releasing NA into brain areas including neocortex, hippocampus, thalamus, subthalamic nucleus and substantia nigra (Atzori et al., 2016). NA and 5-HT are also involved in descending modulation of pain (Pertovaara, 2006; Pertovaara & Almeida, 2006). In addition, experimental data indicates that serotonergic and noradrenergic neuron dysfunction plays a crucial role in the motor and non-motor symptoms in PD (Kish, 2003; Peterson & Li, 2018). In the current study (Study III), rats were intraperitoneally injected with desipramine before i.c. microinjection of 6-OHDA to protect the function of noradrenergic neurons. Because the focus was on the deficiency of dopamine, no further experiment was designed to explore the possible effects of the striatal 6-OHDA lesion on descending modulation of pain by NA or 5-HT in this study.

6.5 DIFFUSE NOXIOUS INHIBITORY CONTROLS (DNIC)

When studying descending control of pain, one specific mechanism that needs to be taken into account in interpretations of results is diffuse noxious inhibitory controls (DNIC) that is one part of the descending inhibitory control systems of pain. The DNIC system is activated by a conditioning noxious stimulus and it exerts inhibitory effect on concurrent nociceptive signals evoked at remote body areas (Le Bars, 2002). Ascending signals in the DNIC system are carried in the ventrolateral funiculi of the spinal cord and its descending inhibitory effect on wide-dynamic range neurons of the spinal dorsal horn is mediated by dorsolateral funiculi (Villanueva & Le Bars, 1995). Dorsal reticular nucleus in the caudal medulla is a key structure in the DNIC loop, whereas the midbrain periaqueductal gray (PAG) or the rostroventromedial medulla (RVM) that are important relays for descending pain controls are not critical for inducing the DNIC effect (Villanueva & Le Bars, 1995). Early studies showed that serotonergic agents could facilitate the DNIC system, while opioids, opioid antagonists and serotonin antagonists could attenuate the DNIC system (Willer et al., 1990; Le Bars et al., 1992; Bannister et al., 2016). More recent studies have shown that also noradrenaline acting on spinal α2-adrenoceptors is involved in DNIC and that the role of serotonin in DNIC is complex and varies depending on whether serotonin acts on pain facilitatory 5-HT3 receptors or pain inhibitory 5-HT7 receptors (Bannister & Dickenson, 2017). Also, dopamine acting on spinal dopamine D2 receptors has been shown to

contribute to the DNIC effect (Lapirot et al., 2011). It has been proposed DNIC is involved in the detection of nociceptive signals as a filter that extracts and amplifies potentially dangerous alarm signals (Villanueva & Le Bars, 1995). Deficiencies of DNIC have shown to underlie, at least partly, the development of chronic pain e.g., in headache, osteoarthritis and fibromyalgia (Lautenbacher & Rollman, 1997; Kosek & Ordeberg, 2000; Renton, 2017). However, one human study reported no alterations of the DNIC effect in PD patients suggesting that DNIC mechanisms do not play an important role in pain in PD (Mylius et al., 2009), whereas activity in descending inhibitory circuits was attenuated in a tonic fashion in animals with experimental PD in the present study. In general, DNIC effect has been demonstrated using short-lasting noxious conditioning stimulation (seconds-minutes), while in the present series of studies noxious conditioning muscle stimulation used to activate descending controls in a tonic fashion was long-lasting (days-weeks). Additionally, while DNIC effect has a short onset (seconds), activation of descending inhibition as revealed by secondary heat hypoalgesia following i.m. injection of HT saline had a long onset (one day; Study I-III). Therefore, descending inhibitory mechanisms studied in this thesis, at least partly, differ from those underlying DNIC. In line with this proposal, thalamic nuclei were shown to contribute to regulation of tonic descending controls assessed in the present study (Study III), whereas there is no published evidence about the contribution of thalamic mechanisms to DNIC. Furthermore, heating-needle stimulation applied at a non-painful temperature of 43°C proved to enhance descending inhibitory controls in the present study (Study II, III), whereas activation of DNIC is considered to require conditioning stimulation at a noxious/painful intensity. This finding further supports the proposal that the enhancement of descending inhibitory controls by 43°C heating-needle stimulation in the present study was not due to activation of DNIC.

6.6 POTENTIAL CLINICAL IMPLICATIONS

From a clinical perspective, the present results support the proposal that dysregulation of descending pain control may contribute to pain in various pathophysiological conditions, including PD. Selective enhancement of descending pain inhibition by intramuscular heating-needle stimulation at a non-painful temperature of 43°C that recruits innocuous C-fibers may provide an alternative treatment method for pain in PD and some other pathophysiological pain conditions through restoration of descending inhibition of pain.

7 CONCLUSIONS

In animal studies:

(1) Under simulated weightlessness and in experimental PD, endogenous descending facilitation of pain is enhanced and endogenous descending inhibition of pain is decreased.

(2) In the spinal dorsal horn, superficial layers are involved in descending facilitation and deep/middle layers in descending inhibition.

(3) Dopaminergic hypoactivity in the thalamic MD and VM nuclei plays an important role in the abnormal descending modulation of pain in experimental PD.

(4) Dopamine D2 receptors in the thalamic VM nucleus are involved in the 43°C heating-needle stimulation induced enhancement of descending inhibition of pain in experimental PD.

In human study:

43°C heating-needle stimulation for 30-45min enhances descending inhibition, and 43°C heating-needle stimulation at acupoints produces greater descending inhibition than that at non-acupoints.

8 FUTURE PROSPECTS

The present series of studies demonstrated that superficial layers of the spinal dorsal horn are involved in descending facilitation and deep/middle layers in descending inhibition (Study I). Earlier studies have shown that multiple neurotransmitters/receptors are involved in mediating descending modulation of pain in the spinal cord dorsal horn (Dickenson, 2019). However, it is still unclear which neurotransmitters/receptors are involved in descending modulation of pain under simulated weightlessness condition. This still needs to be studied.

The present results in healthy subjects indicated that 43°C heating-needle stimulation for 30-45min enhances descending inhibition, and that stronger descending inhibition was produced by the heating-needle stimulation applied at acupoints than control sites (Study II). Future studies are needed to assess whether heating-needle stimulation applied using these stimulus parameters effectively activates descending inhibition of pain also in patients with chronic pain. In addition, by using brain imaging technology, such as functional magnetic resonance imaging (MRI) or positron emission tomography (PET), it may be possible to reveal the central mechanisms underlying the heating-needle stimulation induced pain relief in humans.

In study III, changes in descending modulation of pain and the effect of 43°C heating-needle stimulation on pain modulation were explored in rats with early-stage PD. In future studies, it is necessary to uncover changes in descending modulation of pain in PD rats at different stages of the disease; i.e., not only in the early stage as in the present study, but also at later stages. The present results demonstrated a dopamine deficiency in thalamic MD/VM nuclei as a cause for the increase in descending facilitation and decrease in descending inhibition in PD rats. Earlier studies have shown that other neurotransmitters including serotonin and noradrenaline also play potential role in the pathophysiology of PD (Politis & Niccolini, 2015; Sommerauer et al., 2018). Therefore, future studies are needed to determine whether deficiencies in the serotonin and noradrenaline systems contribute to the abnormality of descending modulation of pain in PD. In addition, while 43°C heating-needle stimulation proved to have therapeutic effects in PD rats through action on descending modulation of pain, it remains to be studied whether this therapeutic effect can be induced with heating-needle stimulation also in PD patients.

In the current series of studies, experiments were performed only in males (both in the animal and human studies). Numerous studies have shown sex-related differences in pain perception and pain modulation (Lei et al., 2011; Lei & You, 2012; Nasser & Afify, 2019). In the future, main results of the present series of studies need to be replicated in females to find out whether there are gender-related differences in the effect of weightlessness and PD on descending

modulation of pain and whether heating-needle stimulation is as effective in recruiting descending pain inhibition in females as males.

REFERENCES

Abdelsalam RM, Safar MM. Neuroprotective effects of vildagliptin in rat rotenone Parkinson's disease model: role of RAGE - NFκB and Nrf2 - antioxidant signaling pathways. J. Neurochem. 2015;133: 700-707.

Aicher SA, Randich A. Antinociception and cardiovascular responses produced by electrical stimulation in the nucleus tractus solitarius, nucleus reticularis ventralis and the caudal medulla, nucleus reticularis ventralis, and the caudal medulla. Pain 1990;42: 103-119.

Aira Z, Barrenetxea T, Buesa I, Martínez E, Azkue JJ. Spinal D1-like dopamine receptors modulate NMDA receptor-induced hyperexcitability and NR1 subunit phosphorylation at serine 889. Neurosci. Lett. 2016;618: 152-158.

Alam ZI, Daniel SE, Lees AJ, Marsden DC, Jenner P, Halliwell B. A generalised increase in protein carbonyls in the brain in Parkinson's but not incidental Lewy body disease. J. Neurochem. 1997;69: 1326-1329.

Ali U, Fan XL, You HJ. Role of muscle spindle in weightlessness-induced amyotrophia and muscle pain. Neurosci. Bull. 2009;25: 283-288.

Alitto HJ, Usrey WM. Corticothalamic feedback and sensory processing. Curr. Opin. Neurobiol. 2003;13: 440-445.

Apkarian AV, Shi T. Squirrel monkey lateral thalamus.I. Somatic nociresponsive neurons and their relation to spinothalamic terminals. J. Neurosci. 1994;14: 6779-6795.

Atzori1 M, Cuevas-Olguin R, Esquivel-Rendon E, Garcia-Oscos F, Salgado-Delgado RC, Saderi N, Miranda-Morales M, Treviño M, Pineda JC, Salgado H. Locus coeruleus norepinephrine release: A central regulator of CNS spatio-temporal activation? Front. Synaptic Neurosci. 2016;8: 25.

Bajic D, Proudfit HK. Projections of neurons in the periaqueductal gray to pontine and medullary catecholamine cell groups involved in the modulation of nociception. J. Comp. Neurol. 1999;405: 359-379.

Bannister K, Dickenson AH. The plasticity of descending controls in pain: translational probing. J. Physiol. 2017;595: 4159-4166.

Bannister K, Lockwood S, Goncalves L, Patel R, Dickenson AH. An investigation into the inhibitory function of serotonin in diffuse noxious inhibitory controls in the neuropathic rat. Eur. J. Pain 2017;21: 750-760.

Basbaum AI, Fields HL. Endogenous pain control mechanisms: Review and hypothesis. Ann. Neurol. 1978;4: 451-462.

Basbaum AI, Fields HL. Endogenous pain control system: brainstem spinal pathways and endorphin circuitry. Annu. Rev. Neurosci. 1984;7: 309-338.

Behbehani MM, Fields HL. Evidence that an excitatory connection between the periaqueductal grey and nucleus raphe magnus mediates stimulation-produced analgesia. Brain Res. 1979;170: 85-93.

Beiske AG, Loge JH, Rønningen A, Svensson E. Pain in Parkinson's disease: prevalence and characteristics. Pain 2009;141: 173-177.

Bell A. the neurobiology of acute pain. Vet. J. 2018;237: 55-62.

Berberich P, Hoheisel U, Mense S. Effects of a carrageenan-induced myositis on the discharge properties of group III and IV muscle receptors in the cat. J. Neurophysiol. 1988;59: 1395-1409.

Berkley KJ, Guilbaud G, Benoist JM, Gautron M. Responses of neurons in and near the thalamic ventrobasal complex of the rat to stimulation of uterus, cervix, vagina, colon, and skin. J. Neurophysiol. 1993;69: 557-568.

Bertrand E, Lechowicz W, Szpak GM, Dynecki J. Qualtitative and quantitative analysis of locus coeruleus neurons in Parkinson's disease. Folia Neuropathol. 1997;35: 80-86.

Besson JM, Chaouch A. Peripheral and spinal mechanisms of nociception. Physiol. Rev. 1987;67: 67-186.

Bester H, Bourgeais L, Villanueva L, Besson JM, Bernard JF. Differential projections to the intralaminar and gustatory thalamus from the parabrachial area: a PHA-L study in the rat. J. Comp. Neurol. 1999;405: 421-449.

Bethel-Brown CS, Morris JK, Stanford JA. Young and middle-aged rats exhibit isometric forelimb force control deficits in a model of early-stage Parkinson's disease. Behav. Brain Res. 2011;225: 97-103.

Bichler Z, Lim HC, Zeng L, Tan EK. Non-motor and motor features in LRRK2 transgenic mice. PloS one 2013;8: e70249.

Blanchet PJ, Brefel-Courbon C. Chronic pain and pain processing in Parkinson's disease. Prog. Neuropsychopharmacol. Biol. Psychiatry 2018;87: 200-206.

Blandini F, Levandis G, Bazzini E, Nappi G, Armentero MT. Time-course of nigrostriatal damage, basal ganglia metabolic changes and behavioural alterations following intrastriatal injection of 6-hydroxydopamine in the rat: new clues from an old model. Eur. J. Neurosci. 2007;25: 397-405.

Bloom F, Battenberg E, Rossier J, Ling N, Guillemin R. Neurons containing beta-endorphin in rat brain exist separately fom those containing enkephalin: immunocytochemical studies. Proc. Natl. Acad. Sci. USA. 1978;75: 1591-1595.

Boccard SGJ, Pereira EAC, Aziz TZ. Deep brain stimulation for chronic pain. J. Clin. Neurosci. 2015;22: 1537-1543.

Boix J, Hieber DV, Connor B. Gait Analysis for Early Detection of Motor Symptoms in the 6-OHDA Rat Model of Parkinson's Disease. Front. Behav. Neurosci. 2018;12: 39.

Bouthenet ML, Souil E, Martres MP, Sokoloff P, Giros B, Schwartz JC. Localization of dopamine D3 receptor mRNA in the rat brain using in situ hybridization histochemistry: comparison with dopamine D2 receptor mRNA. Brain Res. 1991;564: 203-219.

Boyson SJ, McGonigle P, Molinoff PB. Quantitative autoradiographic localization of the D1 and D2 subtypes of dopamine receptors in rat brain. J. Neurosci. 1986;6: 3177-3188.

Branchi I, D'Andrea I, Armida M, Cassano T, Pèzzola A, Potenza RL, Morgese MG, Popoli P, Alleva E. Nonmotor symptoms in Parkin's disease: investigating early-phase onset of behavioral dysfunction in the 6-hydroxydopamine-lesioned rat model. J. Neurosci. Res. 2008;86: 2050-2061.

Brefel-Courbon C, Ory-Magne F, Thalamas C, Payoux P, Rascol O. Nociceptive brain activation in patients with neuropathic pain related to Parkinson's disease. Parkinsonism Relat. Disord. 2013;19: 548-552.

Brefel-Courbon C, Payoux P, Thalama C, Ory F, Quelven I, Chollet F, Montastruc JL, Rascol O. Effect of Levodopa on pain threshold in Parkinson's disease: a clinical and positron emission tomography study. Mov. Disord. 2005;20: 1557-1563.

Buchanan P, Convertino VA. A study of the effects of prolonged simulated microgravity on the musculature of the lower extremities in man: an introduction. Aviat. Space Environ. Med. 1989;60: 649-652.

Buhmann C, Wrobel N, Grashorn W, Fruendt O, Wesemann K, Diedrich S, Bingel U. Pain in Parkinson's disease: a cross-sectional survey of its prevalence, specifics, and therapy. J. Neurol. 2017;264: 758-769.

Campos ACP, Berzuino MB, Hernandes MS, Fonoff ET, Pagano RL. Monoaminergic regulation of nociceptive circuitry in a Parkinson's disease rat model. Exp. Neurol. 2019;318: 12-21.

Capra NF, Ro JY. Human and animal experimental models of acute and chronic muscle pain: intramuscular algesic injection. Pain 2004;110: 3-7.

Carlsson A, Lindqvist M, Magnusson T. 3,4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists. Nature 1957;180: 1200.

Carr KD. Dopaminergic mechanisms in the supraspinal modulation of pain. Pain 1984;2 (Suppl.): S332.

Casale EJ, Light AR, Rustioni A. Direct projection of the corticospinal tract to the superficial laminae of the spinal cord in the rat. J. Comp. Neurol. 1988;278: 275-286.

Casey KL, Minoshima S, Berger KL, Koeppe RA, Morrow TJ, Frey KA. Positron emission tomographic analysis of cerebral structures activated specifically by repetitive noxious heat stimuli. J. Neurophysiol. 1994;71: 802-807.

Celerier E, Rivat C, Jun Y, Laulin JP, Larcher A, Reynier P, Simonnet G. Long-lasting hyperalgesia induced by fentanyl in rats: preventive effect of ketamine. Anesthesiology 2000;92: 465-472.

Chakrabarti S, Regec A, Gintzler AR. Biochemical demonstration of mu-opioid receptor association with Gsalpha: enhancement following morphine exposure. Brain Res. Mol. Brain Res. 2005;135: 217-224.

Chen CCV, Shih YYI, Chang C. Dopaminergic imaging of nonmotor manifestations in a rat model of Parkinson's disease by fMRI. Neurobiol. Dis. 2013;49: 99-106.

Cheng KJ. Neurobiological mechanisms of acupuncture for some common illnesses: a clinician's perspective. J. Acupunct. Meridian Stud. 2014;7: 105-114.

Chiang CY, Chang CT, Chu HC, Yang LF. Peripheral afferent pathway for acupuncture analgesia. Sci. Sin. 1973;16: 210-217.

Choi JW, Kang SY, Choi JG, Kang DW, Kim SJ, Lee SD, Park JB, Ryu YH, Kim HW. Analgesic effect of electroacupuncture on paclitaxel-induced neuropathic pain via spinal opioidergic and adrenergic mechanisms in mice. Am. J. Chin. Med. 2015;43: 57-70.

Chudler EH, Dong WK. The role of the basal ganglia in nociception and pain. Pain 1995;60: 3-38.

Cicchetti F, Drouin-Ouellet J, Gross RE. Environmental toxins and Parkinson's disease: what have we learned from pesticide-induced animal models? Trends pharmacol. sci. 2009;30: 475-483.

Coffield JA, Bowen KK, Miletic V. Retrograde tracing of projections between the nucleus submedius, the ventrolateral orbital cortex, and the midbrain in the rat. J. Comp. Neurol. 1992;321: 488-499.

Cunningham CW, Elballa WM, Vold SU. Bifunctional opioid receptor ligands as novel analgesics. Neuropharmacology 2019;151: 195-207.

Cury RG, Galhardoni R, Fonoff ET, Lloret SP, Ghilardi MGS, Barbosa ER, Teixeira MJ, Andrade DCD. Sensory abnormalities and pain in Parkinson disease and its modulation by treatment of motor symptoms. Eur. J. Pain 2016;20: 151-165.

Derbyshire SW, Jones AK. Cerebral responses to a continual tonic pain stimulus measured using positron emission tomography. Pain 1998; 76: 127-135.

Dickenson AH. The neurobiology of chronic pain states. Anasth. Intens. Care Med. 2019;20: 426-429.

Dieb W, Ouachikh O, Alves S, Boucher Y, Durif F, Hafidi A. Nigrostriatal dopaminergic depletion increases static orofacial allodynia. J. Headache Pain 2016;17: 11.

Ding H, Hayashida K, Sutu T, Sukhtankar DD, Kimura M, Mendenhall V, Ko MC. Supraspinal actions of nociception/orphanin FQ, morphine and substance P in

regulating pain and itch in non-human primates. Br. J. Pharmacol. 2015;172: 3302-3312.

Djaldetti R, Shifrin AMD, Rogowski Z, Sprecher E, Melamed E, Yarnitsky D. Quantitative measurement of pain sensation in patients with Parkinson disease. Neurology 2004;62: 2171-2175.

Domenici RA, Campos ACP, Maciel ST, Berzuino MB, Hernandes MS, Fonoff ET, Pagano RL. Parkinson's disease and pain: Modulation of nociceptive circuitry in a rat model of nigrostriatal lesion. Exp. Neurol. 2019;315: 72-81.

Dubois A, Savasta M, Curet O, Scatton B. Autoradiographic distribution of the D1 agonist [3H]SKF 38393, in the rat brain and spinal cord. Comparison with the distribution of D2 dopamine receptors. Neuroscience 1986;19: 125-137.

Dubner R, Max M. Antidepressants and relief of neuropathic pain: evidence for noradrenergic mechanisms. In: Besson JM, Guilbaud G. Eds, Noradrenergic Agonists for the Treatment of Pain. Excerpta Medica. Elsevier, Amsterdam, 1992; pp 211-218.

Faivre F, Joshi A, Bezard E, Barrot M. The hidden side of Parkinson's disease: Studying pain, anxiety and depression in animal models. Neurosci. Biobeh. Rev. 2019;96: 335-352.

Faull RLM, Laverty R. Changes in dopamine levels in the corpus striatum following lesions in the substantia nigra. Exp. Neurol. 1969;23: 332-340.

Favorov OV, Pellicer-Morata V, DeJongh Curry AL, Ramshur JT, Brna A, Challener TD, Waters RS. A newly identified nociresponsive region in the transitional zone (TZ) in rat sensorimotor cortex. Brain Res. 2019;1717: 228-234.

Fields HL. Is there a facilitating component to central pain modulation? APS J. 1992;1: 71-78.

Fields HL, Basbaum AI. Brainstem control of spinal pain-transmission neurons. Annu. Rev. Physiol. 1978;40: 217-248.

Gao X, Zhang YQ, Wu GC. Effects of dopaminergic agents on carrageenan hyperalgesia after intrathecal administration to rats. Eur. J. Pharmacol. 2001;418: 73-77.

George SR, Zastawny RL, Briones-Urbina R, Cheng R, Nguyen T, Beiber M, Kouvelas A, Chan AS, O'Dowd BF. Distinct distributions of mu, delta and kappa opioid receptor mRNA in rat brain. Biochem. Biophys. Res. Commun. 1994;205: 1438-1444.

Gerdelat-Mas A, Simonetta-Moreau M, Thalamas C, Ory-Magne F, Sloui T, Rascol O, Brefel-Courbon C. Levodopa raises objective pain threshold in Parkinson's disease: a RIII reflex study. J. Neurol. Neurosurg. Psychiatry 2007;78: 1140-1142.

German DC, Manaye KF, White III CL, Woodward DJ, Mclntire DD, Smith WK, Kalaria RN, Mann DMA. Disease-specific patterns of locus coeruleus cell loss. Ann. Neurol. 1992;32: 667-676.

Gibbons CH, Garcia J, Wang N Shih LC, Freeman R. The diagnostic discrimination of cutaneous α-synuclein deposition in Parkinson disease. Neurology 2016;87: 505-512.

Gierthmühlen J, Lienau F, Maag R, Hagenah JM, Deuschl G, Fritzer E, Clein C, Baron R, Helmchen C. Somatosensory processing in a German family with PINK1 mutations: its potential role in Parkinson disease. J. Neurol. Neurosurg. Psychiatry 2009;80: 571-574.

Girault JA, Greengard P. The neurobiology of dopamine signaling. Arch. Neurol. 2004;61: 641-644.

Gomez-Paz A, Drucker-Colin R, Milan-Aldaco D, Palomero-Rivero M, Ambriz-Tututi M. Intrastriatal Chromospheres' Transplant Reduces Nociception in Hemiparkinsonian Rats. Neuroscience 2018;387: 123-134.

Gouardères C, Cros J, Quirion R. Autoradiographic localization of mu, delta and kappa opioid receptor binding sites in rat and guinea pig spinal cord. Neuropeptides 1985;6: 331-342.

Grandi LC, Giovanni GD, Ggalati S. Reprint of “Animal models of early-stage Parkinson's disease and acute dopamine deficiency to study compensatory neurodegenerative mechanisms”. J. Neurosci. Methods 2018;310: 75-88.

Greenfield JG, Bosanquet FD. The brain-stem lesions in Parkinsonism. J. Neurol. Neurosurg. Psychiatry.1953;16: 213-226.

Gubellini P, Kachidian P. Animal models of Parkinson's disease: An updated overview. Rev. Neurol. 2015;171: 750-761.

Guilbaud G, Kayser V, Benoist JM, Gautron M. Modifications in the responsiveness of rat ventrobasal thalamic neurons at different stages of carrageen inproduced inflammation. Brain Res.1986;385: 86-98.

Guilbaud G, Peschanski M, Gautron M, Binder D. Neurones responding to noxious stimulation in VB complex and caudal adjacent regions in the thalamus of the rat. Pain 1980;8: 303-318.

Guo KH, Yamawaki N, Svoboda K, Shepherd GMG. Anterolateral motor cortex connects with a medial subdivision of ventromedial thalamus through cell-type-specific circuits, forming an excitatory thalamo-cortico-thalamic loop via layer 1 apical tuft dendrites of layer 5B pyramidal tract type neurons. J. Neurosci. 2018;38: 8787-8797.

Han JS. Acupuncture: neuropeptide release produced by electrical stimulation of different frequencies. Trends Neurosci. 2003;26: 17-22.

Haque T, Yamamoto S, Masuda Y, Kato T, Sato F, Uchino K, Oka A, Nakamura M, Takeda R, Ono T, Kogo M, Yoshida A. Thalamic afferent and efferent connectivity to cerebral cortical areas with direct projections to identified subgroups of trigeminal premotoneurons in the rat. Brain Res. 2010;1346: 69-82.

Harding-Forrester S, Feldman DE. Chapter 4: Somatosensory maps. Handb. Clin. Neurol. 2018;151: 73-102

Hardy JD, Wolff HG, Goodell H. Experimental evidence on the nature of cutaneous hyperalgesia. J. Clin. Invest. 1950;29: 115-140.

Hardy SG. Projections to the midbrain from the medial versus lateral prefrontal cortices of the rat. Neurosci. Lett. 1986;63: 159-164.

Harris JA, Mihalas S, Hirokawa KE, Whitesell JD, Choi H, Bernard A, Bohn P, Caldejon S, Casal L, Cho A, Feiner A, Feng D, Gaudreault N, Gerfen CR, Graddis N, Groblewski PA, Henry AM, Ho A, Howard R, Knox JE, Kuan L, Kuang X, Lecoq J, Lesnar P, Li Y, Luviano J, McConoughey S, Mortrud MT, Naeemi M, Ng L, Oh SW, Ouellette B, Shen E, Sorensen SA, Wakeman W, Wang Q, Wang Y, Williford A, Phillips JW, Jones AR, Koch C, Zeng H. Hierarchical organization of cortical and thalamic connectivity. Nature 2019;575: 195-202.

Heeringa MJ, Abercrombie ED. Biochemistry of somatodendritic dopamine release in substantia nigra: an in vivo comparison with striatal dopamine release. J. Neurochem. 1995;65: 192-200.

Higgins C, Smith BH, MatthewsK. Evidence of opioid-induced hyperalgesia in clinical populations after chronic opioid exposure: a systematic review and meta-analysis. Br. J. Anaesth. 2019;122: e114-e126.

Hiramatsu M, Inoue K, Ambo A, Sasaki Y, Kameyama T. Long-lasting antinociceptive effects of a novel dynorphin analogue, Tyr-D-Ala-Phe-Leu-Arg psi (CH(2)NH) Arg-NH(2), in mice. Br. J. Pharmacol. 2001;132: 1948-1956.

Horne MK, Tracey DJ. The afferents and projections of the ventro-posterolateral thalamus in the monkey. Exp. Brain Res. 1979;36: 129-141.

Hu ML, Qiu ZY, Hu K, Ding MX. Analgesic neural circuits are activated by electroacupuncture at two sets of acupoints. Evid. Based Complement Alternat. Med. 2016: 3840202.

Iggo A. Non-myelinated afferent fibres from mammalian skeletal muscle. J. Physiol. 1961;155: 52-53.

Ilkiw JL, Kmita LC, Targa ADS, Noseda ACD, Rodrigues LS, Dorieux FWC, Fagotti J, DosSantos P, Lima MMS. Dopaminergic lesion in the olfactory bulb restores olfaction and induces depressive-like behaviors in a 6-OHDA model of Parkinson's disease. Mol. Neurobiol. 2019;56: 1082-1095.

Imbe H, Dubner R, Ren K. Masseteric inflammation-induced Fos protein expression in the trigeminal interpolaris/caudalis transition zone: contribution of somatosensory-vagal-adrenal integration. Brain Res. 1999;845: 165-175.

Ishida Y, Kawai K, Magata Y, Abe H, Yoshimoto M, Takeda R, Hashiguchi H, Mukai T, Saji H. Alteration of striatal [11C]raclopride and 6-[18F]fluoro-l-3,4-dihydroxyphenylalanine uptake precedes development of methamphetamine-induced rotation following unilateral 6-hydroxydopamine lesions of medialforebrain bundle in rats. Neurosci. Lett. 2005;389: 30-34.

Jackson DM, Westlind-Danielsson A. Dopamine receptors: molecular biology, biochemistry and behavioural aspects. Pharmacol. Ther. 1994;64: 291-370.

Jackson-Lewis V, Blesa J, Przedborski S. Animal models of Parkinson's disease. Parkinsonism Relat. Disord. 2012;18: 183-185.

Jardín I, López JJ, Diez R, Sánchez-Collado J, Cantonero C, Albarrán L, Woodard GE, Redondo PC, Salido GM, Smani T, Rosado JA. TRPs in Pain Sensation. Front. Physiol. 2017;8: 392.

Jellinger KA. Pathology of Parkinson's disease. Changes other than the nigrostriatal pathway. Mol. Chem. Neuropathol. 1991;14: 153-197.

Jensen K, Norup M. Experimental pain in human temporal muscle induced by hypertonic saline, potassium and acidity. Cephalalgia 1992;12: 101-106.

Jensen TS, Yaksh TL. Effects of an intrathecal dopamine agonist, apomorphine, on thermal and chemical evoked noxious responses in rats. Brain Res. 1984;296: 285-293.

Johnson M, Bobrovskaya L. An update on the rotenone models of Parkinson's disease: Their ability to reproduce the features of clinical disease and model gene-environment interactions. Neuro. Toxicol. 2015;46: 101-116.

Kaakkola S, Teräväinen H. Animal models of Parkinsonism. Pharmacol. Toxicol. 1990;67: 95-100.

Kellgren J. Observations on referred pain arising from muscle. Clin. Sci. 1938;3: 175-190.

Kirik D, Rosenblad C, Björklund A. Characterization of behavioral and neurodegenerative changes following partial lesions of the nigrostriatal dopamine system induced by intrastriatal 6-hydroxydopamine in the rat. Exp. Neurol. 1998;152: 259-277.

Kish SJ. Biochemistry of Parkinson's disease: is a brain serotonergic deficiency a characteristic of idiopathic Parkinson's disease? Adv. Neurol. 2003;91: 39-49.

Knox M, Fluckey JD, Bennett P, Peterson CA, DupontVersteegden EE. Hindlimb unloading in adult rats using an alternative tail harness design. Aviat. Space Environ. Med. 2004;75: 692-696.

Koblinger K, Jean-Xavier C, Sharma S, Füzesi T, Young L, Eaton SEA, Kwok CHT, Bains JS, Whelan PJ. Optogenetic activation of A11 Region increases motor activity. Front. Neural Circuits 2018;12: 86.

Koechlin H, Coakley R, Schechter N, Werner C, Kossowsky J. The role of emotion regulation in chronic pain: A systematic literature review. J. Psychos. Res. 2018;107: 38-45.

Kosek E, Ordeberg G. Lack of pressure pain modulation by heterotopic noxious conditioning stimulation in patients with painful osteoarthritis before, but not following, surgical pain relief. Pain 2000;88: 69-78.

Krettek JE, Price JL. The cortical projections of the mediodorsal nucleus and adjacent thalamic nuclei in the rat. J. Comp. Neurol. 1977;171: 157-191.

Kuner R. Central mechanisms of pathological pain. Nat. Med. 2010;16: 1258-1266.

Lapirot O, Melin C, Modolo A, Nicolas C, Messaoudi Y, Monconduit L, Artola A, Luccarini P, Dallel R. Tonic and phasic descending dopaminergic controls of nociceptive transmission in the medullary dorsal horn. Pain 2011;152: 1821-1831.

Laughlin TM, Vanderah TW, Lashbrook J, Nichols ML, Ossipov M, Porreca F, Wilcox GL. Spinally administered dynorphin A produces long-lasting allodynia: involvement of NMDA but not opioid receptors. Pain 1997;72: 253-260.

Lautenbacher S, Rollman GB. Possible deficiencies of pain modulation in fibromyalgia. Clin. J. Pain 1997;13: 189-196.

Le Bars D. The whole body receptive field of dorsal horn multireceptive neurones. Brain Res. Rev. 2002;40: 29-44.

Le Bars D, Willer JC, De Broucker T. Morphine blocks descending pain inhibitory controls in humans. Pain 1992;48: 13-20.

LeDoux JE. Emotion circuits in the brain. Annu. Rev. Neurosci. 2000;23: 155-184.

Lei J, You HJ. Variation of pain and vasomotor responses evoked by intramuscular infusion of hypertonic saline in human subjects: Influence of gender and its potential neural mechanisms. Brain Res. Bull. 2012;87 (6): 564-570.

Lei J, You HJ. Endogenous descending facilitation and inhibition differ in control of formalin intramuscularly induced persistent muscle nociception. Exp. Neurol. 2013;248: 100-111.

Lei J, You HJ, Andersen OK, Graven-Nielsen T, Arendt-Nielsen L. Homotopic and heterotopic variation in skin blood flow and temperature following experimental muscle pain in humans. Brain Res. 2008;1232: 85-93.

Lei J, Jin L, Zhao Y, Sui MY, Huang L, Tan YX, Chen YK, You HJ. Sex-related differences in descending norepinephrine and serotonin controls of spinal withdrawal reflex during intramuscular saline induced muscle nociception in rats. Exp. Neurol. 2011;228: 206-214.

Lei J, Sun T, Lumb BM, You HJ. Roles of the periaqueductal gray in descending facilitatory and inhibitory controls of intramuscular hypertonic saline induced muscle nociception. Exp. Neurol. 2014;257: 88-94.

Lemon RN. Descending pathways in motor control. Annu. Rev. Neurosci. 2008;31: 195-218.

Levant B, McCarson KE. D3 dopamine receptors in rat spinal cord: implications for sensory and motor function. Neurosci. Lett. 2001;303: 9-12.

Liu YY, Latremoliere A, Li XJ, Zhang ZC, Chen MY, Wang XH, Fang C, Alexandre C, Gao ZY, Chen B, Ding X, Zhou JY, Zhang YM, Chen CF, Wang KH, Woolf CJ , He ZG. Touch and tactile neuropathic pain sensitivity are set by corticospinal projections. Nature 2018;561: 547-550.

Lorenz J, Casey KL. Imaging of acute versus pathological pain in humans. Eur. J. Pain 2005; 9: 163-165.

Lundberg A. Convergence of excitatory and inhibitory action on interneurones in the spinal cord. UCLA Forum Med. Sci. 1969;11: 231-265.

Lv QY, Wu FZ, Gan XL, Yang XQ, Zhou L, Chen J, He YJ, Zhang R, Zhu BX, Liu LY. The involvement of descending pain inhibitory system in electroacupuncture-induced analgesia. Front. Integr. Neurosci. 2019;13: 38.

Magerl W, Fuchs PN, Meyer RA, Treede RD. Roles of capsaicin-insensitive nociceptors in cutaneous pain and secondary hyperalgesia. Brain 2001;124: 1754-1764.

Mansour A, Meador WJ, Bunzow JR, Civelli O, Akil H, Watson SJ. Localization of dopamine D2 receptor mRNA and D1 and D2 receptor binding in the rat brain and pituitary: an in situ hybridization-receptor autoradiographic analysis. J. Neurosci. 1990;10: 2587-2600.

Marini G, Tredici G. Parafascicular nucleus-raphe projections and termination patterns in the rat. Brain Res. 1995;690: 177-184.

Martikainen IK, Hagelberg N, Jääskeläinen SK, Hietala J, Pertovaara A. Dopaminergic and serotonergic mechanisms in the modulation of pain: In vivo studies in human brain. Eur. J. Pharmacol. 2018;834: 337-345.

Matheus FC, Rial D, Real JI, Lemos C, Takahashi RN, Bertoglio LJ, Cunha RA, Prediger RD. Temporal dissociation of striatum and prefrontal cortex uncouples anhedonia and defense behaviors relevant to depression in 6-OHDA-Lesioned rats. Mol. Neurobiol. 2016;53: 3891-3899.

Matsumoto M, Hidaka K, Tada S, Tasaki Y, Yamaguchi T. Low levels of mRNA for dopamine D4 receptor in human cerebral cortex and striatum. J. Neurochem. 1996;66: 915-919.

Mayer DJ, Mao J, Price DD. The association of neuropathic pain, morphine tolerance and dependence and the translocation of protein kinase C. NIDA Res. Mnogr. 1995;147: 269-298.

McDonald J, Lambert DG. Opioid receptors. BJA Educ. 2015;15: 219-224.

Melzack R, Casey KL. Sensory, motivational, and central control determinants of pain. In: Kenshalo DR, (Ed.) The skin senses. Charles C Thomas, Amsterdam;1968; pp423-443.

Mense S. Nociception from skeletal muscle in relation to clinical muscle pain. Pain 1993;54: 241-289.

Mercado-Reyes J, Almanza A, Segura-Chama P, Pellicer F, Mercado F. D2-like receptor agonist synergizes the μ-opioid agonist spinal antinociception in nociceptive, inflammatory and neuropathic models of pain in the rat. Eur. J. Pharmacol. 2019;853: 56-64.

Meyer RA, Campbell JN, Raja SN. Antidromic nerve stimulation in monkey does not sensitize unmyelinated nociceptors to heat. Brain Res. 1988;441: 168-172.

Miletic V, Coffield JA. Responses of neurons in the at nucleus submedius to noxious and innocuous mechanical cutaneous stimulation. Somatosens. Mot. Res. 1989;6: 567-587.

Millan MJ. Multiple opioid systems and pain. Pain 1986;27: 303-349.

Millan MJ. Descending control of pain. Prog. Neurobiol. 2002;66: 355-474.

Miyanishia K, Choudhurya ME, Watanabea M, Kubob M, Nomotob M, Yanoa H, Tanakaa J. Behavioral tests predicting striatal dopamine level in a rat hemi-Parkinson's disease model. Neurochem. Int. 2019;122: 38-46.

Mokry J. Experimental models and behavioural tests used in the study of Parkinson's disease. Physiol. Res. 1995;44: 143-150.

Monconduit L, Bourgeais L, Bernard JF, Villanueva L. Convergence of cutaneous, muscular and visceral noxious inputs onto ventromedial thalamic neurons in the rat. Pain 2003;103: 83-91.

Moradi M, Yazdanian M, Haghparast A. Role of dopamine D2-like receptors within the ventral tegmental area and nucleus accumbens in antinociception induced by lateral hypothalamus stimulation. Behav. Brain Res. 2015;292: 508-514.

Moustafa AA, Chakravarthy S, Phillips JR, Gupta A, Keri S, Polner B, Frank MJ, Jahanshahi M. Motor symptoms in parkinson's disease: A unified framework. Neurosci. Biobehav. Rev. 2016;68: 727-740.

Murakami S, Miyazaki I, Miyoshi K, Asanuma M. Long-term systemic exposure to rotenone induces central and peripheral pathology of Parkinson's disease in mice. Neurochem. Res. 2015;40: 1165-1178.

Myers RD. Injection of solutions into cerebral tissue. Relation between volume and diffusion. Physiol. Behav. 1966;1: 171-174.

Mylius V, Engau I, Teepker M, Stiasny-Kolster K, Schepelmann K, Oertel WH, Lautenbacher S, Möller JC. Pain sensitivity and descending inhibition of pain in Parkinson's disease. J. Neurol. Neurosurg. Psychiatry 2009;80: 24-28.

Naik DR, Sar M, Stumpf WE. Immunohistochemical localization of enkephalin in the central nervous system and pituitary of the lizard, Anolis carolinensis. J. Comp. Neurol. 1981;198: 583-601.

Nasser SA, Afify EA. Sex differences in pain and opioid mediated antinociception: modulatory role of gonadal hormones. Life Sci. 2019;237: 116926.

Neal Jr. CR, Mansour A, Reinscheid R, Nothacker HP, Civelli O, Watson Jr. SJ. Localization of orphanin FQ (nociceptin) peptide and messenger RNA in the central nervous system of the rat. J. Comp. Neurol. 1999;406: 503-547.

Nolano M, Provitera V, Estraneo A, Selim MM, Caporaso G, Stancanelli A, Saltalamacchia AM, Lanzillo B, Santoro L. Sensory deficit in Parkinson's disease: evidence of a cutaneous denervation. Brain 2008;131(Pt 7): 1903-1911.

Obara I, Przewlocki R, Przewlocka B. Spinal and local peripheral antiallodynic activity of Ro64-6198 in neuropathic pain in the rat. Pain 2005;116: 17-25.

Obeso JA, Rodriguez-Oroz M, Martin C, Alonso F, Zamarbide I, Lanciego JL, Rodriguez-Diaz M. The origin of motor fluctuations in Parkinson's disease: importance of dopaminergic innervation and basal ganglia circuits. Neurology 2004;62: 17-30.

Odeh F, Antal M. The projections of the midbrain periaqueductal grey to the pons and medulla oblongata in rats. Eur. J. Neurosci. 2001;14: 1275-1286.

Olivares-Moreno R, Moreno-Lopez Y, Concha L, Martínez-Lorenzana G, Condés-Lara M, Cordero-Erausquin M, Rojas-Piloni G. The rat corticospinal system is functionally and anatomically segregated. Brain Struct. Funct. 2017;222: 3945-3958.

Ossipov MH, Kovelowski CJ, Wheeler-Aceto H, Cowan A, Hunter JC, Lai J, Malan TP, Porreca F. Opioid antagonists and antisera to endogenous opioids increase the nociceptive response to formalin: demonstration of an opioid kappa and delta inhibitory tone. J. Pharmacol. Exp. Ther. 1996;277: 784-788.

Ossipov MH, Lai J, King T, Vanderah TW, Malan Jr, Hruby VJ, Porreca F. Antinociceptive and nociceptive actions of opioids. J. Neurobiol. 2004;61: 126-148.

Pan Z, Hirakawa N, Fields HL. A cellular mechanism for the biectional pain-modulating actions of orphanin FQ/nociceptin. Neuron 2000;26: 515-522.

Paulson PE, Morrow TJ, Casey KL. Bilateral behavioral and regional cerebral blood flow changes during painful peripheral mononeuropathy in the rat. Pain 2000;84: 233-245.

Paxinos G, Watson C. The Rat Brain in Stereotaxic Coordinates. 4th ed. Elsevier Academic Press, San Diego. 1998

Peirs C, Seal RP. Neural circuits for pain: Recent advances and current views. Science 2016;354: 578-584.

Pertovaara A. Noradrenergic pain modulation. Prog. Neurobiol. 2006;80: 53-83.

Pertovaara A, Almeida A. Descending inhibitory systems. Handb. Clin. Neurol. 2006;81: 179-192.

Peschanski M, Guilbaud G, Gautron M. Posterior intralaminar region in rat: neuronal responses to noxious and nonnoxious cutaneous stimuli. Exp. Neurol. 1981;72: 226-238.

Peterson AC, Li CR. Noradrenergic dysfunction in Alzheimer's and Parkinson's diseases - An overview of imaging studies. Front. Aging Neurosci. 2018;10: 127.

Politis M, Niccolini F. Serotonin in Parkinson's disease. Behav. Brain Res. 2015;277: 136-145.

Polli A, Weis L, Biundo R, Thacker M, Turolla A, Koutsikos K, Chaudhuri KR, Antonini A. Anatomical and functional correlates of persistent pain in Parkinson's disease. Mov. Disord. 2016;31: 1854-1864.

Quiroga-Varela, Aguilar E, Iglesias E, Obeso JA, Marin C. Short- and long-term effects induced by repeated 6-OHDA intraventricular administration: A new progressive and bilateral rodent model of Parkinson's disease. Neuroscience 2017;361: 144-156.

Radhakrishnan R, Moore SA, Sluka KA. Unilateral carrageenan injection into muscle or joint induces chronic bilateral hyperalgesia in rats. Pain 2003;104: 567-577.

Radhakrishnan R, Sluka KA. Deep tissue afferents, but not cutaneous afferents, mediate transcutaneous electrical nerve stimulation-induced antihyperalgesia. J. Pain 2005;6: 673-680.

Reep RL, Corwin JV, Hashimoto A, Watson RT. Efferent connections of the rostral portion of medial agranular cortex in rats. Brain Res. Bull. 1987;19: 203-221.

Renton T. Chronic orofacial pain. Oral Dis. 2017;23: 566-571.

Reynolds DV. Surgery in the rat during electrical analgesia induced by focal brain stimulation. Science 1969;64: 444-445.

Ridet JL, Sandillon F, Rajaofetra N, Geffard M, Privat A. Spinal dopaminergic system of the rat: light and electron microscopic study using an antiserum against dopamine, with particular emphasis on synaptic incidence. Brain Res. 1992;598: 233-241.

Riley DA, Thompson JL, Krippendorf BB, Slocum GR. 1995. Review of spaceflight- and hindlimb suspension unloadinginduced sarcomere damage and repair. Basic Appl. Myogr. 1995;5: 139-145.

Rizzi A, Ruzza C, Bianco S, Trapella C, Calo G. Antinociceptive action of NOP and opioid receptor agonists in the mouse orofacial formalin test. Peptides 2017;94: 71-77.

Ro JY, Capra NF. Evidence for subnucleus interpolaris in craniofacial muscle pain mechanisms demonstrated by intramuscular injections with hypertonic saline. Brain Res. 1999;842: 166-183.

Ro JY, Capra N, Masri R. Development of a behavioral assessment of craniofacial muscle pain in lightly anesthetized rats. Pain 2003;104: 179-185.

Ro JY, Capra N, Masri R. Contribution of peripheral N-methyl-D-aspartate receptors to c-fos expression in the trigeminal spinal nucleus following acute masseteric inflammation. Neuroscience 2004;123: 213-219.

Roeckel LA, Le Coz GM, Gavériaux-Ruff C, Simonin F. Opioid-induced hyperalgesia: cellular and molecular mechanisms. Neuroscience 2016;338: 160-182.

Rukavina K, Leta V, Sportelli C, Buhidma Y, Duty S, Malcangio M, Ray Chaudhuri K. Pain in Parkinson's disease: new concepts in pathogenesis and treatment. Curr. Opin. Neurol. 2019;32: 579-588.

Salpeter SR, Buckley JS, Buckley NS, Bruera E. The use of very-low-dose methadone and haloperidol for pain control in the hospital setting: A preliminary report. J. Palliat. Med. 2015;18: 114-119.

Sauer H, Oertel WH. Progressive degeneration of nigrostriatal dopamine neurons following intrastriatal terminal lesions with 6-hydroxydopamine: a combined retrograde tracing and immunocytochemical study in the rat. Neuroscience 1994;59: 401-415.

Sauerbier A, Jenner P, Todorova A, Chaudhuri KR. Non motor subtypes and Parkinson's disease. Parkinsonism Relat Disord. 2016; 22(Suppl. 1): S41-S46.

Schapira AHV, Chaudhuri KR, Jenner P. Nonmotor features of Parkinson disease. Nat. Rev. Neurosci. 2017;18: 509.

Schomburg ED. Spinal sensorimotor systems and their supraspinal control. Neurosci. Res. 1990;7: 265-340.

Schomburg ED, Steffens H. Comparative analysis of L-DOPA actions on nociceptive and non-nociceptive spinal reflex pathways in the cat. Neurosci. Res. 1998;31: 307-316.

Schrepf A, Harper DE, Harte SE, Wang H, Ichesco E, Hampson JP, Zubieta JK, Clauw DJ, Harris RE. Endogenous opioidergic dysregulation of pain in fibromyalgia: a PET and fMRI study. Pain 2016;57: 2217-2225.

Schröder W, Lambert DR, Ko MC, Koch T. Functional plasticity of the N/OFQ-NOP receptor system determines analgesic properties of NOP receptor agonists. Br. J. Pharmacol. 2014;171: 3777-3800.

Senapati AK, Huntington PJ, LaGraize SC, Wilson HD, Fuchs PN, Peng YB. Electrical stimulation of the primary somatosensory cortex inhibits spinal dorsal horn neuron activity. Brain Res. 2005;1057: 134-140.

Seo BK, Sung WS, Park YC, Baek YH. The electroacupuncture-induced analgesic effect mediated by 5-HT1, 5HT3 receptor and muscarinic cholinergic receptors in rat model of collagenase-induced osteoarthritis. BMC Complement Altern. Med. 2016;16: 212.

Sherrington CS. Flexion-reflex of the limb, crossed extension-reflex and reflex stepping and standing. J. Physiol. 1910;40: 28-121.

Shimura T, Kamada Y, Yamamoto T. Ventral tegmental lesions reduce overconsumption of normally preferred taste fluid in rats. Behav. Brain Res. 2002;134: 123-130.

Simone DA, Sorkin LS, Oh U, Chung JM, Owens C, LaMotte RH, Willis WD. Neurogenic hyperalgesia: Central neural correlates in responses of spinothalamic tract neurons. J. Neurophysiol. 1991;66: 228-246.

Skagerberg G, Lindvall O. Organization of diencephalic dopamine neurones projecting to the spinal cord in the rat. Brain Res. 1985;342: 340-351.

Sluka KA. Stimulation of deep somatic tissue with capsaicin produces long lasting mechanical allodynia and heat hypoalgesia that depends on early activation of the cAMP pathway. J. Neurosci. 2002;22: 5687-5693.

Sluka KA, Price MP, Breese NM, Stuckey CL, Wemmie JA, Welsh MJ. Chronic hyperalgesia induced by repeated acid injections in muscle is abolished by the loss of ASIC3, but not ASIC1. Pain 2003;106: 229-239.

Sommerauer M, Hansen AK, Parbo P, Fedorova TD, Knudsen K, Frederiksen Y, Nahimi A, Barbe MT, Brooks DJ, Borghammer P. Decreased noradrenaline transporter density in the motor cortex of Parkinson's disease patients. Mov. Disord. 2018;33: 1006-1010.

Sumikura H, Takao M, Hatsuta H, Ito S, Nakano Y, Uchino A, Nogami A, Saito Y, Mochizuki H, Murayama S. Distribution of α-synuclein in the spinal cord and darsal root ganglia in an autopsy cohort of elderly persons. Acta Neuropathol. Commun. 2015;3: 57.

Taguchi T, Katanosaka K, Yasui M, Hayashi K, Yamashita M, Wakatsuki K, Kiyama H, Yamanaka A, Mizumura K. Peripheral and spinal mechanisms of nociception in a rat reserpine-induced pain model. Pain 2015;156: 415-427.

Tariq S, Nurulain SM, Tekes K, Adeghate E. Deciphering intracellular localization and physiological role of nociceptin and nocistatin. Peptides 2013;43: 174-183.

Tiberi M, Jarvie KR, Silvia C, Falardeau P, Gingrich JA, Godinot N, Bertrand L, Yang FT, Fremeau RJ, Caron MG. Cloning, molecular characterization, and

chromosomal assignment of a gene encoding a second D1 dopamine receptor subtype: differential expression pattern in rat brain compared with the D1A receptor. Proc. Natl. Acad. Sci. USA. 1991;88: 7491-7495.

Tinazzi M, Recchia S, Simonetto S, Tamburin S, Defazio G, Fiaschi A, Moretto G, Valeriani M. Muscular pain in Parkinson's disease and nociceptive processing assessed with CO2 laser-evoked potentials. Mov. Disord. 2010;25: 213-220.

Torres ES, Zelikowsky M, Richter F, Hean S, Masliah E, Chesselet MF, Fanselow MS. Mice overexpressing human alpha synuclein under the Thy1-promotor show increased fear conditioning and altered responses in anxiety related behavior. Neuroscience Abstr. 2010;750.25/H18.

Treede RD, Meyer RA, Campbell JN. Myelinated mechanically insensitive afferents from monkey hairy skin: heat-response properties. J. Neurophysiol. 1998;80: 1082-1093.

Tsou K, Khachaturian H, Akil H, Watson SJ. Immunocytochemical localization of pro-opiomelanocortin-derived peptides in the adult rat spinal cord. Brain Res. 1986;378: 28-35.

Uchida M, Kobayashi O, Yoshida M, Miwa M, Miura R, Saito H, Nagakura Y. Coexistence of Alterations of Gastrointestinal Function and Mechanical Allodynia in the Reserpine-Induced Animal Model of Fibromyalgia. Dig. Dis. Sci. 2019;64: 2538-2547.

Uddin Z, MacDermid JC. Quantitative Sensory Testing in Chronic Musculoskeletal Pain. Pain medicine (Malden, Mass). 2016;17: 1694-1703.

Ungerstedt U. 6-Hydroxy-dopamine induced degeneration of central monoamine neurons. Eur J Pharmacol. 1968;5: 107-110.

Vallone D, Picetti R, Borrelli E. Structure and function of dopamine receptors. Neurosci. Biobehav. Rev. 2000;24: 125-132.

Van der Kraan M, Tatro JB, Entwistle ML, Brakkee JH, Burbach JPH, Adan RAH, Gispen WH. Expression of melanocortin receptors and pro-opiomelanocortin in the rat spinal cord in relation to neotrophic effects of melanocortins. Mol. Brain Res. 1999;63: 276-286.

Van Dijken H, Dijk J, Voom P, Holstege JC. Localization of dopamine D2 receptor in rat spinal cord identified with immunocytochemistry and in situ hybridization. Eur. J. Neurosci. 1996;8: 621-628.

Van Tol HHM, Wu CM, Guan HC, Ohara K, Bunzow J, Civelli OR, Kennedy J, Seeman P, Niznik HB, Jovanovic V. Multiple dopamine D4 variants in the human population. Nature 1992;358: 149-152.

Villanueva L, Le Bars D. The activation of bulbo-spinal controls by peripheral nociceptive inputs: diffuse noxious inhibitory controls. Biol. Res. 1995;28: 113-125.

Wall PD, Woolf CJ. Muscle but not cutaneous C-afferent input produces prolonged increases in the excitability of the flexion reflex in the rat. J. Physiol. 1984;356: 443-458.

Wang XF, Li S, Chou AP, Bronstein JM. Inhibitory effects of pesticides on proteasome activity: implication in Parkinson's disease. Neurobiol. Dis. 2006;23: 198-205.

Wei H, Viisanen H, Pertovaara A. Descending modulation of neuropathic hypersensitivity by dopamine D2 receptors in or adjacent to the hypothalamic A11 cell group. Pharmacol. Res. 2009;59: 355-363.

White K, Targett M, Harris J. Gainfully employing descending controls in acute and chronic pain management. Vet. J. 2018;237: 16-25.

Willer JC, Le Bars D, De Broucker T. Diffuse noxious inhibitory controls in man: involvement of an opioidergic link. Eur. J. Pharmacol. 1990;182: 347-355.

Williams-Gray CH, Worth PF. Parkinson's disease. Medicine 2016;44: 542-546.

Witting N, Svensson P, Gottrup H, Arendt-Nielsen L, Jensen TS. Intramuscular and intradermal injection of capsaicin: a comparison of local and referred pain. Pain 2000;84: 407-412

Wood PB. Role of central dopamine in pain and analgesia. Expert Rev. Neurother. 2008;8: 781-797.

Wronski TJ, Morey-Holton ER. Skeletal response to simulated weightlessness: a comparison of suspension techniques. Aviat. Space Environ. Med. 1987;58: 63-68.

Xiao WH, Bennett GJ. Effects of mitochondrial poisons on the neuropathic pain produced by the chemotherapeutic agents, paclitaxel and oxaliplatin. Pain 2012;153: 704-709.

Xiao Y, Lei J, Ye G, Xu H, You HJ. Role of thalamic nuclei in the modulation of Fos expression within the cerebral cortex during hypertonic saline-induced muscle nociception. Neuroscience. 2015;304: 36-46.

Yam MF, Loh YC, Tan CS, Adam SK, Manan NA, Basir R. General pathways of pain sensation and the major neurotransmitters involved in pain regulation. Int. J. Mol. Sci. 2018;19: 2164.

Yang YX, Wood NW, Latchman DS. Molecular basis of Parkinson's disease. Neuroreport 2009;20: 150-156.

You HJ, Dahl Morch C, Chen J, Arendt-Nielsen L. Simultaneous recordings of wind-up of paired spinal dorsal horn nociceptive neuron and nociceptive flexion reflex in rats. Brain Res. 2003;960: 235-245.

You HJ, Cao DY, Yuan B, Arendt-Nielsen L. Sex differences in the responses of spinal wide-dynamic range neurons to subcutaneous formalin and in the effects of different frequencies of conditioning electrical stimulation. Neuroscience 2006;138: 1299-1307.

You HJ, Colpaert FC, Arendt-Nielsen L. Long-lasting descending and transitory short-term spinal controls on deep spinal dorsal horn nociceptive-specific neurons in response to persistent nociception. Brain Res. Bull. 2008;75: 34-41.

You HJ, Lei J, Niu N, Yang L, Fan XL, Tjølsen A, Li Q. Specific thalamic nuclei function as novel 'nociceptive discriminators' in the endogenous control of nociception in rats. Neuroscience 2013;232: 53-63.

You HJ, Lei J, Sui MY, Huang L, Tan YX, Tjølsen A, Arendt-Nielsen L. Endogenous descending modulation: spatiotemporal effect of dynamic imbalance between descending facilitation and inhibition of nociception. J. Physiol. 2010;588: 4177-4188.

You HJ, Lei J, Ye G, Fan XL, Li Q. Influence of intramuscular heat stimulation on modulation of nociception: complex role of central opioid receptors in descending facilitation and inhibition. J. Physiol. 2014;592: 4365-4380.

You HJ, Lei J, Xiao Y, Ye G, Sun ZH, Yang L, Niu N. Pre-emptive analgesia and its supraspinal mechanisms: enhanced descending inhibition and decreased descending facilitation by dexmedetomidine. J. Physiol. 2016;594: 1875-1890.

Yuan B, Morrow TJ, Casey KL. Responsiveness of ventrobasal thalamic neurons after suppression of S1 cortex in the anesthetized rat. J. Neurosci. 1985;5: 2971-2978.

Zambito Marsala S, Tinazzi M, Vitaliani R, Recchia S, Fabris F, Marchini C, Fiaschi A, Moretto G, Giometto B, Macerollo A, Defazio G. Spontaneous pain, pain threshold, and pain tolerance in Parkinson's disease. J. Neurol. 2011;258: 627-633.

Zengin-Toktas Y, Ferrier J, Durif F, Llorca PM, Authier N. Bilateral lesions of the nigrostriatal pathways are associated with chronic mechanical pain hypersensitivity in rats. Neurosci. Res. 2013;76: 261-264.

Zhang DX, Carlton SM, Sorkin LS, Willis WD. Collaterals of primate spinothalamic tractneurons to the periaqueductal grey. J. Comp. Neurol. 1990;296: 277-290.

Zhang X, Ashton-Miller JA, Stohler CS. A closed-loop system for maintaining constant experimental muscle pain in man. IEEE Trans. Biomed. Eng. 1993;40: 344-352.

Zhu LX, Li CY, Yang B, Ji CF, Li WM. The effect of neonatal capsaicin on acupuncture analgesia. Acupunct. Res. 1990;15: 285-291.