CLINICAL PAIN MANAGEMENT
second edition

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Chronic Pain

Edited by Peter R Wilson, Paul J Watson,
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Clinical Pain Management

Chronic Pain

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Clinical Pain Management

Chronic Pain

2nd edition

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Series preface

Since the successful first edition of *Clinical Pain Management* was published in 2002, the evidence base in many areas of pain medicine has changed substantially, thus creating the need for this second edition. We have retained the central ethos of the first volume in that we have continued to provide comprehensive coverage of pain medicine, with the text geared predominantly to the requirements of those training and practicing in pain medicine and related specialties. The emphasis continues to be on delivering this coverage in a format that is easily accessed and digested by the busy clinician in practice.

As before, *Clinical Pain Management* comprises four volumes. The first three cover the main disciplines of acute, chronic, and cancer pain management, and the fourth volume covers the practical aspects of clinical practice and research. The four volumes can be used independently, while together they give readers all they need to know to deliver a successful pain management service.

Of the 161 chapters in the four volumes, almost a third are brand new to this edition while the chapters that have been retained have been completely revised, in many cases under new authorship. This degree of change reflects ongoing progress in this broad field, where research and development provide a rapidly evolving evidence base. The international flavor of *Clinical Pain Management* remains an important feature, and perusal of the contributor pages will reveal that authors and editors are drawn from a total of 16 countries.

A particularly popular aspect of the first edition was the practice of including a system of simple evidence scoring in most of the chapters. This enables the reader to understand quickly the strength of evidence which supports a particular therapeutic statement or recommendation. This has been retained for the first three volumes, where appropriate. We have, however, improved the system used for scoring evidence from a three point scale used in the first edition and adopted the five point Bandolier system which is in widespread use and will be instantly familiar to many readers (www.jr2.ox.ac.uk/bandolier/band6/b6-5.html).

We have also retained the practice of asking authors to highlight the key references in each chapter. Following feedback from our readers we have added two new features for this edition: first, there are key learning points at the head of each chapter summarizing the most salient points within the chapter; and second, the series is accompanied by a companion website with downloadable figures.

This project would not have been possible without the hard work and commitment of the chapter authors and we are deeply indebted to all of them for their contributions. The volume editors have done a sterling job in diligently editing a large number of chapters, and to them we are also most grateful. Any project of this magnitude would be impossible without substantial support from the publishers – in particular we would like to acknowledge our debt to Jo Koster and Zelah Pengilley at Hodder. They have delivered the project on a tight deadline and ensured that a large number of authors and editors were kept gently, but firmly, "on track."

Andrew SC Rice, Douglas Justins, Toby Newton-John,
Richard F Howard, Christine A Miaskowski
London, Newcastle, and San Francisco

I would also like to add my personal thanks to the Series Editors who have given their time generously and made invaluable contributions through the whole editorial process from the very outset of discussions regarding a second edition in deciding upon the content of each volume and in selecting Volume Editors. More recently, they have provided an important second view in the consideration of all submitted chapters, not to mention stepping in and assisting with first edits where needed. The timely completion of the second edition would not have been possible without this invaluable input.

Andrew SC Rice
Lead Editor

Introduction to Clinical Pain Management: Chronic Pain

Chronic pain has traditionally had the negative connotation of psychogenic etiology and an arbitrary time domain. It has also been a pejorative term to the extent that chronic pain syndrome was deliberately omitted from the IASP *Taxonomy of Chronic Pain Syndromes*. This new volume gathers together the scientific and clinical evidence that confirms chronic pain as an identifiable syndrome, the final common path of many etiologies. Consistent with any clinical syndrome, there are common neurophysiological, neuroanatomical, and functional changes throughout the organism regardless of the precipitating factors. These changes are addressed in the early chapters of this volume. In addition, there is physical, psychological, and psychiatric deconditioning resulting from central and peripheral nervous system dysfunction. Socioeconomic impairment and reduction in quality of life almost invariably accompany these changes.

There has also been a recent paradigm shift from the curative medical model of pain in which symptoms are expected to resolve once the underlying pathologic process is treated medically or surgically to a model which emphasizes patient autonomy, symptom management, and functional restoration. This volume addresses this new model of chronic pain in those specific conditions where applicable. It also explores the conceptually distinct rehabilitation model, in which it is recognized that the underlying pathology may be incurable or untreatable. The goals now involve minimizing the adverse effects of the pain and maximizing function and quality of life.

Fundamental changes in practitioners' responsibilities to patients and society are occurring as a result of philosophical and legal advances related to chronic pain. Previously implied rights of patients now have been formalized in various intractable pain acts of several jurisdictions. The classical doctrine of *primum non nocere* (first do no harm) is being challenged ethically and legally under these circumstances. Experts in these fields explore these changing ethical and legal climates in early chapters.

This volume contains 46 chapters in three parts. The first part, General Considerations, comprises 14 chapters that cover subjects ranging from basic neurophysiology through clinical evaluation to the ethical, legal, and societal aspects of this disease as described above. Part II, entitled Management – therapies, contains 9 chapters that address pharmacological, psychological, behavioral, interventional (invasive) and alternative/complementary/placebo issues. Part III has 23 chapters that describe both specific and nonspecific pain syndromes and their management. The subjects discussed include general neuropathic pain syndromes, specific pain syndromes and regional pain (neck, back, joints, chest, abdomen, and pelvis), and issues related to pain at the extremes of age.

Chronic pain now covers a vast scientific and clinical arena, and has become a medical specialty in its own right. Scientific rationale and therapeutic options are much better described now than at any time in the past. This volume gathers the available evidence-based information on diagnosis and management in an accessible format without overwhelming detail. Where evidence-based data are not available, the authors provide thoughtful advice based on scientific experience and clinical wisdom. It is inevitable in a volume such as this that there will be omissions, for which we must accept responsibility. Nevertheless, we believe that this volume is an essential resource for all clinicians whose patients have chronic pain and scientists who challenge traditional assumptions.

Peter R Wilson, Paul J Watson, Jennifer A Haythornthwaite, and Troels S Jensen
Rochester, Leicester, Baltimore, and Aarhus

How to use this book

SPECIAL FEATURES

The four volumes of *Clinical Pain Management* incorporate the following special features to aid the readers' understanding and navigation of the text.

Key learning points

Each chapter opens with a set of key learning points which provide readers with an overview of the most salient points within the chapter.

Cross-references

Throughout the chapters in this volume you will find cross-references to chapters in other volumes in the *Clinical Pain Management* series. Each cross-reference will indicate the volume in which the chapter referred to is to be found.

Evidence scoring

In chapters where recommendations for surgical, medical, psychological, and complementary treatment and diagnostic tests are presented, the quality of evidence supporting authors' statements relating to clinical interventions, or the papers themselves, are graded following the Oxford Bandolier system by insertion of the following symbols into the text:

[I] Strong evidence from at least one published systematic review of multiple well-designed randomized controlled trials

[II] Strong evidence from at least one published properly designed randomized controlled trial of appropriate size and in an appropriate clinical setting

[III] Evidence from published well-designed trials without randomization, single group pre-post, cohort, time series, or matched case-controlled studies

[IV] Evidence from well-designed non-experimental studies from more than one center or research group

[V] Opinions of respected authorities, based on clinical evidence, descriptive studies or reports of expert consensus committees.

Oxford Bandolier system used by kind permission of Bandolier: www.jr2.ox.ac.uk/Bandolier

Where no grade is inserted, the quality of supporting evidence, if any exists, is of low grade only (e.g. case reports, clinical experience, etc).

Other textbooks devoted to the subject of pain include a tremendous amount of anecdotal and personal recommendations, and it is often difficult to distinguish these from those with an established evidence base. This text is thus unique in allowing the reader the opportunity to do this with confidence.

Reference annotation

The reference lists are annotated with asterisks, where appropriate, to guide readers to key primary papers, major review articles (which contain extensive reference lists), and clinical guidelines. We hope that this feature will render extensive lists of references more useful to the reader and will help to encourage self-directed learning among both trainees and practicing physicians.

A NOTE ON DRUG NAMES

The authors have used the international nonproprietary name (INN) for drugs where possible. If the INN name differs from the US or UK name, authors have used the INN name followed by the US and/or UK name in brackets on first use within a chapter.

Abbreviations

4-AP	4-aminopyridine
5-FU	5-fluorouracil
5-HT	5-hydroxytryptamine
ABC	American Botanical Council
ACC	anterior cingulate cortex
ACE	angiotensin-converting enzyme
Ach	acetylcholine
ACOG	American College of Obstetricians and Gynecologists
ACR	American College of Rheumatology
ACTH	adrenocortical trophic hormone
ACh	acetylcholine
ADL	activities of daily living
AED	antiepileptic drug
AFP	atypical facial pain
AHPA	American Herbal Products Association
AIDS	acquired immunodeficiency syndrome
ALJ	Administrative Law Judges
AMA	American Medical Association
AMPA	α-amino-3-hydroxyl-5-methyl-4-isoxazole
AO	atypical odontalgia
AOJ	atlanto-occipital joint
APC	adenoma prevention with celecoxib
APF	anti-proliferative factor
APPROVe	Adenomatous Polyp Prevention on Vioxx
ARF	acute renal failure
ASA	acetylsalicylic acid
ATN	antiretroviral toxic neuropathy
BDI	Beck Depression Inventory
BDNF	brain-derived neurotrophic factor
BDZ	benzodiazepine
BMS	burning mouth syndrome
BOI	burden of illness
BPI	Brief Pain Inventory
BSI	Brief Symptom Inventory
BTP	breakthrough pain
BZD	benzodiazepine
C	cytosine
CABG	coronary artery bypass graft
CAD	coronary atherosclerotic disease
CAM	complementary and alternative medicine
CARF	Commission on Accreditation of Rehabilitation Facilities
CART	Classification and Regression Tree
CBC	complete blood count
CBT	cognitive-behavioral therapy
CCI	chronic constriction injury
CCK	cholecystokinin
CCR2	chemotactic cytokine receptor 2
CD	Crohn's disease
cDNA	complementary DNA
CES	cauda equina syndrome
CES-D	Center for Epidemiological Studies-Depression
CFS	chronic fatigue syndrome
CGRP	calcitonin gene-related peptide
CHF	congestive heart failure
CI	confidence interval
CIN	cervical intraepithelial neoplasia
CLBP	chronic lower back pain
CMI	cell-mediated immunity
CMV	cytomegalovirus
CNCP	chronic noncancer pain
CNMP	chronic nonmalignant pain
CNS	central nervous system
COMM	current opioid misuse measure
COMT	catechol-O-methyltransferase
COOA	combined opioid–opioid analgesia
COPD	chronic obstructive pulmonary disease
COX	cyclooxygenase
CP/CPPS	chronic prostatitis/chronic pelvic pain syndrome
CPP	chronic pelvic pain
CPQ	Chronic Pain Questionnaire
CPSP	central poststroke pain; or chronic postsurgical pain
CRD	colorectal distension
CRP	chronic regional pain
CRPS	complex regional pain syndrome
CSA	Controlled Substances Act
CSF	cerebrospinal fluid
CSM	Committee on Safety of Medicines
CSQ	Coping Strategies Questionnaire
CT	computed tomography
CTN	classical trigeminal neuralgia

CTTH	chronic tension-type headache
CVA	cerebrovascular accident
CWP	chronic widespread pain
d4T	stavudine
DAP	depolarizing after potentials
DAS	Disease Assessment Score
DBS	Deep brain stimulation
ddC	zalcitabine
ddI	didanosine
DDwR	disk displacement with reduction
DDwoR	disk displacement without reduction
DEA	Drug Enforcement Administration
DHE	dihydroergotamine
DILS	diffuse infiltrative lymphocytosis
DLF	dorsolateral funiculus
DM	diabetes mellitus
DMARD	disease-modifying antirheumatic drugs
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DNIC	diffuse noxious inhibitory control
DPN	diabetic peripheral neuropathy
DREZ	dorsal root entry zone
DRG	dorsal root ganglion
DSM	Diagnostic and Statistical Manual of Mental Disorders
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders IV
DSP	distal symmetrical polyneuropathy
EAA	excitatory amino acids
EDDP	ethylidine-dimethyl-diphenylpyrrolidine
EECP	enhanced external counter pulsation
EEG	electroencephalogram
ELBW	extremely low birth weight
EMDR	eye movement desensitization and reprocessing
EMG	electromyogram
EMLA	eutectic mixture of local anesthetics
EP	episodic pain
ERCP	endoscopic retrograde cholangiopancreatography
ERK	extracellular signal-regulated kinase
ES	effect size
ESBY	Electrical Stimulation versus Coronary Bypass Surgery
ESCOP	European Scientific Cooperative of Phytotherapy
ESR	erythrocyte sedimentation rate
ETTH	episodic tension-type headache
FABQ	Fear Avoidance Beliefs Questionnaire
FAP	functional abdominal pain
FBSS	failed back surgery syndrome
FCE	functional capacity evaluation
FDA	Food and Drug Administration
FDR	false discovery rate
FHM	familial hemiplegic migraine
fMRI	functional magnetic resonance imaging
FMS	fibromyalgia syndrome
FRA	flexor reflex afferents
FSH	follicle-stimulating hormone
G	guanine
GABA	gamma aminobutyric acid
GABA-A	gamma-aminobutyric acid A
GABA-b	gamma aminobutyric acid-b
GAD	generalized anxiety disorder; or glutamic acid decarboxylase
GBP	gabapentin
GBS	Guillain–Barré syndrome
GDNF	glial-derived neurotrophic factor
GH	growth hormone
GI	gastrointestinal
GnRH	gonadotropin-releasing hormone
gp120	glycoprotein 120
GP	general practitioner
GPRD	General Practice Research Database
GS	gastrocnemius-soleus
GTN	glyceryl trinitrate
HAART	highly active antiretroviral therapy
HADS	Hospital Anxiety and Depression Scale
HIV	human immunodeficiency virus
HIZ	high intensity zone
HLA	human leukocyte antigen
HMO	health maintenance organization
HPA	hypothalamic–pituitary–adrenal
HPV8	human papilloma virus 8
HRR	hazard rate ratio
HSAN	hereditary sensory and autonomic neuropathy
HTEA	high thoracic epidural anesthesia
HZ	herpes zoster
HZV	herpes zoster virus
IAP	intermittent acute porphyria
IASP	International Association for the Study of Pain
IBS	irritable bowel syndrome
IC	interstitial cystitis
ICD-10	International Classification of Diseases, 10th edition
ICER	incremental cost–effectiveness ratio
ICN	intercostobrachial neuralgia
ICU	intensive care unit
IDET	intradiscal electrothermotherapy
IGF	insulin-like growth factor
IHS	International Headache Society
IL-1β	interleukin-1β
IL-1Ra	interleukin-1 receptor antagonist
IL-6	interleukin-6
IMET	Individualized Medication Effectiveness Tests

INCB	International Narcotics Control Board
IP	incident pain
IRIS	immune reconstitution inflammatory syndrome
ISSVD	International Society for the Study of Vulvovaginal Disease
i.t.	intrathecal
ITB	intrathecal baclofen
ITDD	intrathecal drug delivery
IUD	intrauterine devices
IVOT	intravenous opioid (sensitivity) testing
IVR	intravenous regional
JCAHO	Joint Commission on the Accreditation of Healthcare Organizations
JFS	juvenile fibromyalgia syndrome
JNK	c-Jun-N-terminal kinase
LAAJ	lateral atlanto-axial joint
LANSS	Leeds Assessment of Neuropathic Symptoms and Signs
LBP	low back pain
LIF	leukemia inhibitory factor
LIMA	left internal mammary artery
Lng-IUS	levonorgestrol-releasing intrauterine system
LTP	long-term potentiation
LUNA	laparoscopic uterine nerve ablation
M6G	morphine-6-glucuronide
MBSR	mindfulness-based stress reduction
MCIC	minimum clinically important change
MCS	motor cortex stimulation
MCSF	macrophage colony-stimulating factor
MEDAL	Multinational Etoricoxib and Diclofenac Arthritis Long-term
MFP	myofascial pain
MMPI	Minnesota Multiphasic Personality Inventory
MOH	medication overuse headache
MPA	medroxyprogesterone acetate
MPI	Multidimensional Pain Inventory
MPQ	McGill Pain Questionnaire
MR	magnetic resonance
MRI	magnetic resonance imaging
mRNA	messenger RNA
MS	multiple sclerosis
MSP	musculoskeletal pain
MT	mindful therapies
MVAS	million visual analog scale
NAPQI	*N*-acetyl-*p*-benzoquinoneimine
NCPB	neurolytic celiac plexus block
NCS	nerve conduction studies
NE	noradrenaline; or norepinephrine
NGF	nerve growth factor
NHL	non-Hodgkin's lymphoma
NHS	National Health Service
NICE	National Institute for Clinical Excellence
NIH	National Institutes of Health
NMDA	*N*-methyl-D-aspartic acid
NNH	number needed to harm
NNT	number needed to treat
NO	nitric oxide
NOP	neuropathic orofacial pain
NPS	Neuropathic Pain Scale
NPV	negative predictive value
NRS	numeric rating scale
NRTI	nucleoside reverse transcriptase inhibitors
NSAID	nonsteroidal anti-inflammatory drug
NTG	nitroglycerin
NYHA	New York Heart Association
OA	osteoarthritis
OAM	Office of Alternative Medicine
OCP	oral contraceptive pill
ODER	opioid dose escalation rate
OECD	Organisation for Economic Cooperation and Development
OEI	opioid escalation index
OIH	opioid-induced hyperalgesia
OR	opioid rotation; or odds ratio
OT	opioid tolerance
PADT	Pain Assessment and Documentation Tool
PAG	periaqueductal gray
PAG/PVG	periaquaductal or periventricular gray
PAR	pain relief
PASS	Pain Anxiety Symptoms Scale
PCA	patient-controlled analgesia
PCPT	Posttraumatic Chronic Pain Test
PCR	polymerase chain reaction
PD	Parkinson's disease
PDN	painful diabetic neuropathy
PDPN	painful diabetic peripheral neuropathy
PDQ	Pain Disability Questionnaire
PENS	percutaneous electrical nerve stimulation
PET	positron emission tomography
PGIC	Patient Global Impression of Change
PHN	postherpetic neuralgia
PID	pelvic inflammatory disease
PKC	protein kinase C
PML	progressive multifocal leukoencephalopathy
PMP	Pain Management Program
PMR	percutaneous revascularization; or progressive muscle relaxation
PNL	partial sciatic nerve ligation
PNS	peripheral nervous system
POMS	Profile of Mood States
PPI	proton-pump inhibitors
PPV	positive predictive value
PT	physical therapy
PTSD	posttraumatic stress disorder
PVD	peripheral vascular disease

QALY	quality adjusted life year
QOL	quality of life
qPCR	quantitative polymerase chain reaction
QS	quality scale
QSART	quantitative sudomotor axon reflex test
QST	quantitative sensory testing
RA	rheumatoid arthritis
RAP	recurrent abdominal pain; or refractory angina pectoris
rCBF	regional cerebral blood flow
RCT	randomized controlled trial
RDC	research diagnostic criteria
RF	radiofrequency
rhNGF	recombinant human growth factor
RNA	ribonucleic acid
rRNA	ribosomal RNAs
RR	relative risk
RSD	reflex sympathetic dystrophy
RSO	resting sweat output
RVM	rostral ventromedial medulla
SCI	spinal cord injury
SCL-90R	Symptom Checklist 90-Revised
SCS	spinal cord stimulation
SDR	selective dorsal rhizotomy
SEP	somatosensory evoked potential
SF-36	Short-Form-36
SFS	Spinal Function Sort
SHBPS	Saskatchewan Health and Back Pain Survey
SIF	sacral insufficiency fracture
SIP	Sickness Impact Profile
SLE	systemic lupus erythematosus
SNF	skilled nursing facility
SNI	spared nerve injury
SNL	spinal nerve ligation
SNP	single nucleotide polymorphisms
SNRI	serotonin noradrenaline reuptake inhibitor
SNT	spinal nerve transection
SP	substance P
SPECT	single photon emission computed tomography
SPID	sum of the differences in pain intensity
SPS	Shingles Prevention Study
SR	sustained release
SSR	sympathetic skin response
SSRI	selective serotonin reuptake inhibitor
STAI	State-Trait Anxiety Inventory
STAR	Screening Tool for Addiction Risk
STN	symptomatic trigeminal neuralgia
SUPPORT	Study to Understand Prognosis, Preferences for Outcomes, and Risks of Treatment
T	thymine
TC	treatment control
TCA	trichloroacetic acid; or tricyclic antidepressant
tDCS	transcranial direct current stimulation
TDF	transdermal fentanyl
TENS	transcutaneous electrical nerve stimulation
TMD	temporomandibular disorder
TMJ	temporomandibular joint
TMR	transmyocardial revascularization
TN	trigeminal neuralgia
TNF	tumor necrosis factor
TNFα	tumor necrosis factor-α
TPBS	three-phase bone scan
TRP	transient receptor potential
TSK	Tampa Scale for Kinesiophobia
TST	thermoregulatory sweat test
TTH	tension-type headache
TTX	tetrodotoxin
TTX-r	tetrodotoxin-resistant
TTX-S	tetrodotoxin-sensitive channel
U	uracil
UC	ulcerative colitis
UMNS	upper motor neuron syndrome
VAS	visual analog scale
VC	ventrocaudalis
VDCC	voltage-dependent calcium channel
VMpo	ventral medial posterior
VPL	ventroposterolateral
VPM	ventroposteriomedial
VSCC	voltage-sensitive calcium channel
VZV	varicella zoster virus
WDR	wide dynamic range
WHO	World Health Organization
WHYMPI	West Haven-Yale Multidimensional Pain Inventory
WLC	waiting list control
WLQ	Work Limitations Questionnaire
WOMAC	Western Ontario and McMaster Universities Osteoarthritis Index
ZJ	zygapophysial joint

PART I

GENERAL CONSIDERATIONS

1

Applied physiology: neuropathic pain

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KEY LEARNING POINTS

- Much information about neuropathic pain models is gleaned from studies in animal models.
- Damage to peripheral nerves causes phenotypic and excitability changes.
- Inflammatory mediators can produce excitation of neurons in the peripheral nervous system (PNS) and central nervous system (CNS).
- Nerve injury can lead to cell death and anatomical reorganization.
- A loss of inhibitory mechanisms and increase in excitatory mechanisms are associated with increased activity in the spinal cord in neuropathic pain.
- Microglia are activated in neuropathic pain and release pronociceptive substances which can activate neurons in the spinal cord.
- Supraspinal sites have increased excitatory influences on spinal nociceptive processing following nerve injury.

INTRODUCTION

Neuropathic pain is a form of chronic pain defined as "Pain arising as a direct consequence of a lesion or disease affecting the somatosensory system."[1] The spectrum of neuropathic pain is associated with a variety of disease states (**Table 1.1**),[2,3] but it is important to recognize that neuropathic pain is a relatively frequent, but unusual and by no means inevitable, consequence of those disorders.

Various patterns of neuropathic pain are recognized and it may be spontaneous in nature (continuous or paroxysmal) or evoked by sensory stimuli. These patterns may coexist in the same patient and are not necessarily unique to any disease entity. Neuropathic pain is also usually associated with various phenomena associated with disturbances in sensory function and it is possible to broadly classify neuropathic pain patients on the basis of their sensory phenotype, for example in postherpetic neuralgia.[4] Therefore, pain may exist in the context of sensory loss (anesthesia dolorosa) or more unusually in the presence of hypersensory phenomena (e.g. allodynia (**Figure 1.1**), hyperalgesia (**Figure 1.1**), and hyperpathia). Occasionally, a mixed picture of disordered sensory function may be evident depending on which areas are tested.

While the biological advantage to the organism of nociceptive pain is readily identifiable, it is less easy to do so for neuropathic pain and it is probable that, in broad terms, neuropathic pain is a result of a pathological process representing a disordered regenerative response to neuronal damage. For example, in patients with the hyper-sensory subtype of neuropathic pain, the mechanistic implication of allodynia is that elements of the sensory nervous system which normally signal innocuous

Table 1.1 A classification of the more frequent disorders associated with neuropathic pain, with examples.

Cause of neuropathy	Examples
Trauma	Phantom limb
	Spinal cord injury
	Surgical
	Peripheral nerve injury
Infection/inflammation	Postherpetic neuralgia
	HIV
Cancer	Invasion/compression of neural structures by tumor
Drugs	Vinca alkaloids
	Taxols
	Ethanol
	Antiretroviral drugs
Ischemic injury	Poststroke pain
	Metabolic neuropathies, i.e. diabetic neuropathy
Compression	Trigeminal neuralgia
	Sciatica
Demyelination	Multiple sclerosis
	Charcot–Marie–Tooth

sensation have begun to encode painful stimuli, while in hyperalgesia the structures which normally subserve nociception have become hyperexcitable.

Before exploring what is known about the pathophysiology of neuropathic pain, three major caveats as to the nature of the existing literature need to be stated. First, the overwhelming bulk of the literature related to neuropathic pain mechanisms has emerged from rodent studies in which the major outcome measure is hypersensitivity of spinal withdrawal reflexes evoked by sensory stimuli. Thus, in this chapter, it will actually only be possible to discuss the putative mechanisms of evoked hypersensitivity, a relatively minor component of the spectrum of clinical neuropathic pain. Second, since it is also not currently possible to directly measure pain in experimental animals, the putative pain mechanisms which are to be discussed can only be interpreted in the context of responses to nerve injury which are possibly, but not certainly, related to pain. Third, the vast majority of research into neuropathic pain mechanisms has concentrated on changes in the peripheral nerve or spinal cord following peripheral nerve injury. Although knowledge is accumulating regarding alterations in the brain following peripheral nerve injury, much less is known about the significance of these changes. Therefore, this chapter will focus mainly on peripheral and spinal mechanisms of neuropathic pain.

ANIMAL MODELS OF NEUROPATHIC PAIN

Unravelling the mechanisms involved in neuropathic pain requires the use of laboratory animal models that replicate as far as possible, with the above caveats, the different pathophysiological changes present in patients. For reasons of reproducibility and simplicity, most studies of neuropathic pain are based upon animal models of traumatic nerve injury, usually in the rat sciatic nerve (**Figure 1.2**).

Rodent models of neuropathy

The most commonly used nerve injury models are: the chronic constriction injury (CCI) of sciatic nerve,[7] the partial sciatic nerve ligation (PNL) model,[8] the spinal nerve ligation (SNL)/transection model (**Figure 1.2**),[9] and the spared nerve injury (SNI) model.[6] All models are associated with the development of hypersensitivity to thermal (heat and cold), and mechanical stimuli which are used experimentally as correlates of hyperalgesia and allodynia symptoms in neuropathic pain patients.[10] However, the relevance of these measures to the human condition is questionable.

The CCI model consists of the loose ligation of the sciatic nerve with chromic gut sutures. An inflammatory reaction develops and consequentially damage to most A-fibers and some C-fibers. It is likely that there is a significant inflammatory component in the development

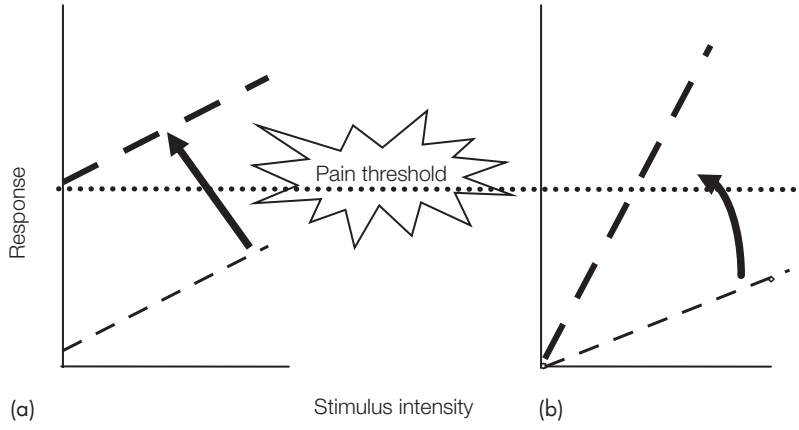

Figure 1.1 Graphical representation of (a) allodynia, a painful response to a normally innocuous stimuli and (b) hyperalgesia, an increased response to a normally painful stimulus. Stimulus intensity versus response relationship for noxious and innocuous stimuli. © The Board of Management and Trustees of the British Journal of Anaesthesia. Adapted from Bridges *et al.*, 2001[5] by permission of Oxford University Press/ British Journal of Anaesthesia.

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Figure 1.2 Rodent models of nerve injury. Many rodent models are based upon injury to the peripheral, usually sciatic, nerve. Schematic drawing of partial sciatic nerve injury (PSNL), chronic constriction injury (CCI), spared nerve injury (SNI), and spinal nerve ligation or transection (SNL/SNT) of the L5 and L6 spinal nerves. Adapted from Decosterd and Woolf, 2000[6] by permission of the International Association for the Study of Pain.

of the painful neuropathy.[11] In the PNL model, a tight ligation is created around 33–50 percent of the sciatic nerve, leaving the rest of the nerve "uninjured."[8] The SNL model traditionally consists of injury to the L5 and L6 spinal nerves, which contribute to the sciatic nerve.[9] However, a transection of the L5 spinal nerve alone results in comparative symptoms and hence some experimenters now use this as a modified SNL model.[5] This model is favorable to mixed injury models as it allows the examination of cellular responses of injured afferents (with cells in the L5/L6 dorsal root ganglia (DRG)) versus uninjured afferents (in the L4 DRG), and their relative importance in neuropathic pain.[12] The spared nerve injury model involves tight ligation and lesion of the tibial and common peroneal nerves.[6] This model allows testing of distinct regions of the hindpaw which are either innervated by injured or uninjured neurons, as well as separating degenerating neurons from uninjured neurons to a greater level.

Although commonly used and reproducible, there are shortcomings of these animal models which need to be considered. First, while neuropathic pain can be a devastating consequence of nerve injury in humans, the majority do not develop neuropathic pain following nerve injuries,[3] whereas most animals do develop reflex hypersensitivity in response to the above injuries. Therefore, the aforementioned animal models do not precisely mirror the "normal" human response to nerve injury. Second, for good ethical reasons, most animal models of neuropathic pain study the animals for a period of weeks, whereas the clinical course of neuropathic pain presenting to a pain relief clinic is often measured in years. Finally, as with all animal models, it is difficult to know what is actually perceived by the animal. To date, the behavioral manifestation of pain in rodent models of neuropathic pain has relied largely on measuring alterations in cutaneous sensory thresholds via measurement of reflex withdrawal thresholds to stimuli, such as punctuate mechanical (such as von Frey filaments),[13] which are not without their shortcomings, heat (such as the infrared heating device[14]) or cooling (such as the application of acetone) stimuli. Whilst these hypersensory phenomena do occur in a subset of humans with neuropathic pain, they are more usually observed in response to mechanical rather than thermal stimuli. (It must be noted that because the terms hyperalgesia and allodynia are defined in terms of pain, and we cannot yet measure pain in rodents, the use of these terms in the context of animal studies is inappropriate. We will therefore use the term "hypersensitivity" in the context of animal studies.)

Therefore, there is a need for the development of more clinically relevant animal models of neuropathic pain, as well as more complex behavioral tests designed to measure a spontaneous ongoing pain phenotype, and pain comorbidity.

Recent developments in rodent models of neuropathy

In recent years, scientists have worked to rectify the limitations of animal models, including development of models that more closely represent individual disease states. For example, as a model of peripheral diabetic neuropathy, a single injection of streptozotocin induces diabetes in the rat and is associated with the development of reflex hypersensitivity.[15] To model trigeminal neuralgia, chronic constriction injury of the infraorbital branch of the trigeminal nerve has been described.[16] In order to reproduce some features of postherpetic neuralgia, varicella zoster virus-infected fibroblasts are injected into the hindpaw and retrogradely transported to the cell bodies of sensory neurons in the DRG.[17, 18, 19] Similarly, the mechanisms by which the HIV virus could directly interact with the nervous system to produce peripheral neuropathic pain are being investigated by studying the effects of the HIV-envelope protein, glycoprotein 120 (gp120) *in vivo*.[20, 21, 22] Gp120 is thought to be key to the production of neurological disorders associated with HIV infection via the activation of the chemokine receptors CXCR4 and CCR5 expressed by neurons and glial cells.[23] Finally, drug-induced neuropathies are becoming more prevalent clinically with painful peripheral neuropathy presenting as an

unfortunate side effect of treatment with chemotherapeutics, including taxols and vinca alkaloids, or with antiretroviral agents which form part of the highly active antiretroviral therapy (HAART) for the treatment of HIV disease. Rats treated systemically with such drugs develop signs of a neuropathic phenotype and are therefore important, clinically relevant models that are currently being investigated for the understanding of underlying mechanisms.[22, 24, 25, 26, 27] The aforementioned models are important as they model some aspects of the diseases most frequently associated with neuropathic pain.

The majority of neuropathic pain models were originally described in rats, but more recently have adapted to the mouse. The translation of these models from rat to mouse is important as novel transgenic tools, useful for the study of neuropathic pain, are further developed.

Behavioral tests of pain phenotype

In addition to new models, work is being conducted to improve the range of behavioral tests employed *in vivo* (**Figure 1.3**). For example, spontaneous exploratory activity assessed in the open field paradigm is classically used as a measure of anxiety-related behavior in rodents.[28] This test has been used as a measure of locomotor activity in pain models[29] and more recently, additional measures of thigmotactic behavior indicate the presence of altered exploratory behavior in rodent models of pain without the presence of locomotor deficits. This behavior is sensitive to clinically employed analgesics, such as gabapentin and morphine,[19, 27] suggesting the thigmotaxis to be correlated to a nonstimulus-evoked pain-like behavior in rodents be it spontaneous pain or pain comorbidities.

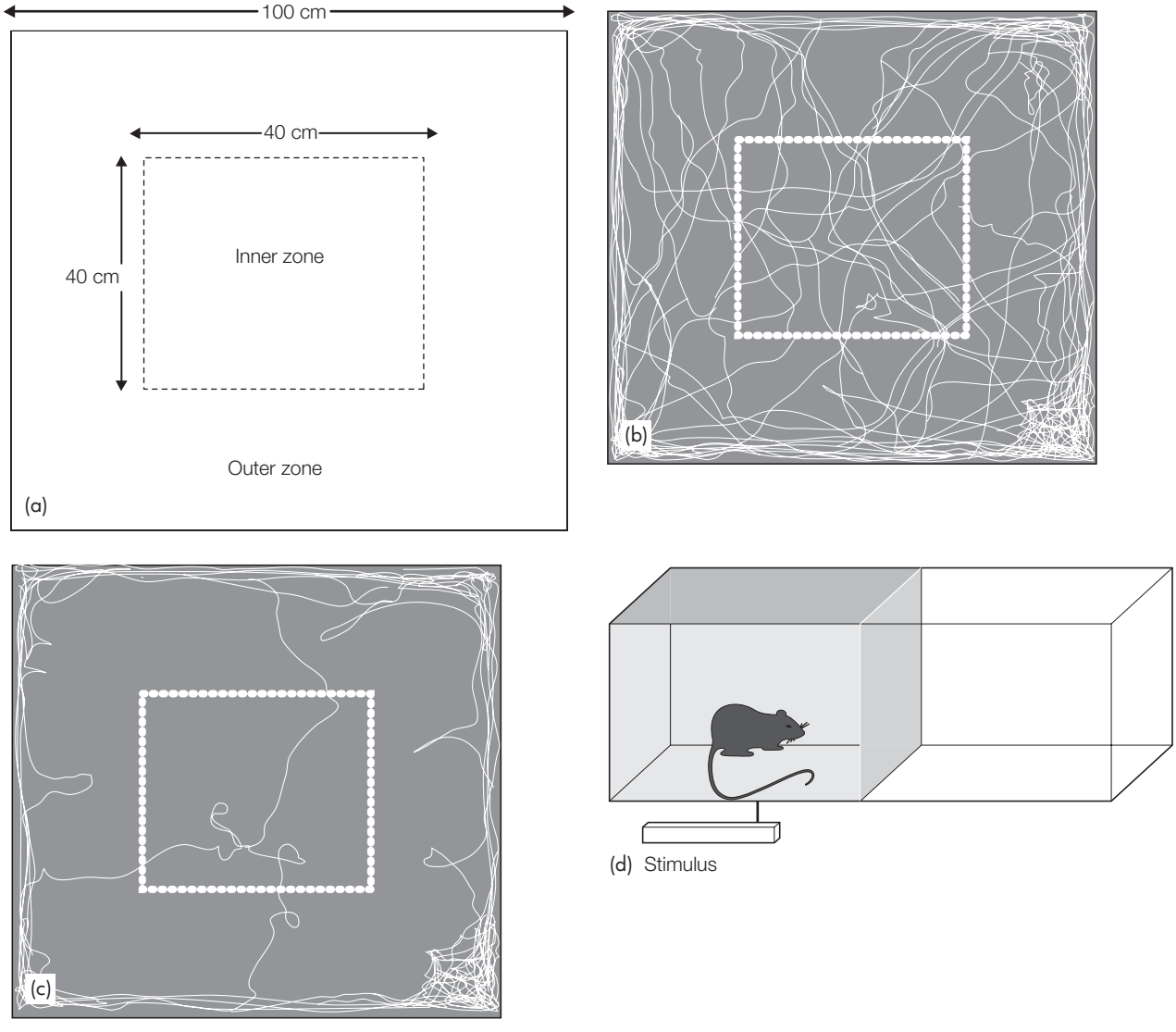

Figure 1.3 Examples of behavioral paradigms adapted for the assessment of pain conditions in rodents. (a–c) The open field paradigm in which neuropathic rats display thigmotactic (wall hugging) behavior: (a) open field arena; (b) naive rat; (c) rat with nerve injury. (d) The dark/light box: place preference paradigm in which rats chose between the aversive noxious stimulus or the aversive light compartment.

Further types of test involve active escape and avoidance of preferred environments (such as a dark versus light arena) in association with noxious stimuli.[30] These tests involve conflicting choices in which the animal must choose an adverse environment over the presence of a noxious stimulus and appear to respond well to analgesic drugs.[31] Alternatively, place preference paradigms associate a place with a preferable treatment such as delivery of an analgesic drug. However, the development of the latter paradigm in relation to neuropathic pain is ongoing and their utility remains to be proven. It is important to remember the effects of species variability[32] and therefore care must be taken to establish the suitability of tests in rodents.

MECHANISMS OF NEUROPATHIC HYPERSENSITIVITY

A variety of pain-related phenomena, both central and peripheral, have been associated with peripheral nerve injury (**Table 1.2**). These are generally not mutually exclusive and it is entirely possible that any one of these (or more likely a combination) contribute to symptomatology in individual patients suffering from neuropathic pain. It is therefore inappropriate to attempt to generate a unifying hypothesis of pathophysiology for all neuropathic pain states. The next challenge is to diagnose which of these phenomena may be operative in an individual patient and to interpret each symptom within the mechanistic framework arising from work with neuropathic pain models. In this regard, neuropathic pain is ideally suited to the mechanistic-based approach to treatment.[33, 34]

Peripheral mechanisms

PRIMARY AFFERENT EXCITABILITY

In normal primary afferent neurons, it is rare for firing threshold to be reached without the input of a stimulus.

Table 1.2 An overview of pathophysiological events which are likely to be related to the generation of neuropathic pain.

Peripheral nervous system	Central nervous system
Sensitization and spontaneous activity in sensory neurons	Central sensitization
Abnormal ion channel expression	Spinal reorganization
Altered neuronal biochemistry	Changes in inhibitory systems
Sensory neuron apoptosis	Glial cell activation
Immune–neuronal interactions	Alterations in descending modulation
Loss of trophic support for neurons	Cortical reorganization

However, following a nerve injury, many injured axons and associated cell bodies in the DRG undergo an increase in their intrinsic electrical excitability. As a result they begin to generate impulse discharge spontaneously or with only minimal stimulation linked to the injury site.[35] This has been termed ectopic discharge[36] and has also been demonstrated in humans, suffering from neuropathic pain.[37] Ectopic discharge originating in the peripheral nervous system (PNS) can result in excess spontaneous and stimulus-evoked electrical impulses feeding into the central nervous system (CNS) (**Figure 1.4**).[39] Ectopic afferent activity may also trigger and maintain central sensitization amplifying the afferent signal from the remaining afferents that innervate the partly denervated skin and deep tissues leading to tenderness to touch ("tactile allodynia").[38]

Furthermore, oscillations in resting membrane potential in primary sensory neurons are thought to contribute to their ectopic potential. A small number of A-fibers (10 percent) exhibit subthreshold membrane oscillations in their resting state or under depolarization conditions. An increase in these oscillations is observed in sensory neurons from axotomized rats.[40] Due to the sensitivity of such oscillations to tetrodotoxin (TTX), a role for changes in sodium channel function in the nerve in DRG has been proposed. Increases in oscillations lead to increased ectopic activity in these neurons that may underlie paresthesiae, dysesthesiae, as well as frank pain.

Abnormal discharges may also arise at the site of nerve injury, at other points along the nerves or in the cell body in the DRG.[41] Myelinated and unmyelinated primary afferent axons may become spontaneously active after nerve injury.[38, 42] Wallerian degeneration of an injured, spontaneously active myelinated fiber allows cross-excitation of neighboring unmyelinated fibers (termed "ephaptic transmission") inducing ectopic discharge even in an uninjured axon.[43, 44] Such ectopic discharge present in both low-threshold mechanoreceptors and in nociceptors may contribute to allodynia and hyperalgesic components of neuropathic pain.

Sodium channels

Sodium (Na^+) channels are critical to the physiology of excitable membranes. There are significant alterations in the expression of Na^+ channels in the cell bodies and the terminal neuroma of peripheral nerves following nerve injury. Such accumulation of Na^+ channels in the neuroma of cut sensory axons[45] are thought to generate ectopic discharge (**Figure 1.5**).[46]

There are many different and distinct voltage-gated Na^+ channels, of which at least six are expressed by primary afferent neurons within the DRG.[47] These can be defined by their sensitivity to TTX. In the DRG, TTX-sensitive channels (TTX-s) are expressed predominantly by A-fibers. In contrast, TTX-resistant (TTX-r) channels are expressed by a subset of primary afferent neurons specifically in the smaller C-fibers associated with

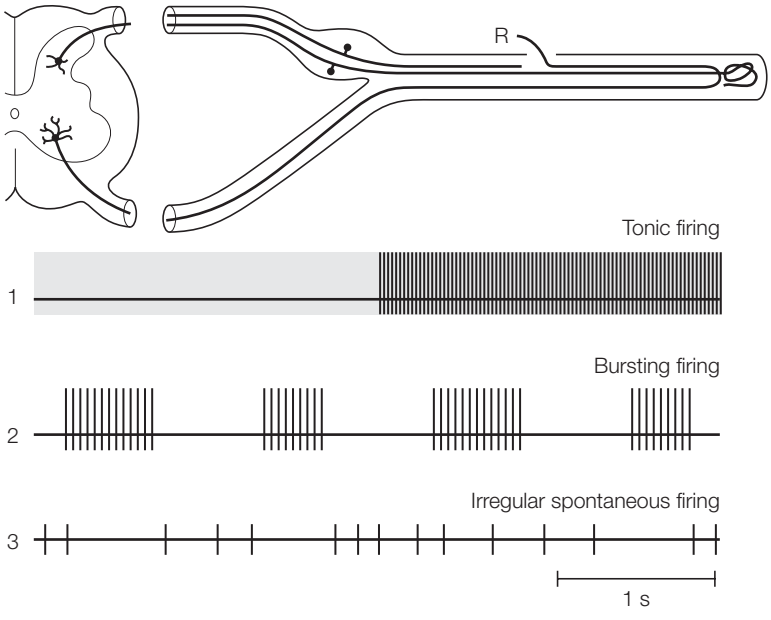

Figure 1.4 Patterns of spontaneous ectopic discharge recorded from sensory neurons ending in a neuroma. Fine axon bundles were microdissected from an injured nerve and placed on a recording electrode (R). Spontaneously active fibers fire tonically (1), in bursts (2), or irregularly (3). Intracellular recording from a dorsal root ganglion neuron with ectopic burst discharge (asterisks, spike height is truncated). One burst is shown in detail below. Bursts are triggered when ongoing membrane potential oscillations reach threshold and are maintained by postspike depolarizing after potentials (DAP). The burst initiates a hyperpolarizing shift which stops firing and resets the oscillations. Reprinted from Devor, *Melzack and Wall's Textbook of Pain*. 2005, 5th Edition © 2005 Elsevier Ltd,[38] adapted from Amir and Devor 1992.[39] Used with permission from The American Physiological Society and Elsevier.

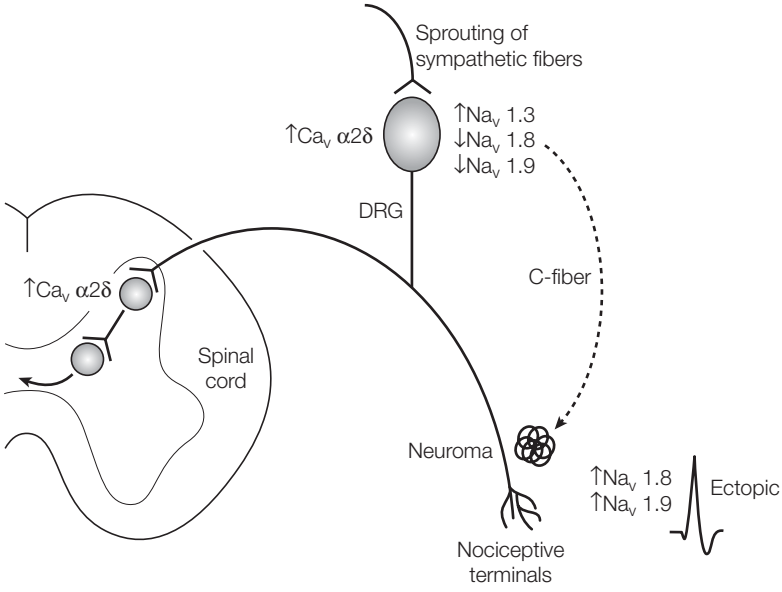

Figure 1.5 Alterations in Na^+ and Ca^{2+} channel subunits in the peripheral nervous system (PNS) following nerve injury. There is an increase in the expression of tetrodotoxin sensitive Nav1.3 channels and the calcium channel α2δ-1 (Cavα2δ-1) subunits in dorsal root ganglion (DRG) neuron cell bodies. The tetrodotoxin-resistant Na^+ channel subunits Nav1.8 and Nav1.9 decrease in the DRG and are also redistributed from the DRG neuron cell bodies to peripheral axons at the site of injury. Sprouting of sympathetic nerve fibers in the DRG also act to sensitize peripheral afferents. These changes are thought to result in spontaneous ectopic discharges and lower the threshold for mechanical activation that leads to hypersensitivity.

nociception.[48] Following peripheral nerve injury, there is a reorganization of ion channel expression in DRG neurons.[36] Some sodium channels subtypes are diminished, whilst others appear *de novo* and others are translocated to different parts of the neuron. For example, there is an up-regulation of the TTX-s channels $Na_v1.3$ (not normally expressed by DRG cells) and $Na_v1.7$, and a down-regulation of the TTX-r channels $Na_v1.8$ and $Na_v1.9$. As $Na_v1.8$ and $Na_v1.9$ produce slowly inactivating currents, their decreased expression may lead to a hyperpolarizing shift in resting potential, increasing the fraction of TTX-s channels available for activation.[47, 49] Electrophysiological studies demonstrate a reduced density of TTX-r currents and a shift in the voltage dependence of activation to a more negative potential in the following nerve injury.[49] In contrast, up-regulation of $Na_v1.3$ results in a switch in the properties of the TTX-s currents in DRG neurons, with the emergence of a rapidly repriming current, which could sustain frequent ectopic discharges and lead to hyperexcitability in the cell.[50] In support of this, TTX produces dose-dependent inhibition of ectopic activity[51] and reduced mechanical hypersensitivity in the spinal nerve transection (SNT) model.[52] In partial nerve injuries, the intact afferent neurons show little or no change in the expression of $Na_v1.8$, although there is a redistribution of these channels from their cell bodies in the DRG to their axons,[53] which may explain the neuroma hypersensitivity. These findings were corroborated in immunohistochemical studies of tissue taken from patients suffering from neuropathic pain following traumatic brachial plexus avulsion[54] and in human sensory nerves localized close to the injury site and within the neuroma.[55]

A Na^+ channel subunit that has received more attention in recent years is the $Na_v1.7$ channel. $Na_v1.7$ is expressed, almost exclusively, in DRG, particularly in small C-fiber nociceptors and to a lesser extent in medium-sized Aδ and large Aβ cells.[56] The $Na_v1.7$ channel underlies a fast TTX-s current with slow repriming kinetics and slow inactivation. Significantly, the $Na_v1.7$ channel has been localized to sensory endings, such that both its distribution and physiology may predispose it to a major role in transmitting painful stimuli. A mutation in the human gene encoding $Na_v1.7$ resulting in sensory neuron hyperexcitability is thought to be associated with the development of neuropathic pain in primary erythermalgia.[57, 58] However, experimentally the role for $Na_v1.7$ in neuropathic pain is unclear as mice lacking this channel develop signs of neuropathic pain as normal.[59]

The mechanism contributing to the changes in Na^+ channel expression in peripheral nerve injury is unclear, but the influence of growth factors appears to be a crucial factor. For example, in the absence of nerve growth factor (NGF), DRG neurons *in vitro* increase $Na_v1.3$ expression and decrease $Na_v1.8$ expression.[60] NGF is a member of the neurotrophin family of polypeptides, which are produced by peripheral target tissue during embryonic development, are required for peripheral sensory neurons for survival and can influence the morphology, excitability, and synaptic plasticity of sensory neurons in adulthood.[61] Additionally, glial-derived neurotrophic factor (GDNF), a member of a second family of growth factors, normalizes $Na_v1.3$ expression, reduces ectopic discharge in A-fibers, and reduces hypersensitivity[62] when delivered to the injured nerve. $Na_v1.9$ expression is similarly reliant on GDNF.

Therapeutic agents that exhibit use-dependent block of sodium channels show efficacy against painful peripheral neuropathy in the clinic. Systemic administration of lidocaine and other sodium-channel blockers relieves painful symptoms of postherpetic neuralgia, painful diabetic neuropathy, idiopathic trigeminal neuralgia, and other conditions.[63] Topical lidocaine also relieves pain in postherpetic neuralgia.[64] Sodium channel blockade is also a likely mechanism through which at least some drugs which also have efficacy in epilepsy (e.g. phenytoin and carbamazepine) might suppress neuropathic pain and the well-established efficacy of tricyclic antidepressants (TCA) may be due, at least in part, to their ability to block sodium channels.[65]

Potassium channels

There is a large variety of K^+ channels[66] and their significance in pain signaling is far from understood. Classic voltage-gated K^+ channels, often called delayed rectifiers, have six transmembrane domains and can be divided into nine gene subfamilies. The K_V1 subfamily is the most explored among subtypes of sensory neurons.[67] $K_V1.1$ and $K_V1.2$ are present in large-diameter sensory neurons, whereas $K_V1.4$ is present in most small sensory neurons that express $Na_V1.8$, making it the candidate nociceptive delayed rectifier. The activation of voltage-gated K^+ channels ultimately decreases the excitability of a cell. Thus, K^+ channels are prime molecular targets for suppressing hyperactive neurons, and might, therefore, prove useful in suppressing hypersensitivity.

Other K^+ channels that figure prominently in excitation of neurons, are the M channel (*KCNQ* gene), the H channel- (*HCN* gene) and calcium-activated K channels. All these channels are thought to be present on some populations of sensory neurons.[68, 69, 70] However, their relevance to pain is largely unknown.

Calcium channels

Activation of voltage-dependent calcium channels (VDCC) is critical for neurotransmitter release. Calcium ion channels have also been shown to influence the generation of hypersensitivity and in particular, a role for N-type Ca^{2+} channels has been shown. N-type, but not P- or Q-type, Ca^{2+} channel antagonists can attenuate hypersensitivity to mechanical and heat stimuli in models of neuropathic pain.[71, 72] Furthermore, cannabinoid receptor agonists, known to have analgesic effect in nerve injury models, attenuate Ca^{2+} flux at N-type channels.[73]

A calcium channel subunit that has received much attention of late is the $\alpha_2\delta$-1 subunit. This subunit is upregulated in rat DRG neurons, on central afferents terminals and on neurons within the spinal dorsal horn following nerve injury (**Figure 1.5**).[74, 75] This is correlated with pain behavior following peripheral nerve injury suggesting that $\alpha_2\delta$-1 may contribute to neuroplasticity in neuropathic pain. In support of this, transgenic mice that constitutively overexpress $\alpha_2\delta$-1 in neuronal tissues demonstrate pain behavior and exaggerated and prolonged dorsal horn neuronal responses to peripheral mechanical and thermal stimulation.[76] Furthermore, the $\alpha_2\delta$-1 subunit is thought to be the site of action of gabapentin[77, 78] and pregabalin,[79] which are effective in relieving signs of hypersensitivity in animal models[80] and neuropathic pain in man.[64, 81]

ALTERATIONS IN SENSITIVITY TO STIMULI

Transient receptor potential ion channels

Transient receptor potential (TRP) ion channels are sensory transducers, many of which are expressed in nociceptive primary sensory neurons where they are involved in generating chemical- and thermal-evoked pain sensations.[82] In particular, TRPV1 responds to noxious heat (temperatures $>43^\circ C$) and the pungent ingredient in hot chilli peppers, capsaicin, producing the classic burning sensation. In contrast, TRPA1 responds to cold temperatures ($<18^\circ C$) and to the irritant, mustard oil, also producing a burning sensation.

Following nerve injury, the phenotype of cells expressing TRP channels fundamentally changes so that TRPV1

and TRPA1 are also expressed by neurons of a non-nociceptive phenotype. Expression of TRPV1 has been shown to decrease in injured nociceptive neurons, while they increase in the neighboring uninjured neurons.[83] This includes novel expression in large diameter, low threshold A-fibers which may indicate a phenotypic switch contributing to symptoms of neuropathic pain. Similarly, TRPA1 expression is increased in a subset of small diameter primary sensory neurons following nerve injury likely inducing cold hypersensitivity.[84] Interfering with TRPA1 channel function using antisense knockdown technology abolishes hypersensitivity to a cold stimulus following spinal nerve ligation in the rat.[85] Therefore, targeting specific TRP channels may prove useful as analgesic strategies in the future.

THE ROLE OF PERIPHERAL INFLAMMATORY MEDIATORS

Nerve injury, trauma, and/or infection evoke a cascade of cellular events in the PNS, including a neuroinflammatory response with the release of chemical mediators, including many proinflammatory cytokines and chemokines.[86, 87] Cytokines and chemokines (small chemoattractant cytokines) are growth factor proteins secreted primarily from leukocytes as part of the immune and inflammatory response[88] and have been demonstrated to play a role in the pathogenesis of pain.[87] These factors can act on neurons to induce changes in gene expression, which in turn lead to the emergence of abnormal electrical activity, known to be essential for the manifestation of neuropathic pain behavior. Following nerve trauma, tumor necrosis factor-α (TNFα) is released from Schwann cells and infiltrating and resident macrophages, and in turn stimulates the sequential production and release of interleukin-1β (IL-1β) and interleukin-6 (IL-6) (**Figure 1.6**).[86] Accordingly, neutralizing antibodies to TNFα and IL-1β reduce behavioral signs of experimental neuropathic pain[90, 91] and IL-6 knockout mice fail to exhibit neuropathic pain after nerve injury.[92]

Intact and injured sensory neurons are known to express receptors which respond to TNFα, IL-1β, and IL-6. However, the direct mechanism of neuronal sensitization remains to be fully determined. Indirect evidence suggests an action of TNFα on neuronal sodium or calcium channels,[93] whereas IL-1β may be involved in a complex signaling cascade that leads to the production of pronociceptive compounds (such as nitric oxide, NGF, and prostaglandins) from immune cells or Schwann cells. Such substances lead to changes in gene expression and neuronal excitability in intact nociceptors.[94] The gp130 cytokines, IL-6 and leukemia inhibitory factor (LIF), have been shown to be crucial in the up-regulation of key modulators of sensory processing, such as brain-derived neurotrophic factor (BDNF), galanin, and substance P following nerve injury.[94] The chemokine CCL2 (MCP-1) is another injury-induced factor that accumulates within sensory neurons in models of neuropathic pain[22] and contributes to macrophage recruitment. CCL2 has been implicated in the maintenance of neuropathic pain and knockout mice for the receptor, CCR2, fail to develop signs of neuropathic pain.[95] Recent developments in the understanding of the importance of nonneuronal cells and inflammatory mediators in the response to damage of the peripheral nervous system has greatly aided the understanding of peripheral mechanisms of neuropathic pain.

CELL DEATH IN THE PNS

Many forms of nerve injury can also produce death of sensory neurons.[96] Apoptosis may be a result of mitochondrial dysfunction[97] and has been associated with a number of neuropathies.[96, 98, 99] Mitochondria-dependent apoptosis is activated by a number of factors including reactive oxygen species, ceramide, and nitric oxide,[100] which have been implicated in the pathophysiology of neuropathies. These factors cause the release of cytochrome C from mitochondria leading to the formation of the apoptosome complex and subsequent activation of effector caspases. Alternatively, apoptotic pathways can be activated via stimulation of death receptors, such as TNFR1[100] which can act via the JNK (c-Jun-N-terminal kinase) pathway to activate effector caspases. In support of this, TNFα is released in response to chemotherapeutic agents that produce painful peripheral neuropathy,[101] following direct nerve injury,[102] and in response to HIV-gp120 *in vitro*[103] and caspases have been shown to be important in neuropathic responses in various models of neuropathy.[20, 96, 104, 105] It is thought that the activation of these pathways may be involved in neuropathic pain even though there may be a prolonged latent phase of apoptosis, before cell death.

Spinal cord mechanisms

The sensory input from primary sensory neurons is transferred, via their central axons, to second-order neurons in the dorsal horn of the spinal cord. The synaptic contacts made between afferent central terminals and dorsal horn neurons are highly organized, both topographically and functionally to maintain accurate transfer of information regarding the peripheral noxious stimuli. Following peripheral nerve lesions, synaptic processing in the spinal cord can be subject to diverse forms of functional, chemical, and structural plasticity that are highly involved in the production of hypersensitivity to sensory input. Increased synaptic efficacy (the phenomenon of central sensitization), loss of inhibitory mechanisms, alterations in synaptic contacts, and the activation of nonneuronal cells all play major roles in producing increased pain sensitivity in neuropathic pain. This chapter will address each of these areas in turn.

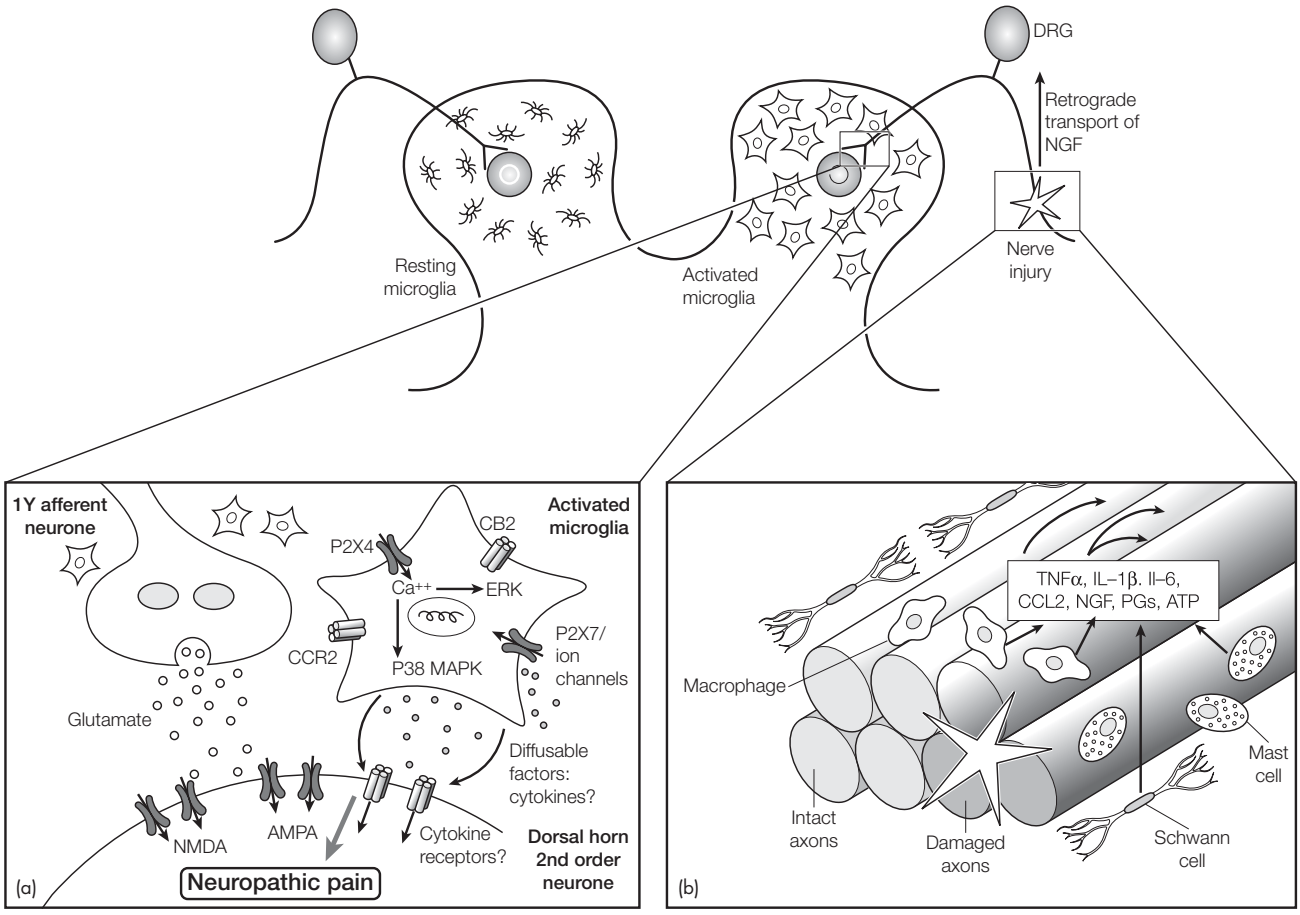

Figure 1.6 The immune system in neuropathic pain. Overview of the effect of the immune system on primary sensory neurons and the spinal cord after peripheral nerve injury. (a) Representation of a mixed nerve injury in which injured and uninjured axons are juxtaposed. The site of injury is typified by the recruitment and proliferation of nonneuronal elements (such as Schwann cells, mast cells, and macrophages), which release factors including the cytokines TNFα, IL-1δ, IL-6, the chemokine CCL2, prostaglandins (PGs) and growth factors, including nerve growth factor (NGF) that initiate and maintain sensory abnormalities after injury. These factors might either induce activity in the axons they act on or be transported retrogradely to cell bodies in the dorsal root ganglion (DRG), where they alter the gene expression of neurons. (b) The effect of the immune system in the spinal cord following peripheral nerve injury with a focus on microglial activation. A primary afferent neuron terminal is flanked by microglial cells that maintain and survey the environment in the spinal cord. In neuropathic pain states, the microglia are activated, probably by the release of transmitters or modulators from primary afferents. The activated microglia release several proinflammatory cytokines, chemokines, and other agents that modulate pain processing by affecting either presynaptic release of neurotransmitters and/or postsynaptic excitability. The release of inflammatory mediators (such as tumor necrosis factor-α (TNFα), interleukin-1β (IL-1β), interleukin-6 (IL-6), nitric oxide (NO), ATP, and prostaglandins (PGs) initiates a self-propagating mechanism of enhanced cytokine expression by microglial cells. This leads to an increase in intracellular calcium, and activation of the p38 and MAPK/ERK pathway. AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; CCR2, CCL2 receptor; CX3CR1, fractalkine receptor; EAA, excitatory amino acids; ERK, extracellular signal-regulated kinase; FPRL1, formyl peptide receptor-like 1; MHC, major histocompatibility complex; NGF, nerve growth factor; NK1R, neurokinin-1 receptor; NMDA, *N*-methyl-D-aspartic acid; P2 × 4, P2 × 7, ionotropic purinoceptors; p38MAPK, p38 mitogen-activated protein kinase. Adapted with permission from Macmillan Publishers Ltd: *Nature Reviews Neuroscience*[86] © 2005 and reprinted from *Trends in Neuroscience*, 28, Tsuda M, Inoue K, Salter MW, Neuropathic pain and spinal microglia: a big problem from molecules in "small" glia, 101–7, © 2005, with permission from Elsevier.[89]

EXCITATORY MECHANISMS

The afferent barrage associated with peripheral nerve injury is associated with the development of a sustained state of hyperexcitability of dorsal horn neurons, a process dubbed central sensitization.[106, 107] In addition to events such as lowering of activation thresholds of spinal neurons, central sensitization is characterized by the appearance of "wind-up."[108, 109, 110] Wind-up is characterized by an increasing response to repeated C-fiber

volleys, and may contribute to hyperalgesia in humans. However, the exact relationship of the relatively short-lived phenomenon of wind-up and the persistent state of central sensitization remains to be fully elucidated.[111]

The excitatory amino acid glutamate is the major excitatory neurotransmitter released at the central terminals of primary afferent nociceptive neurons following noxious stimulation. Glutamate acts at a number of post-synaptic receptors, including metabotropic (mGluRs) and the ionotropic α-amino-3-hydroxyl-5-methyl-4-isoxazole (AMPA), kainate and *N*-methyl-D-aspartic acid (NMDA) receptors. A large body of evidence suggests that the NMDA receptor subtype is the most intimately involved in central sensitization associated with inflammation and nerve injury.[110] For glutamate to exert its effects, receptor phosphorylation and the removal of an Mg^{2+}-dependent ion channel block are critical events in activating the NMDA receptor. NK1 (substance P), AMPA (glutamate), and trkB (BDNF) receptors and the activation of intracellular serine/threonine and tyrosine kinase signalling cascades are all involved in this permissive process.[112, 113]

NMDA receptors are also involved in the maintenance of central sensitization. Nerve injury induces increased release of excitatory amino acids into the spinal dorsal horn which is associated, in an NMDA receptor-dependent manner, with increased intracellular calcium concentration ($[Ca^{2+}]_i$) in dorsal horn neurons.[114] Initial NMDA receptor activation contributes to further increased concentrations of glutamate and aspartate, representing a continual positive feedback loop which maintains sensitization. The increased $[Ca^{2+}]_i$ could also form a positive feedback loop, potentially through indirect activation of protein kinase C (PKC), a hypothesis supported by the antihypersensitivity effect of a PKC inhibitor in the SNL model of neuropathic pain,[115] as well as the evidence that deletion of genes for isoforms of adenylate cyclase, protein kinase A, and protein kinase C all impair the development of pain hypersensitivity in transgenic mice.[116, 117] Activity-dependent central sensitization is displayed by many cells in both the superficial and deep laminae of the dorsal horn. However, in the context of pain hypersensitivity, the effect of sensitization appears to be particularly important for lamina I spinothalamic or spinoparabrachial projection neurons, particularly those expressing the NK_1 receptor.[118, 119]

In addition to Ca^{2+} influx through the NMDA ion channel inducing heterosynaptic potentiation in dorsal horn neurons, activation of voltage-gated calcium channels can enhance excitatory transmission through NMDA receptor-independent mechanisms.[120] For example, neurotrophins such as BDNF, acting through their cognate Trk receptors, facilitate synaptic transmission,[121, 122] partly through a NMDA receptor independent mechanism. Synaptic transmission may also be enhanced by cytokines, such as TNFα, which may be released from glial cells in the dorsal horn.[123] Pharmacological studies support a role for NMDA receptors in neuropathic pain. Pre- and postinjury intraperitoneal administration of the NMDA receptor antagonist MK-801 prevented hypersensitivity in the CCI model[124] and electrophysiological data also demonstrates that MK-801 significantly reduces the hyperresponsiveness to noxious stimulation after peripheral nerve injury.[125]

The agonist action of glutamate at the NMDA receptor can be modulated by glycine.[126] Antagonizing the glycine modulatory site of the NMDA receptor prevents development of hypersensitivity following peripheral nerve injury and attenuates wind-up in isolated spinal cord neurons.[127] Coadministration of a glycine/NMDA receptor antagonist and morphine has also been demonstrated to attenuate pain behavior in an animal model of trigeminal neuralgia.[128]

SPINAL INHIBITORY SYSTEMS

γ-Aminobutyric acid and glycine

The γ-aminobutyric acid (GABA) pathway forms a major inhibitory neurotransmitter system in the CNS. Depression of such spinal inhibitory mechanisms are thought to be important for sustained enhancement of excitatory transmission and central sensitization.[129] In support of this, administration of GABA-mimetics reduces neuropathic hypersensitivity and antagonism of the GABA receptors is associated with hypersensitivity.[130] Moreover, peripheral nerve injury results in a substantial loss of GABA-mediated inhibitory currents,[131] decreased extracellular levels of GABA,[132] a decrease in dorsal horn levels of the GABA synthesizing enzyme glutamic acid decarboxylase (GAD) 65 kDa,[131] and decreased GABA receptor levels in the spinal cord, probably due to degeneration of the primary afferent neuron terminals on which the receptor is localized.[133] Apoptosis in the dorsal horn following nerve injuries may correlate to selective death of GABAergic inhibitory interneurons[131] due to excessive glutamate release or a result of cell death-inducing signals within the spinal cord.[134] All of the above factors likely promote a functional loss of GABAergic transmission in the superficial dorsal horn.

GABAergic and/or glycinergic inhibition are important factors in the maintenance of orderly information processing by preventing the generation of synchronized wave activity in the CNS. Synchronous neuronal activity leading to oscillatory Ca^{2+} waves can be evoked in the spinal dorsal horn network by the potassium channel blocker 4-aminopyridine (4-AP) after pretreatment with blockers of $GABA_A$, glycine, and AMPA/kainate receptors.[135] This may correlate to reduced inhibition and increased neuronal excitability observed in dorsal horns of animals with neuropathic pain.[136] Theoretically, such synchronous activation of larger parts of the dorsal horn network would lead to pain that violates the innervation patterns of peripheral nerves or dorsal roots characterized by violation of sensory modality borders (e.g. allodynia,

where normally nonnoxious stimuli are perceived as painful) and somatotopic borders (radiating pain or mirror-image pain). Therefore, disinhibition as a result of altered GABA and glycine signaling may lead to waves of excitability and could underpin neuropathic pain. However, further studies will be required to evaluate under what physiological and pathophysiological conditions crossing of somatotopic and sensory modality borders occurs in spinal dorsal horn.[135]

Opioid system

The endogenous opioid system is also dysregulated following nerve injury. Evidence supports a loss of μ-opioid receptors in the DRG[137] and in the spinal cord following nerve injury.[40, 138, 139] Spinal opioid receptors are localized predominantly on the presynaptic terminals of primary afferents in the superficial dorsal horn[138] and therefore this may reflect degeneration of primary afferent neurons. Additionally, increased cholecystokinin (CCK) mRNA synthesis by DRG neurons[140] and increased expression of the CCK_B receptor in the superficial dorsal horn following peripheral axotomy may potentially decrease the antinociceptive effects of opioids due to opioid antagonistic properties of CCK.[141] These changes may all contribute to the reduced potency of peripherally or spinally delivered opioids in neuropathic pain (**Figure 1.7**).[142]

Cannabinoid system

The endogenous cannabinoid system has received much interest within the field of neuropathic pain due to the fact that unlike the opioid system, spinally expressed cannabinoid receptors are unaffected following nerve injury.[143] In such, manipulation of the cannabinoid system has been effective in alleviating signs of neuropathic pain in animal models of neuropathic pain[5, 22, 144, 145] representing a possible therapeutic advantage of cannabinoids over opioids in neuropathic pain.

ANATOMICAL REORGANIZATION

Tactile mechanical allodynia is thought to be mediated by Aβ-fiber afferents.[146] However, the mechanisms by which this occurs are yet to be fully understood. Several studies using bulk labeling and single afferent fiber-filling techniques have demonstrated that following a peripheral nerve lesion, the central axons of injured Aβ-fibers sprout from their normal termination sites in the deeper laminae of the dorsal horn (laminae II and IV) into lamina II of the dorsal horn, which is normally restricted to C-fiber and Aδ nociceptors.[147, 148] This synaptic rearrangement means that second-order dorsal horn neurons that normally receive predominantly high threshold sensory input, now receive inputs from low threshold mechanoreceptors. Such misinterpretation of information within the spinal cord may result in low threshold sensory information being interpreted as nociceptive, leading to the emergence of hypersensitivity after peripheral nerve injury. The outgrowth of central Aβ-fiber terminals is prevented by NGF and GDNF treatment, presumably by provision of trophic support for damaged C-fibers, suggesting an important role for neurotrophins in the regulation of this manifestation of structural plasticity.[149] However, some studies have raised concerns about the specificity of bulk-labeling techniques and the sampling of intracellular labeled intact and injured afferents,[150, 151] such that the labeling may actually be due to damaged C-fibers abnormally taking up the label. However, in favor of the sprouting theory, stimulation of Aβ-fibers in injured nerves can produce activation of neurons in lamina II measured electrophysiologically and by expression of c-Fos.[152, 153] Nevertheless, further work is required to resolve the basis for the differences in these anatomical studies, and to determine the extent to which sprouting of Aβ-fibers contributes to tactile hypersensitivity after peripheral nerve injury.

THE ROLE OF NONNEURONAL CELLS

Peripheral nerve injury produces molecular and cellular changes that result in multiple forms of neuronal plasticity and anatomical reorganization at various levels of the peripheral and central nervous systems. Oligodendrocytes, astrocytes, and microglia form a large group of CNS glial cells. Although often underappreciated, a substantial body of evidence has accumulated showing that peripheral nerve injury leads to activation of glia in the spinal cord implicating astrocytes and particularly microglia.[89, 123]

Microglia are immune-derived cells and represent 5–10 percent of glia in the CNS.[154] Microglia are said to be resting under normal conditions and do not actively influence nociceptive processing. However, microglia become activated by events such as CNS injury, microbial invasion, and in some pain states. Following peripheral nerve lesions, spinal microglia appear to migrate to the relevant spinal segments, thus increasing the local microglial population, and become activated involving a stereotypic series of changes including morphological alteration (they become hypertrophic and ameobiod), gene expression, and function. Moreover, activated microglia produce and release various chemical mediators, including proinflammatory cytokines, chemokines, and other potentially pain-producing substances, that can produce immunological actions and can also act on neurons to alter their function (**Figure 1.6**).[89, 155] The status of microglia in the spinal cord has been examined in a variety of nerve injury models and substantial evidence, both direct and indirect, indicates that microgliosis fundamentally contributes to the pathophysiology of neuropathic pain.[20, 22, 156, 157, 158] This is supported by several studies that have shown specific microglial inhibitors and/or modulators, such as fluorocitrate and minocycline block, and/or reverse neuropathic states.[21, 22, 159, 160]

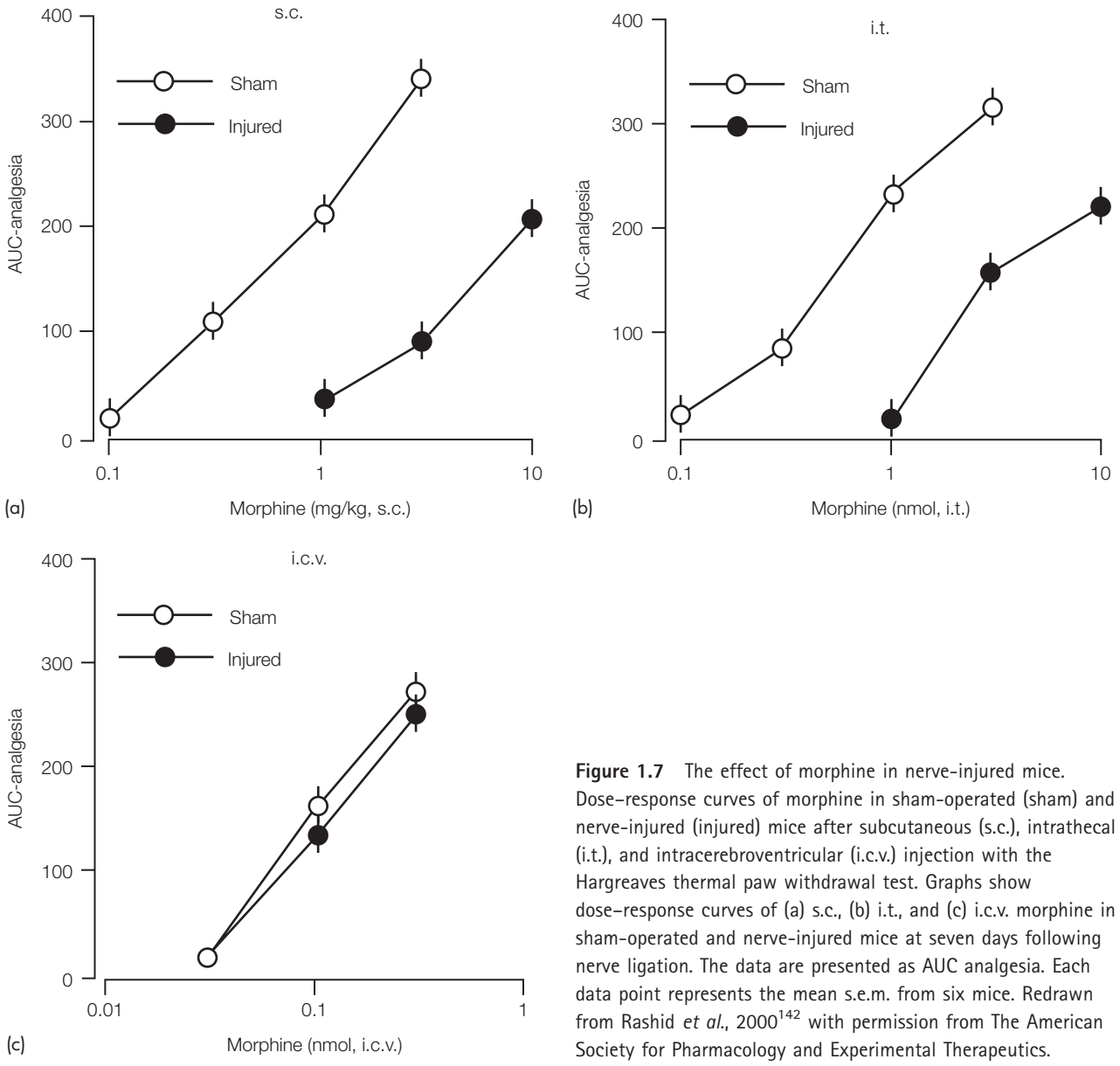

Figure 1.7 The effect of morphine in nerve-injured mice. Dose–response curves of morphine in sham-operated (sham) and nerve-injured (injured) mice after subcutaneous (s.c.), intrathecal (i.t.), and intracerebroventricular (i.c.v.) injection with the Hargreaves thermal paw withdrawal test. Graphs show dose–response curves of (a) s.c., (b) i.t., and (c) i.c.v. morphine in sham-operated and nerve-injured mice at seven days following nerve ligation. The data are presented as AUC analgesia. Each data point represents the mean s.e.m. from six mice. Redrawn from Rashid *et al.*, 2000[142] with permission from The American Society for Pharmacology and Experimental Therapeutics.

It is not clear what factors activate spinal microglia in peripheral neuropathic pain states. Several molecules have been implicated, including macrophage colony-stimulating factor (MCSF),[161] IL-6,[162] substance P, ATP, and the chemokines, fracktalkine,[163] and CCL2.[164] Activated microglia express various molecules allowing them to respond to such stimuli, including the ATP gated ligand-gated cation channels, P2 × 4,[165] and P2 × 7,[166] and the chemotactic cytokine receptor 2 (CCR2), a receptor for CCL2/MCP-1. Recent evidence suggests that ATP-stimulated microglia signal to lamina I neurons via their release of BDNF, causing a depolarizing shift in the neuronal anion reversal potential inverting the polarity of currents activated by GABA. This means that GABA now results in excitation of the cell as opposed to inhibition.[158] Evidence for a role of CCR2 in nerve injury-induced hypersensitivity[95] comes from mutant mice lacking the receptor. However, as CCR2 is also up-regulated in the peripheral nerve, at the site of the nerve injury and in the DRG, it is unclear whether spinal microglia expressed CCR2 is responsible. The cannabinoid receptor subtype CB_2 may also be expressed by spinal microglia after nerve injury and therefore cannabinoids may play a role as modulators of neuropathic pain via actions on microglia.[167] Accordingly, systemically administered CB_2 agonists can inhibit nerve injury-evoked pain behaviors.[95, 168] However, CB_2 agonists might act in the periphery and therefore the role of microglial CB_2 receptors is, at present, unclear.[169, 170]

The recruitment of microglia is commonly associated with the activation (phosphorylation) of p38 MAP (MAP) kinase and MAP kinase ERK (extracellular signal-regulated kinase) in the spinal cord. Phosphorylation of p38 is probably a key intracellular signal in the microglial response in neuropathic pain[157, 171] and the sequential

activation of ERK in neurons, then microglia, and finally astrocytes in a neuropathic pain model[172] suggests that microgial activation might be the first step in a cascade of immune responses in the CNS.[86, 94] The aforementioned molecules expressed by activated microglia in neuropathic pain states, or associated intracellular signaling cascades may be potential analgesic targets.

Supraspinal mechanisms

DESCENDING MODULATION

In addition to the peripheral and spinal mechanisms discussed, supraspinal mechanisms are thought to play an important role in neuropathic pain.[173, 174] The periaqueductal gray (PAG) is the most characterized part of a CNS circuit that controls nociceptive transmission at the level of the spinal cord.[175] The PAG integrates inputs from areas such as the limbic forebrain, diencephalon, amygdala, and hippocampus with ascending nociceptive input from the dorsal horn[176] and is therefore associated with the affective and autonomic responses to pain.

The PAG is closely associated with the brainstem including the rostral ventromedial medulla (RVM), and is critical in the descending modulation of spinal activity through monoaminergic and other pathways.[177] Likely via anatomically distinct pathways, the PAG and RVM can exert both facilitatory and inhibitory influences on the spinal cord.[178] The balance of these two supraspinal pathways and primary afferent input, ultimately determines the excitability of spinal neurons.[174] Under pathological conditions, enhancement of descending facilitatory controls to the spinal cord are likely to allow excitatory influences to predominate to maintain spinal central sensitization (**Figure 1.8**).

Facilitatory cells within the RVM are classed as ON cells, whereas cells that have inhibitory influences on the spinal cord are termed OFF cells.[179] Following nerve injury, there is enhanced descending excitatory drive from the RVM[180] which may represent a central compensatory mechanism for the loss of normal sensory input following peripheral nerve damage.[174] The brainstem areas involved are also implicated in autonomic responses, emotions, and sleep. Therefore, these same pathways likely underpin the well-established links between these states and pain, and may provide a basis for an affective component of pain.[181]

Various transmitter pathways are implicated in descending control mechanisms. For example, CCK, an antianalgesic peptide, may contribute to RVM neuron excitability.[182] Intra-RVM CCK produces reversible thermal and tactile hypersensitivity in naive rats[141] and prevents both the activation of OFF cells and the antinociception produced by systemic morphine.[183] Additionally, although thought mainly to play an inhibitory role in supraspinal systems,[184] supraspinal serotonergic inputs to the spinal cord originating in the RVM may play a role in facilitatory influences following peripheral nerve injury.[185] The 5HT3 receptor, localized to a novel group of small diameter afferents, and a larger

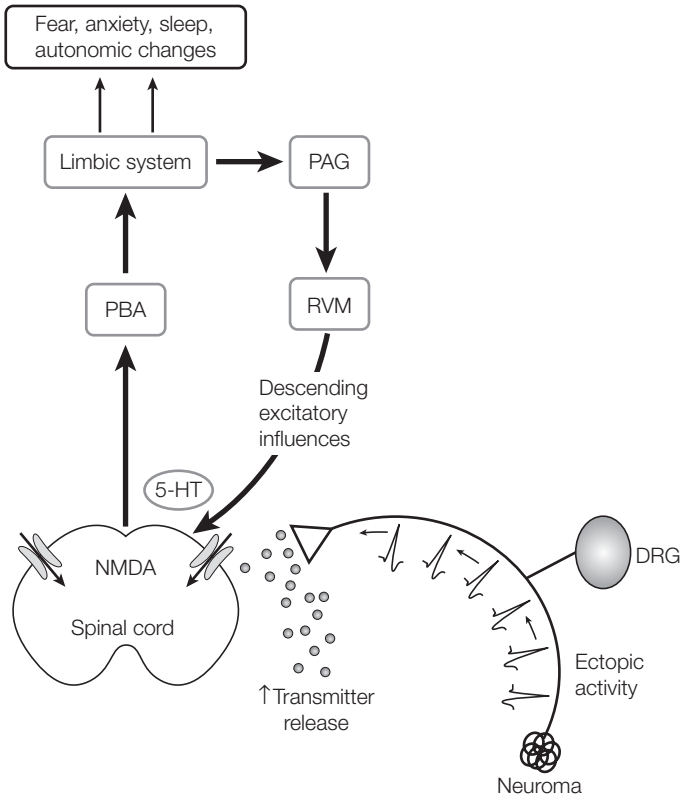

Figure 1.8 Overview of supraspinal involvement in neuropathic pain. Peripheral nerve injury induces spontaneous ectopic activity at the site of injury and the dorsal root ganglion (DRG) resulting in increased release of glutamate and neuropeptides (such as substance P) to the spinal cord, thereby promoting sensory transmission in the spinal cord. Centrally, there is increased function of the *N*-methyl-D-aspartic acid (NMDA) receptor and enhanced descending activity from the rostral ventromedial medulla (RVM) serotonergic excitatory pathways. All these mechanisms can contribute to the development of abnormal pain accompanying nerve injury. Plasticity is seen in the expression and function of ion channels (e.g. Na^+ channels) and neurotransmitters (e.g. substance P). Sprouting of sympathetic nerve fibers in the DRG act to sensitize peripheral afferents. Adapted from Suzuki and Dickenson, 2005,[174] by permission of S Karger AG, Basel.

number of presumed A-delta afferent fibers,[186] has been implicated as the target receptor of this system. Ondansetron, a $5HT_3$ antagonist exerts influences particularly on punctate mechanical responses after nerve injury.[187] Additionally, a preliminary clinical study suggests that block of $5HT_3$ receptors has clinical utility in the treatment of pain.[188]

Finally, evidence suggests that cannabinoids produce their antinociceptive effect at least in part by recruiting the PAG–RVM modulatory system.[189] CB_1 receptors are densely expressed in the PAG, and microinjection of CB_1 agonists into the PAG or RVM produces antinociception.[190] CB_1 receptors are also known to be expressed on rostrocaudally directed fibers in the dorsolateral funiculus, a major tract for descending control systems.[169, 170]

IMAGING OF THE BRAIN IN NEUROPATHIC PAIN

Recent advances in human brain imaging techniques offer an exciting opportunity to examine brain processes in experimental and clinical pain conditions. This has allowed insights into neural correlates of pain and led to a much greater understanding of the pain matrix,[191, 192] which includes brain structures, such as the anterior cingulate cortex (ACC), insula, frontal cortices, S1, second somatosensory cortex (S2), and amygdala.[193]

Neural correlates of allodynia have been examined in various conditions, including patients with neuropathic pain, central pain, or experimentally provoked allodynia. However, the existing data are controversial with some suggesting that allodynia is processed differently than nociceptive pain and others suggesting they share a common neural basis. Areas shown to be involved in allodynia include the parietal association cortex,[194] medial thalamus, putamen, and prefrontal cortex.[195] The ACC, which is almost always activated during acute pain in normal subjects and is involved in the affective (cognitive–evaluative) component of pain, has been differentially associated with processing of allodynia.[196, 197, 198, 199] This suggests that A-β-mediated pain may have a unique cortical representation in some situations which may aid further understanding of the phenomenon that is tactile allodynia. The amygdala, which plays an important role in fear-conditioning and affective disorders, such as anxiety and depression,[200] is activated by a diverse range of persistent nociceptive stimuli in the rat.[201, 202] Evidence suggests a role for the amygdala in the affective–emotional pain response in a rodent model of neuropathy involving GABAergic systems.[203] The amygdala has also been linked to spontaneous pain in humans suffering from postherpetic neuralgia.[204] Such studies highlight the involvement of a number of brain areas in pain responses in neuropathic pain conditions. However, further work using brain imaging techniques is required before our understanding of such systems is complete.

CONCLUSIONS

This brief overview of mechanisms of neuropathic pain outlines the complex nature of the response of the nervous system to a peripheral nerve injury. There is little doubt that a combination of mechanisms, involving peripheral, spinal, and supraspinal mediated events, contribute to the manifestation of neuropathic pain in any one individual. Eventually, it may be possible to improve the ethos of clinical management protocols so that they will move away from disease-based treatment towards symptom or, ultimately, mechanism-based therapies.[34] However, this will require a better understanding of mechanisms involved in neuropathic pain and reliable convenient tools for their assessment in the clinic.[33] It must be emphasized that the majority of preclinical studies employ animal models of nerve injury and measure associated hypersensitivity, which is only evident in a subset of patients with neuropathic pain. Therefore, improvement of animal models and behavioral tests will possibly unravel more therapeutically relevant mechanisms. Advances in technology have led to new approaches for the identification of novel targets involved in neuropathic pain. For example, microarray technology generates data regarding a large number of genes which can lead to the investigation of promising novel targets in neuropathic pain.[205] Additionally, our understanding of genetics may uncover genetic variation in the susceptibility of individuals to develop neuropathic pain,[206] which can also aid our understanding of specific mechanistic alterations and "genetically tailor" analgesics based on an individual's pharmacogenetic profile.

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2

Applied physiology: persistent musculoskeletal pain

HANS-GEORG SCHAIBLE

KEY LEARNING POINTS

- In normal joint and muscle, pain is only elicited by intense tissue-threatening (noxious) stimuli. Inflammation and other deep tissue pathologies cause a state of hyperalgesia and pain that often becomes chronic. Under these conditions, pain is elicited by physiological stimuli.
- Muscle and joint nerves possess nociceptors which are exclusively or preferentially excited by noxious stimuli, and silent nociceptors which do not respond to stimuli under normal conditions.
- During pathological processes, such as inflammation muscle and joint nociceptors, are sensitized to mechanical stimuli. This peripheral sensitization is an important mechanism of primary hyperalgesia.
- Peripheral sensitization is induced and maintained by inflammatory mediators acting on the nociceptive terminals, and by changes of the intrinsic response properties of the neurons.
- In the central nervous system, nociceptive stimulation of deep tissue is encoded in neurons exclusively driven by deep input, and by neurons that show convergent inputs from deep tissue and skin.
- Peripheral sensitization induces a state of hyperexcitability in the central nociceptive system (central sensitization) that increases the gain of central nociceptive processing at spinal, thalamic, and cortical levels. Spinal hyperexcitability contributes to primary and accounts for secondary hyperalgesia.
- Descending systems control the nociceptive processing at the spinal level. During peripheral inflammation descending inhibition increases and reduces central sensitization. Descending facilitation may support secondary hyperalgesia.
- Pain treatment should target peripheral, as well as central, sensitization.

PAIN IN THE MUSCULOSKELETAL SYSTEM

Pain in the musculoskeletal system is of major clinical importance because it is frequent and often chronic. In general, the deep somatic tissue is a major site of injury (e.g. sport injuries), acute and chronic inflammatory processes (e.g. rheumatoid arthritis), and degenerative disease (e.g. osteoarthritis, osteoporosis). Because many of the pathological changes in the musculoskeletal system are not reversible, symptomatic pain treatment is one of

the most important tasks in clinical medicine. It should limit suffering and maintain the ability to use the motor system properly.

Pain sensation in muscle and joint

The major sensation from deep tissue, such as joint and muscle, is pain. In the absence of disease, we are not aware of sensory processes in the deep tissue. However, sensory information from muscle and joint continuously controls the activity of the motor system and is involved in the sense of movement and position.[1] Pain significantly influences the motor control system and usually forces the patient to restrict movements.

Deep tissue pain is often dull and aching, and poorly localized, and is thus different from cutaneous pain which may be sharp and precisely localized.[2] In particular, muscle pain is often aching and cramping and often referred to other deep tissue, such as other muscles, tendon, fascia, joint, and ligaments.[3] In the normal deep tissue, acute and short-lasting pain sensations can be elicited by tissue-threatening mechanical stimuli, showing the excitation of nociceptors in deep tissue structures (see below under Nociceptors of deep tissue and peripheral sensitization). Clinically relevant pain in deep tissue is different. It usually appears as hyperalgesia or persistent pain at rest.[2, 4, 5, 6] In the state of hyperalgesia, noxious stimuli cause stronger pain than normal, and pain is even evoked by mechanical stimuli whose intensity does not normally elicit pain, i.e. movements in the working range and gentle pressure, e.g. during palpation. Clinically relevant muscle pain often appears as a combination of ongoing muscle pain, tenderness, soreness (tenderness and stiffness), weakness, and paresthesias (sensation of pressure and tension) in the muscle.[7, 8]

Some decades ago and again more recently, in order to gain more insight into the nature and origin of deep tissue pain, experimental invasive sensory testing was carried out in conscious humans. For example, pain in the normal joint can be elicited when noxious mechanical and chemical stimuli are directly applied to the fibrous structures, such as ligaments and fibrous capsule. No pain is elicited by stimulation of cartilage and stimulation of normal synovial tissue rarely evokes pain.[5] Stimulation of fibrous structures with innocuous mechanical stimulation can evoke pressure sensations.[5] In the muscle, pain can be elicited by noxious mechanical stimulation and also by high intensity thermal stimulation (48°C).[9] Collectively these data show good correlation between the impact of noxious stimuli and the evoked pain sensations at least in the normal deep tissue. Accordingly, recordings from deep tissue afferents have revealed that deep tissue nociceptors reliably encode noxious stimuli.

Differences between cutaneous and deep tissue pain sensations have been pointed out. In addition to differences in pain sensation, autonomic responses to noxious stimuli can be different. In contrast to cutaneous pain, muscle pain typically elicits a drop in blood pressure, as well as sweating and nausea.[10]

Causes of clinically relevant pain in deep tissue

Considerations on clinically relevant pain in the deep tissues include several questions: (1) Which pathological processes cause pain? (2) From which structures is pain evoked? This is being particularly discussed for osteoarthritic pain. (3) Does pain reflect nociception and how much is it associated with psychological and social factors? This seems to be extremely relevant for the large number of patients with low back pain.

In general, inflammatory conditions cause similar pain symptoms in all somatic deep tissues, namely hyperalgesia with increased responses to noxious stimuli and occurrence of pain upon innocuous mechanical stimulation. Inflammatory conditions are frequent in joints and often chronic, such as during rheumatoid arthritis. Initially, the synovial tissue and the articular and periarticular soft tissues are the most important sites of inflammation, but with time the joint undergoes structural changes, such as cartilage degradation, pannus formation, and bone deformation. Presumably all of these changes may contribute to pain generation, and mechanical as well as inflammatory factors may contribute to the activation of the nociceptive system.

Pain during degenerative osteoarthritis (OA) shows similarities and differences to inflammatory arthritic pain. Osteoarthritic pain is usually localized to the joint with OA, but it can be referred (e.g. hip OA may cause knee pain). It varies in intensity and is usually worsened by exercise (weight-bearing, movement) and relieved at rest. It is usually episodic, but may be constantly present in advanced OA. A particular quality of OA pain is pain at night.[11] The site of OA pain and the nature of OA pain are under discussion because the cartilage is not innervated[12] and because there is a poor correlation between radiological signs (narrow joint space and osteophytes) and the occurrence of joint pain.[11] Some recent studies used magnetic resonance (MR) imaging and found that painful OA knee joints exhibit more MR abnormalities than nonpainful OA joints. These are synovial hypertrophy and synovial effusions, as well as subchondral bone marrow edema lesions (which may increase intraosseal pressure).[13] These data and the observation of inflammatory cells in the sublining tissue[11] suggest that pain may be evoked by inflammatory mechanisms that appear from time to time (possibly corresponding to painful episodes in chronic OA). At later stages, capsular fibrosis and muscle contracture around the joint may contribute to OA pain. Quite clearly, however, factors such as obesity, perceived helplessness, and other psychological factors influence OA pain as well.[11]

Specific causes of muscle pain are acute trauma (tear and blow), overload (e.g. exercise, particularly eccentric contraction), and myositis. Muscle pain is also elicited by ischemic contractions and by muscle spasm. A particular muscle pain syndrome is the referred pain elicited from painful trigger points in the muscle. Pain syndromes involving muscular pain are fibromyalgia and the myofascial pain syndrome, but in these cases no clear muscle pathophysiology has been established. On the other hand, significant muscular diseases, such as slow cell death in muscle during muscle dystrophy do not cause pain.[10, 11]

Another site of clinically relevant pain in deep tissue is the bone. Frequent causes of bone pain are trauma, fracture, and also degenerative disorders, such as osteoporosis and bone metastases.[14, 15] Research into neuronal mechanisms of bone pain has been very sporadic. However, recent studies on cancer pain have focused on mechanisms of bone pain,[14] and the bone near the joint may be one site at which osteoarthritic pain is generated, as mentioned above.[11, 13]

INNERVATION OF JOINT, MUSCLE, AND BONE

Joints are supplied by branches descending from main nerve trunks or their muscular, cutaneous, and periosteal branches. A typical joint nerve contains thick myelinated Aβ- (group II), thinly myelinated Aδ- (group III), and a high proportion (∼80 percent) of unmyelinated C- (group IV) fibers. The latter are either sensory afferents or sympathetic efferents (each ∼50 percent).[12] Aβ fibers terminate as corpuscular endings of the Ruffini-, Golgi-, and Pacini-type in fibrous capsule, articular ligaments, menisci, and adjacent periosteum.[1] Articular Aδ- and C-fibers terminate as noncorpuscular or free nerve endings in the fibrous capsule, adipose tissue, ligaments, menisci, and the periosteum.[16] Using staining for nerve fibers and neuropeptides, endings were also identified in the synovial layer. The cartilage is not innervated.[12]

Muscle nerves contain axons from motoneurons, sensory neurons, and postganglionic sympathetic neurons. For example, in the nerve of cat gastrocnemius-soleus (GS) muscle, about one-third of the axons are myelinated (∼60 percent of these are from motoneurons and ∼40 percent are sensory) and two-thirds of the fibers are unmyelinated. In the latter group, ∼50 percent of the units are sensory and ∼50 percent are sympathetic efferent. Thick myelinated afferents terminate as organized endings (muscle spindles, tendon organs), whereas Aδ- and C-fibers terminate as free nerve endings. Most of these endings are located in the wall of arterioles in the muscle belly and in the surrounding connective tissue.[7]

A large proportion of articular and muscular sensory neurons are peptidergic. The major neuropeptides in joint and muscle nerves are substance P, calcitonin gene-related peptide (CGRP), and somatostatin. Neurokinin A, galanin, enkephalins, and neuropeptide Y have also been localized in joint afferents. Neuropeptides influence the inflammatory process in the periphery and modify spinal processing of joint and muscle input. They may also act on the primary afferent neurons themselves (see below under Molecular mechanisms of peripheral sensitization). However, these neuropeptides are not specific for deep afferents.[7, 12]

ACTIVATION OF THE NOCICEPTIVE SYSTEM BY NOXIOUS DEEP TISSUE STIMULATION UNDER NORMAL AND INFLAMMATORY CONDITONS

Nociceptors of the deep somatic tissue encode noxious stimuli applied to the normal tissue. This is important for the protection of the tissue against damage. Noxious stimuli, such as twisting of a joint, cause immediate motor responses and a conscious pain experience both of which are parts of a strategy to avoid further damage. It is thought that loss of sensory mechanisms causes damage of the joint such as in Charcot's joint. Essentially, treatment of joint pain should not impair the normal nociceptive function.

Importantly, significant changes of the nociceptive processing are induced by inflammation and tissue injury which are called peripheral sensitization (sensitization of primary afferents) and central sensitization (development of hyperexcitability of nociceptive neurons in the central nervous system). **Figure 2.1** summarizes the structures of the nociceptive system and the sequence of inflammation-evoked events in the nociceptive system. Inflammation leads to peripheral sensitization which in turn causes the development of hyperexcitability in the spinal cord.[7, 12] Ascending axons in the spinothalamic tract activate the lateral and medial thalamocortical system which evoke the conscious pain sensation with its sensory discriminative and the affective components.[17] In parallel, ascending projections to the brainstem are activated. The activation of the brainstem contributes to the activation of the brain by noxious stimuli, but it also acts back on the spinal cord through descending systems.[18, 19, 20]

NOCICEPTORS OF DEEP TISSUE AND PERIPHERAL SENSITIZATION

Mechanosensitivity of peripheral nociceptors in the normal joint and muscle

In single-fiber recordings, primary afferent neurons have been classified according to their mechanosensitivity. These recordings showed types of primary afferent neurons that encode noxious stimuli applied to joint and muscle and are thus suitable for signaling noxious

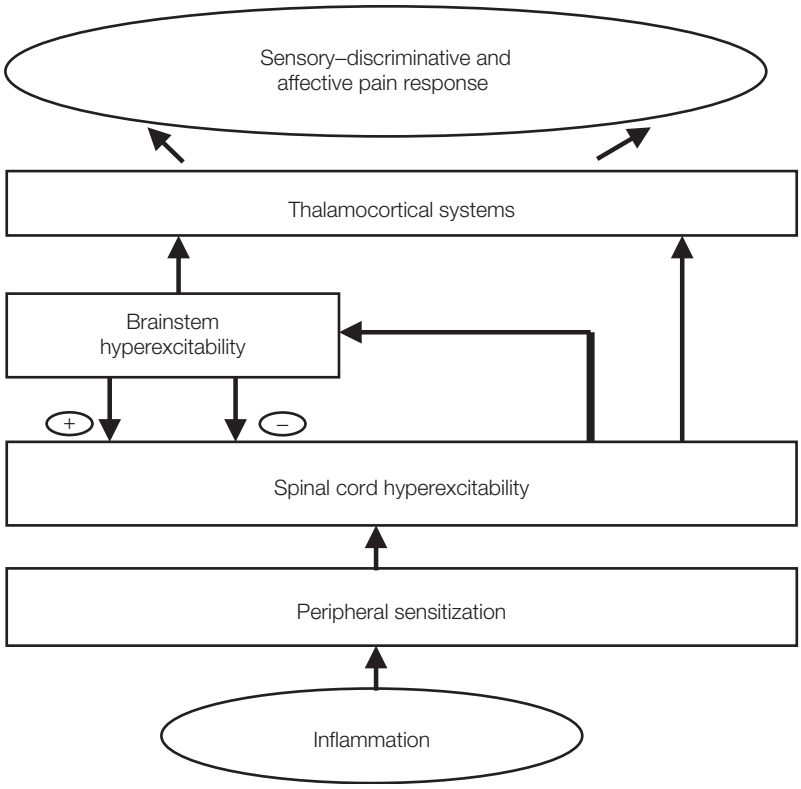

Figure 2.1 Sequence of neuronal events induced by inflammation in deep tissue.

mechanical events which cause pain sensations in awake individuals.

In the joint nerve, more than 50 percent of the Aδ-fibers and most C-fibers with a detectable receptive field are able to encode noxious mechanical stimuli applied to the joint. These fibers are either weakly activated by innocuous stimuli and strongly activated by noxious stimuli, or they are exclusively activated by noxious stimuli.[21, 22] Innocuous stimuli are light to moderate pressure and movements within the normal working range of the joint. Noxious stimuli are high-intensity pressure (that causes pain when applied to humans) and movements against the resistance of the tissue beyond the limit of the normal working range. Fibers activated by these noxious mechanical stimuli are thought to be the nociceptors which cause pain upon twisting the normal joint against the resistance of the tissue. Some Aδ-fibers and a significant proportion of C-fibers do not respond to any mechanical stimulus applied to the normal joint. These fibers are "initially mechanoinsensitive" or "silent nociceptors" that are only activated during inflammation (see below under Changes of mechanosensitivity during inflammation (peripheral sensitization)).[23, 24, 25] In contrast, most Aβ-fibers and about half of the Aδ-fibers are low threshold units that are strongly activated by innocuous pressure, such as light pressure and movements in the working range.[26, 27] Their responses to innocuous stimuli might be used to control movements and to prevent unphysiological movements. Although these units may show their highest discharge rate upon noxious stimuli, they do not discriminate between innocuous and noxious stimuli. In fact, the most adequate innocuous mechanical stimulus can evoke a stronger response than a noxious mechanical stimulus, e.g. a noxious movement into another direction.[26, 27]

In the muscle nerve, numerous sensory Aδ- and C-fibers are only activated by noxious mechanical stimuli. These muscle nociceptors do not respond to everyday stimuli, such as weak local pressure, contractions, and muscle stretch within the physiological range. They require potentially noxious stimuli to be readily activated, and the best stimulus is noxious squeezing of the muscle belly or tendon at intensities that elicit pain in humans. Nociceptors may also respond to unphysiological stretch and maximal contraction. The threshold of a nociceptor may lay below frankly tissue-damaging intensities (small response to moderate pressure). Similar units have been found in the cat, dog, rat, and humans.[28, 29, 30, 31, 32, 33] Electrical stimulation of such nerve fibers in human muscle nerves evokes cramp-like sensations. Electrical stimulation frequencies of 5–6 Hz are required to elicit pain sensations.[34] Why such high frequencies are needed is unknown. It may be speculated that a small number of muscle afferents drive spinal cord neurons only during temporal facilitation, either because they form fewer synapses on neurons than cutaneous afferents or because descending inhibition of nociceptive spinal cord neurons is stronger for deep input than for cutaneous input (see below under Descending influences on spinal neurons with deep input).

Only a proportion of the sensory units with free nerve endings in muscle nerves are nociceptors. Other slowly

conducting units are more sensitive and respond strongly to physiological stimuli, such as stretch and contraction. These low threshold units are considered to be ergoreceptors. Presumably they are important for respiratory and circulatory adjustments during physical exercise.[7] In addition, as in the joint nerve, some units are mechanoinsensitive and may thus be silent nociceptors, because they respond to intra-arterial injection of bradykinin into the muscle.

Changes of mechanosensitivity during inflammation (peripheral sensitization)

An inflamed joint hurts during movements in the working range and during palpation, and pain may occur under resting conditions. An inflamed muscle exhibits tenderness. An important mechanism for the heightened pain sensitivity is an increase of mechanosensitivity in afferent fibers supplying inflamed tissue. During development of inflammation in the joint, some low threshold Aβ-fibers show transiently increased responses to joint movements in the initial hours of inflammation. They do not develop resting discharges. Importantly, the majority of Aδ- and C-fibers show increased mechanosensitivity. Many low threshold Aδ- and C-fibers show increased responses to movements in the working range and to noxious movements. Most strikingly, a large proportion of high threshold afferents are sensitized such that they begin to respond to movements in the working range of the joint. The units may develop ongoing discharges in the resting position.[23, 24, 25] Increased mechanosensitivity has also been found during chronic forms of arthritis, suggesting that mechanical sensitization is an important neuronal basis for chronic persistent hyperalgesia of the inflamed joint.[35]

Furthermore, initially mechanoinsensitive afferents (silent nociceptors) are sensitized and become mechanosensitive.[23, 24, 25] While these fibers are not activated even by noxious mechanical stimulation of the normal joint, they can develop mechanosensitivity within one to four hours after onset of inflammation. Then they show responses to movements of the joint, even to innocuous ones, and one can identify a receptive field upon mechanical stimulation of the inflamed tissue. Thus, during inflammation, there is a recruitment of further nociceptive sensory neurons for signaling of noxious events. Silent nociceptors have also been identified in cutaneous nerves in humans and in visceral nerves.[36, 37, 38] In particular, studies in skin nerves of humans have shown that silent nociceptors are particularly important for neurogenic inflammation and for the induction of central sensitization.[39, 40]

In muscle nerve, inflammation enhances the proportion of Aδ-fibers showing resting discharges, as well as the discharge rate in spontaneously active fibers. It is likely that these changes produce spontaneous pain and dysesthesias of the inflamed muscle. In addition, mechanical threshold significantly drops in numerous sensory C-fibers.[41] Thus, similar processes are seen as in afferent fibers of the joint. In addition, in the muscle, ischemic conditions may play an important role in pain generation. Interruption of blood supply to a resting muscle is not painful unless it lasts for long periods of time. Indeed, ligation of arteries to the muscle does not activate Aδ- and C-fibers within the first five minutes.[42] Long-lasting complete interruption of blood supply may cause resting discharges in muscle afferents within 15–60 minutes followed by a block of action potential generation or conduction.[7] However, if the muscle is forced to contract under ischemic conditions, severe pain develops rapidly. During ischemia, a small percentage of sensory C-fibers respond to contraction, although these units do not or only minimally respond to contraction when the blood supply is intact.[42, 43]

Molecular mechanisms of peripheral sensitization

Primary afferent neurons are equipped with numerous ion channels and receptors for mediators. Stimuli applied to the sensory endings open ion channels, and the resulting ion currents depolarize the endings. The generation of this receptor potential is called transduction. When the depolarization reaches a certain threshold, voltage-gated ion channels are opened that generate action potentials which are conducted along the axon to the spinal cord. The generation of the action potential is called transformation. Thus, the responsiveness of neurons depends on transduction mechanisms and on the triggering of action potentials.[44, 45]

The elicitation of an action potential by a stimulus is called activation. The previous sections have described changes of mechanosensitivity upon inflammation which are called sensitization (see above under Nociceptors of deep tissue and peripheral sensitization). After sensitization, action potentials are elicited at lower stimulus energies, and thus a nociceptive neuron may respond to normally innocuous stimuli, in addition to showing an augmented response to noxious stimuli.

Sensitization involves a number of different molecular mechanisms. It results from the effect of numerous inflammatory mediators that bind to receptors in the membrane of the sensory endings, but changes of the intrinsic properties of the neurons also contribute to sensitization. The latter conclusion is derived from findings that dorsal root ganglion or trigeminal neurons from inflamed tissue maintain enhanced excitability even when the neurons are removed from the ganglion and acutely dissociated several days after inflammation of joint[46] or muscle.[47] In whole cell patch clamp recordings from these neurons, enhanced excitability could be identified by a decrease of the rheobase, an increase in the slope of the

stimulus response function assessed with depolarizing current injection, and a decrease in the duration of the action potential after hyperpolarization. Most likely, changes in the activation of voltage-gated K^+ currents play an important role.[46, 47]

As mentioned, nociceptors are equipped with numerous receptors for inflammatory mediators. Binding of mediators to membrane receptors (many of which are coupled to G proteins) activates intracellular second messenger systems. These in turn activate intracellular processes that increase the sensitivity of the ion channels that are involved in stimulus transduction and/or transformation. Mediators are thus able to excite and/or sensitize primary afferent neurons for mechanical and chemical stimuli. These mediators also produce vascular and other changes in the tissue and thus contribute to the inflammatory process itself.

Effects of mediators on joint afferents have been previously summarized in detail elswhere.[48] As far as it has been tested, the effects on muscular afferents are comparable. Mediators that have effects on joint afferents include classical inflammatory mediators such as bradykinin, prostaglandins E_2 and I_2, and serotonin, purinergic compounds, neuropeptides, cytokines, and others. Common observations are that these mediators (1) affect Aδ- and/or C-fibers, not Aβ-fibers, (2) have an effect only in subpopulations of the units, (3) may or may not affect high threshold, as well as low threshold Aδ- and C-fibers, and (4) cause some initially mechanoinsensitive afferent fibers to be sensitized and become mechanosensitive.

Bolus injection of bradykinin, an algesic mediator, into joint and muscle arteries may cause an immediate short-lasting activation (less than one minute) of joint and muscle afferents, but thereafter there is a sensitization for mechanical stimuli of joint and muscle afferents that lasts minutes even when bradykinin did not excite the neuron.[49, 50] Both PGE_2 and PGI_2 cause ongoing discharges and/or sensitization to mechanical stimulation of the joint. The effect of PGE_2 has a slow onset and a duration of minutes, the action of PGI_2 begins quickly and has a short duration.[51, 52, 53, 54] In addition, these PGs sensitize joint and muscle afferents to the effects of bradykinin whether or not they have an excitatory effect by themselves.[42, 55] PGE_2 and bradykinin together can cause a stronger sensitization to mechanical stimulation than bradykinin or PGE_2 alone.[49] Conversely, nonsteroidal anti-inflammatory drugs (NSAID), such as aspirin and indometacin, which block PG synthesis, reduce spontaneous discharges from acutely and chronically inflamed joints, and attenuate the responses to mechanical stimulation.[56, 57] Serotonin also sensitizes joint afferents to mechanical stimuli,[58, 59] and Aδ- and C-fibers muscle afferents to the action of bradykinin and to excitation by mechanical stimuli.[55] Combined i.m. application of bradykinin and serotonin causes muscle pain in humans.[60]

ATP,[61, 62] adenosine,[62] capsaicin, and anandamide[63, 64] excite a proportion of joint afferents (the latter indicate the presence of the TRPV1 receptor). Capsaicin also causes muscle pain in humans.[65] Effects have also been observed for neuropeptides. Indeed, substance P[66] and VIP[67, 68] increased, whereas somatostatin[69] and endomorphin[70] reduced mechanosensitivity in numerous afferents; the peptides galanin,[71] neuropeptide Y,[72] and nociceptin[73] sensitized some neurons and reduced responses in other neurons. Whether the different patterns of peptide effects (excitation or inhibition) are dependent on the functional state of the neuron is not known at the moment. It was proposed that the simultaneous presence of different neuropeptides regulates excitability of the afferent fibers.

Of particular importance for the progress of arthritis are cytokines such as TNFα, interleukin-1β, and interleukin-6. Cytokines play an important role in neuropathic pain,[74] but, for example, IL-6 is also able to induce a long-lasting sensitization of C-fibers of the joints to mechanical stimulation.[75] Finally, mechanosensitivity can also be influenced by quite different compounds. For example, it was shown that responses of nociceptive articular afferents are reduced by gabapentin,[76] a compound used for the treatment of neuropathic pain, and by intra-articular injection of elastoviscous hyaluronan solutions.[77]

Recordings from afferent fibers from inflamed joints revealed that the proportion of neurons that show an effect of a given mediator can be different from the proportion of responsive afferents from normal joints. This may result from changes of receptor expression. Some data indicate that receptor expression in dorsal root ganglion (DRG) neurons can change in the course of arthritis (e.g. down-regulation of mu-opioid receptors[70] or biphasic regulation of somatostatin receptors[78]). However, changes of the neurons may also result from changes of the milieu in the tissue innervated. Disease processes are dynamic and, therefore, it is likely that different cells and molecules are important at different times in a chronic inflammatory or degenerative process[11] or during growth of bone cancer.[14] Hence the molecular mechanism of nociception may change over time. This aspect needs much more attention.

SPINAL PROCESSING OF INPUT FROM DEEP TISSUE AND CENTRAL SENSITIZATION

Nociceptive spinal cord neurons with joint and muscle input

Neurons with input from joint and muscle are located in the superficial and deep dorsal horn. This distribution matches the spinal termination of joint and muscle afferents which project to the superficial dorsal horn and, in particular Aβ- and Aδ-fibers, to the deep dorsal horn.[7, 12] Neurons with nociceptive information

from joint[12, 79, 80] and muscle[81, 82, 83, 84] are either exclusively driven by input from deep tissue, or they exhibit convergent inputs from skin and deep structures. Neurons exclusively driven from deep tissue are excited by pressure applied to the deep tissue, but not by mechanical stimulation of the overlying skin. Their receptive fields are not restricted to a specific structure such as only a joint or a muscle belly. Rather they include a joint and adjacent muscles. Many of these neurons are high-threshold and require noxious pressure onto joint and/or muscle to be activated. Neurons with joint input may be activated by noxious movements, such as twisting of the joint against resistance of the tissue, like the articular nociceptors. The remaining neurons are wide dynamic range neurons which respond with increasing frequency when stimulus intensity is increased from the innocuous to the noxious range. Neurons with convergent inputs from deep tissue and skin are excited by mechanical stimuli applied to deep tissue (muscle, tendons, joint structures) and by mechanical stimulation of the skin. Often receptive fields in the deep tissue are located more rostral than cutaneous receptive fields thus allowing determination of both receptive fields. Most of these neurons are wide dynamic range neurons which respond to innocuous and noxious pressure onto deep tissue in a graded fashion. They may be activated by movements in the working range, but show much stronger responses to painful movements.

Neurons with input from joint and muscle project to different supraspinal sites (cerebellum, spinocervical nucleus, thalamus, reticular formation) subserving the generation of the conscious pain response and adaptations to pain (see **Figure 2.1**), or they project to intraspinal (segmental) interneurons and motoneurons.[7, 12] Spinal and supraspinal motor reflexes regulate movements and exert protective functions including flexor reflexes upon nociceptive stimulation.[1] Noxious stimulation of joint afferents[12, 85] and muscle afferents[7, 8] can evoke nociceptive withdrawal reflexes. During acute chemical stimulation of the knee and electrical stimulation of muscle nerves[86] and during inflammation in the joint,[85, 87] spinal motor reflexes are enhanced. In line with this, it has been thought that noxious stimulation of the muscle causes reflex muscle spasms and that muscle spasms will enhance the pain in the muscle – thus establishing a vicious circle. However, during myositis, a decrease rather than an increase of the reflex activation of motoneurons was observed,[8] and during experimental joint inflammation some γ-motoneurons developed progressive inhibition rather than facilitation.[87] Thus, the reflex pattern is modified during inflammation. Patients with painful muscles exhibit low rather than enhanced EMG activity[8] indicating that prolonged nociceptive stimulation actually induces a reduction of motor reflexes, followed by atrophy and loss of force.[8, 12] However, muscle spasms may be elicited from painful trigger points in adjacent muscles, and by articular dysfunction and ligamentous strain.[8]

Development of spinal hyperexcitability during peripheral inflammation

In the course of joint or muscle inflammation, spinal cord neurons with deep input develop a state of hyperexcitability, which is also called central sensitization (see **Figure 2.1**). Central sensitization is characterized by typical neuronal changes: (1) Spinal cord neurons with high threshold show a decrease of their excitation threshold, such that they are activated by innocuous stimuli applied to the inflamed tissue. (2) Both high threshold and wide dynamic range neurons show a marked increase of their responses to noxious stimulation of the inflamed tissue. This increased responsiveness to stimuli applied to inflamed tissue contributes to primary hyperalgesia at the site of inflammation. (3) With a similar time course, the neurons also show enhanced responses to mechanical stimuli applied to adjacent and even remote healthy tissue, and the total receptive field may expand.[80, 88, 89] These changes indicate that the sensitivity of the spinal cord neuron is increased so that previous subthreshold inputs are now sufficient to excite the neuron. The sensitization of neurons with expansion of receptive fields has the consequence that a stimulus activates more neurons in a segment.[90] Central sensitization can persist during chronic inflammation. In rats with unilateral arthritis,[79] as well as in rats suffering from chronic polyarthritis,[91] spinal cord neurons appear on average more sensitive and have expanded receptive fields in deep tissue and skin.

Pronounced spinal changes evoked by persistent inflammation were also observed when c-Fos was used to label activated neurons. During polyarthritis induced by injection of Freund's complete adjuvcant, numerous neurons in the dorsal and also in the ventral horn of several segments expressed c-Fos.[92] Increased c-Fos staining was also elicited by palpation of bones that were infiltrated with cancer cells, thus strongly suggesting that central sensitization is involved in bone pain.[14]

The changes described in the spinal cord are likely to account for deep referred pain and secondary hyperalgesia that are induced in humans by noxious stimulation of deep tissue.[93] Numerous pathological conditions, such as inflammation and osteoarthritis, seem to be associated with central sensitization. When a noxious stimulus, e.g. intramuscular injection of 6 percent NaCl, is applied to a muscle, the area in which pain is felt is larger during pathological conditions, such as osteoarthritis.[94] This suggests that the spinal cord is indeed in a state of hyperexcitability.

Sensitized nociceptive afferents from inflamed tissue play a key role in initial sensitization. Obviously these afferents not only evoke enhanced synaptic activation of spinal cord neurons to stimulation of inflamed tissue, but they also trigger the processes that increase sensitivity of spinal cord neurons. Interestingly, the stimulation of primary afferents from deep tissue (muscle and joint)

evokes more prolonged facilitation of a nociceptive flexor reflex than stimulation of cutaneous afferents,[86] and capsaicin injection into deep tissue elicits more prolonged hyperalgesia than injection of capsaicin into the skin,[95] suggesting that deep input is particularly able to induce long-term changes in the nociceptive system. In addition to afferent and spinal mechanisms, descending pathways influence central sensitization.

Molecular mechanisms of spinal sensitization

In experiments, central sensitization has been observed during peripheral inflammation and also in models of neuropathic pain. Research in humans suggests that central sensitization may indeed be present in a number of different pain states, such as inflammation, osteoarthritis, fibromyalgia, migraine attacks, and others. Concerning molecular mechanisms of central sensitization, several points must be made. First, an important basis for central sensitization is the potential of nociceptive spinal cord neurons to undergo neuroplastic changes. The latter can, for example, be shown with defined protocols of electrical nerve stimulation. Electrical stimulation of C-fibers can induce wind-up of the responses to electrical nerve stimulation[96] (a short-lived increase of responsiveness, however, not outlasting the stimulation protocol) or a long-term potentiation[97] (a persistent increase of synaptic responses to electrical stimulation outlasting the conditioning stimulus). Second, while for central sensitization under inflammatory conditions mainly an increase of excitatory mechanism is being discussed, in the case of neuropathic pain loss of inhibition (e.g. by apoptosis of inhibitory interneurons) has been proposed as an important mechanism.[98] Third, again in studies on neuropathic pain, an involvement of both neurons and glial cells has been shown.[99] These data suggest that different mechanisms may contribute to central sensitization in different pain states. Ongoing research has to dissect out which mechanisms are particularly important for different pain states.

In the case of inflammation in joint and muscle, the contribution of transmitters and receptors to central sensitization has mainly been studied. Once inflammation develops in the joint, the intraspinal release of glutamate[100] (the main transmitter of nociceptive afferents) and neuropeptides (cotransmitters in primary afferents and interneurons) is enhanced. While only noxious compression of the normal joint enhances the intraspinal release of substance P, neurokinin A, and CGRP above baseline, these excitatory peptides are intraspinally released even by innocuous compression when the joint is inflamed.[101, 102, 103] Intraspinal release of substance P is also evoked by palpation of bone with cancer.[14] In addition, the intraspinal milieu is altered by (enhanced) release of further mediators. For example, prostaglandin E_2 is tonically released above baseline within the dorsal and ventral horn.[104] This is likely to result from an up-regulation of spinal COX-2, that is already present at three hours after induction of knee joint inflammation.[104] Thus, as a presynaptic mechanism, a cocktail of transmitters and/or modulators is released in the spinal cord under inflammatory conditions that is likely to influence the synaptic processing.

Glutamate activates AMPA/kainate (non-*N*-methyl-D-aspartic acid (NMDA)) receptors and NMDA receptors. Both glutamate receptor types have been implicated in the generation and maintenance of inflammation-induced spinal hyperexcitability. Application of antagonists at AMPA/kainate and NMDA receptors prevents the development of hyperexcitability in the course of joint inflammation[80] and muscle inflammation.[8] Importantly, antagonists at both receptor types can also reduce responses of the neurons to mechanical stimulation of the joint after inflammation is established,[80] even in a chronic model of inflammation.[12] The excitatory neuropeptides facilitate the responses of spinal cord neurons to mechanical stimulation of joint and muscle, further the development of inflammation-evoked hyperexcitability, and "open" synaptic pathways such that more neurons respond to stimulation.[8] However, antagonists at neuropeptide receptors are less antinociceptive than antagonists at glutamate receptors.[105, 106, 107] Topical application of PGE_2 to the spinal cord surface facilitates the responses of spinal cord neurons to mechanical stimulation of the joint similar to knee joint inflammation.[108] Topical application of the COX inhibitor indometacin to the spinal cord before inflammation attenuated the development of hyperexcitability.[108] Thus spinal PGs are involved in inflammation-evoked spinal hyperexcitability.

DESCENDING INFLUENCES ON SPINAL NEURONS WITH DEEP INPUT

From brainstem nuclei, impulses "descend" onto the spinal cord and influence the transmission of pain signals at the dorsal horn.[18, 19, 20] The periaqueductal gray (PAG) matter is a key region for descending inhibition. It receives inputs from the hypothalamus, cortical regions, and the limbic system and projects to the rostral ventromedial medulla (RVM), which includes several subnuclei. Neurons in RVM then project along the dorsolateral funiculus (DLF) to the dorsal horn. OFF cells of RVM exert descending inhibition of nociception, but ON cells facilitate nociceptive mechanisms at the spinal dorsal horn. Spinobulbospinal loops are significant in setting the gain of spinal processing.[19]

A particular form of descending inhibition of wide dynamic range neurons is the diffuse noxious inhibitory controls (DNIC). When a strong noxious stimulus is applied to a given body region, nociceptive neurons with input from that body region send impulses to structures located in the caudal medulla (caudal to RVM) and this

triggers a centrifugal inhibition (DNIC) of nociceptive wide dynamic range neurons located throughout the neuraxis.[109]

Most spinal cord neurons with joint and muscle input are tonically inhibited by descending inhibitory systems that modulate spinal cord activity.[81, 110] These neurons are also inhibited by DNIC.[109] Tonic descending inhibition,[110] as well as DNIC,[109, 111] are increased during acute inflammation, but may be normalized in the chronic stage of inflammation.[111, 112] Interestingly, inhibition is mainly observed on neurons with input from the inflamed region (thus attenuating primary hyperalgesia), but processing in neurons with input from neighboring tissues may rather be enhanced, thus facilitating secondary hyperalgesia.[19]

SUPRASPINAL NEURONS WITH INPUT FROM JOINT AND MUSCLE

The thalamus and cortex contain nociceptive neurons that are activated by nociceptive deep input from muscles and joints. Most of these neurons have convergent inputs from skin and deep tissue, but small proportions of neurons respond only to noxious stimulation of muscle and tendon.[113, 114, 115] In the thalamus, such neurons are located in the ventrobasal complex, in the posterior complex[114] and in the medial nucleus.[116] Similarly, the somatosensory cortex contains a large proportion of neurons that respond to noxious stimulation, and a small proportion of these neurons is driven by deep input.[7, 117]

In polyarthritic rats, a large proportion of neurons in the ventrobasal complex respond to movements and gentle pressure on to inflamed joints and often long-lasting discharges were noted, whereas only few neurons respond to these stimuli in normal rats. Some neurons also displayed paroxysmal discharges. Furthermore, neurons in the nucleus centralis lateralis acquire input from the inflamed joint which is not present in normal animals.[118] Similarly, neurons in superficial cortical layers that do not respond to joint stimulation in normal rats start to respond to joint stimulation in polyarthritic rats.[119, 120] These findings indicate substantial neuroplasticity at the thalamocortical level that may contribute to inflammatory deep tissue pain. It is unknown whether these alterations mirror the altered spinal processing or whether additional elements of neuroplasticity are generated in the thalamus and cortex themselves.

CONCLUSIONS

There is considerable experimental evidence for substantial changes in the nociceptive processing during disease processes in the deep tissue such as joint, muscle, and bone. Available data from patients show considerable convergence of experimental and clinical data, and they indicate that different levels of the neuraxis are rational targets for analgesic treatment. Still much more research is required in order to better understand and treat chronic pain because current treatments are often not sufficient. There may be several reasons for that. Long-term molecular changes in the nociceptive systems are still poorly understood. Furthermore, chronic pain often seems to be a state in which nociceptive and neuropsychological components interact. This interaction should be better explored and form the basis of an "integrative" treatment strategy.

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3

Applied physiology: persistent visceral pain

TIMOTHY J NESS

KEY LEARNING POINTS

- Persistent visceral pain is common and represents a state of hypersensitivity.
- The substrates of visceral sensation differ from cutaneous sensation.
- Inflammation, stress, and altered neurological substrates due to neuropathic or developmental processes can all lead to visceral hypersensitivity.

INTRODUCTION

Persistent visceral pain is a common clinical experience, but only recently have scientific studies defined its mechanisms. It is poorly understood and much of our knowledge of it is an extrapolation from acute pain studies. Pain arising from the internal organs of the body is uniquely different from pain that arises from the surface of the body in relation to the neuroanatomical substrates involved, in relation to the responses evoked by visceral stimuli, and in relation to the modifying effects of both internal and external factors. Most painful disorders associated with the viscera represent conditions of hypersensitivity made manifest by these same internal and external factors. There are underlying similarities that have been observed between multiple visceral sensory systems such that an understanding of one particular system may improve understanding of other systems. This chapter attempts to summarize what is known about persistent visceral pain by placing an emphasis on the mechanisms of visceral hypersensitivity. These mechanisms will be contrasted and compared with what is known about the more extensively studied superficial pains. This summary builds on previous reviews by this author of similar topics and general statements are referred to those reviews.[1, 2, 3, 4]

THE EXPERIENCE OF VISCERAL PAIN

When visceral pains are experienced, they are often associated with poor and unreliable localization. They are generally deep and diffuse and often the only localization of pathology comes with a physical examination in which manipulations directly stimulate the painful organ. Whereas superficial sensations from a specific site are always reliably localized to the same site and do not migrate to other body areas in the absence of nerve injury, the same cannot be said for visceral pain. Visceral pain can be felt in several different areas at the same time or can migrate throughout a region even though pathology is localized within a single organ. Visceral pain

is classically described as being referred to other sites and this referral has two separate components: (1) the sensation is transferred to another site (e.g. angina can be felt in the neck and arm) and (2) other sites become more sensitive to inputs applied directly to those other sites (e.g. flank muscle becomes sensitive to palpation when passing a kidney stone). This latter phenomenon is a form of secondary hyperalgesia which can involve both somatic and other visceral structures.

Based on clinical experience, stimuli which can lead to the production of pathological visceral pain can be categorized into four groups:

1. acute mechanical stretch/distension of visceral structures;
2. ischemia of visceral structures;
3. chemical stimuli from a local pathological process (e.g. an infiltrating tumor);
4. functional alterations leading to atypical patterns of afferent activity.

Visceral pains may also occur secondary to iatrogenic damage of the viscera and their associated nerves produced by interventional therapies, surgery, chemotherapy, and/or radiation. There is a poor correlation between the amount of visceral pathology and the intensity of associated pain. For example, very extensive processes with ongoing tissue damage (e.g. ulcerative colitis or gastric perforation) may produce little or no pain in some individuals, while minimally discernable pathology may produce out-of-control pain in others.

VISCERAL HYPERSENSITIVITY DISORDERS

The observation that pathology and symptomatology may not correlate is readily apparent in numerous visceral pain disorders. Some disorders, such as chronic pancreatitis, have definable pathology, but alterations in pain appear out of proportion to objective radiographic or laboratory findings. Other disorders, such as irritable bowel syndrome, noncardiac chest pain, and postcholecystectomy syndrome, appear to have no grossly apparent histopathological basis for the discomfort and pain. Instead, visceral discomfort and pain in such conditions are termed functional and are associated with altered patterns/pressures associated with motility, production of gas, and ingestion of food or beverage. Hence, natural visceral stimuli in the physiologic range can be associated with discomfort and pain in the absence of obvious visceral pathology.

Hypersensitivity to somatically applied stimuli is typically associated with histological evidence of ongoing tissue damage/inflammation. Exception to this statement are neuropathic pain disorders in which there may be a history of nerve injury, but no apparent local histopathological changes. In this case, routine tissue examination would suggest that neuropathic pain disorders are functional.

With an increased sophistication of testing related to visceral disorders, there may prove to be identifiable markers or imaging studies that allow for a reduced reliance on subjective reports of sensation. An example of this comes from the painful bladder disorder, interstitial cystitis (IC). In general, the urothelium of patients with the nonulcerative form of IC appears normal on routine cystoscopic and microscopic examination. It takes a highly sophisticated analysis to discern any quantitative differences between the tissues of IC patients and normal healthy controls such that most measures have been deemed to be of little use in diagnosis. However, when the urothelium of IC patients is examined using a scanning electron microscope, defects in the urothelial surface and tight junctions are common[5] and a laboratory marker for a factor that suppresses urothelial cell proliferation may prove diagnostic for the disorder.[6] Until similar subtleties of evaluation become routine, the current state-of-the-art for diagnosis of painful visceral disorders requires full consideration of the entire constellation of signs, symptoms, and tests.

Psychophysical studies have demonstrated evidence for hypersensitivity in virtually all clinically relevant visceral pain disorders. This includes hypersensitivity to gastric distension in patients with functional dyspepsia,[7] intestinal and rectal distension in patients with irritable bowel syndrome,[8, 9] biliary and/or pancreatic duct distension in patients with postcholecystectomy syndrome or chronic pancreatitis,[10] and bladder distension in patients with interstitial cystitis.[11] In these studies, pain could be evoked at intensities of stimulation lower than those required to produce the same quality and intensity of sensation in a healthy population. A more sophisticated testing of visceral sensitivity using random order, graded distension of the rectum in irritable bowel patients suggest that the population of subjects is heterogenous,[12] with subgroups demonstrating hypersensitivity and others hypervigilance.

Dissociating potential psychological modifiers of sensory reports from other more physiological pathologies has proven to be difficult. It represents a sometimes insurmountable methodological problem and, perhaps more importantly, due to observations related to the phenomenon of stress-induced hyperalgesia (where psychological factors alter physiological responses) it may not be appropriate to perform such a dissociation. Psychophysical studies related to visceral sensation in normal healthy subjects have suggested a basis for some of the emotional factors that may affect pain reports. Strigo *et al.*[13] compared sensations evoked by balloon distension of the esophagus with thermal stimulation of the skin overlying the sternum and found that greater anxiety was evoked by esophageal distension. Furthermore, they found unpleasantness ratings were higher when the esophageal stimulus was administered and a stronger

affective component to the visceral sensation was measured using the McGill Pain Questionnaire.

Other psychophysical studies of experimental visceral pain sensation in humans have identified that a sensitization process occurs with repeated stimulation of the gut[14, 15] and of the urinary bladder[16] consistent with observations in nonhuman animals, where repeated presentation of the same visceral stimuli produces increasing vigor of neuronal, cardiovascular, and visceromotor reflex responses.[17, 18]

Clinically, there are three entities that are accepted as potential sources of painful hypersensitivity: (1) inflammation; (2) stress (anxiety); and (3) altered neural function that may be due to injury during critical periods of development (e.g. the neonatal period) or more direct neuropathic processes. These will be discussed below after a description of the anatomy of visceral pain.

SUBSTRATES OF SENSATION

Peripheral pathways

The peripheral nervous system pathways of abdominal visceral sensation have been defined in humans and are summarized in **Figure 3.1**. Most viscera have a dual, or in some cases triple, source of afferents travelling via the vagus nerve, the pelvic nerve, and/or via the splanchnics (nerves travelling in association with sympathetic efferent fibers). Spinal visceral afferent fibers have their cell bodies in dorsal root ganglia and central terminals in the superficial dorsal horn of the spinal cord (lamina I and II), deeper laminae (IV, V), the intermediolateral cell column and sacral parasympathetic nucleus (pelvic nerve), and in the area around and dorsal to the central canal (lamina X). It is notable that visceral primary afferents differ significantly from cutaneous primary afferents in both number and pattern of distribution. Grossly, peripheral axons of visceroceptive primary afferents are diffusely organized into web-like plexuses rather than forming distinct peripheral nerve entities. Afferents with endings in a specific visceral site may have cell bodies in the dorsal root ganglia of ten or more spinal levels in a bilaterally distributed fashion. In contrast, afferents arising in cutaneous structures travel to a limited number (three to five levels) of unilaterally located dorsal root ganglia. Individual visceroceptive afferent C-fibers have been demonstrated to branch within the spinal cord and to spread over ten or more spinal segments and to branch into superficial, deep, and even contralateral spinal dorsal horn laminae.[19] Individual cutaneous afferent C-fibers, on the other hand, have been demonstrated to form tight unilateral baskets of input to localized spinal cord segments and terminate predominantly in superficial laminae.[19]

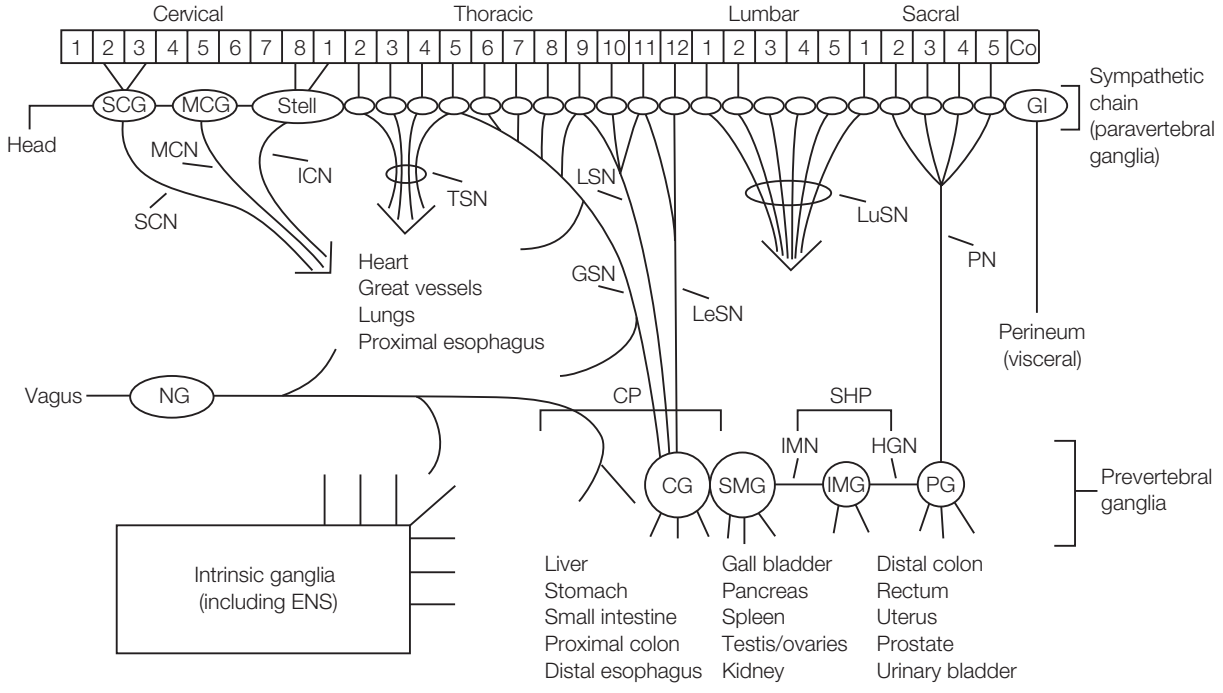

Figure 3.1 The nervous supply of the viscera in humans. Abbreviations are as follows: SCN, MCN, ICN, superior middle and inferior cardiac nerves; TSN, GSN, LSN, LeSN, LuSN, thoracic, greater, lesser, least, lumbar splanchnic nerves; PN, pelvic nerve; IMN, intermesenteric nerve; HGN, hypogastric nerve; SCG, MCG, superior and middle cervical ganglia; Stell: stellate ganglion; CG, celiac ganglion; SMG, IMG, superior and inferior mesenteric ganglia; PG, pelvic ganglion; GI, ganglion impar; CP, SHP, celiac and superior hypogastric plexuses; NG, nodose ganglion; ENS, enteric nervous system. Adapted from Ness and Gebhart, 1990.[1]

Spinal dorsal horn neurons

When quantitatively examined, spinal dorsal horn neurons with visceral inputs are located in the dorsal horn of the spinal cord (lamina I, II, V), the intermediolateral cell column and sacral parasympathetic nucleus (pelvic nerve), and in lamina X. These neurons have multiple, convergent inputs from other viscera, from joints, from muscle, and from cutaneous structures. Convergent receptive fields for these neurons are therefore large with diffuse inputs. This is considered the basis of referral of visceral sensation to somatic sites (e.g. myocardial ischemia typically radiates to the left shoulder and upper arm; the pain is not felt at the source – the heart). In contrast, neurons with exclusively cutaneous input are commonly identified in the spinal dorsal horn, in particular from glabrous skin. Taken together, these results suggest an imprecise organization of visceral primary inputs that would be consistent with an imprecise localization by the central nervous system. Viscero–visceral convergence and secondary hyperalgesia are common enough phenomena that, when coupled with the baseline diffuse character of visceral sensations, there prove to be diagnostic difficulties for both patients and physicians when the possibility of more than one pathology exists.

Unique spinal pathway

The traditional pain pathway for the transmission of information from the dorsal horn of the spinal cord to the brain is via the anterolateral quadrant white matter of the spinal cord. Based on lesion and tracing studies, tracts located within these sites include the spinothalamic, spinoreticular, spinomesencephalic, and spinohypothalamic tracts. This area is clearly important for cutaneous pain sensation because lesions of the anterolateral spinal white matter lead to pinprick analgesia in contralateral dermatomes below the level of the lesion. However, recently researchers have demonstrated that surgical lesions of the dorsal midline of the spinal cord have profound effects on visceral pain-related responses in humans, primates, and rodents. Specifically, a punctate thoracic midline myelotomy in humans has been demonstrated to relieve cancer-related pelvic and abdominal pain.[20, 21, 22, 23, 24, 25] Similar lesions in nonhuman primates reduce the activity of thalamic neurons evoked by colorectal distension[26] and in rats, similar lesions reduce or abolish thalamic neuronal responses and/or behavioral responses to colorectal distension,[27, 28] duodenal distension,[29] pancreatic stimulation,[30] and hypersensitivity following lower extremity osteotomy.[31] Not all ascending information related to the viscera travels by this midline route: dorsal midline lesions abolished visceral inputs to the nucleus gracilis of the medulla,[32] but did not affect inputs to the ventrolateral medulla.[28] Spinal neurons with viscerosomatic convergence and axonal extensions into the dorsal columns have been demonstrated for primates[33] and rats.[20, 32] In rats, acute inflammation of the colon, produced by the topical application of mustard oil, resulted in increased responses of these postsynaptic dorsal column neurons to colorectal distension.[34] Using that model, Palacek and Willis[35] demonstrated that the dorsal midline pathway may be necessary for the augmentation of reflex responses that occur secondary to visceral inflammation, but not for the basal reflex responses.

Supraspinal terminations of visceral input

Standard anatomical and electrophysiological tracing methods have established widespread distribution of visceral input to the brain. The axons of second-order spinal neurons that receive visceral input have been shown to ascend the spinal cord to the brain with sites of termination in the medulla, pons, mesencephalon, hypothalamus, and thalamus. Neurons excited by visceral stimuli have likewise been identified at these same sites with extensive characterizations of neurons located within the ventral posterolateral, dorsomedial, and submedius nuclei of the thalamus, the locus coeruleus, parabrachial nucleus, ventrolateral medulla, and numerous brain stem and limbic sites.[36, 37, 38, 39, 40, 41, 42] Higher-order neurons excited by visceral stimuli have also been demonstrated to be present in the somatosensory and ventrolateral orbital cerebral cortices.[43, 44, 45] A lack of visceral sensation has been noted in neurosurgical patients who have sustained damage to their frontal lobes.[46, 47, 48]

Functional imaging of humans during visceral stimulation has revealed some consistencies, but the most common finding is that there is a multitude of sites which demonstrate increased regional blood flow in response to visceral stimulation. Rectal distension and urinary bladder distension both produce increased bloodflow in select areas of the thalamus, hypothalamus, mesencephalon, pons, and medulla (for example, Ref. 49). Cortical sites of processing include the anterior and midcingulate cortex, the frontal and parietal cortices and in the cerebellum.[50] The most illustrative imaging study to date comparing visceral pain sensation with cutaneous pain sensation is that of Strigo *et al.*[51] These investigators matched the intensity of pain sensation produced by esophageal distension with that produced by heating of the skin overlying the sternum. Whereas both cutaneous and esophageal pain sensations were associated with activation of the secondary somatosensory cortex, the parietal cortex, the thalamus, basal ganglia, and cerebellum, there was a higher activation of the anterior insular cortex bilaterally when cutaneous stimuli were used and the esophageal stimulus selectively activated the ventrolateral prefrontal cortex. Esophageal pain produced a broader bilateral cortical activation and produced activation of a more anterior locus of the anterior cingulate cortex than

cutaneous pain. This all suggests some shared components of sensation from the same segmental structures, but also a selective activation of some structures by different types of pain.

MODELS OF VISCERAL PAIN

Human models

Stimuli which have been employed in experimental studies of visceral nociception in human subjects include electrical stimuli, chemical stimuli, thermal stimuli, ischemia, and mechanical stimuli. Electrical stimulation produces reports of pain in humans and has been used to evoke cerebral potentials, in order to assess visceral sensory pathways. Chemical stimuli have been applied topically, intravascularly, or via physiological pathways (e.g. excreted agents) in order to define the endogenous substances responsible for an altered sensitivity to mechanical or environmental stimuli (e.g. acidity of urine) which may occur spontaneously or secondary to inflammation. Thermal stimuli (hot or cold) have been administered using hot or cold solutions instilled into visceral lumens and utilized to test for normal sensation and function, but rarely have been sources of clinical pain.[52] Ischemia of visceral structures has been produced by the occlusion of visceral vasculature. Experimentally and clinically, the effects of such occlusion are dependent upon collateral bloodflow and metabolic activity of the selected organ. Venous congestion has mixed ischemic and mechanical components and so could also be a source of pain. The most commonly utilized experimental visceral stimuli are mechanical stimuli, such as the probing and stretch of visceral structures or the distension of hollow organs using fluids or foreign bodies. Mechanical stimuli may mimic what is observed in certain pathological pain states (e.g. bowel obstruction) and the pattern of mechanical stimulation may be important as it has been proposed to be the source of pain in functional bowel disorders.

Due to the fact that the hollow organs of the gastrointestinal tract are readily accessible through natural orifices, the earliest clinical studies of visceral sensation used balloon distension of esophagus, stomach, small bowel, large bowel, and rectum as their visceral stimuli. The advantages of balloon distension of hollow organs are many, foremost being that balloon distension reproduces pathologically experienced pain in humans in terms of intensity, quality, and area to which the sensation is referred. Hollow organ distension at constant pressure produces sensations and responses that are reliably reproducible and easily controlled by the experimenter.

Nonhuman animal models

There are over 50 different models of visceral pain that have been described, but only a few have been utilized in more than one laboratory.[2] The recent past has seen a development of models that approximate physiological and behavioral responses similar to that of human visceral pain. One of the earliest models, the chemically induced writhing model in rodents, is produced by injecting irritant chemicals into the peritoneal cavity. This model has found less utility with the development of other models since the intraperitoneal injections did not selectively activate specific viscera, frequently yielded false positives when used to screen potential analgesic drugs, and are ethically questionable as they are associated with a persistent stimulus from which the animal cannot escape. Current models of visceral pain are more likely to utilize mechanical (e.g. distending) stimuli of controllable duration or chemical stimuli applied directly to relevant targets, thus permitting selectivity with respect to site of stimulation.

Balloon distension of hollow organs, principally along the gastrointestinal tract, is the most widely used experimental stimulus of the viscera. As noted above, experimental balloon distension of the gastrointestinal tract in humans has been established to reproduce pathologically experienced pain in terms of intensity, quality, and the area to which the sensation is referred. Whereas distending stimuli have been established as adequate for hollow organs, occlusive, ischemic, and irritant stimuli have been tested as adequate stimuli in other organs. Because inflammation of the urinary bladder is commonly associated with reports of pain and urgency in humans, experimental models of bladder irritation, including a model of cystitis, have been developed in rodents.[53] Kidney stones are undeniably painful in humans and a model of artificial ureteral calculosis has been developed in rats.[54] Occlusion of blood supply to most viscera is associated with pain and ischemia/anoxia is thus considered an adequate stimulus in the viscera. Accordingly, models of coronary artery occlusion and ischemia of abdominal visceral organs have been reported.[55]

Visceral pain is not a unitary entity and so there is a need for more than one visceral pain model. It is difficult to equate pain due to infection of the normally sterile urinary bladder with painless colons containing a sewer of the same infective organisms. Some differences between organ systems are clearly developmental in that organs which derive from midline structures (i.e. the gut) are associated with bilateral sensations, highly generalized responses and processing bilaterally within the spinal dorsal horn. In contrast, those organs which derive from unilateral structures (i.e. kidneys, ureters) generally have lateralized sensations, more regionalized responses, and lateralized spinal processing. The use of multiple models and multiple types of noxious stimuli applied to different organ systems allow us to distinguish the generalities of visceral pain from its mechanistic specifics.

MECHANISMS OF VISCERAL HYPERSENSITIVITY

Inflammation as a mechanism

A potent modifier of behavioral, neuronal, autonomic, and motor responses to visceral stimulation in experimental models inflammation has been commonly used to produce visceral hypersensitivity. The presence of inflammation in visceral structures frequently, but not universally, leads to reports of pain and sensitivity to mechanical and chemical stimuli. Cystitis, esophagitis, gastritis, duodenitis, ileitis, colitis, and proctitis all have evidence of mucosal inflammatory changes, as a hallmark finding. However, profound inflammatory changes of the mucosal lining, such as occurs with ulcerative colitis, may present with nonpainful, bloody stools.

Inflammation produces profound changes in the responsiveness of subsets of previously unresponsive visceral primary afferents and the term "silent" afferents has been coined.[56] These afferents are normally nonreactive to most stimuli, but in the presence of products of inflammation become spontaneously active and highly reactive to mechanical stimuli, such as distension. Silent afferents have been frequently noted in visceral structures forming up to 50 percent of the neuronal sample,[57] but are only infrequently noted in cutaneous structures. The lack of sensitivity of the viscera at baseline may relate to the sparcity of active visceral afferents which are quantitatively fewer per unit area than similar measures of cutaneous afferents. Because they are few, increased activity may be necessary in order to cross a threshold for perception.

Spinal neurons responsive to visceral stimuli also change their responsiveness to visceral stimuli in the presence of inflammation and when other sensitizing manipulations have been performed (see, for example, Refs 58, 59). Whether this is due to increased afferent activity, altered intrinsic properties of dorsal horn neurons, or altered modulatory influences within the central nervous system is unknown. It is likely that all of these separate mechanisms contribute in some way to the final sensitized state.

Whereas acute inflammation is often obvious with the hallmark features of redness, swelling, pain, and warmth, the more subtle changes related to chronic inflammation are often difficult to identify. Progressive fibrosis, mast cell infiltration, and altered oxidative stress markers all suggest that an ongoing indolent inflammatory process may be present that has sensory consequences equal to that of acute inflammation. Mast cell infiltration has been implicated in the hypersensitivity states of irritable bowel syndrome[60] and interstitial cysititis,[61] which has, in turn, prompted treatment with antihistamines and cromolyn-related compounds with mixed benefits. Mast cells have been observed to cluster around nerve bundles[62] and have been noted to express estrogen receptors[63] which, in turn, suggests a mechanism for menstrual cycle-associated exacerbations of some visceral pains.

More subtle than the histologically identifiable alterations in cell distribution are the biochemical changes that indicate a low level of chronic inflammation. Alterations in measures of oxidative stress have been observed in several hypersensitivity disorders, such as fibromylagia[64] and chronic fatigue syndrome,[65] and form a basis for sensory changes in the absence of histological changes. Use of antioxidant/micronutrient therapies (e.g. vitamins C and E, selenium) has had reported utility in the treatment of painful visceral disorders, such as chronic pancreatitis.[66]

Stress as a mechanism

Cutaneous and visceral sensation appears to differ in relation to the effect of stress on the magnitude of responses to stimulation. Although stress-induced analgesia (or hypoalgesia) has been a long-recognized phenomenon associated with cutaneous sensation, it would appear that stress-induced hyperalgesia is the correlate phenomenon associated with visceral sensation. Clinically, stressful life events have been viewed as classic triggers for the evocation of diffuse abdominal complaints of presumed visceral origin.[9] It is the rule, rather than the exception, that stressful life events, unless coupled with other major physiological events such as pregnancy, lead to an exacerbation of underlying pain disorders. A prominent role for stress in the pathophysiology and presentation of multiple clinical pain states, including irritable bowel syndrome, Crohn's disease, interstitial cystitis, rheumatoid arthritis, and psoriasis, has been well documented.[67, 68, 69, 70, 71] Using IC as a specific example, more than 60 percent of IC patients report symptom exacerbation by both acute and chronic stress, and clinical studies have shown that acute stress increases bladder pain and urgency in these individuals.[72, 73, 74, 75, 76] Not only is there a significant positive relationship between stress and the IC symptoms of pain and urgency, but as severity of the disease increases, the relationship between stress and symptom manifestation becomes even more evident.[76]

In nonhuman animal models, acute exposure to numerous stressors (footshock, water avoidance, forced swimming, cold water swim) can produce stress-induced analgesia.[77] However, in these same model systems when the stress is perceived as uncontrollable, chronic, or unpredictable, it may induce long-term pathophysiological changes presumed to be the mechanisms of stress-induced hyperalgesia. It is notable that stress-induced analgesia and stress-induced hyperalgesia can be apparently coexistent. Classic behavioral stressors, such as restraint or cold-water swim, produce an elevation in thresholds for the evocation of responses to thermal stimuli (stress-induced analgesia), but the same animals

have an increased vigor of visceromotor responses to colorectal distension (visceral hyperalgesia[78, 79]). This phenomenon appears to be associated with early-in-life events,[78] genetics,[80] and can be modified by gonadal hormones, neurokinins, corticotrophin-releasing factor, and mast cell function. Robbins *et al.*[81] has demonstrated similar phenomena in association with urinary bladder sensation and function.

A neurophysiological correlate of the phenomenon of stress-induced hyperalgesia was demonstrated by Qin *et al.*[82, 83, 84] In their studies, they injected glucocorticoids or aldosterone into the amygdala, manipulations which are known to produce increased measures of anxiety in animal subjects. These manipulations also produced a hypersensitivity to visceral stimulation as measured by an increased vigor of both visceromotor responses and responses of spinal dorsal horn neurons to colon or urinary bladder distension. This suggests the potential for a chicken–egg relation, where visceral pain (which produces anxiety) may activate the mechanisms of stress-induced hyperalgesia, thereby leading to visceral hypersensitivity which produces more anxiety, and on and on. Logically, a cotreatment of both pain and anxiety would seem to have the greatest utility.

Developmental changes as a mechanism

The neonatal period is a critical period of development related to visceral sensation and function. Multiple lines of convergent evidence suggest that neural monitoring and control systems are rapidly developing at both peripheral and central sites during the mid- to late-neonatal period. For example, in rats, the spinobulbospinal reflexes associated with micturition develop at two to three weeks postpartum and are associated with alterations in transient glutamatergic receptor expression.[85] Neurotrophins, which in rats are at minimal levels at birth in visceral tissues such as the urinary bladder, increase during development and have maximal levels present two weeks after birth with subsequent reductions with additional development.[86] Taken together, these findings indicate that the viscera and the neural structures associated with sensation and motor function are rapidly changing in the neonatal period and infancy periods and, as such, are susceptible to modifications by factors, such as inflammation. Control systems related to nociceptive processing are also developing at the same time[87] and are associated with progressive increases in glycinergic and GABAergic spinal inhibitory influences from birth through infancy.[88] A postnatal switch in GABAergic control of nociceptive reflexes has been observed to occur[89] and altered expression of μ-opioid receptors occurs in the same time period.[90] In rats, inflammation of the hindpaw has been demonstrated to produce primary afferent terminal expansion within lamina II,[91] III, and IV[92] of the spinal dorsal horn when performed in the neonatal, but not adult periods, and so a similar expansion of primary afferent terminals due to visceral inflammation would seem likely. A series of studies by Al-Chaer *et al.*,[93, 94] demonstrated that similar long-lasting effects resulting from neonatal exposure to nociceptive stimuli also occur in visceral pain systems: neonatal exposure to either repetitive colorectal distension (CRD) or repetitive application of mustard oil to the colorectal region resulted in increased abdominal withdrawal reflexes to CRD, increased responses of primary afferents to CRD, and increased spinal neuronal responses to CRD in rats tested as adults. Importantly, these effects did not require an identifiable change in colonic histopathology in the adult animals. Randich *et al.*[95] noted similar persistent developmental and experiential influences following neonatal inflammation of the bladder.

At the present time, there has been only a limited amount of work performed in humans, but seminal studies by Fitzgerald,[96, 97] and expanded on by others (for review, see Ref. 98), have provided evidence that early-in-life exposure to painful cutaneous stimuli can lead to later-in-life increases in sensitivity to the same stimuli. For example, extremely low birth weight (ELBW) infants who received multiple painful procedures as part of their neonatal care demonstrated some characteristics of autonomic hyperresponsiveness to needle sticks or other painful stimuli,[99] were more prone to clinical somatization,[100] and reported more pain as a component of their overall health status during adolescence.[101] These findings coupled with the nonhuman animal data are consistent with the view that neonatal events could prime an organism to respond with enhanced sensory reactions to inflammation and related painful stimuli as adults.

Neuropathic changes as a mechanism

A possible source of visceral hypersensitivity could be previous injury of the nerve pathways associated with visceral sensation. Interventional neurolytic procedures and intra-abdominal surgical interventions undoubtedly injure these nerve pathways, but there has been a long-unstated assumption that this form of nerve injury is without perceived consequence. The validity of such an assumption is certainly not established and there is evidence of visceral sensory consequences in models of neuropathic pain.[102] It will take future epidemiologic studies to properly assess such possibilities.

CONCLUSIONS

Visceral pain differs from other pains in many ways. This is not to say that there are no similarities. Primary afferent cell bodies associated with visceral nociception reside within dorsal root ganglia and the initial processing of sensory information occurs at the level of the dorsal horn

of the spinal cord or in the brain stem (e.g. vagal and trigeminal inputs). Many sites of higher processing in the brain are activated by both noxious visceral and noxious somatic stimuli. Where visceral pains differ from somatic pain is in the encoding properties of visceral primary afferent transducers and in their distribution to and within the central nervous system. The final consequence of these dissimilarities is a difference in localization and a difference in the magnitude of emotional and autonomic responses to visceral stimuli. Persistent visceral pain is also different from other pains in that it represents a hypersensitivity state that may be induced/exacerbated by inflammation, stress, developmental changes, and/or nerve injury. Due to its differences from other types of pain, the treatment of visceral pain may need to differ and may need to address the mechanisms of hypersensitivity rather than simple organ pathology. At present, clinical practice is the use of the same therapeutics for pain of any type. With additional information, it may become possible to determine treatments that are selective for persistent visceral pains.

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4

Genetics of chronic pain: crucial concepts in genetics and research tools to understand the molecular biology of pain and analgesia

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KEY LEARNING POINTS

- The genetics of pain is a complex trait, due to a combination of genetic and environmental factors.
- Methodological and technological developments in genetics make the study of the genetics of pain tenable.
- Measurement in pain genetics research is a unique and critical consideration.
- Common genetic influences on analgesic drugs are a critical field of pain research.

NOTE

This chapter is organized into two sections: the first section provides an introduction to concepts of and tools for the study of genetics and genomics; the second section delves into research-related issues unique to the study of pain.

CONCEPTS AND TOOLS IN GENETICS AND GENOMICS

INTRODUCTION

With few exceptions, all of the cells in the human body contain the same genetic material in the form of deoxyribonucleic acid (DNA). DNA is composed of a collection of functional units termed *genes* (collectively referred to as a *genome*) that provide the instructions for the synthesis of all ribonucleic acid (RNA)-based transcripts (an intermediary of most gene expression). In turn, these RNA transcripts provide the basis for the translation of all human proteins. However, different cell types each synthesize (or "express") a unique subset of the total possible RNA species and proteins encoded for by DNA. These differences in expression are the primary basis for the different cell types (as defined both in terms of their structure and function), as well as the cooperative assembly of various cell types into tissues and organs. The occurrence of changes in the nucleotide composition of a DNA molecule (a nucleotide is the "quantum unit" of

DNA), either through variations or mutations, can modify gene expression and resultant protein synthesis, thus altering cell structure and function. These genotypic changes lead to phenotypic changes that are observed as neuronal abnormalities, altered pain sensations, and/or a variety of medical conditions.

The Human Genome Project has increased our understanding of the contribution of genetics to health and human disease. While its initial goal was the mapping of the human genome, the project has expanded to map the genomes of many organisms. In addition, the Human Genome Project will determine the common population variations in given genomes, as well as their expression at both the RNA (transcriptome) and protein (proteome) levels. An in-depth description of the Human Genome Project can be found at www.genome.gov.

GENES AND CHROMOSOMES

Genes determine hereditary traits through the provision of precise instructions for cellular activity. Genes are both the functional and physical unit of heredity passed from parent to offspring. A gene is composed of a linear segment of DNA that encodes instructions for the synthesis of RNA molecules, which in turn provide the instructions for the synthesis of proteins. Each DNA molecule contains from tens to thousands of genes.

The nucleic acid sequence of a DNA molecule is encoded by four repeating nucleotide bases: adenine (A), cytosine (C), guanine (G), and thymine (T) (**Figure 4.1**). A DNA molecule is composed of two strands of nucleotides with the nucleic acids facing inward in an antiparallel fashion and the sugar-phosphate backbone forming a ladder-like structure which twists for stability. Nucleotide bases on each side of the ladder are linked by hydrogen bonds to form a base pair, with adenine coupled with thymine and guanine coupled with cytosine.

DNA molecules are wound around histone protein complexes that provide structural support and regulatory functions. This structure permits a remarkable amount of compaction, which results in condensed superstructures called chromosomes. Nucleated cells of humans have 23 pairs of morphologically distinct chromosomes, with one chromosome of a pair inherited from each parent. There are 22 autosomes and a pair of sex chromosomes. The chromosomes that form each pair are termed *homologs*, and with the exception of a set of genes harbored in the sex chromosomes, each chromosome pair provides two copies of each gene. The two copies of the gene are called *alleles*. The two alleles are referred to as *homozygous* if their sequence is the same and *heterozygous* if each allele's sequence is different. Chromosomes can be isolated from cells, stained and visualized by microscopy with the total chromosomal set of a cell termed a *karyotype* (**Figure 4.2**). Publication of the human genome sequence in 2000 provided more precise estimates of the position of genes and has largely superseded the use of karyotype analysis (i.e. chromosome banding). However, gross chromosomal abnormalities such as extra, missing, or broken chromosomes are still commonly identified by examining the karyotype.

In addition to the nuclear genome, DNA is contained in mitochondria. The mitochondrial genome is a compact circular DNA molecule and exists in multiple copies within each mitochondrion. The number of mitochondria found in each cell type is dependent on the energy requirements of that cell. Neurons are among the most mitochondrion-rich cell types. The human mitochondrial genome is composed of 37 genes, including 24 genes that encode RNA end-products (2 ribosomal RNAs (rRNA) and 22 transfer RNAs (tRNA)). Mitochondrial genomes are transmitted matrilineally to offspring as the only gamete that contains both cytoplasm and organelles is the human egg.

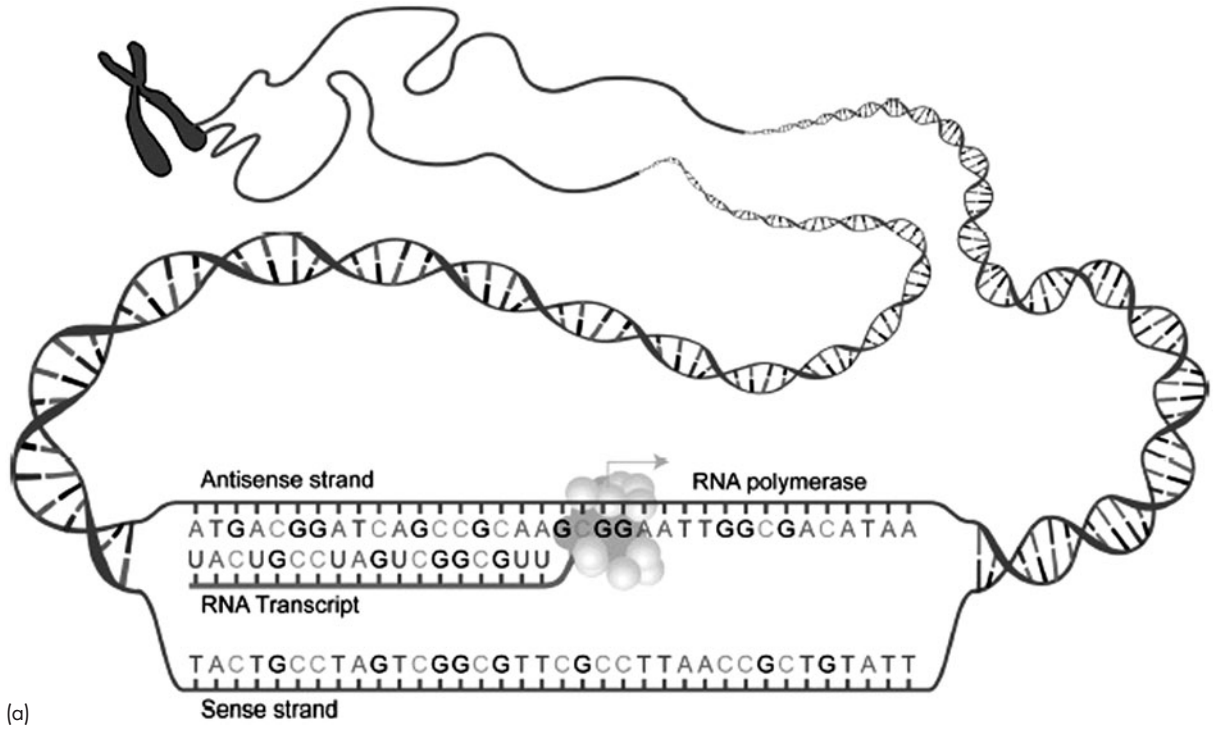
GENE EXPRESSION AND PROTEIN SYNTHESIS – REGULATION OF GENE EXPRESSION

Genes are transcribed into complementary single-stranded molecules of genetic material composed of RNA (**Figure 4.1**). The primary differences between DNA and RNA are the presence of a hydroxyl group at the 2′-position of the ribose sugar and the use of uracil (U) to replace thymine as the base complementary to adenine. Messenger RNA is then translated into the amino acid sequence of a protein at a cellular structure called the ribosome.

Subsets of RNA molecules serve as the end-product of gene expression. One subset, the rRNA genes, expresses only RNA which combines with proteins to form ribosomes that participate in protein translation of messenger RNA (mRNA). The other subset is tRNA genes whose products participate in protein synthesis by donating amino acids to a growing protein polypeptide chain. Only a small fraction of each RNA transcript is translated.

Most genes are organized into two main regions, the promoter and the coding regions. The promoter region lies immediately upstream of the coding region. A specific sequence of nucleotides in the promoter region interacts with protein complexes termed transcription factors in a dynamic manner to determine each gene's unique expression pattern (i.e. timing, quantity). In addition, transcription factors provide genes with the ability to interact with and respond to changes in the cellular environment.

The coding region is composed of exons (i.e. the portions of genes that are included in the mature mRNA) and introns (i.e. the portions of genes that are initially transcribed but are later processed, or spliced, out of the mature mRNA). The outer ends of genes are often untranslated and are termed the 5′- (the upstream or beginning) and 3′- (the downstream or end) regions



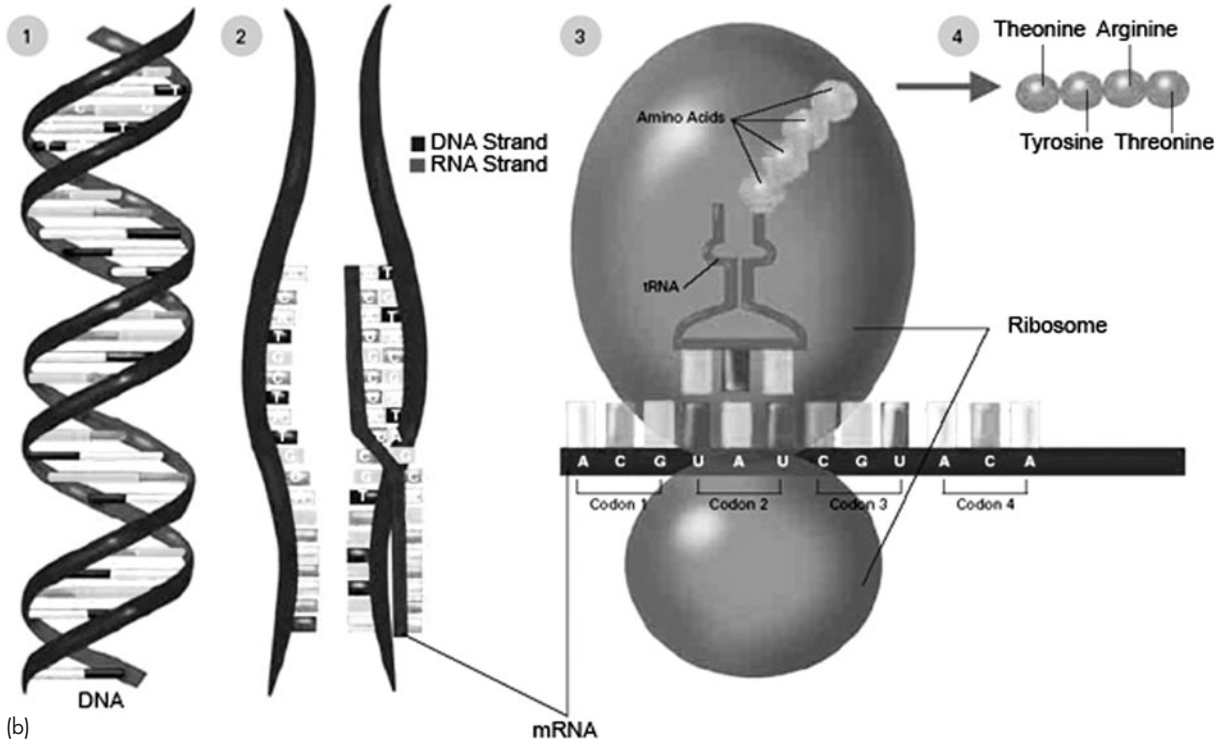


Figure 4.1 The structure of DNA contains information that is *transcribed* into RNA and subsequently *translated* into proteins. (a) The DNA molecule consists of two long strands of nucleotides that are complementary and which coil into a double helix for stability. The two strands (i.e. sense, antisense) are held together by hydrogen bonds between complementary nucleic acid bases: A with T, G with C. The double helix opens and one side is transcribed into a single complementary strand of mRNA. (b) The resulting mRNA is then translated into amino acids (read in units of three adjacent nucleotides, or *codons*) and a peptide chain is formed at a cell structure called the ribosome. The amino acid chain may be further processed to form a mature protein. (a) Reprinted from National Human Genome Research Institute. *Online Education Kit: Bioinformatics: Finding Genes*. Bethesda, MD, USA: National Human Genome Research Institute, last updated: April 27, 2007. Available from: www.genome.gov/25020001. (b) Reprinted from National Institute of General Medical Sciences. The New Genetics. Bethesda, MD, USA: National Institute of General Medical Sciences, 2006: 13. Available from: http://publications.nigms.nih.gov/thenewgenetics/index.html.