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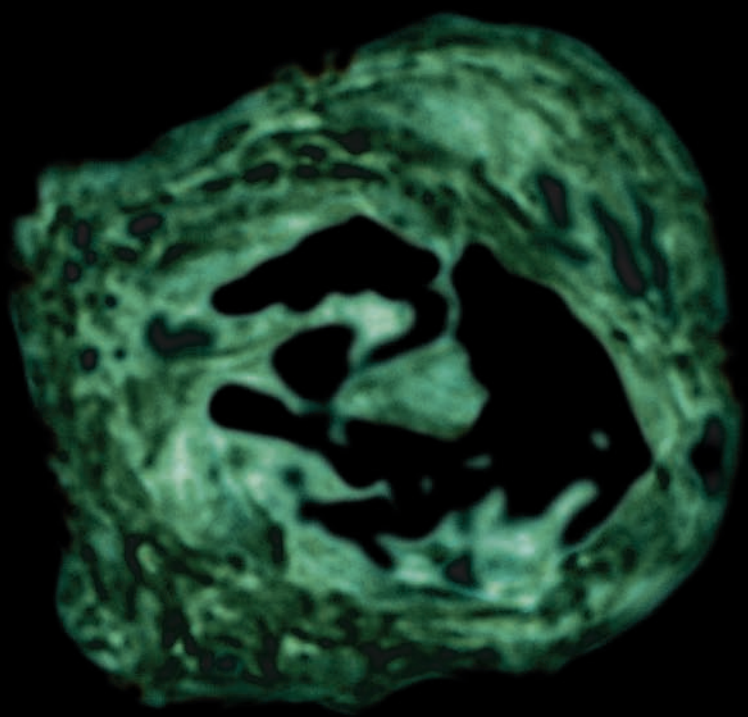
2008

Wrist ligaments-
innervation patterns and ligamento-muscular reflexes



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WRIST LIGAMENTS

INNERVATION PATTERNS AND LIGAMENTO-MUSCULAR REFLEXES

Elisabet Hagert

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Stockholm 2008

This thesis is the result of a research collaboration between Karolinska Institutet and Umeå University, Institute of Integrative Medical Biology, Section of Anatomy and the University of Gothenburg, Sahlgrenska Academy, Institute of Clinical Sciences.

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I believe a leaf of grass is no less than the journey work of the stars,
And the pismire is equally perfect, and a grain of sand, and the egg of the wren,
And the tree-toad is a chef-d'oeuvre for the highest,
And the running blackberry would adorn the parlors of heaven,
And the narrowest hinge in my hand puts to scorn all machinery.

- Walt Whitman, Song of Myself

To Ulf, Sofia and my parents

Abstract

Objectives: The principal role of wrist stability is to facilitate the dexterous hand in a multitude of functions. To achieve joint stability, in general, a fine interaction of both static and dynamic elements is of essence. While static joint stability is constituted by the anatomical congruity of joint surfaces and the ligamentous restraints acting to limit joint translations, the dynamic aspects of joint stability chiefly concern sensorimotor control of the muscular forces acting on a joint. Sensorimotor functions require a presence of sensory end organs in the joint, afferent projections from the joint to the spinal cord for fast neuromuscular control, as well as supraspinal integration of sensorimotor information. The wrist joint, with its complex anatomy and ability to facilitate hand functions, is hypothesized to possess innate sensorimotor functions, making it ideal for research on the dynamics of joint neuromuscular control.

Methods: By using staining for HTX and the immunohistochemical markers p75, PGP 9.5, S100 and trkB, the general morphology, ligament composition and presence of nerves and mechanoreceptors was analyzed in the dorsal radiocarpal (DRC), dorsal intercarpal (DIC), scaphotriquetral (STq), scapholunate interosseous (SLI), scaphotrapeziotrapezoid (STT), radioscaphoid (RS), scaphocapitate (SC), radioscaphocapitate (RSC), long radiolunate (LRL), short radiolunate (SRL), ulnocarpal (UC), ulnolunate (UL), lunotriquetral interosseous (LTqI), triquetrocapitate (TqC) and triquetrohamate (TqH) wrist ligaments (Papers I-III). Using ultrasound technique, a fine-wire electrode was inserted into the SLIL on 9 healthy volunteers. The ligament was thereafter stimulated and the EMG activity in four forearm muscles recorded while the wrist was kept in isometric flexion, extension, radial, and ulnar deviation (Paper IV).

Results: Wrist ligaments have a variable degree of innervation, which is reflected in the general composition of the ligament. The radiovolar ligaments are primarily dense collagenous structures with little or no innervation. The dorsal and triquetral ligaments, on the other hand, have large loose connective tissue regions where nerves, mechanoreceptors and vessels abound. After stimulation of the SLIL, muscle reactions (excitatory or inhibitory) were observed in the forearm muscles at various time intervals. An immediate ligamento-muscular reaction was observed in extensor carpi radialis brevis (ECRB) in flexion and in flexor carpi radialis/ulnaris (FCR/FCU) in extension, radial and ulnar deviation. Later cocontraction reactions, with simultaneous activation of agonist/antagonist muscles, occurred around 150 ms after stimulation of the SLIL.

Conclusions: Dense ligaments with sparse innervation are suggested to be primarily mechanically important ligaments. The dorsal and triquetral ligaments are, on the other hand, regarded as sensory important ligaments. These all emanate from the triquetrum and are, thus, able to signal in all wrist positions and motions. The immediate reactions in antagonist muscles are likely to have joint protective functions. Later cocontraction reactions indicate an integrated supraspinal control to stabilize the wrist joint.

Key words: *wrist ligaments, mechanoreceptors, proprioception, sensorimotor functions, neuromuscular control, ligamento-muscular reflexes, immunohistochemistry.*

List of publications

This thesis is based on the following manuscripts, which will be referred to in the text by their Roman numerals.

I. Hagert E, Ljung B-O, Forsgren S.
General innervation pattern and sensory corpuscles in the scapholunate interosseous ligament.
Cells Tissues Organs 2004;177:47-54.

II. Hagert E, Forsgren S, Ljung B-O.
Differences in the presence of mechanoreceptors and nerve structures between wrist ligaments may imply differential roles in wrist stabilization.
Journal of Orthopaedic Research 2005 Jul;23(4):757-63.

III. Hagert E, Garcia-Elias M, Forsgren S, Ljung B-O.
Immunohistochemical Analysis of Wrist Ligament Innervation in Relation to Their Structural Composition.
Journal of Hand Surgery (Am.) 2007;32A:30-36.

IV. Hagert E, Persson JKE, Werner M, Ljung B-O.
Evidence of Wrist Ligamento-Muscular Reflexes. A Neurophysiological Study.
Submitted.

Table of contents

List of abbreviations

ACL	Anterior cruciate ligament
AIN	Anterior interosseous nerve
BDNF	Brain-derived neurotrophic factor
DIC	Dorsal intercarpal ligament
DIN	Dorsal interosseous nerve
DRC	Dorsal radiocarpal ligament
DRG	Dorsal root ganglion
DRUJ	Distal radioulnar joint
dSLIL	Dorsal scapholunate interosseous ligament
ECRB	Extensor carpi radialis brevis muscle
ECRL	Extensor carpi radialis longus muscle
ECU	Extensor carpi ulnaris muscle
EMG	Electromyography
FCR	Flexor carpi radialis muscle
FCU	Flexor carpi ulnaris muscle
HTX	Hematoxylin-eosin
IP	Internal portion
IR	Immunoreactive, immunoreactivity
LRL	Long radiolunate ligament
LTqIL	Lunotriquetral interosseous ligament
MH	Meniscus homologue
NGF	Nerve growth factor
NF	Neurofilament protein
p75	Low-affinity nerve growth factor receptor p75
PAP	Peroxidase-antiperoxidase
PGP 9.5	Protein Gene Product 9.5

PIN	Posterior interosseous nerve
pLTqIL	Palmar lunotriquetral interosseous ligament
PN	Propriospinal neuron
RMS	Root mean square
RS	Radioscaphoid ligament
RSC	Radioscaphocapitate ligament
S100	S-100 protein
SC	Scaphocapitate ligament
SL	Scapholunate
SLIL	Scapholunate interosseous ligament
SRL	Short radiolunate ligament
STq	Scaphotriquetral ligament
STT	Scaphotrapeziotrapezoid ligament
TFCC	Triangular fibrocartilage complex
TqC	Triquetrocapitate ligament
TqH	Triquetrohamate ligament
trkA	Tyrosine kinase receptor A
trkB	Tyrosine kinase receptor B
UC	Ulnocarpal ligament
UL	Ulnolunate ligament
UTq	Ulnotriquetral ligament

GLOSSARY

Certain nomenclature will appear frequently in this thesis, and a brief definition is therefore included to assist the reader in understanding the core terminology of proprioceptive and sensorimotor research.

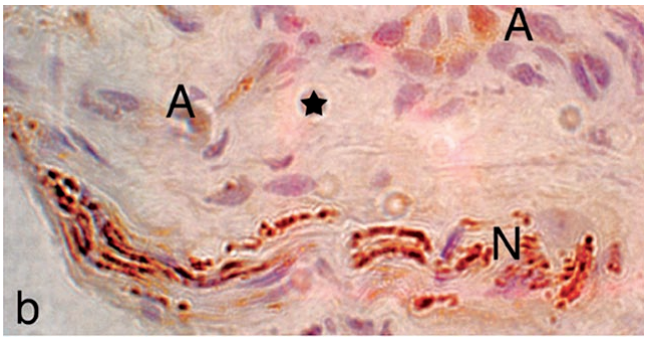
Carpal kinematics	[Gr: *kinein*, to move.] The study of wrist motions, without consideration of the forces causing the motion.
Carpal kinetics	[Gr: *kinesis*, act of moving.] The study of how the wrist sustains load, and how forces cause movement.
Kinesthesia	[Gr: *kin-* to move, *-esthesia*, to sense.] The conscious sensation of joint motion.
Ligamento-muscular reflexes	Reactions in muscles around a joint after influence by afferent sensory information from joint ligaments.
Mechanoreceptor	Sensory nerve ending responding to mechanical stimuli, i.e. changes in joint position and velocity.
Neuromuscular control	The unconscious efferent response to an afferent signal (i.e. from mechanoreceptors) concerning dynamic joint stability.
Proprioception	[Lat: *proprio-*, one's own, *-ception*, to perceive.] Pertaining to the conscious and unconscious perception of movement, posture and joint position.
Sensorimotor functions	The field of proprioception dealing specifically with joint control. The definition entails the total integration of sensory, motor and central processes pertaining to joint stability.
Sensory corpuscle	[Lat: *corpuscle* -small body.] Denoting a specialized neural ending that elicits afferent impulses when stimulated. The mechanoreceptor (see above) is a type of sensory corpuscle.

Sources: 1) Dorland's Medical Dictionary; 2) Proprioception and Neuromuscular Control in Joint Stability (eds. Lephart SM, Fu FH).

THESIS AT A GLANCE

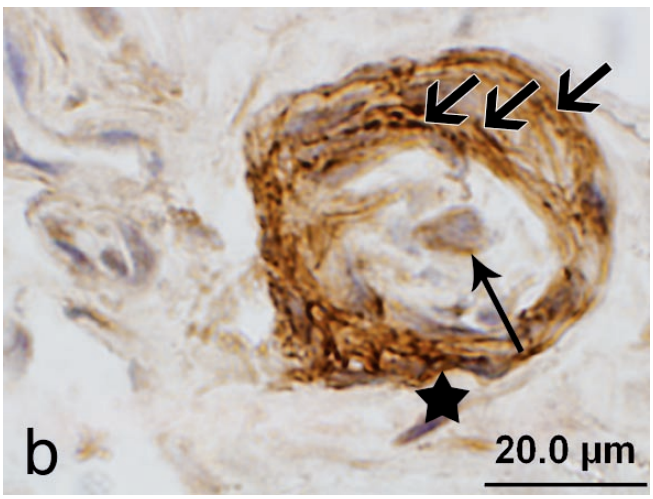
I. Evidence of innervation in the SLIL

Is the biomechanically important scapholunate interosseous ligament (SLIL) innervated?
Material: 9 SLIL specimens.
Methods: HTX stain and PGP 9.5, S100 and p75 immunohistochemistry; light-microscopy.
Conclusions: Nerves and mechanoreceptors are present in the SLIL.



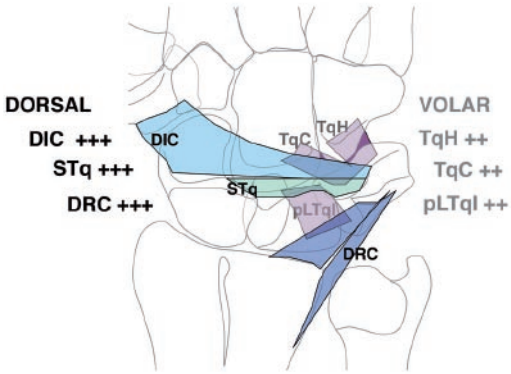
II. Differential innervation of wrist ligaments

Are other ligaments in the wrist innervated?
Material: 7 wrist ligaments from 5 normal, fresh specimens.
Methods: HTX stain and PGP 9.5, S100, p75, trkA and trkB immunohistochemistry; light-microscopy.
Conclusions: There is a varying degree of innervation (nerves/mechanoreceptors) in the wrist ligaments.



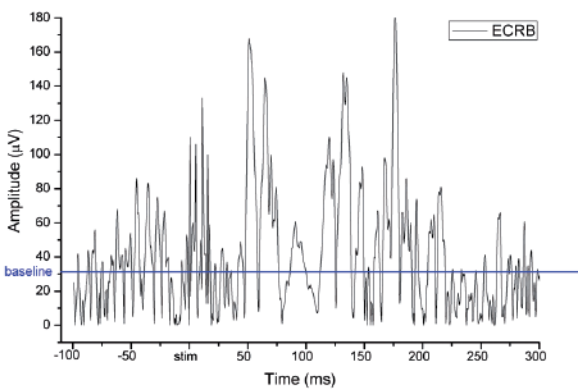
III. Mechanically and sensory important ligaments

Is there a correlation between ligament structure and degree of innervation?
Material: 14 wrist ligaments from 5 fresh-frozen cadaver specimens.
Methods: HTX stain and PGP 9.5, S100 and p75 immunohistochemistry, light and confocal microscopy.
Conclusion: The volar radial ligaments are dense structures with little/no innervation. The dorsal and triquetral ligaments have abundant innervation, and are considered sensory important ligaments.



IV. Wrist ligamento-muscular reflexes

Will stimulation of the SLIL elicit muscle reactions in the forearm muscles?
Material: 9 healthy volunteers.
Method: Electrical stimulation of the SLIL while monitoring EMG activity in four forearm muscles (ECRB, ECU, FCR, FCU), with the wrist in isometric flexion, extension, radial and ulnar deviation.
Conclusion: Immediate reactions in antagonist muscles are seen, suggesting joint protective functions. Later cocontractions of agonist/antagonist muscles indicate a supraspinal neuromuscular control to stabilize the wrist joint.



INTRODUCTION

"True stability results when presumed order and presumed disorder are balanced. A truly stable system expects the unexpected, is prepared to be disrupted, waits to be transformed."
- Tom Robbins

Wrist stability

The principal role of wrist stability is to facilitate the dexterous hand in a multitude of functions, from delicate finger manipulations and handling of tools, to lifting or sustaining heavy loads. To achieve joint stability, in general, a fine interaction of both static and dynamic elements is of essence. While static joint stability is constituted by the anatomical congruity of joint surfaces and the ligamentous restraints acting to limit joint translations, the dynamic aspects of joint stability chiefly concern proprioceptive control of compressive and directional muscular forces acting on a joint.

Traditionally, studies on the wrist have focused on the anatomical and mechanical dimensions of joint stability. Several exquisite publications have sought to bring an understanding to the complex nature of wrist kinetics and kinematics,[55,58,70,91,111] principally through experimental models exploring the biomechanics of wrist stability or clinical interpretations of wrist dysfunctions following injury or disease. Through these publications, a thorough understanding of wrist joint anatomy, kinetics and kinematics is now evident.[29]

The dynamic influences of muscles in wrist stability are, however, still largely unknown. Although publications exist on the physiological and architectural properties of the primary wrist movers,[10,16,56] the role of proprioception in wrist neuromuscular control has not been delineated.

Proprioception

The term *proprioception* is derived from Latin, "*proprius*", belonging to one's own, and "*-ception*", to perceive. The term was first established by the 1932 Nobel laureate in Physiology or Medicine, Sir Charles Scott Sherrington, who, in 1906,[89] defined proprioception as sensations arising in the deep areas of the body, contributing to conscious sensations (*"muscle sense"*), total posture (*"postural equilibrium"*) and segmental posture (*"joint stability"*).[53] Since then, the term proprioception has been used to intend any or all sensations pertaining to the locomotor system. As a result, there are multiple misperceptions on the concept and science of proprioception. To clarify this issue, the field of proprioception has received its own taxonomy, with sub-classifications relating to various fields of somatosensory, postural and neuromuscular control.[53]

A more precise expression for the function of proprioception in joint stability is, therefore, *sensorimotor function*. The term implies an interaction between sensory information arising in the joint and the motors (muscles) acting on the joint. More specifically, sensorimotor function entails the total integration of sensory, motor and central (spinal/supraspinal) adaptations in processes pertaining to functional joint stability.

Prerequisites for joint sensorimotor function

Based on the definition of sensorimotor function as the integration of sensory, motor and central processes, certain anatomical and physiological criteria, in short, must be fulfilled[49,93] (fig. 1):

a. Presence of *sensory end organs* (nerves and mechanoreceptors) in joint ligaments and/or joint capsule,
b. *neural projections* from the joint to the spinal cord (via the dorsal root ganglia),
c. local and segmental *interconnections to efferent α-motoneurons* for rapid joint control, as well as
d. *supraspinal relay* of proprioceptive information to the brain stem (medulla), cerebellum and somatosensory/motor cortex, for complete integration of sensorimotor functions.
e. Some researchers also propagate the presence of *local proprioceptive reflexes through γ-motoneurons*, where muscle spindles in the muscles around the joint are influenced directly by afferent information from the joint, through a so called *fusimotor effect*, allowing regulation and pre-programming of the muscles around the joint.[47]

The wrist joint, with its complex anatomy and ability to facilitate hand functions, is hypothesized to possess innate sensorimotor functions, making it ideal for research on the dynamics of joint neuromuscular control.

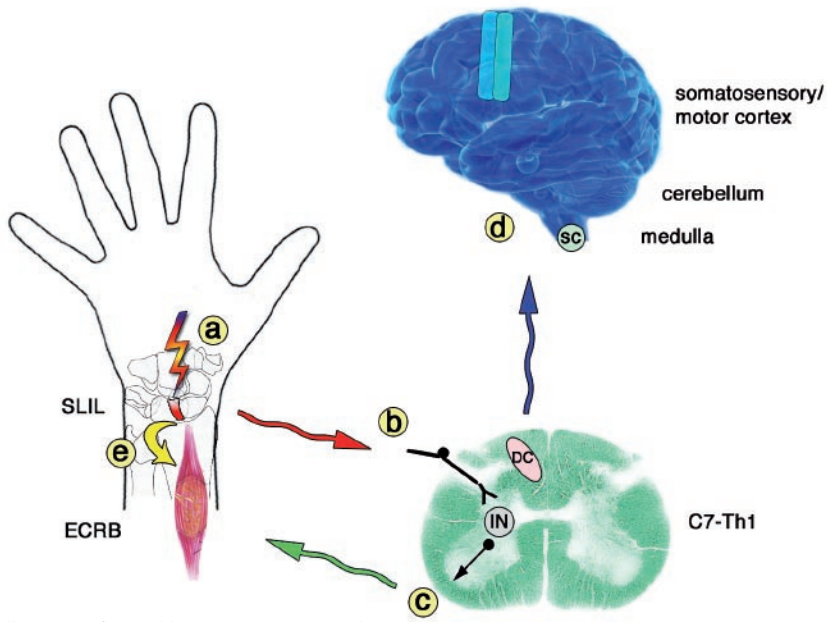


Figure 1
Schematic illustration of a possible wrist sensorimotor pathway.
(a) Sensory end organs in the wrist ligaments react to changes in joint rotation, velocity and/or noxious stimuli, signaling (b) neural projections from the joint, via the dorsal root ganglia, to the spinal cord, (c) where local and segmental interconnections to efferent α-motoneurons take place for rapid neuromuscular control, and, additionally, (d) supraspinal relay of proprioceptive information occurs along the dorsal column to the medulla, where it ascends along the spinocerebellar tracts to the cerebellum and sensory/motorcortex, for complete integration of sensorimotor functions. Some researchers also propagate the presence of local proprioceptive reflexes through γ-motoneurons (e), where muscle spindles in the muscles around the joint are influenced directly by afferent information from the joint, through a so called fusimotor effect, allowing regulation and pre-programming of the muscles around the joint.

AIMS OF THE INVESTIGATION

Based on the hypothesis that the wrist has innate sensorimotor functions, this thesis was aimed at investigating aspects pertaining specifically to the wrist joint, namely the presence of sensory end organs in wrist ligaments and afferent neural projections from ligaments to muscles acting on the joint (ligamento-muscular reflexes). In this context, the following questions were posed:

I. Are the wrist ligaments purely mechanically stabilizing structures, or is there evidence of sensorimotor functions as signified by the presence of sensory end organs (nerves/mechanoreceptors)?

II. If nerves and mechanoreceptors are present, how are they distributed within the ligament, and is the distribution equal throughout the wrist?

III. Does an innervated ligament influence the activity of the muscles acting on the wrist through ligamento-muscular reflexes?

IV. If ligamento-muscular reflexes can be recorded, when do they appear and what functions might they have in wrist neuromuscular control?

MATERIAL AND METHODS

"Nature composes some of her loveliest poems for the microscope and the telescope."
- Theodore Roszak

IMMUNOHISTOCHEMICAL AND MORPHOLOGICAL STUDIES (Papers I-III)

Wrist ligaments

In Paper I, the scapholunate interosseous ligament (SLIL) was harvested intraoperatively from four patients (2 women/2 men, mean age 52 years) undergoing total wrist arthrodesis due to radiocarpal osteoarthritis and, additionally, from five patients (2 women/3 men, mean age 61.4 years) who underwent thoracoscapular disarticulation due to malignant sarcoma of the upper arm.
In Paper II, a total of seven wrist ligaments were collected from this latter group of patients who had undergone disarticulation of the arm. The specimens were harvested within 0.5-6 hours after amputation.
In Paper III, the material was based on a cadaver population, where a total of fourteen wrist ligaments were collected from five fresh-frozen specimens (3 men, mean age 71, and 2 women, approximately similarly aged).
The wrist ligaments studied in Papers I-III were, in total, the dorsal radiocarpal (DRC), dorsal intercarpal (DIC), scaphotriquetral (STq), SLIL (entire SLIL in Papers I-II, dorsal SLIL in Paper III), scaphotrapeziotrapezoid (STT), radioscaphoid (RS), scaphocapitate (SC), radioscaphocapitate (RSC), long radiolunate (LRL), short radiolunate (SRL), ulnocarpal (UC), ulnolunate (UL), lunotriquetral interosseous (LTqI, entire in Paper II and palmar LTqI in Paper III), triquetrocapitate (TqC) and triquetrohamate (TqH) (fig. 2).

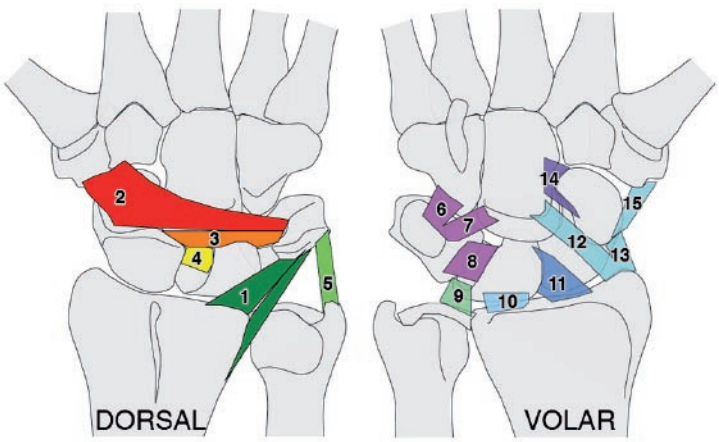


Figure 2
Schematic drawing of the wrist ligaments analyzed using immunohistochemical techniques (Papers I-III). The dorsal radiocarpal (1), dorsal intercarpal (2), scaphotriquetral (3), scapholunate interosseous (4), ulnocarpal (5), triquetrohamate (6), triquetrocapitate (7), palmar lunotriquetral (8), ulnolunate (9), short radiolunate (10), long radiolunate (11), radioscaphocapitate (12), radioscaphoid (13), scaphocapitate (14), scaphotrapeziotrapezoid (15) ligaments.

Immunohistochemical procedures

The ligaments were fixed overnight in a solution of 4% formaldehyde in 0.1 M phosphate buffer, pH 7.0. After rinsing, series of 7-8 µm thick sections were cut with a cryostat and subsequently processed for immunohistochemistry or demonstration of morphology. The tissues were stained with hematoxylin (HTX)-eosin for analysis of general tissue morphology. Peroxidase anti-peroxidase (PAP) staining was used as immunohistochemical method, hereby delineating the various regions of mechanoreceptors and nerve fascicles. Normal swine serum was used as normal serum. As primary antibodies polyclonal rabbit antibodies against Protein Gene Product (PGP) 9.5, S-100 protein (S100) and low-affinity nerve growth factor receptor p75 (p75). In Paper II, antibodies against tyrosine kinase receptors (trk) A and B were also used. After rinsing, incubation with secondary antibodies (swine anti-rabbit) was performed, followed by additional rinsing and exposure to peroxidase-labeled immunoglobulin (Ig) G. The slides were dehydrated and mounted in a microscopy mounting medium. When staining for PGP 9.5 and trkA, the slides were initially pretreated with acid potassium permanganate ($KMnO_4$), according to the methodology described by Hansson and Forsgren.[40]
In Paper III, the immunohistochemical procedure was complemented with EnVision detection. This technique implies the use of microwave antigen retrieval to reveal epitopes that may be hidden by the formaldehyde fixation.
For control purposes, incubation with primary antibodies was either omitted or replaced with normal rabbit serum, resulting in the elimination of specific stainings. A positive control was also performed, where the primary antibodies were used on tissues with known neural contents, resulting in appropriate immunoreactions (IR). In Paper II, blocking peptides were used against trkA and trkB, to additionally verify the staining of these antibodies.
For further details regarding the immunohistochemical procedures, see Papers I-III.

Imaging and analysis

Serial sections of ligaments were examined using light-microscopy techniques. The larger ligaments examined in Paper III were suture-marked to allow precise orientation of each ligament. These ligaments were sectioned at each end (proximal-distal or radial-ulnar) and each section mounted on the same slide, to allow concurrent comparison of the various regions of the ligament.
In Papers II-III, the degree of innervation was semiquantitatively assessed by analyzing serial sections of ligaments in various locations with regard to the presence of mechanoreceptors and nerve fascicles. If a specimen was found to be without innervation, additional sectioning, staining and analysis was performed.
In Paper III, the autofluorescent component of eosin in HTX-eosin staining was also used to study HTX slides using a fluorescence laser confocal microscope system (courtesy of the Karolinska Institutet Visualization Core Facility). This technique facilitated the analysis of ligament composition, as described in this paper.

NEUROPHYSIOLOGICAL STUDY (Paper IV)

Volunteers

For the neurophysiological experiment, nine healthy volunteers, 4 women and 5 men (mean age 26, range 21-28 years), were included. All subjects were healthy, with no history of wrist trauma or hand disability, and they participated voluntarily after signing consent forms. The dominant hand was examined in all subjects.

Ultrasound techniques

Electrode positioning
Ultrasound depiction of the SLIL is an established imaging modality,[24,44] and was used to ensure a correct positioning of the stimulating electrode in this experiment. A fine-wire stainless steel electrode with a distal uninsulated hook was pre-inserted into a puncture needle. Following identification of the dorsal region of the SLIL, and using sterile conditions, the needle was inserted into the dSLIL (fig. 3). As the needle was retracted, the hook of the electrode was maintained in the ligament. Imaging confirming correct needle position was obtained from all subjects.

Muscle imaging
To ensure a correct positioning of the surface electrodes to be used in the ensuing electromyographic (EMG) experiment, ultrasound imaging was also used to identify the extensor carpi radialis brevis (ECRB), extensor carpi ulnaris (ECU), flexor carpi radialis (FCR) and flexor carpi ulnaris (FCU) muscles. With the forearm in pronation, mimicking the position to be used in the experiment, the center of each muscle belly was identified with the muscle both relaxed and contracted, and a skin marker placed at the correct position.

Electromyographic procedure

EMG preparations
After cleaning with alcohol, skin abrasion and, if necessary, shaving, the Ag-AgCl surface registration electrodes were positioned according to Perotto and Delagi,[80] with the recording electrode at the skin marker described above, and the reference electrode placed about 20 mm proximally. The ground surface electrode was placed between the stimulating and the registration electrodes, and the stimulating anode electrode was placed on the dorsum of the hand, just distally to the insertion point of the stimulating cathode electrode located in the ligament. The electrodes were connected to a four-channel electromyograph (for additional details, see Paper IV).

Stimulation procedure
Four 1 ms square pulses were delivered into the dSLIL by the fine-wire electrode inserted into the ligament. The sensory threshold was determined in each subject as the minimum current strength felt in five out of ten stimulations. The experimental stimulus was 2-3 times the sensory threshold (mean 2.0 mA), and consistently below the threshold of pain.

Experimental protocol
The subject was seated with the arm in an armrest, with the shoulder slightly abducted and flexed, flexed elbow and pronated forearm. The wrist was positioned outside the armrest, to allow unconstrained wrist motion.
In a pilot study preceding the experiment, a negative control was performed where the ligament was stimulated while the subject relaxed the arm, which did not result in any reflex EMG activity. Previous studies have also reported that background muscle activity is necessary to detect any changes in muscle pattern,[50,102] hence the subjects performed isometric wrist flexion, extension, radial and ulnar deviation, respectively, while the motion was resisted manually by one of the examiners.
The dSLIL was stimulated with thirty consecutive stimulations at 2-second intervals, in each wrist position. The raw EMG signals were amplified, band pass-filtered at 20 to 10000 Hz and digitally sampled at a rate of 2.5 kHz from 100 ms before to 700 ms after the stimulus.

Statistical analysis

The average EMG data from the thirty stimulations was exported to and mounted in Microsoft Excel®. The data was divided into specific time intervals, where the pre-stimulus 100 ms interval was regarded as baseline (t_1), and the post-stimulus EMG (t_2) was divided into 20 ms time intervals, until 500 ms after stimulation.
The Excel sheets were thereafter imported in OriginPro®, where the data was rectified to allow analysis of the absolute EMG amplitude recorded. The root mean square (RMS) of each time interval was calculated, and each time interval after stimulation was compared to the baseline value using student's t-test (t_1-t_2). To reduce the risk of chance or mass significance, only statistically significant ($p<0.05$) changes in amplitude (μV) occurring in a majority of subjects (five or more) were noted as peaks of EMG activity.

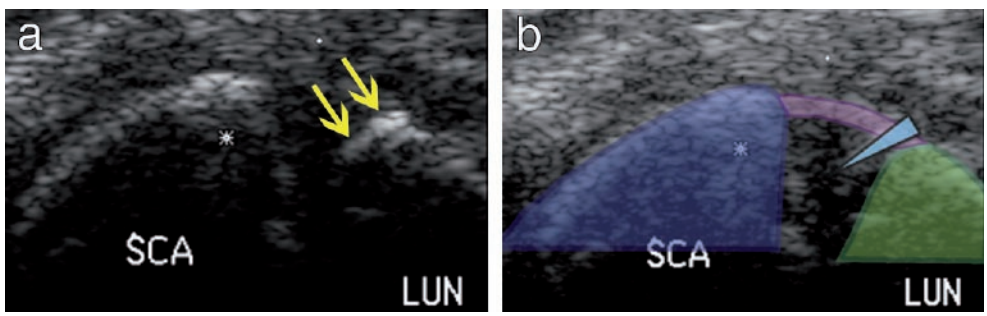


Figure 3
a) A transverse-dorsal ultrasound image of the dorsal surfaces of the scaphoid (SCA) and lunate (LUN). The inserted needle-point is visualized (arrows) in the scapholunate interval, positioned in the dorsal region of the SLIL. b) Colorization of image (a), scaphoid (blue), lunate (green) and dorsal SLIL (purple). The needlepoint is graphically illustrated in the ligament (blue arrow).

RESULTS

"All our knowledge has its origins in our perceptions." - Leonardo da Vinci

IMMUNOHISTOCHEMICAL STUDIES (Papers I-III)

General ligament composition

The general morphology of the wrist ligaments examined in Papers I-III was analyzed using HTX and, occasionally, p75 staining. In all three studies, the specimens exhibited a structural composition typically encountered in ligaments.
The core of the ligament, the *fascicular region*, consisted of parallel and densely packed collagen fibers, where no blood vessels or nerve fascicles could be found. Surrounding this fascicular region was a layer of loose connective tissue, the *epifascicular region*, throughout which nerves and vessels were seen coursing. When present, nerve fascicles, fine nerve fibers and mechanoreceptors were frequently seen in the vicinity of arterioles (fig. 4). The degree of innervation could, thus, be related to the degree of vascularity observed in the ligament.

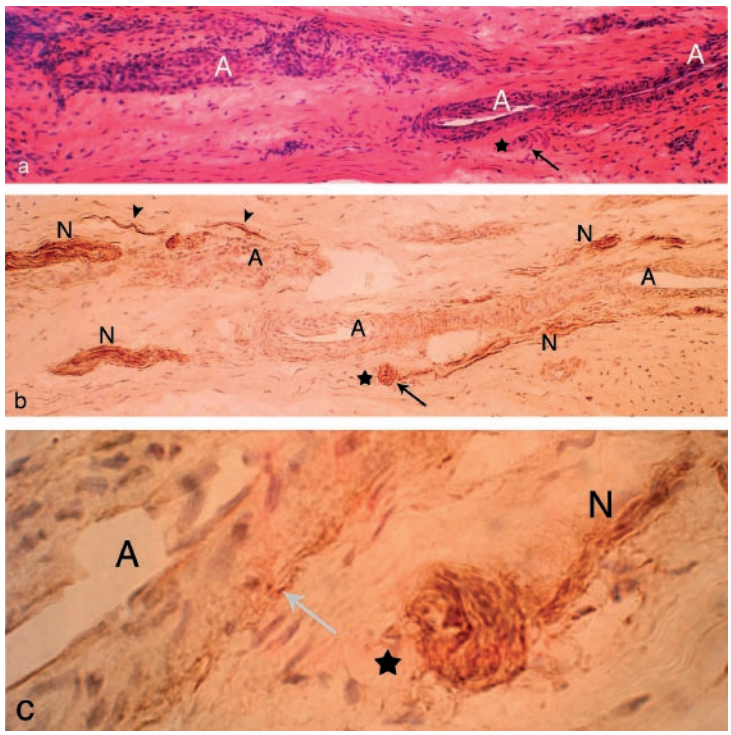


Figure 4
Overview of the SLIL, showing arterioles (A), several large (N) and small (arrowheads) nerve fascicles and a sensory corpuscle (arrow). a) HTX-eosin stain. b) p75. Strong p75 IR is seen in the nerve fascicles and the corpuscle. c) Higher magnification of a part of (b), where perivascular p75 IR (arrow) can be observed. Note the nerve fascicle in continuation with the corpuscle. (★) indicates corresponding regions in the images. Magnification x100 a-b, x400 c.

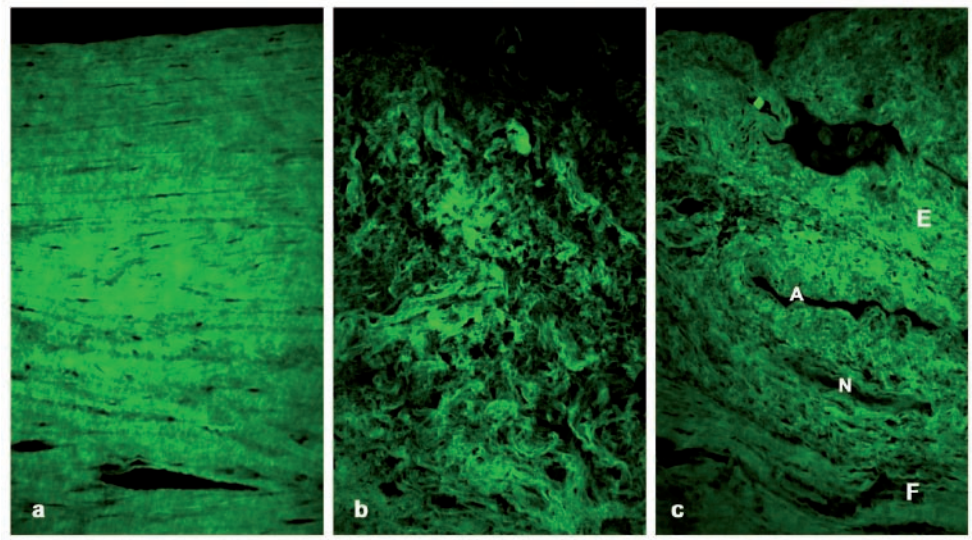


Figure 5
Comparison of the variable structural composition found in wrist ligaments, as seen in HTX stains using autofluorescent technique (magnification x10). a) Section of a UL ligament showing predominately densely packed parallel collagen fibers. b) Section of a SRL ligament consisting of disorganized loose connective tissue and no apparent collagenous structure. c) Section of an STq ligament with a vascularized (A) and innervated (N) epifascicular region (E) located close to the surface of the ligament and collagenous structures found in the deeper fascicular layers (F).

In Paper III, the ligament composition in sections stained for HTX was additionally examined using fluorescence laser confocal microscope. Using the lowest magnitude in the microscope (x2.5), varying ratios of fascicular to epifascicular regions in the wrist ligaments were revealed (fig. 5). Certain ligaments consisted predominately of dense fascicular regions, with only marginal epifascicular areas. These ligaments were rarely seen to be innervated with nerve fascicles and/or mechanoreceptors. Other ligaments, contrarily, had large epifascicular regions and, correspondingly, a pronounced presence of innervation. The SRL differed markedly from the other ligaments, in that it primarily consisted of disorganized loose connective tissue and no distinct collagenous structure. The presence of innervation and the degree of vascularity in the ligaments were, thus, both correlated to the magnitude of the epifascicular regions.

Patterns of innervation

To elucidate neural structures in the wrist ligaments, and aid in the classification of mechanoreceptors/sensory corpuscles, serial sections of each ligament were stained with different immunohistochemical markers, targeted toward different neural and perineurial structures. The specific antibodies were chosen based on their staining characteristics, as previously reported in studies on human and animal mechanoreceptors and nerve endings.[4,17,38,61,101,112]

Table 1. Classification of mechanoreceptors - based on Freeman & Wyke (1967) and Halata (1975), modified by E. Hagert

Type	Eponym/Name (descriptive)	Characteristics	Neurophysiological trait	Role in joint function	IR patterns in mechanoreceptors
I	Ruffini (dendritic)	Coil-shaped. Partial encapsulation. Arborizing nerve branches with bulbous terminals. 50-100 µm.	Slowly adapting Low-threshold	Static joint position Changes in velocity/ amplitude	Central axon – *PGP9.5, S100* Terminal nerve branches – *PGP9.5, trkB* Incomplete capsule – *p75*
II	Pacini (lamellated)	Rounded, ovular corpuscle. Thick lamellar capsule. 20-50 µm.	Rapidly adapting Low-threshold	Joint acceleration/ deceleration	Central axon – *PGP9.5, S100* Thick capsule – *p75*
III	Golgi-like (grouped dendritic)	Large, spherical. Partial encapsulation. Groups of arborizing and terminal nerve endings. >150 µm.	Rapidly adapting High-threshold	Extreme ranges of joint motion	Terminal nerve branches – *PGP9.5, S100, trkB* Incomplete capsule – *p75*
IV	Free nerve endings	Varicose appearance, often close to arterioles. Groups or single fibers.	Aδ fibers - fast C fibers - slow	Noxious, nociceptive, inflammatory	Axon – *PGP9.5, trkB*
V	Unclassifiable	Variable size, appearance, and degree of encapsulation.	Unknown	Unknown	Incomplete capsule - *p75* Variable IR pattern

Abbreviations:
Protein Gene Product 9.5 (PGP9.5); S-100 protein (S100); tyrosine kinase receptor B (trkB); low-affinity neurotrophic receptor p75 (p75); immunoreactions (IR).

The precise immunoreactions seen in different structures will be described below. In summary, the following general reactions were observed in Papers I-III (see table 1):
- PGP 9.5, a general nerve marker, was seen staining the axons of nerve fascicles, the central axon of the corpuscle as well as the terminal nerve branches within corpuscles.
- S100 marked the S-100 protein in Schwann cells, as well as in nerve fascicles and intra-corpuscular nerve endings.
- p75 was of great importance in identifying corpuscles, as it stained the perineurial cells of nerve fascicles, and, thus, the perineurial capsule of corpuscles.
- TrkB, which serves as a receptor for brain-derived neurotrophic factor (BDNF) and neurotrophin (NT)-4, was also observed in nerve fascicles and terminal nerve endings within the corpuscles. Specific IR toward trkA, a receptor for nerve growth factor (NGF) and NT-3, was, however, neither observed in nerve fascicles nor corpuscles.

Ruffini receptor ending
The Ruffini ending was first described by Italian histologist Angelo Ruffini in the 19th century. Synonyms for this ending are dendritic or spray ending. The Ruffini ending was the most prevalent of all sensory corpuscles observed in Papers I-III. This corpuscle is ovular in shape, approximately 50 µm in diameter, with an incomplete p75 IR perineurial capsule (fig. 6b). The afferent axon usually enters the corpuscle on the long side, where the continuation (the central axon) demonstrates PGP 9.5 and S100 IR (fig. 6a,d). The central axon arborizes into terminal dendritic nerve endings, which are visualized when staining for PGP 9.5, p75, trkB and S100 (fig. 6a-d).

Pacini corpuscle
In 1831, the Italian anatomist Filippo Pacini discovered the corpuscle that carries his name, the Pacini corpuscle. Though the eponym is primarily used for this receptor, it is at times referred to as a lamellated sensory corpuscle, indicating the thick lamellar capsule that characterizes this nerve ending. In the ligament, this receptor is quite small with a diameter of 20-50 µm. While the Pacini corpuscle was present in the wrist ligaments, it was quite rare as compared to the Ruffini ending. Histologically, a Pacini corpuscle consists of an afferent and central axon that was visualized when staining for PGP 9.5 (not shown), trkB and S100 (fig. 7a,b). The central axon is surrounded by layers of p75 IR perineurial lamellae, which constitute the distinguishing and characteristic thick capsule of the Pacini corpuscle (fig. 7c).

"Golgi Tendon Organ"
Named after Camillo Golgi, 1906 Nobel Laureate in Physiology or Medicine, the expression "Golgi tendon organ" is, in fact, a misnomer in terms of ligament innervation, and should be reserved for the specialized nerve ending found in the myotendinous junction.
The nerve ending intended in the ligament is a type of spray ending, belonging to the same family as the Ruffini ending.[94] More suitable names for this receptor include large Ruffini ending, grouped dendritic nerve ending or "Golgi-like".[39,103] It is characterized by a large (>150 µm/diam), partially encapsulated receptor with groups of arborizing nerve endings at its core. The capsular reactions were p75 IR, while the nerve endings in the separate compartments within the corpuscle were demarcated when staining for PGP 9.5, trkB and S100. It was rarely found in the wrist ligaments, only occasionally seen in DRC and DIC (see fig. 3, Paper II).

Unclassifiable corpuscles
A large number of corpuscles observed in the wrist ligaments were neither classifiable as Pacini nor Ruffini. These were receptors of variable sizes and appearances, often with incomplete p75 IR capsules, and their exact role in joint proprioception remains unknown.

Nerve fascicles
Nerve fascicles of varying dimensions were frequently observed in the vicinity of arterioles. The axon of the nerve fascicles were distinctly PGP 9.5 IR and, to an extent, trkB IR (fig. 7a). p75 IR was principally located in the perineurium of nerve fascicles, and S100 IR primarily seen in the Schwann-related cells (fig. 7b).

Arterioles
p75 IR was distinctly seen in the walls of arteries and arterioles, usually in the border between media and adventitia, as well as in within the adventitia (fig. 4b,c).

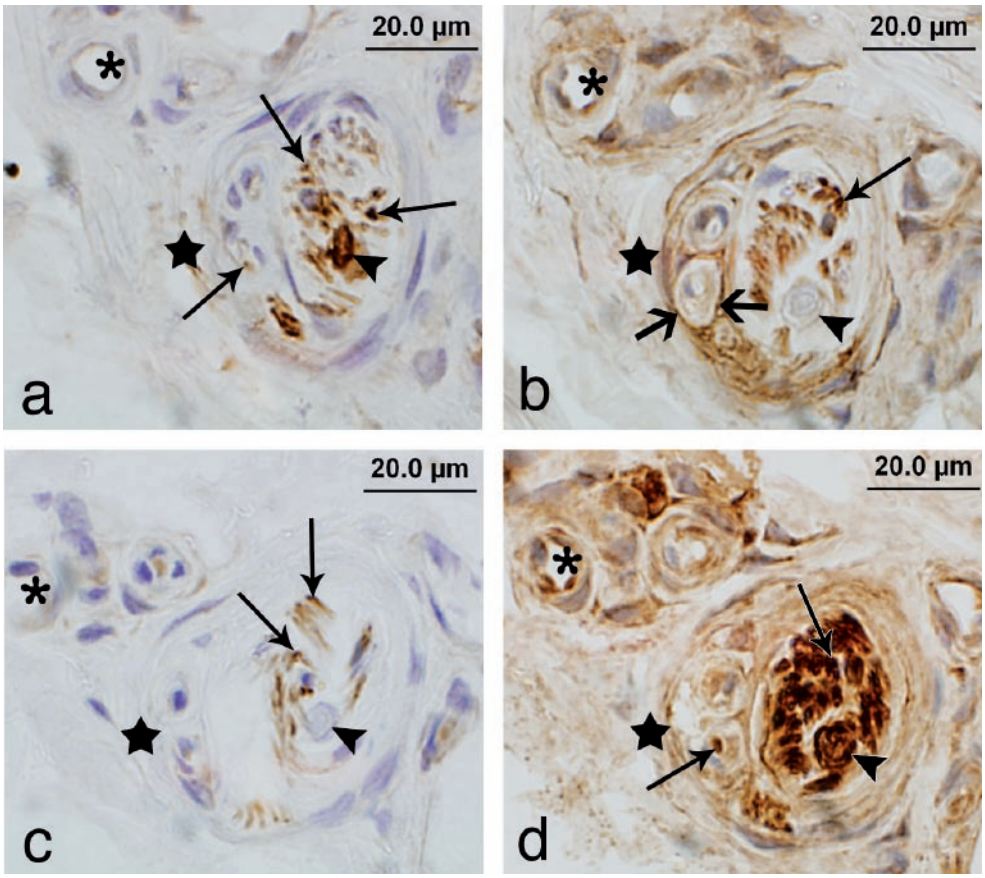


Figure 6

Ruffini ending in a DRC ligament, as seen in sections stained for PGP 9.5 (a), p75 (b), trkB (c) and S100 (d). The incomplete capsule is p75 IR (b). The central axon (arrowhead) of the corpuscle is clearly visualized by showing specific IR in (a) and (d). Terminal nerve endings (thin arrows) within the corpuscle are seen in all stainings. A small blood vessel (*) is seen in the vicinity. (★) indicates equivalent regions in the outer border of the corpuscle.

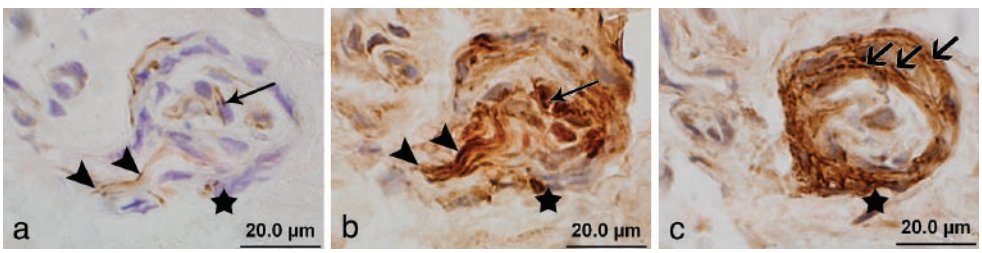


Figure 7

Serial sections of a Pacini corpuscle in a DRC ligament. (★) indicates corresponding regions in the images. (a) and (b): A thin nerve fascicle (arrowheads) in continuation with the corpuscle is visualized in sections processed for trkB (a) and S100 (b), which continues in the corpuscle as the central axon (thin arrow). The characteristic thick capsule, with several perineurial layers (thick arrows), is seen displaying strong p75 IR (c).

Distribution of innervation

In Paper II, the first indication that wrist ligaments may have varying degrees of innervation was found. In this study, the DRC, DIC and SLIL were consistently richly innervated as compared to the other ligaments studied. Based on these findings, Paper III aimed at examining a larger number of biomechanically important wrist ligaments, with the aim of delineating specific innervation patterns in relation to ligament structure.

Distribution within the ligament
Nerves and mechanoreceptors were most frequently found close to the insertions into bone, and, as described above, consistently found in the *epifascicular region* of ligaments. Furthermore, since the ratio of fascicular to epifascicular regions varied, it follows that the degree of innervation varied, with rich innervation found in ligaments with large epifascicular regions, and limited or non-existing in ligaments with predominant fascicular regions.

Distribution in the wrist
The wrist ligaments were found to vary distinctly with regard to degree of innervation. The dorsal wrist ligaments, DRC-DIC-STq-dSLI, all had a *pronounced* innervation, with nerves and mechanoreceptors in all sections studied from all specimens. The volar triquetral ligaments, TqC-TqH-pLTqI, had an *intermediate* innervation, with nerves and mechanoreceptors in 3 of 5 studied specimens. The remaining volar ligaments were more sparsely innervated, with only nerves and/or occasional mechanoreceptors found (for details, see table I, Paper III).

NEUROPHYSIOLOGICAL STUDY (Paper IV)

General patterns of muscle reactions

When analyzing the significant muscle reactions occurring in a majority of subjects, it became apparent that changes in EMG activity, recorded as changes in amplitude, appeared at various time intervals after stimulation of the dSLIL. If the post-stimulus value (median value of all subjects with statistically significant amplitude change) for a time interval was positive, this indicated an increase in EMG activity, an excitation. A negative value, on the other hand, denoted a decrease, an inhibition, as compared to the pre-stimulus value (for details, see Table I, Paper IV).

General patterns observed
Immediate reaction. The primary reaction was consistently seen in the antagonist muscle(s), as related to each wrist position. This reaction appeared within 20 ms after stimulation.
Reciprocal activation. The immediate reaction was frequently followed by a corresponding activation in the agonist muscle(s) for each wrist position, from 20-60 ms after stimulation, with or without concurrent antagonist activation.
Cocontraction phase. Around 150 ms after stimulation, a longer period of activation in two or more forearm muscles was observed. This reaction usually lasted 100 ms and occurred

simultaneously in agonist and antagonist muscles, thus, a *cocontraction* of forearm muscles. The largest changes in EMG amplitude were seen in this phase, which appeared in the primary motor of the current wrist position.
Receding reaction. In the final phases of recorded muscle activity, from around 250 ms after stimulation, phases of small or no excitations, as well as frequent inhibitions, were observed.

Specific reaction patterns for each wrist position

Extension
The wrist was, in average, in 40° wrist extension throughout the experiment. The *immediate reaction* was monophasic and seen in the FCR and FCU. This was followed by a *reciprocal activation* of the ECRB and ECU. The main *cocontraction phase* occurred in all four forearm muscles from around 60 to 180 ms, with the largest amplitude change observed in the ECRB during this phase. A second cocontraction phase, with smaller amplitude reactions, was seen around 250 ms. The final phase of *receding activity* demonstrated inhibitory reactions in the FCR, ECU and ECRB.
The simultaneous muscle reactions occurring in all four forearm muscles are illustrated in figure 8a. For details of each muscle reaction in extension, see figure 3, Paper IV.

Flexion
The experiment was performed with 20 to 33° wrist isometric flexion. The *immediate reaction* occurred in the ECRB within 20 ms after stimulation. A *reciprocal activation* followed in the FCR and FCU from 20 to 60 ms, and again from 120 to 200 ms where the largest amplitude change was observed in the FCU. A brief *cocontraction phase* was seen in the ECU and FCR at 240-260 ms. The *receding activity* was characterized by inhibitory reactions in the FCR and FCU, and sporadic excitatory responses in the FCR (see fig. 8b for an overview, fig. 6, Paper IV for details).

Radial deviation
The wrist was kept in an average of 22° radial deviation with slight extension. In radial deviation, the largest amplitudes in the *immediate reaction* phase were observed in the FCR and FCU. This was immediately followed by a *cocontraction phase* in the ECRB, ECU and FCR from 20 to 60 ms. A second cocontraction phase is seen in all wrist muscles from 100 to 160 ms, and a third, smaller cocontraction, in the ECRB and FCR at 250 ms. The *receding activity* was primarily one of inhibition in the ECRB and ECU, with occasional excitatory reaction in the FCR (fig. 8c for an overview, fig. 8, Paper IV for details).

Ulnar deviation
The average isometric ulnar deviation position was 33° from neutral. The *immediate reaction* was observed in the FCR and FCU. Following this, the reaction patterns differed markedly in ulnar deviation. The characteristic reaction was one of *inhibition* of the primary motor, the ECU. A total of five inhibitory phases were observed in the ECU from 80 to 420 ms after stimulation. Only sporadic excitatory responses were seen in the FCR and ECRB, and no periods of *cocontraction* (fig. 8d for an overview, fig.10, Paper IV for details).

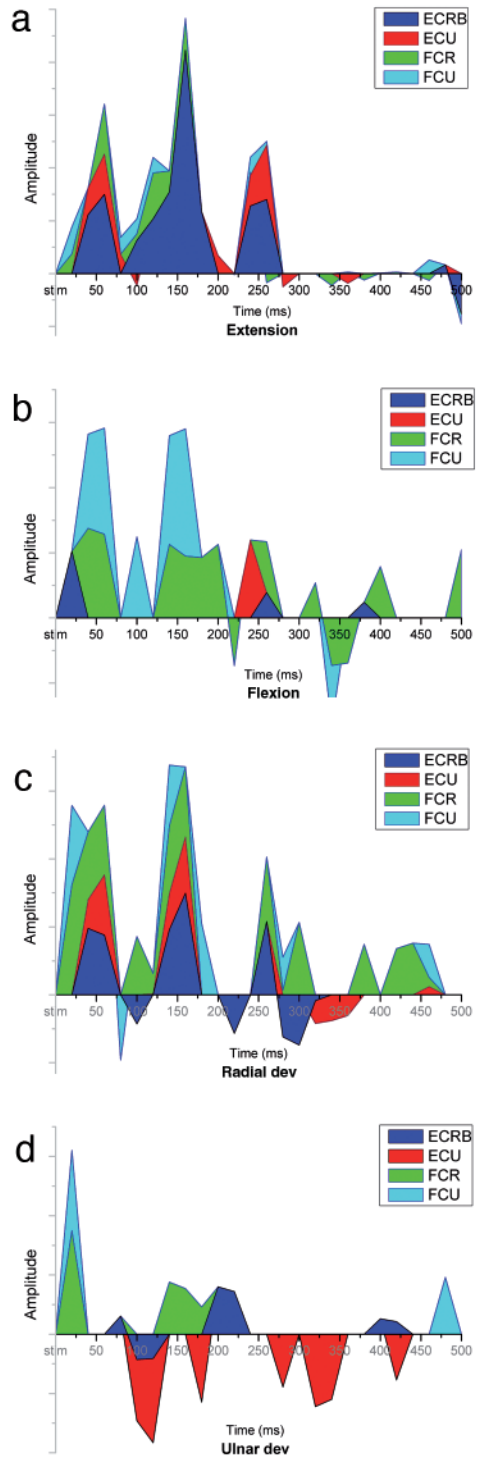


Figure 8
Graphic illustration of the EMG amplitude (Y-axis) in all forearm muscles along a time line (X-axis), depicting the simultaneous muscle activity in ECRB-ECU-FCR-FCU following stimulation of the ligament with the wrist in isometric extension (a), flexion (b), radial deviation (c), and ulnar deviation (d).

SUMMARY OF RESULTS

The results of Papers I-IV can be summarized as follows:

- The wrist ligaments, in general, consist of a core of densely packed parallel collagen bundles, a *fascicular region*, surrounded by a layer of loose connective tissue, the *epifascicular region*, throughout which nerves, mechanoreceptors and vessels course.

- By using various immunohistochemical markers, nerve fascicles and mechanoreceptors of both Ruffini, Pacini and large dendritic (Golgi-like) types were identified; Ruffini ending being the predominant type observed in the wrist ligaments.

- When present, the innervation was consistently found in the epifascicular region, and close to the ligament insertion into bone. The innervation of a wrist ligament thus correlated with the density of innervation of its epifascicular region.

- The radiovolar ligaments were found to be sparsely innervated with large fascicular regions. The dorsal ligaments, DRC, DIC and SLIL, as well as the volar triquetral ligaments, TqC, TqH and pLTqI, on the other hand, presented with large epifascicular regions and distinct innervation.

- After stimulation of the SLIL, patterns of changes in EMG reactivity (excitatory or inhibitory) were observed in the forearm muscles at various time intervals.

- An *immediate* ligamento-muscular reaction was observed in the ECRB in flexion and in the FCR/FCU in extension, radial and ulnar deviation, A later *cocontraction* reaction, with simultaneous activation of agonist/antagonist muscles, occurred around 150 ms after stimulation of the SLIL.

DISCUSSION

"Surely there is grandeur in knowing that in the realm of thought, at least, you are without a chain; that you have the right to explore all heights and depth; that there are no walls nor fences, nor prohibited places, nor sacred corners in all the vast expanse of thought."
- Robert Green Ingersoll

The prerequisites for joint sensorimotor functions, as outlined in the introduction, are in short: the presence of sensory end organs in joint ligaments and/or joint capsule, neural projections from the joint to the spinal cord, local spinal interconnections for fast joint control and supraspinal integration of sensorimotor information. The presence of sensory end organs in wrist ligaments and evidence of ligamento-muscular reflexes support the hypothesis of innate wrist sensorimotor functions. The results of Papers I-IV will, hereby, be discussed in a general context of wrist sensorimotor functions, as well as in relation to possible clinical relevance and future perspectives.

INNERVATION PATTERNS IN WRIST LIGAMENTS

The consistent presence of nerve fascicles and mechanoreceptors within the epifascicular regions, and the presence of densely innervated regions close to the bony insertion of the ligaments correlates with previous findings on sensory nerve endings in the knee, shoulder and elbow joints of humans.[46,72,82] The midportion of the long DRC and DIC ligaments were also found to contain a certain number of mechanoreceptors, findings which have been confirmed in a later study regarding the innervation of the DRC.[105] While the predominant mechanoreceptor type observed overall was the Ruffini ending, all four types of nerve endings were identified in the wrist ligaments. The distinct pattern of mechanoreceptor populations within the ligament is likely to reflect the innate functions of these ligaments. The possible role of the various mechanoreceptor types in relation to wrist function will be briefly discussed.

Ruffini ending
Microneurographic recordings from Ruffini endings in cat knee intra-capsular ligaments have revealed that this is a slowly-adapting, low-threshold receptor, which is constantly reactive during joint motion.[31] Additionally, these endings were found to react to axial loading and tensile strain in the ligament, but not to perpendicular compressive joint forces, revealing their importance in signaling joint position and rotation, rather than direct pressure. The observation that Ruffini endings were the most common mechanoreceptor type in the wrist ligaments,[36,37,57] suggests a primary importance of wrist sensorimotor function in monitoring wrist positions and motions.

Golgi-like receptor
The Golgi-like receptor was only rarely identified, and, when found, only present in the DRC or DIC.[36] Its presence in the DRC has been additionally later verified.[57] This large receptor belongs to the same family as the Ruffini ending, the "spray-endings", and they have even been suggested to be variations of the same receptor.[94] As such, the Golgi-like ending is also a slowly-adapting receptor, but, contrary to the Ruffini, has a high-

threshold to mechanical stress, making it completely inactive in the immobile joint, but important in the detection of extreme ranges of joint movement.[49]
The DRC and DIC are among the longest ligaments in the wrist, averaging 20 and 36 mm, respectively.[113] The DRC originates from the dorsoulnar and distal rim of the radius, attaches to the distal dorsal lunate and inserts onto the dorsal triquetrum. The DIC (with its proximal STq portion) originates from the triquetrum, courses via the dorsal lunate to insert onto the dorsal scaphoid, as well as, frequently, onto the trapezium and trapezoid.[75,76] While the DRC is considered important in stabilizing the wrist in flexion and pronation,[114] the DIC is attributed importance in maintaining transverse stability of the proximal carpal row, as well as indirect stability of the dorsal midcarpal joint space.[8] These ligaments have, furthermore, been described by Viegas et al[113] as having a "lateral-V" configuration, where the DRC/DIC ligament complex (together with the dorsal interosseous ligaments) is proposed to indirectly stabilize the scaphoid and lunate throughout all ranges of wrist motion while accommodating the change in the distance from the distal radius to the carpus. Ordinarily this would be an impossible task for one ligament alone without restricting wrist motion.[114] The finding of Golgi-type endings in the DRC and DIC ligaments is, therefore, not surprising, since this receptor is important in monitoring tensile strain in the ligament during ultimate angles of joint motion.

Pacini corpuscle
The Pacini corpuscle differs from the spray-endings, in that it is a rapidly-adapting, high-threshold receptor sensitive to joint acceleration/deceleration, that is able to sense mechanical disturbances occurring even at a distance.[64] Contrary to the Ruffini ending, it is sensitive to compressive but not tensile forces.[32] Hence, it is believed to be important in signaling during potentially damaging joint motions, and it is also regarded as one of the few mechanoreceptors that may contribute to osseoperception.[64]
The Pacini corpuscle has been identified as the most abundant receptor in the lateral ankle ligaments,[69,71] making it ideal for rapid signaling of potentially damaging joint perturbations in a ligament system frequently subjected to distortion and injury. In the wrist, however, the Pacini corpuscle was only occasionally identified, suggesting that monitoring of joint velocity and onset/termination of motion is of less importance in wrist sensorimotor stability.

Location within the ligament
The greatest density of mechanoreceptor populations is generally found in the ligament-bone interface, indicating that this is a key area for monitoring ligament and joint disturbances. Studies on the ligament-bone region in the human anterior cruciate ligament (ACL) have shown greater ligament stiffness and higher yield strength, as compared to the mid-substance of the ligament.[11] The ligament-bone strain distribution, furthermore, displays a complex combination of both compressive and tensile components.[100] The presence of a multitude of receptor types in this region, thus, permits a sensorimotor monitoring of joint activities relating to both tension (triggering Ruffini endings) and compression (triggering Pacini corpuscles). Since ligaments are more resistant to strains close to their insertions, this serves to ensure triggering of mechanoreceptors only by potentially noxious motions, while they remain silent during ordinary joint activity.[97,99] The ligament-bone interface, therefore, has ideal anatomical

and physiological properties for joint protective sensorimotor functions.
Contrarily, the mid-substance of the ligament is more pliant and less resistant to strain and is thus more readily responsive to joint disturbances. The DRC has been shown to have approximately a quarter of its innervation in the mid-region,[105] and our findings show mechanoreceptors in both DRC and DIC midsubstance.[36,37] In relation to the discussion on the dorsal wrist ligaments above, the findings of mechanoreceptors throughout the entire length of the DRC and DIC ligaments supports the notion that they have important functions in the constant sensorimotor monitoring of wrist motions.

DISTRIBUTION OF INNERVATION IN THE WRIST

To date, there are seven publications on the distribution of sensory nerve endings in the wrist joint[35-37,45,57,81,105] and four on the innervation of the distal radioulnar ligaments and the triangular fibrocartilage complex (TFCC).[12,33,78,90] In the studies that have quantified the density of nerve endings, there is one aspect in common – while the majority (though not all) of the ligaments have some neural structures present, there is distinct evidence of a varying degree of innervation in the wrist. Although this present thesis concerns the innervation of the wrist joint (carpus), for the sake of completion, the innervation of the distal radioulnar joint (DRUJ) ligaments will also be presented. The collective distribution pattern reported in the abovementioned studies on the wrist and the DRUJ is illustrated in figure 9, and the details of the richly innervated ligaments will be discussed in relation to anatomical location and implication on wrist function.

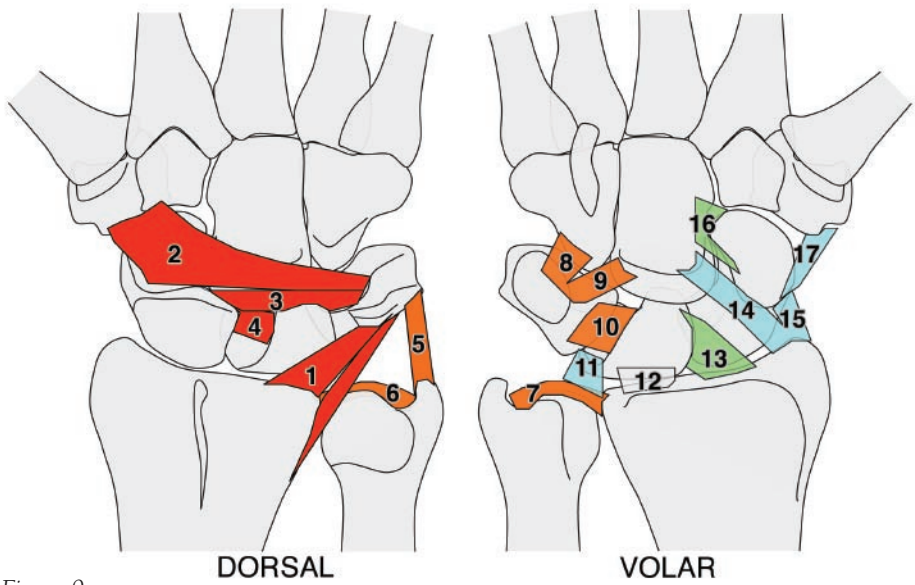


Figure 9
Distribution of mechanoreceptors (R) and nerves (N) in the wrist ligaments, including TFCC. Pronounced innervation (red) = (R)+(N) consistently found in all specimens studied; intermediate (orange) = (R)+(N) in a majority of specimens studied; limited (blue) = (R) in approximately 20%; occasional (green) = no (R) found, only (N); rare (grey) = no (R), only single (N).
Ligaments: dorsal radiocarpal (1), dorsal intercarpal (2), scaphotriquetral (3), scapholunate interosseous (4), ulnotriquetral (5), dorsal radioulnar (6), volar radioulnar (7), triquetrohamate (8), triquetrocapitate (9), palmar lunotriquetral (10), ulnolunate (11), short radiolunate (12), long radiolunate (13), radioscaphocapitate (14), radioscaphoid (15), scaphocapitate (16), scaphotrapeziotrapezoid (17).

Dorsal and triquetral wrist ligaments
All of the dorsal wrist ligaments – dSLI, DRC and DIC (including its proximal STq portion) – have been found to have a pronounced sensory innervation, with mechanoreceptors and nerve endings found in all specimens studied,[35-37,57,105] making these ligaments the most innervated of all wrist ligaments presently analyzed. The DRC, in particular, has been thoroughly examined, since it is consistently found to have an abundance of sensory nerve endings.
The volar triquetral ligaments – pLTqI, TqC and TqH – have an intermediate pattern of innervation, with sensory nerve endings (mechanoreceptors and nerve fascicles) found in a majority of specimens studied.[37]

Triangular Fibrocartilage Complex (TFCC)
The presence of nerve endings in the TFCC[12,33,78,90] and the ulnotriquetral (also referred to as the ulnocarpal or ulnar collateral) and ulnolunate ligaments[36,37] have been analyzed using various staining techniques and light-microscopy. However, the published studies have used different definitions of the anatomical subregions of the TFCC, making it difficult to succinctly compare outcomes of the various studies. One common and general trait, however, is the presence of nerve endings in the ulnar region of the TFCC; more precisely, in the ulnotriquetral (UTq) ligament, the "internal portion" (IP) and the meniscus homologue (MH).

Triquetrum and ulnar wrist
In summary, apart from the dSLI, *all* of the ligaments with a consistent sensory innervation are found in relation to the triquetrum and the ulnar part of the wrist – the DRC, DIC, STq, pLTqI, TqC, TqH, UTq, MH and IP.

Ulnar control column
The ulnar part of the wrist, as constituted by the distal ulna, triquetrum and ulnar distal carpal row (capitate/hamate), has been described by Weber in 1984 as the *control column* of the wrist,[111] indicating a kinematic and kinetic control function throughout global wrist motion.
The most proximal part of the control column is the distal ulna and the distal radioulnar articulation, DRUJ. It is well-recognized that the distal ulna acts as a keystone in the DRUJ, where the ulnar head is the pivot point around which the distal radius rotates 160° or more.[34] From the ulnar styloid process, the volar ulnocarpal ligaments extend vertically toward the triquetrum, creating a ligamentous extension and prolongation of the osseous keystone, the ulna.[86]
The triquetrum is considered the center of the control column.[111] On its volar aspect, the triquetrum has strong connections to the hamate and lunate by the volar TqHC and pLTqI ligaments. This fan-shaped ligamentous arrangement provides a volar stability both within the proximal carpal row, as well as across the midcarpal joint, making it important in both flexion/extension, ulnar/radial deviation as well as dart-throwing motions of the wrist.[73,87] Dorsally, the triquetrum is the center of attachment for the long dorsal wrist ligaments, the DRC and DIC. The importance of these ligaments in providing dorsal wrist stability throughout wrist motions has been described in detail above.
When reviewing the sensory innervation of the ligaments of the wrist and distal radioulnar joint, it becomes apparent that the triquetrum is the hub from which ligaments

with important sensorimotor functions spread out into the wrist, thus providing proprioceptive information throughout all aspects of wrist motion. Furthermore, the ligamentous attachments from the ulna to the triquetrum were also found to be richly innervated with sensory nerve endings. Hence, in analogy to the ulna being the osseous keystone of the DRUJ, the triquetrum and its ligamentous attachments should be considered the sensorimotor keystone of the entire wrist joint.

WRIST NEURAL PROJECTIONS

To adequately discuss the neural projections and possible proprioceptive pathways from the wrist joint, a brief overview of the nerves contributing to the innervation of the wrist joint is first demanded (see fig. 10).

Innervation of the wrist joint

The dorsal wrist capsule and the dorsal wrist ligaments are primarily innervated by the terminal branch of the posterior interosseous nerve (PIN), namely, the *dorsal interosseous nerve* (DIN), which arborizes in the fourth extensor compartment and distributes branches to the majority of the dorsum of the wrist.[23,115] Additionally, the dorsal sensory branches of the radial nerve and the dorsal sensory branches of the ulnar nerve innervate the dorsoradial and dorsoulnar aspects of the wrist, respectively.

While the innervation of the dorsal wrist is quite constant and predictable, the innervation pattern of the volar wrist is more diverse and several dissection studies have been published on the anatomical variances as related to surgical denervation of the wrist.[9,18,22,26,109] In general, the volar wrist capsule and the radioscapholunate (RSL) ligament receive branches from the anterior interosseous nerve (AIN), whereas the volar carpal ligaments appear to receive their innervation from the lateral antebrachial cutaneous nerve, the volar cutaneous branch of the median nerve, deep branches from the ulnar nerve, as well as endings from the AIN.

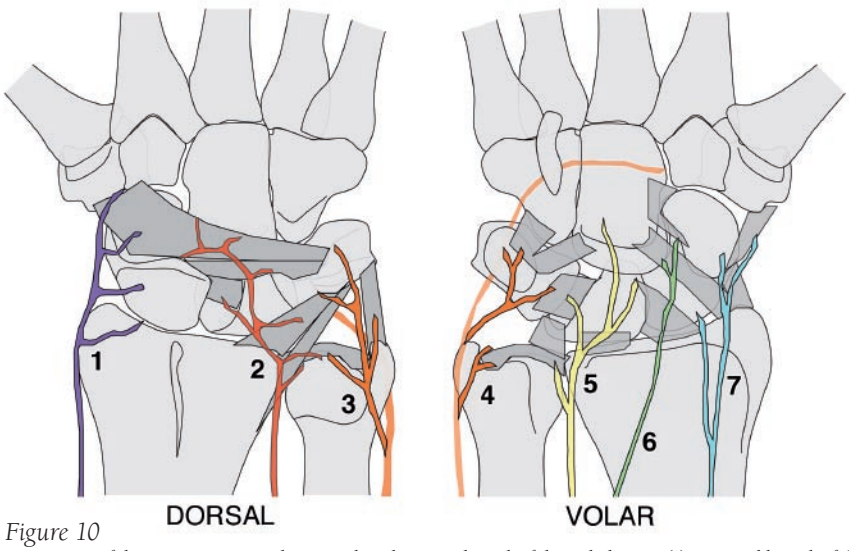


Figure 10

Innervation of the wrist joint. Dorsal nerves: dorsal sensory branch of the radial nerve (1), terminal branch of the posterior interosseous nerve, the dorsal interosseous nerve (2), dorsal sensory branch of the ulnar nerve (3). Volar nerves: deep branches from the ulnar nerve (4), terminal branch of the anterior interosseous nerve (5), volar cutaneous branch of the median nerve (6), lateral antebrachial cutaneous nerve (7).

Innervation of the distal radioulnar joint
The innervation to the DRUJ and TFCC appears to be three-fold, with the dorsal region primarily innervated by the DIN, the ulnar region primarily by the dorsal sensory branch of the ulnar nerve and the volar region by branches from the ulnar nerve.[33,90] While dissection studies have shown AIN branches in the vicinity of the volar DRUJ capsule,[23,26] detailed microscopic studies have not been able to show AIN afferents in the DRUJ ligaments.[33] The same holds true for the medial antebrachial cutaneous nerve, which has an extra-articular course in the DRUJ,[26,115] but lacks intra-articular contributions.[33,78]

Neural projections from the wrist joint
The precise neural projections from the human wrist joint to the spinal cord have not been delineated. Recently, however, the spinal projections of sensory neurons in the rat wrist joint have been analyzed.[52] In this study, retrograde neurotransport was used to identify NGF (using CGRP IR) and neurofilament (NF) reactive neurons in the sensory dorsal root ganglia (DRG). Interestingly, the low-affinity NGF receptor, p75, was stained for in Papers I-III to specifically identify sensory corpuscles in the wrist ligaments. Hence, the presence of NGF reactivity in the DRG may strengthen the notion that the reactive DRG neurons indeed constitute wrist proprioceptive afferent projections.
DRG neurons emanating from the rat wrist joint, with anticipated nociceptive and proprioceptive functions, were primarily found at the level of C7-Th1, with a predominance of sensory projections at the C8 level. In the human, the most distal branches to emanate from C8 would correspond to the terminal branches of the PIN (the DIN) and the dorsal sensory branch of the ulnar nerve, which are the primary afferents to the richly innervated ligaments dorsally and ulnarly in the wrist. Hence, given similar topography in the human spinal cord, the findings of specific proprioceptive neurons in the rat DRC would suggest there might also be afferent neural projections from the sensory nerve endings in the ligaments of the human wrist joint to proprioceptive DRG neurons.

WRIST SENSORIMOTOR REFLEXES

Substantial information regarding the sensory innervation of wrist ligaments has been presented. However, in order to verify a presumed sensorimotor function, the study presented in Paper IV was instigated to investigate the existence of wrist ligamento-muscular reflexes. Various reflex patterns were evident after stimulating the SLIL, with time-related changes in the EMG activity of forearm muscles. While the exact pathways of these reactions were not delineated in Paper IV, previous publications may lend support to hypothetical discussions.

Possible proprioceptive pathways

Immediate joint reflexes
An initial monophasic muscular reaction, occurring within the first 20 ms, was consistently recorded from the antagonist muscle(s) in each respective wrist position. Hence, in wrist flexion, the primary reaction was observed in the ECRB, whereas in wrist

extension, radial and ulnar deviation, the primary reaction was seen in the FCR and FCU. The first study on joint ligamento-muscular reflexes was conducted at Södersjukhuset, Stockholm in 1958, where Palmer[79] was able to present evidence of joint protective reflexes elicited after traction on the medial knee ligament. Similar reflexes have subsequently been confirmed in the human knee, shoulder and elbow joints.[20,47,83] Controversy exists regarding protective reflexes, however, since the efficacy of a defensive reflex is entirely dependent on the immediacy of the ligamento-muscular reaction. Hence, in order to be joint protective, an adequate reaction would need to be equivalent in response time to a monosynaptic stretch reflex, i.e. the patellar reflex, where the antagonist muscles act as first line of defense to break a potentially damaging joint motion.

Experiments on the excitation of human flexor motoneurons have shown that stimulation of the median and/or ulnar nerves at the level of the wrist joint elicit monosynaptic excitations of the FCR and FCU, occurring within 20 ms after stimulation.[66] The ulnar nerve, in particular, was believed to be important in contributing proprioceptive feedback to spinal motoneurons.[62] These reaction times are similar to the immediate reactions observed in Paper IV, indicating that these too may be of monosynaptic origin.

Cocontraction reactions

Following the immediate reflex, a pattern of co-activation of agonist and antagonist muscles, i.e. a cocontraction, was observed. These reactions generally commenced around 40 ms after stimulation, with peak amplitudes of EMG activity around 150 ms, the largest amplitudes being observed in the primary motor for each wrist position. The later onset latency of these reactions, suggest that they differ from the immediate responses described above.

Feline[5] and simian[42] experiments on the complex spinal integration of peripheral and central commands have revealed the presence of specific propriospinal neurons (PNs) at the level of C3-C4. PNs are affected both by a feed-forward inhibition from the cerebral cortex, which serves to control the specificity of arm and hand movements, as well as by a feed-back inhibition from peripheral afferents, which control the velocity, onset and termination of motions.[63] PNs, furthermore, have axons that project directly to forelimb motoneurons (C6-Th1), thus enabling fast motor commands.[62] Strong evidence is now emerging that similar propriospinal neurons are present in humans.[84] The polysynaptic interactions that occur at PNs are typically characterized by a delay in onset latency, as compared to monosynaptic reflexes, and generally result in a co-activation of both agonist and antagonist muscles.[84] These reactions may, hence, serve to explain the first cocontraction reactions observed in wrist forearm muscles at about 40 ms after stimulation.

More advanced supraspinal control of motion, however, generally demand longer time relays. Studies on cortical somatosensory evoked potentials from the median and radial nerves,[106] as well as on the effects of transcranial magnetic stimulation on reflexes in the forearm muscles,[19] indicate afferent and efferent time-intervals, respectively, that correspond with the later onset cocontractions observed in Paper IV, suggesting that these reactions are modulated by transcortical pathways for the complete integration of sensorimotor information.[68]

Functional implications of sensorimotor reflexes

Immediate reflex response
The primary response in extension, radial and ulnar deviation was found in the FCR and FCU. In these three wrist positions, the dorsal SLIL is in a state of maximum elongation,[108] thereby leaving the ligament susceptible to disruption and damage. The simultaneous contraction of the FCR and FCU as paired antagonists would, therefore, effectively alleviate a potentially damaging extension or deviation motion. Similarly, the immediate response in flexion was found in the primary antagonist, ECRB. The short onset latency and the specific recruitment of muscle groups to prevent potentially damaging wrist positions, suggests that the immediate reactions that were recorded have wrist joint protective functions.

Cocontraction reactions
The long onset latency of the cocontraction reaction implies that it has an importance in the long-term control of joint neuromuscular stability, as opposed to the simple joint protective reflex described above. Global contractions of agonist/antagonist muscles around a joint will generate a general joint stiffness, thereby effectively reducing the risk of joint damage,[98]and is a joint protective strategy which has been well documented in the knee joint.[41,65]
Furthermore, the delicate balance of cocontraction is believed important in maintaining smooth joint motions. This ability to sustain an adequate joint equilibrium has been shown to be impaired in ACL deficient knees,[13] where inadequate neuromuscular recruitment results in changes in knee kinematics that are potentially harmful to the joint.[88]
In relation to the wrist joint, cadaver studies of SLIL injuries and dissociations have revealed functional changes in the excursions of the primary muscles controlling wrist stability.[104] Following sectioning of the SLIL and widening of the SL interval, the radial wrist movers, in particular the FCR, have a significant increase in their moment arms, causing an increase in the load distribution through the radial carpus and further displacement of the scaphoid.[104] Contrarily, with the SLIL intact, the FCR is considered an important dynamic stabilizer of the scaphoid.[59]
With the wrist in extension, flexion and radial deviation, the majority of muscle reactions observed in Paper IV were found in the FCR. Hence, in the intact wrist, the FCR appears to have an important role in the neuromuscular stability of the wrist joint, whereas following SLIL injury, where the proprioceptive feedback is assumed to be lost, FCR strengthening exercises may have an adverse and detrimental effect on carpal integrity.
In Paper IV, cocontractions were primarily observed occurring at later onset latencies in the ECRB and FCR. These reactions were most distinct in extension and radial deviation, but they were also seen in ulnar deviation and, occasionally, in flexion. Studies on the spinal control of wrist flexor and extensor muscles have revealed that the ECR and FCR muscles have an intricate and unique interaction in humans.[7] Excitation of Ia and Ib inhibitory interneurons (which inhibit *antagonist* muscles) are known to be elicited from the ECR to the FCR during voluntary wrist extension.[110] During voluntary wrist flexion, however, this activity is decreased, whereas when there is cocontraction of the agonist and antagonist muscles this inhibition is completely abolished. These actions indicate a descending control of the inhibitory interneurons to facilitate the cocontractions of

opposing muscles that are needed to globally stabilize the wrist joint.
Furthermore, in all other joint systems but the wrist, another group of interneurons, the Renshaw cells, act on a spinal level to inhibit *agonist* muscles.[7] This synergistic inhibition, however, does not hold true for the ECR-FCR interaction, where the respective Renshaw cells, despite being antagonists, inhibit one another.[6] This action is believed to be important in allowing the ECR-FCR to act as antagonists during wrist flexion-extension, but as agonists during wrist radial deviation.[7]
These intricate interactions of inhibitory interneurons and Renshaw cells on the spinal control of human wrist muscles, illuminate the complexity of wrist neuromuscular control, and may, in part, provide a physiological explanation to the concurrent cocontraction reactions frequently seen in the ECRB and FCR in Paper IV.

CLINICAL IMPLICATIONS

Acute ligament injuries
The abundant presence of sensory nerve endings in wrist ligaments implies a loss of sensory information in the event of ligament disruption. While the most commonly recognized ligament injury is that of the SLIL, disruptions of secondary stabilizers to the scaphoid are also of importance.[92] Recently, disruptions of the DRC ligament have been recognized in an arthroscopic series of patients with persistent wrist pain after injury, where DRC disruptions were commonly found in combination with SLIL, LTqIL and TFCC ligament injuries.[95] Injuries to any and all of these ligaments will, undoubtedly, affect the sensorimotor function of the wrist joint.
If recognized early, SLIL injuries may be the subject of primary repair,[30] which is beneficial, as an early adaptation of injured ligaments may allow a reinnervation of nerve endings, encouraging both ligament healing[43] and proprioceptive restoration.[107] Acute wrist ligament injuries are, however, grossly underestimated, and arthroscopic examinations of patients with persistent wrist pain after trauma have revealed a substantial incidence of ligament injuries despite normal initial radiographs.[1,2] An optimal treatment of wrist ligament injuries is, thus, dependent on a more aggressive approach in evaluating the "sprained" wrist, preferably by the use of early MRI and/or diagnostic arthroscopic evaluations in patients with persistent wrist pain.

Chronic overuse syndromes
The role of ligaments in chronic neuromuscular disorders has been the focus of several publications in the last years.[48,98,99] Studies on changes in ligament mechanical and sensory properties after load and stress have revealed significant structural alterations, including ligament creep (elongation over time), inability to adequately trigger ligamento-muscular reflexes[98] and acute inflammatory reactions.[25] Even modest loading of wrist ligaments in grip and push-up positions have been shown to cause an increase in ligament laxity, which demand 24 hours of rest to be fully restored.[14] If repetitive loading persists, substantial creep will develop, with risk of chronic inflammatory changes in ligaments,[99] resulting in permanent ligament scarring[25] and subsequent chronic pain conditions. Patients with unilateral partial untreated SLIL injuries have been shown to

have persistent pain, reduction of grip strength and an inability to return to work even several years after injury.[77] Unilateral SLIL injury is also related to bilateral changes in carpal kinematics,[15] and patients with chronic unilateral wrist pain, furthermore, display bilateral deficits in motor control, probably on the basis of persistent abnormal supraspinal sensorimotor integration.[96] Similar conditions can be seen in patients with inherent physiological joint laxity, where alterations in joint kinematics may result in susceptibility to joint injury.[27,28]
It is important to acknowledge that microtrauma due to repetitive loading might be the cause of chronic wrist pain. To avoid developing chronic neuromuscular disorders, patients in high-risk occupations need adequate periods of rest, as well as a variability in vocational activities.[98] By recognizing the emerging research on chronic joint overuse as a cause of musculoskeletal disorders, patients may be properly diagnosed and instructed in preventive measurements, and, when indicated, supported in their rights to workers' compensation.

Proprioception re-education
The concept of proprioception re-education has been a golden standard in the rehabilitation treatments following ACL injuries for the last two decades. Prospective and randomized studies have validated the importance of proprioceptive training in enhancing knee function, both in surgically[60] and conservatively treated patients.[3,51] Rehabilitation strategies following wrist ligament injuries, however, have only been described in two publications,[21,54] and neither describe neither proprioception re-education nor neuromuscular training as part of the program.
While proprioception re-education in the knee joint is principally aimed at restoration of quadriceps/hamstring function, postural control and normalized range of motion,[97] a similar program for the wrist would likely need to focus on the conscious positioning of the wrist using visual stimuli, coordination of finger-wrist motions during grip and pinch, selective strengthening exercises of the forearm muscles (depending on the nature of the wrist injury), as well as cutaneous kinesthetic stimulations to compensate the loss of articular afferents.
Neuromuscular training has also been advocated as a means to prevent injury in athletes, where preemptive training has been show to reduce the risk of injury during contact sports.[67,74] Similarly, one would anticipate a sensorimotor training program to be of value to both athletes and musicians with a high demand on functional wrist stability.

FUTURE PERSPECTIVES

An immensity of work remains to be done before we may truly understand the complex nature of wrist sensorimotor functions. Among these are the following:

Additional studies on wrist ligamento-muscular reflexes. The SLIL is but one of several richly innervated and important ligaments in the wrist joint, and how other ligaments contribute to neuromuscular joint stability remains to be clarified. For instance, what part does the TFCC and DRUJ ligaments play in the integration of forearm/wrist motions? How do the important dorsal wrist ligaments contribute afferent information to the forearm motors?

Analysis of wrist sensorimotor function in patients following wrist ligament injuries, as compared to healthy controls.

Further research to understand the dynamic muscular control of the wrist joint, in particular in determining the complex mechanisms of forearm muscles recruited to act both as agonists and antagonists, depending on the position of the wrist.

Elucidation of the role of the nerves innervating the wrist joint, as compared to the possible role of cutaneous innervation, in wrist kinesthesia and proprioceptive feedback.

Investigations on human wrist neural projections at the level of the spinal cord.

Sensorimotor re-education and integration of wrist cortical representations, as investigated using fMRI.

Development of adequate neuromuscular rehabilitation programs for the enhancement of proprioception re-education following wrist injuries, as well as in the intent of preventing injury.

Santiago Ramón y Cajal, the esteemed Spanish neuroscientist, wrote:

"*It is important to realize that if certain areas of science appear to be quite mature, others are in the process of development, and yet others remain to be born.*"[85]

So it appears that while many areas of wrist research are in the prime of life, at times bordering on senescence, the field of wrist proprioceptive research is but a toddler, taking its first anticipatory, excited and stumbling steps toward future developments and understandings.

CONCLUSIONS

- Wrist ligaments are variably innervated with mechanoreceptors and nerve fascicles, and the degree of innervation reflects the general composition of the ligament.

- Ligaments with little or non-existing innervation have large fascicular regions, with densely packed collagen fibers, suggesting these are mechanically important ligaments.

- Contrarily, ligaments with pronounced innervation have large epifascicular regions, where nerves and mechanoreceptors abound, indicating these are sensory important ligaments.

- The sensory important ligaments emanate from the triquetrum into the wrist, and are able to monitor and signal in all wrist positions and motions. The triquetrum, with its ligamentous attachments, should, thus, be regarded as the sensorimotor keystone of the wrist.

- Ligamento-muscular reflexes are elicited at various time-intervals after electrical stimulation of the SLIL. The muscular activity of the forearm muscles is altered as a consequence of these reflexes.

- The immediate reactions in antagonist muscles are likely to have joint protective functions. Later cocontraction reactions indicate integrated supraspinal control to stabilize the wrist joint.

In summary, these findings provide unambiguous evidence of innate wrist sensorimotor functions, and constitute a solid foundation for further research on the dynamics of neuromuscular joint control.

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- Albert Schweitzer

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