



**AALBORG UNIVERSITY**  
DENMARK

**Aalborg Universitet**

## **Studies on itch and sensitization for itch in humans**

Andersen, Hjalte Holm

*DOI (link to publication from Publisher):*  
[10.5278/vbn.phd.med.00103](https://doi.org/10.5278/vbn.phd.med.00103)

*Publication date:*  
2017

*Document Version*  
Publisher's PDF, also known as Version of record

[Link to publication from Aalborg University](#)

*Citation for published version (APA):*  
Andersen, H. H. (2017). *Studies on itch and sensitization for itch in humans*. Aalborg Universitetsforlag. <https://doi.org/10.5278/vbn.phd.med.00103>

### **General rights**

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal -

### **Take down policy**

If you believe that this document breaches copyright please contact us at [vbn@aub.aau.dk](mailto:vbn@aub.aau.dk) providing details, and we will remove access to the work immediately and investigate your claim.



**STUDIES ON ITCH AND SENSITIZATION  
FOR ITCH IN HUMANS**

**BY  
HJALTE HOLM ANDERSEN**

**DISSERTATION SUBMITTED 2017**



**AALBORG UNIVERSITY**  
DENMARK





***STUDIES ON ITCH AND SENSITIZATION  
FOR ITCH IN HUMANS***

**PHD THESIS**

*by*

Hjalte Holm Andersen



**AALBORG UNIVERSITY**  
DENMARK

Dissertation submitted

Dissertation submitted: October 2017

PhD supervisor: Prof. Lars Arendt-Nielsen  
Aalborg University

PhD committee: Associate Professor, PhD Anne Estrup Olesen  
Aalborg University

Professor, Dr. Med. Martin Metz  
Charité – Universitätsmedizin

Professor Earl Carstens  
University of California

PhD Series: Faculty of Medicine, Aalborg University

Department: Department of Health Science and Technology

ISSN (online): 2246-1302

ISBN (online): 978-87-7210-083-8

Published by:  
Aalborg University Press  
Skjernvej 4A, 2nd floor  
DK – 9220 Aalborg Ø  
Phone: +45 99407140  
aauf@forlag.aau.dk  
forlag.aau.dk

© Copyright: Hjalte Holm Andersen

Printed in Denmark by Rosendahls, 2017



## CV

Hjalte has obtained a B.Sc. in medicine and M.Sc. in translational medicine from Aalborg University, Denmark. Hereafter he enrolled as a PhD Fellow working in the Laboratory of Cutaneous Pain Research, under the supervision of Prof. Lars Arendt-Nielsen at Center for Sensory-Motor Interactions. He is interested in human somatosensation, particularly peripheral and spinal mechanisms of itch, pain, chemesthesis, and thermosensation. His current research explores means to model, manipulate and evaluate sensory symptoms, primary afferent reactivity, and neuronal sensitization processes in humans, chiefly by surrogate models of sensitization, standardized psychophysical methods and vasomotor imaging. Hjalte has presented his work at 7 major international conferences and is a reviewer for a several journals including Journal of Investigative Dermatology, Experimental Neurology, Acta Dermato-Venereologica, Clinical Journal of Pain, and Biomarkers in Medicine. Hjalte was awarded the Novo Nordisk 2014 Scholarship Stipend, the EliteForsk Travel Stipend 2016, and the 2016 Philip Spiegel Award from the International Association for the Study of Pain.



# ENGLISH SUMMARY

Chronic itch, like chronic pain, is a common clinical problem, which is associated with a markedly reduced quality of life for the affected patients. Itch is a major symptom in several of the most prevalent dermatologic diseases (e.g., atopic dermatitis, psoriasis or urticaria) but it also occurs in a variety of non-cutaneous conditions (e.g., related to peripheral neuropathy, renal insufficiency or cholestasis). Chronic itch is often difficult to treat, has a detrimental impact on sleep quality as well as concentration, and is consistently linked to increased rates of anxiety and depression.

Mechanistically, itch and pain are complexly entwined. While analgesic opioids facilitate itch, scratch-induced pain can abolish itch, thus suggesting an antagonistic relationship between the two sensations. At the same time, however, there is substantial overlap between pruritic and algogenic peripheral mediators as well as receptors, and strikingly similar patterns of neuronal sensitization for itch and pain have been documented. Recent evidence suggests that itch arise from at least two distinct peripheral cutaneous pruriceptive subpopulations, which are subsets of a larger population of neurons that also respond to various noxious stimuli (nociceptors). Thus much of the psychophysical research methodology developed in the pain field can be converted and used to increase our understanding of itch – and in particular to explore the sensory neuronal features that are unique to itch.

Within this context, the aim of this PhD-project was to explore itch as a somatosensory modality using histaminergic and non-histaminergic models of itch and itch sensitization in humans in three purposes: 1) to assess itch topography and itch sensitization in healthy controls (first study), 2) to evaluate the antipruritic effect of capsaicin-induced epidermal nerve-ablation in a mechanistic proof-of-concept study (second study), 3) To explore potential pathway-specific itch- and cutaneous pain sensitization in patients with chronic itch due to atopic dermatitis (third study).

Results from the first study suggested that von Frey monofilament stimuli below the mechanical pain threshold can be used to assess itch sensitization (hyperknesis) and that there is considerable heterogeneity in chemical and mechanically evoked itch sensitivity between spinally versus trigeminally innervated areas. Results from the second study demonstrated profound antipruritic effects of high-concentration capsaicin pretreatment and suggested that the two most commonly applied models of itch in humans rely entirely on capsaicin-responsive cutaneous fibers. Finally, the

third study revealed pathway-specific non-histaminergic itch sensitization as well as mechano-nociceptive sensitization occurring both intra- and extra-lesionally in patients with atopic dermatitis.

In conclusion, histaminergic and non-histaminergic models of itch and itch sensitization are versatile and useful tools in both human experimental and clinical itch research towards improved understanding of the mechanisms behind acute and chronic itch.

# DANSK RESUME

Kronisk kløe er som kroniske smerter et udbredt klinisk problem, som for den ramte patient er associeret med reduceret livkvalitet. Kløe er et hyppigt symptom ved flere af de mest almindelige dermatologiske sygdomme (f.eks. atopisk eksem, psoriasis og urticaria), men forekommer også i mange øvrige sygdomstilstande (f.eks. relateret til perifær neuropati, nyreinsufficiens eller kolestase). Kronisk kløe er ofte vanskeligt at behandle og kan være ødelæggende for søvn og koncentrationsevne. Kronisk kløe er desuden regelmæssigt blevet forbundet med øget forekomst af angst og depression.

Kløe og smerte er som sensoriske fænomener mekanistisk nært beslægtede. Opioid-analgetika faciliterer typisk kløe, og kradse-induceret smerte kan kortvarigt lindre kløe, hvilket antyder et antagonistisk forhold mellem de to sensoriske modaliteter. Omvendt er der et substantielt overlap imellem kløe- og smerte-inducerende signalstoffer samt receptorer for primære afferente nerver, og der forefindes en slående lighed imellem den måde, hvorpå sensibilisering for kløe og smerte kommer til udtryk. Ny forskning har vist, at kløe opstår på baggrund af aktivitet i mindst to separate, perifære, kutane, pruriceptive nerve-signalbaner. Disse perifære kløe-nervefibre tilhører en undergruppe af en større fiber-population, som også transmitterer signaler efter kutane stimuli, der typisk opfattes som værende smertefulde (kaldet nociceptorer). Derfor er det nærliggende at megen af den psykofysiske forskningsmetodologi, som er udviklet inden for smerteområdet, kan konverteres og genanvendes med henblik på opnå en bedre forståelse for kløe – og i særdeleshed for de sensoriske karakteristika, som er unikke for kløe.

I den kontekst har nærværende ph.d.-projekt undersøgt kløe som en somatosensorisk modalitet ved anvendelse af histaminerge og ikke-histaminerge kløe- og kløesensibiliserings-modeller med tre formål: 1) at undersøge topografi af kløe-sensitivitet samt kløesensibilisering i raske (første studie), 2) at undersøge den antipruritiske effekt af capsaicin-induceret kutan desensibilisering i et mekanistisk proof-of-concept studie (andet studie) og 3) at belyse potentielt signalbane-specifik kløe- og smerte-sensibilisering i patienter med kronisk kløe grundet atopisk eksem (tredje studie).

Resultater fra det første studie viste, at stimuli med von Frey monofilamenter under den mekaniske smertetærskel kan anvendes til at undersøge kløesensibilisering (hyperknesis), og at der er stor anatomisk heterogenitet i kløe-sensitivitet til mekaniske og kemiske stimuli imellem spinalt og trigeminalt innerverede områder.

Resultater fra det andet studie demonstrerede en markant antipruritisk effekt af høj-koncentrations topisk capsaicinbehandling og viste, at de to hyppigst anvendte humane kløe-provokationsmodeller begge virker via capsaicin-sensitive kutane nervefibre. Afslutningsvist afdækkede det tredje studie signalbane-specifik ikke-histaminerg kløe-sensibilisering samt mekano-nociceptiv sensibilisering, der forekommer både i intra- og ekstra-læsionel hud hos patienter med atopisk eksem.

Det konkluderes, at histaminerge og ikke-histaminerge kløeprovokationer og sensibiliseringsmodeller er alsidige og nyttige forskningsmetoder inden for humaneksperimentel og klinisk forskning i forhold til at opnå en bedre forståelse for de mekanismer, som forårsager akut og kronisk kløe.



# ACKNOWLEDGEMENTS

This PhD project would not have been possible without the help and support of a number of people to whom I am immensely thankful. First and foremost, I express my most sincere gratitude to my PhD supervisor Lars Arendt-Nielsen. Beyond his crucial scientific guidance as well as introducing me to the intricacies of academia, Lars leads by example and has provided the perfect balance between careful instruction and encouragement of self-reliance. Secondly, I would like to thank Jesper Elberling whom I have come to refer to as my clinical supervisor. He has tirelessly provided clinical context and knowledge for my projects. Jesper also has a contagious passion and curiosity for human physiology and skin pathobiology so our regular discussions have been both motivating and productive.

There are numerous people in Department of Health Science and Technology whom have provided more indirect contributions for which I am grateful. These include Thomas Graven-Nielsen, Shellie Boudreau and Rogerio Hirata for organizing always rewarding and pleasant bimonthly scientific meetings and journal clubs. Moreover, Parisa Gazerani has helped me with practical and scientific advice for several of my side projects and Carsten Mørch has kindly tolerated and answered my statistics questions. Also, I would like to acknowledge the help and kind disposition of the administrative staff at Center for Sensory-Motor Interactions, especially from Susanne L. Nielsen, Lone S. Andersen, and Charlotte Villadsen.

I sincerely thank all those who helped me with the studies that constitute this thesis as well as side studies, i.e., those with whom I discussed ideas, hypotheses and study designs, and all those who participated in often long (itching and painful) experiments and pilots. In addition, I would also like to acknowledge Gil Yosipovitch and his entire research group for their kindness and help during my research stay at University of Miami and Antoinette van Laarhoven from Leiden University, for the fruitful collaboration we had during and after her stay in Aalborg. The staff at Dermatology Center North and in particular Henrik Sølvsten, Anne T. Funding, Dorte Lybæk, and Hans B. Lomholt, are thanked for their help in relation to recruitment of patients for one of the studies included in the thesis. My sincere gratitude to all my friends at or associated with SMI/CNAP – I am really fortunate to have spent so many enjoyable hours with you during the last three years.

I also wish to express my gratitude to my family. My mom Annette, my dad Gert, and my three sisters Liv, Freja and Siri, for their always caring and supportive attitude and interest.

Finally and most highly treasured, Kira, who have been an invaluable support during this period. She has tolerated my tendency to bring work home, my absentmindedness in periods where I was preoccupied with research, and has sat through rehearsals and carefully evaluated every one of my scientific presentations. Without her love and never-failing encouragement this thesis would likely never have materialized.

I wholeheartedly thank all those mentioned as well as those of you whom have eluded my memory.

- Hjalte

September 2017, Aalborg

# ABBREVIATIONS

**AD** – Atopic dermatitis

**AUC** – Area under the curve

**CCL 1/11/17** – C-C-motif chemokine ligand 1/11/17

**CGRP** – Calcitonin gene-related peptide

**CMi** – Mechano-insensitive C-fiber

**FLPI** – Full-field laser perfusion imaging

**GLMS** – Generalized labeled magnitude scale

**H<sub>1/4</sub>** – Histamine receptor 1 / 4

**IL 4/13/31** – Interleukin 4/13/31

**MRGPR A/D** – Mas-related G-protein coupled receptor A / D

**NGF** – Nerve growth factor

**NRS** – Numerical rating scale

**PAR-2** – Proteinase-activated receptor 2/4

**PmC** – Polymodal C-fiber

**PoC** – Proof-of-concept

**QST** – Quantitative sensory testing

**SCORAD** – Scoring atopic dermatitis

**SLIGKV** – Ser-Leu-Ile-Gly-Lys-Val-NH<sub>2</sub>

**TRP V1/A1** – Transient receptor potential subfamily vanilloid 1 / ankyrin 1

**VAS** – Visual analogue scale

**VIP** – Vasoactive intestinal polypeptide

**YLD** – Years lived with disability

# PREFACE

This PhD thesis is comprised of work conducted between December 2014 and December 2017 at the Center for Sensory-Motor Interaction, Faculty of Medicine, Aalborg University, Denmark. The work was in part funded through grants from Grosser L. Foghts Fond, Aase of Ejnar Danielsens Fond and Kong Kristian X's Fond. Between March 2017 and August 2017, work relating to the thesis was carried out at Department of Dermatology, University of Miami, US, under the mentorship of Prof. Gil Yosipovitch. This stay abroad was funded through an EliteForsk travel stipend, granted by the Danish Ministry of Science, Technology and Innovation. The PhD dissertation constitutes a contribution to the understanding of itch, itch sensitization as well as the relationship between itch and pain in humans. From a neurophysiological viewpoint pruriception and nociception are often indistinguishable, and itch and pain even definitionally overlap. Yet, as sensory experiences they are quite clearly distinct.

The aims of this PhD project were to explore acute human surrogate models of itch and itch sensitization as well as to use such models and assessment techniques in a pharmaceutical proof-of-concept context and to explore itch sensitization occurring in patients with moderate-to-severe chronic itch due to atopic dermatitis.

The first chapter presents the necessary introductory knowledge on the clinical challenge of itch, human itch neurophysiology, and states the aims of the project in addition to providing an overview of the dissertation. The second chapter explores methods of itch provocation and depicts the methodology used to psychophysically assess itch, pain and itch sensitization as well as cutaneous vasomotor responses. The third chapter describes the applicability of human surrogate itch models for testing of drugs with potential itch-relieving properties and shows profound antipruritic effectiveness of a marketed neuropathic pain pharmaceutical. The fourth chapter explores sensitization observed in chronic itch patients with emphasizes on intra- and extra-lesional non-histaminergic itch sensitization found in patients with atopic dermatitis. Lastly, the thesis is completed in final fifth chapter with a brief conclusion and future perspectives. Throughout the dissertation the gaps in our current understanding of itch as a somatosensory modality are highlighted and, where relevant to the results of the thesis, methods to address these gaps in humans are proposed. The primary content of this dissertation is based on four original papers, which have been published in international peer-reviewed journals.

# TABLE OF CONTENTS

<b>Chapter 1. Introduction .....</b>	<b>17</b>
1.1. Itch – a clinical challenge .....	17
1.2. Itch – a basic scientific challenge .....	18
1.2.1. Itch coding hypotheses .....	19
1.2.2. Uncharted territory .....	25
1.3. Itch sensitization .....	26
1.3.1. Assessment of itch sensitization .....	28
1.3.2. Mechanical itch dysesthesias as proxies of sensitization .....	28
1.4. Aims of PhD project .....	30
1.4.1. Papers and dissertation overview .....	30
<b>Chapter 2. Human surrogate models of acute itch.....</b>	<b>33</b>
2.1. Human surrogate models of sensory symptomatology.....	33
2.2. Methods of itch provocation in humans .....	34
Itch psychophysics.....	35
2.2.1. Itch intensity .....	35
2.2.2. Itch quality .....	37
2.2.3. Itch sensitization features .....	38
2.3. Vasomotor responses to itch provocations .....	42
2.4. Topographical considerations.....	45
<b>Chapter 3. Topical capsaicin-induced sensory desensitization .....</b>	<b>49</b>
3.1. Topical capsaicin-induced desensitization .....	49
3.2. Topical capsaicin and itch .....	50
3.3. Antipruritic potential of 8% topical capsaicin .....	51
3.3.1. Pruriceptive fibers affected by capsaicin.....	53
3.3.2. Preferential non-histaminergic antipruritic efficacy of 8% capsaicin .....	54
<b>Chapter 4. Itch sensitization in patients with atopic dermatitis.....</b>	<b>57</b>
4.1. Atopic dermatitis .....	57
4.1.1. Skin alterations in atopic dermatitis .....	58
4.1.2. Itch in atopic dermatitis .....	59
4.1.3. Emerging drugs for atopic dermatitis .....	60

4.2. Sensitization in patients with pain .....	60
4.3. The sensory correlates of itch sensitization in atopic dermatitis .....	63
4.3.1. Thermosensory changes .....	64
4.3.2. Mechanical itch dysesthesias .....	65
4.3.3. Sensitivity to pruritogens .....	67
4.4. Potential clinical utility of psychophysical tests for itch sensitization .....	71
<b>Chapter 5. Conclusion .....</b>	<b>73</b>
5.1 Future perspectives .....	73
<b>Literature list .....</b>	<b>75</b>

# CHAPTER 1. INTRODUCTION

## 1.1. ITCH – A CLINICAL CHALLENGE

For most people itch is considered to be in the realm of sensory peculiarities like tingling or paraesthesia. Beyond the occasional nuisance from a mosquito bite, an innocuous rash, exposure to a toxic plant, or just wearing an unpleasant woolen sweater etc., most people can go through life largely unbothered by itch. However, for the estimated 8.2-16.7% of the population<sup>1-4</sup>, who suffer from chronic itch this is far from the case<sup>5</sup>. This group includes patients with a wide range of medical conditions, such as atopic dermatitis (AD), urticaria, psoriasis, prurigo, bullous pemphigoid, cholestasis and chronic renal failure, as well as several neuropathic-, infectious-, neoplastic-, autoimmune - and drug-induced conditions<sup>5-7</sup>.

The unfamiliar reader might ask, “Well it’s just itch, how bad can it be?” The answer to that question is twofold; firstly, chronic itch is rarely present alone but is almost always associated with other sensory symptoms, most prominently sensations of pain, heat, pricking and other sensory dysesthesias<sup>8-14</sup>. For example, in AD, 57.3-87% of the patients report pain from the lesional skin areas<sup>8,10</sup>. Secondly, the clinical spectrum of itch conditions is wide-ranging: e.g., a mild case of contact dermatitis that resolves spontaneously, a patient with nostalgia paraesthetica who wakes up 10 times a night during itch bouts<sup>15</sup>, a patient with AD who scratches until drawing blood every day<sup>16,17</sup>, or in a particularly extreme case, a patient with post-herpetic itch reported to have scratched through the skin and skull bone of her forehead and into her brain parenchyma<sup>18</sup>. Despite sparse research, chronic itch is consistently found to significantly reduce quality of life, for instance related to disturbed sleep, attention and sexual function<sup>9,11,16,19,20</sup>. Itch is also consistently linked to increased rates of anxiety, depression and even suicidal ideation<sup>21-24</sup>.

In 2016, the Global Burden of Disease Consortium published a study on the disease categories that imposed the largest burden of disease globally, measured in years lived with disability (YLD)<sup>25</sup>. Skin diseases, excluding cutaneous neoplasms, were ranked as the fifth leading global cause of YLDs, above diseases categories such as diabetes, migraine and osteoarthritis (numbers 6, 7 and 13, respectively). Naturally, not the entire YLD burden imposed by skin diseases is related to itch, but a very significant proportion is. This becomes quite evident when looking into the specific diagnoses; amongst those contributing most YLDs in the skin disease category are: atopic dermatitis, psoriasis and urticaria (numbers 1, 2 and 6 in YLDs, respectively

<sup>25</sup>). These are three conditions that include itch as a major symptom, and where itch is very often the primary complaint of the patients <sup>10,26,27</sup>. Additionally, a myriad of non-dermatological disorders, such as systemic and neuropathic diseases are associated with significant itch <sup>5,28–30</sup>. The socioeconomic implications are correspondingly significant. A study from 2001 estimated that the yearly-incurred cost of AD in the US alone was around \$1.7 billion at that time, and since then the prevalence of the disease has increased significantly <sup>31–33</sup>. This cost is to a large extent driven by a high disease prevalence and a poor efficacy of the available therapeutic options <sup>31,32,34</sup>.

In most clinical scenarios, chronic itch is difficult to treat, and with a few exceptions, conditions associated with chronic itch do not respond well to antihistamines <sup>35–39</sup>. As of 2017, no ‘general use’ antipruritics are available, and the development of efficacious pharmaceutical treatments has proven arduous and ineffective <sup>37,38,40</sup>. This is perhaps because the human neurobiology and pathophysiology of itch is relatively poorly elucidated despite numerous recent advancements, particularly within the last decade <sup>37,41–48</sup>. While various disease-specific biological drugs are slowly emerging, the treatment of most chronic itch conditions of the skin is still first and foremost focused on targeting inflammatory lesions <sup>38,49,50</sup>. However, it is frequently observed, for instance in AD, that even significant remission of the lesions does not necessarily relieve the associated itch <sup>16,26,36,51</sup>. In fact, in the case of AD, the objective lesional evaluation and the subjective severity of itch are surprisingly poorly correlated <sup>51</sup>. This observation, amongst others, has led to the suggestion that neuronal sensitization may contribute significantly to the sensory symptomatology of the disease, as is widely accepted to be the case for many chronic pain conditions <sup>13,14,52–54</sup>. Another factor impeding the management of chronic itch is the scarcity of randomized controlled trials with antipruritics, which has forced clinicians to resort to use of off-label drugs <sup>37,38,40</sup>. Lastly, the fact that an effective non-histaminergic model of itch has only recently been established and mechanistically explored <sup>42,55–58</sup> means that prior human proof-of-concept (PoC) studies on itch have mostly explored the effect of potential antipruritics in relation to the clinically less relevant histaminergic itch pathway, thus reducing the actual predictive power of the earlier PoC studies <sup>59</sup>.

## 1.2. ITCH – A BASIC SCIENTIFIC CHALLENGE

Itch and pain, although distinct in perception, are highly entwined sensory modalities with numerous similarities. Both are perceived as unpleasant, both tend



to spread in chronic conditions and both causes central sensitization-associated sensory signs (termed *alloknesis* and *hyperknesis* for itch and *allodynia* and *hyperalgesia* for pain, see *section 1.3.2.*)<sup>60,61</sup>. Moreover, both sensations prompt a reflex response with hypothesized evolutionary functions. For pain, the nociceptive withdrawal reflex prevents or limits injury, and for itch, the scratch reflex, which is essentially a self-inflicted, weak cutaneous pain that temporarily decreases itch, aids removal of irritants or insects and triggers a focal investigation of the affected area<sup>55,57,62</sup>. Lastly, a number of treatment opportunities represent commonalities; as with pain, itch occasionally responds to, e.g., topical capsaicin, anticonvulsants, local anesthetics and counter-stimulation<sup>37,60</sup> and oppositely, a frequent side effect of opioid analgesics is severe itch.

With such notable differences and similarities, it is not surprising that itch and pain have been studied extensively using comparative methodology. This has resulted in prolonged and ongoing debate related to how itch is transmitted and distinctly recognized from tactile and pain sensation. In the paragraphs below, this ongoing debate is recapitulated with the aims of introducing: 1) the basic concepts of cutaneous itch and pain neurophysiology, 2) the currently occupied positions in the discussion, and 3) the historical scientific background for the contemporary understanding of itch.

### 1.2.1. ITCH CODING HYPOTHESES

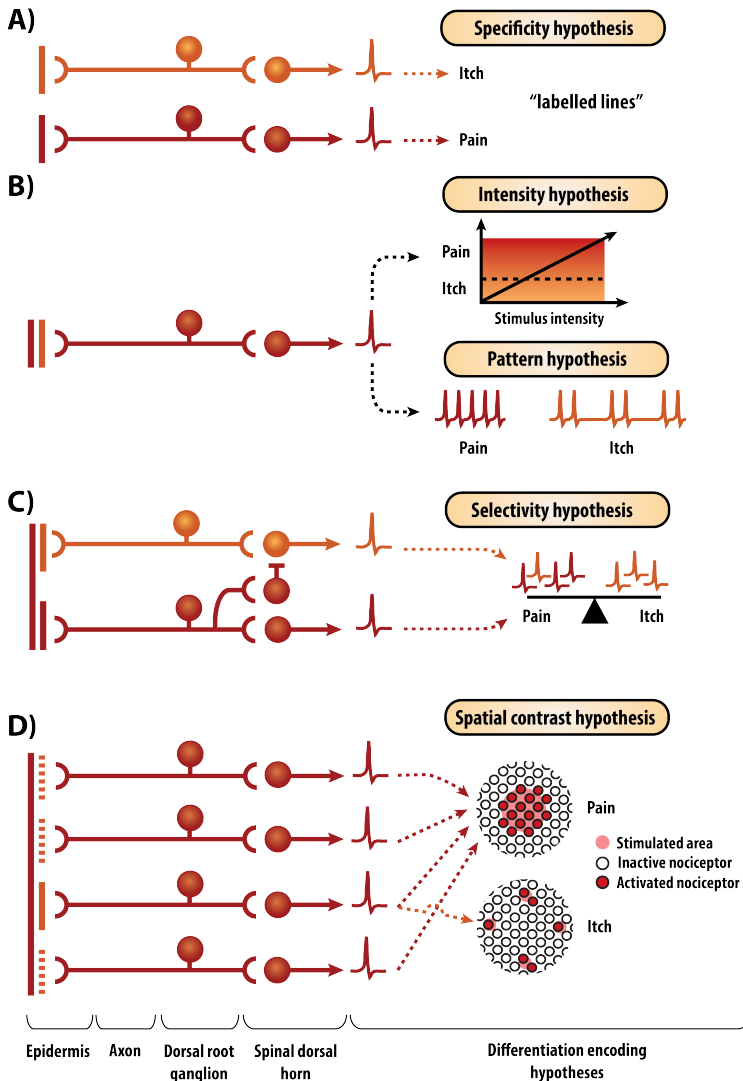
Max von Frey famously referred to itch as ‘pain’s little brother’ and was the first to formulate a coherent concept on the neurophysiology of itch in relation to pain<sup>63</sup>. His suggestion that itch results from mildly painful stimuli, known as the *intensity hypothesis*, (Fig. 1) considers itch to constitute a submodality of pain. This notion was supported by circumstantial evidence: e.g., detection of itch in response to mild punctuate mechanical stimuli, contralateral abolition of both itch and pain sensations by an anterolateral ascending tract cordotomy, and later the finding that unmyelinated peripheral fibers conveyed both sensations<sup>64</sup>. Of note, this proposed concept reversed von Frey’s earlier viewpoint that itch sensation was subserved by touch nerves and the notion that each cutaneous sensation relies on a specialized sensory nerve end organ, which extended on the Müllerian doctrine of specific nerve energy and thus represents a kind of *labeled line* (Fig. 1A) progenitor idea<sup>65–67</sup>.

After Henry Dale, who was later awarded the Nobel Prize, discovered histamine in 1910, a reliable chemical itch provocation became available<sup>68</sup>. Lewis studied histamine-induced skin responses extensively but paid less attention to the evoked

itch<sup>69</sup>, while Bickford was the first to describe “itchy skin”, the perceptual correlate of spinal itch sensitization<sup>70</sup> (later dubbed *alloknesis* and *hyperknesis*<sup>71–73</sup>). In the 1950s, Cormia and Kuykendall observed itch in response to heat stimuli in partly anesthetized human skin<sup>74,75</sup>. Notably, they also performed some of the first meticulous psychophysical studies on itch using histamine injections and observed lowered itch thresholds in lesional skin of patients with itch as well as hyperknesis<sup>74,75</sup>. A few years later, Keele and Armstrong found that low histamine concentrations applied to exposed dermo-epidermal nerves caused itch while higher concentrations evoked pain. They also found that deeper injections of histamine never produced itch but only pain<sup>76,77</sup>. Nominally, these observations were compatible with the idea of intensity coding, with itch being a strictly cutaneous feature.

However, many parallel and simple findings were at odds with the intensity hypothesis. For instance, pain can arise from most tissues while itch is restricted to the skin and adjoining mucosa; reflex responses are completely different for itch and pain (scratching versus withdrawal); many pain-induction modalities never seems to evoke itch (e.g., cold, heat, deep pressure); opioids selectively reduce pain while causing or aggravating itch; first-line pain relievers such as non-steroidal anti-inflammatory drugs work well for minor pains but not itch<sup>60</sup>; and patients often report severe itch exacerbations which are not associated with a transition to pain<sup>6,38,78,79</sup>.

In 1960, Wall and Cronly-Dillon published a study of neurography recordings in cats during various stimuli, including cowhage provocations (*mucuna pruriens* spicules), and proposed that different temporal discharge patterns composed in the central nervous system could give rise to sensory quality distinction (*pattern hypothesis*, Fig. 1B)<sup>80</sup>. Notably, this idea of a characteristic temporal discharge pattern being a key differentiation feature of itch was proposed as early as 1941<sup>64</sup> and rekindled by a recent study in non-human primates<sup>81</sup>. However, this pattern hypothesis is unsupported by psychophysical data<sup>82</sup>, and the potential central filtering mechanisms are largely unaccounted for.



**Figure 1. Proposed neuronal encoding hypotheses to explain differentiation between itch and pain sensations.** Red = nociceptive signaling, orange = pruriceptive signaling. Vertical bars left of the receptive fields represent noxious (red) and pruritic (orange) stimuli. **A)** Two separate 'labeled lines' respond solely to nociceptive or pruriceptive stimuli. **B)** A single primary afferent population detects both nociceptive and pruriceptive stimuli and encodes information based on stimulus intensity (top) and/or as a particular firing pattern (bottom), e.g. with higher inter-burst intervals. **C)** Amongst nociceptive fibers a subgroup is also itch-selective, and when this subgroup is preferentially stimulated, itch rather than pain arises. If a substantial proportion of non-itch-selective nociceptors are co-activated only pain is perceived due to spinal inhibitory processing (red interneuron). **D)** All cutaneous nociceptors are potential pruriceptors if they are activated with sufficiently high spatial contrast, i.e., if a few units are activated whilst neighboring fibers remain silent.

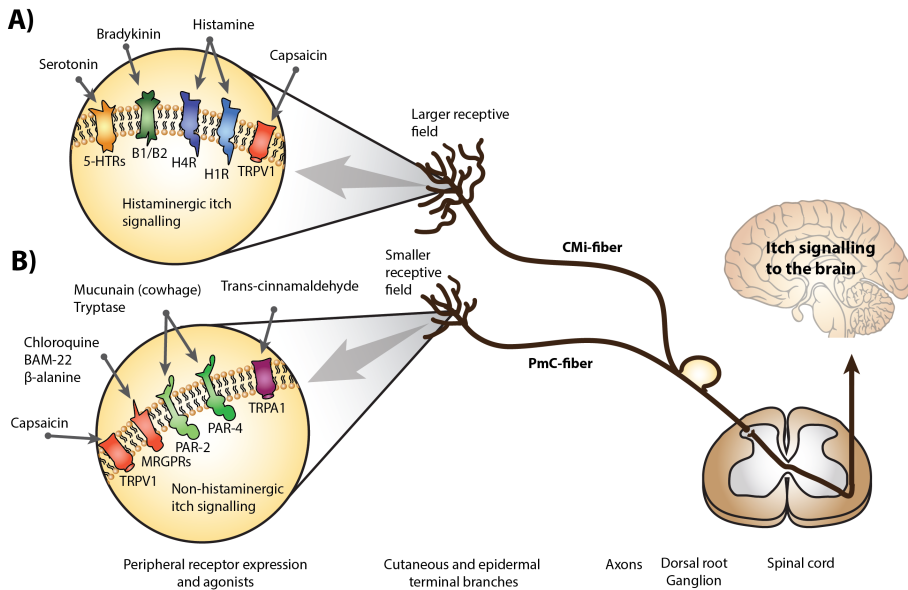
### 1.2.1.1 Histaminergic itch

Following methodological improvements of the microneurography technique it became evident that two functionally distinct classes of unmyelinated nociceptive afferents existed: mechano-heat sensitive ‘polymodal’ C-fibers (PmC) and mechano-insensitive (often previously referred to as ‘silent’) C-fibers (CMi)<sup>83–88</sup>. The two subclasses differ not only in responsiveness to mechanical stimuli but also in their conduction velocities, the sizes of their receptive fields, and the degree to which they exhibit activity-dependent slowing<sup>86,89,90</sup>. This finding had an impact on itch neurophysiology when a subsequent seminal study showed a striking correspondence between the firing of specific CMi-fibers and subjective itch sensations following histamine iontophoresis<sup>91,92</sup>. This result strongly indicated that a subpopulation of CMi-fibers with very low conduction velocities specifically mediated itch (*specificity hypothesis / labeled line*, Fig. 1A). Subsequently, however, it was shown that these histamine-sensitive CMi-fibers (along with other C-nociceptors) also responded to various allergens subjectively causing pain<sup>93,94</sup>. Thus a *selectivity hypothesis* (Fig. 1C) was proposed, suggesting that itch is perceived only when predominate activation of “itch-selective” histamine-sensitive C-fibers occurs, whereas less specific nociceptive fiber activation results in pain even if the itch-selective fibers are also activated<sup>93,95,96</sup>. Since pain quite clearly mediates segmental inhibition of itch, such a system must in essence be wired similarly to a labeled line system with the exception that the peripheral pruriceptor of the *selectivity hypothesis* also detects nociceptive stimuli but both project onto a central pruriceptive pathway (Fig. 1C)<sup>97</sup>. For histaminergic human itch transduction, this remains the status quo; CMi-fibers expressing histamine-receptors 1 and 4 (H<sub>1/4</sub>), convey itch evoked by histamine, and probably other pruritogens, in what appears to be a selective manner (Fig 2A).

### 1.2.1.2 Nonhistaminergic itch

While histamine is the most commonly studied mediator of itch, it had been shown early on and quite convincingly that histamine cannot account for the itch observed in numerous clinical conditions<sup>26,37,38,98</sup>. This is very clear in AD, where several clinical trials have demonstrated a lack of antipruritic effect of antihistamines, and experimental studies have shown patients to be equally or even less sensitive to extra-lesional histaminergic provocations compared to healthy controls<sup>26,28,35,36,99</sup>. Less than a decade ago, neuroscientists began to reappraise the properties of cowhage as an itch inducer<sup>42,55–58</sup>. Shelley and Arthur reported already in the 1950s that cowhage-induced itch seemed rather distinct from that evoked by histamine both in terms of quality and because it produced no discernable cutaneous neurogenic flare<sup>100–102</sup>. Thus, this type of itch was more compatible with that occurring in patients where no signs of histaminergic activity (wheal or flare) were

evident. Shelly and Arthur also successfully extracted the active itch-inducing enzyme called mucunain<sup>100,101</sup>, which in 2008 was shown to work by engaging the proteinase-activated receptors 2/4 (PAR-2/4) expressed on epidermal C-fibers<sup>58</sup>. Within the last decade, comparative microneurographic, psychophysical and vasomotor imaging studies on itch induced by cowhage and histamine provocations have been conducted<sup>42,55,57,62,103</sup>. These studies are very well aligned and confirm that while a subset of CMi-fibers transmit histaminergic itch, cowhage-induced itch is almost exclusively conveyed by PmC-fibers<sup>42,57,103</sup>. This also matches up closely with the vasomotor findings (see *section 2.4*), since the capacity to generate a secondary neurogenic flare is predominantly a CMi-fiber feature, as well as psychophysical data showing that cowhage-induced itch is completely recalcitrant to antihistamines<sup>56,57</sup>. Thus a notion of two distinct peripheral subpathways for itch has emerged, provisionally referred to as ‘histaminergic’ and ‘nonhistaminergic’ itch<sup>57,104,105</sup> (Fig. 2A and B). This *ad hoc* taxonomy is somewhat suboptimal when, as frequently done, used in reference to the neuronal pathways *per se*<sup>38,106</sup> – ‘histaminergic’ itch because many other pruritogens could activate the same peripheral afferents without engaging histamine-receptors nor being blocked by antihistamines, and ‘nonhistaminergic’ because it is solely a definition by negation. In this context, it should also be emphasized that there is not a clear separation between histamine-responsive CMi-fibers and cowhage-responsive PmC-fibers in rodents<sup>107</sup>, highlighting that notable species differences exist for neurophysiology of itch and pain and underlining the need for translation of mechanisms elucidated in rodents<sup>41</sup>. Because microneurography experiments have consistently shown that essentially all PmC-fibers respond to various pain-evoking stimuli as well as cowhage<sup>42,81,103</sup>, the explanatory encoding models mentioned above have recently been rendered insufficient, and the discussion of the differentiation encoding of itch and pain has resurfaced<sup>96,108–110</sup>.



**Figure 2.** An overview of peripheral induction of itch by activation of receptors on mechano-insensitive (CMI) and polymodal (PmC) C-nociceptors. Only agonists applied as pruritogens in humans are shown. Pruriceptive CMI-fibers (A) express e.g. 5-HTRs, B1/B2, H1/4R and TRPV1 and conduct histaminergic itch, while subgroups of PmC-fibers (B) express MRGPRs, PAR-2/4, and TRPA1 as well as TRPV1 and transmit non-histaminergic itch. This illustration is simplified; there is evidence suggesting that both bradykinin and serotonin receptors are also expressed on PmC-fibers. 5-HTRs: 5-hydroxytryptamin receptors; B1/2: bradykinin receptor B1/B2; BAM-22: Bovine adrenal medullary peptide-22; H1R/4R: histamine receptor H1/H4; MRGPRs: Mas-related G-protein-coupled receptors; PAR-2/4: protease-activated receptor type 2/4; TRPA1/V1: transient receptor potential ankyrin 1/vanilloid 1. In part derived from<sup>105</sup>.

### 1.2.1.3 The spatial contrast hypothesis

The cowhage-sensitive PmC-fibers are clearly not itch-specific nor ‘itch-selective’ nociceptors but largely polymodal, and their activation gives rise to pain under certain circumstances and itch under others<sup>42,110,111</sup>. For instance, experiments with autoclaved cowhage spicules (unable to activate PAR-2/4) coated with capsaicin are capable of inducing intense itch, even though capsaicin almost exclusively elicits pain when administered by intradermal injection or transdermally. Perhaps the most adequate encoding model for explaining these observations relates to the peculiar spatial aspects of itch, which is strictly confined to the superficial skin layers and adjoining mucosa<sup>42</sup>. The hypothesis, tentatively articulated in the 1990s<sup>97,112</sup>, has since come to be referred to as the *spatial contrast hypothesis*. It suggests that for itch to be evoked, C-nociceptor activation simply has to occur in a sufficiently

scattered spatial pattern<sup>42,110</sup>. That is, if just a few nociceptive endings are discharging while nearby neighboring units remain silent, itch is felt, whereas if more uniform nociceptor activation from a skin area occurs, pain is perceived (Fig. 1D). Such an encoding mode would also account for scratch-induced itch relief, which would create denser nociceptor activation in the relevant area. For this hypothesis to hold true principally, activation of any sparsely distributed subgroup of C-nociceptors regardless of the affected area should cause itch while being relatively dispensable for pain<sup>110</sup>. Indirect evidence supports this precondition. For example, histamine-sensitive C-fibers constitute approximately 5-10% of all C-fibers, pruriceptive C-fibers expressing Mas-related G-protein coupled receptor A1 (MRGPR A1) constitute 5% of the entire C-fiber population, and chloroquine-responsive fibers represent 12.8% of the total DRG population in mice<sup>92,93,113,114</sup>. These 3 subpopulations thus inherently create a high spatial contrast signaling pattern and their activation give rise to itch. Psychophysical evidence for the spatial contrast hypothesis is currently lacking or is of a circumstantial nature<sup>115,116</sup>.

#### 1.2.1.4 Implications of itch encoding

The academic pursuit for knowledge on neuronal encoding of itch is certainly not without potential clinical implications. If itch is conveyed in a manner, which does not involve primary afferent C-fibers with a certain degree of specificity or selectivity for pruriception, then development of a pharmaceutical general purpose antipruritic drug acting on the peripheral level is difficult to envision<sup>110,117</sup>. In such a scenario, itch in each inflammatory dermatosis would perhaps result from a complex mix of inflammatory mediators engaging various minority populations of C-fibers, which in turn would also exhibit sensitization and altered transducer expression. Oppositely, if an encoding relying on, e.g., spatial contrast proves accurate, then, at least for conditions of peripheral neuropathic itch and pain, treatment targets would be virtually identical<sup>28,118</sup>. Improved knowledge of the spinal filtering mechanisms responsible for tuning itch versus pain perception following a cutaneous insult might yield potential treatment loci, for instance related to the inhibitory interneurons proposed in the specificity hypothesis and elucidated in several rodent studies<sup>108,119,120</sup>.

### 1.2.2. UNCHARTED TERRITORY

Beyond the neuronal encoding of itch, a number of fairly basic features of the pruriceptive system remain entirely or largely unexplored in humans. These include: locognosia for itch; spatial and temporal summation properties of itch (including

potential differences in the spatial acuity for histaminergic and non-histaminergic itch); the detailed topographic sensitivity for itch; the organization of pruriceptive C-nociceptors in human skin; the primary afferent substrate for mechanically evoked itch; the mechanism by which thermal counter-stimulation can profoundly augment/decrease itch; the afferents involved in generating scratch suppression of itch; the significance of descending inhibition on the pruriceptive system; the importance, in humans, of numerous molecular pruriceptive transducers recently discovered in rodents; the mechanisms behind the opioidergic modulation of itch; the interactions between acute itch sensitivity and stress, exercise, circadian rhythm and sleep deprivation; the potential importance of reduced segmental gating as an aggravating mechanism in patients with chronic itch; and the peripheral and central mechanisms underlying sensitization for itch.

### 1.3. ITCH SENSITIZATION

In association with pain and itch both in inflammatory and neuropathic conditions, peripheral nociceptors may exhibit increased sensitivity to a variety of stimuli. In parallel, the spinal processing of nociceptive and non-nociceptive signals from the periphery can be facilitated in the central nervous system directly or by means of disinhibition<sup>53,121–123</sup>. The International Association for the Study of Pain defines sensitization in the context of pain as: “*increased responsiveness of nociceptive neurons*”<sup>123</sup>. These sensitization processes are thought to contribute to the aggravation and chronification of pain in patients and to mediate clinical symptoms such as hyperalgesia and allodynia to mechanical and thermal stimuli<sup>54,123</sup>. Converging lines of evidence indicate that largely parallel sensitization processes occur for itch. Firstly, chemical responses of C-fibers are characterized by tachyphylaxis. Thus, even in inflammatory dermatoses, continued endogenous release of pruritogens alone can hardly explain the chronic spontaneous itch<sup>111,124,125</sup>. Secondly, the patterns of pain- and itch-evoked dysesthesias are highly similar in terms of spatiotemporal properties<sup>71,73</sup>. Lastly, several lines of indirect evidence, e.g., poor correlation between lesional severity and itch<sup>16,51</sup>, itch in response to normally innocuous mechanical stimuli (such as certain fabrics)<sup>14,126</sup>, examples of significant antipruritic effect of centrally acting GABAergic, serotonergic and noradrenergic drugs<sup>127</sup>, and altered expression of molecular transducers on epidermal C-fibers<sup>128</sup>, suggest neuronal sensitization as an important factor in chronic itch conditions.



Within the field of pain research the molecular mechanisms as well as psychophysical manifestations of sensitization have been extensively studied<sup>54,121,123</sup> but very little is known about the mechanisms causing sensitization specifically for itch. However, the processes leading to sensitization for itch appear to largely overlap with the processes leading to sensitization for pain<sup>129,130</sup>. A thorough outline of the molecular mechanisms leading to neuronal sensitization is not within the scope of this dissertation, but briefly, two processes are involved:

1) *Peripheral sensitization* involves local inflammatory signaling from prostaglandins, interleukins, histamine, tumor necrosis factor alpha and growth factors such as nerve growth factor (NGF) released from the immune cells and keratinocytes<sup>131</sup>. In addition neuropeptides such as calcitonin gene-related peptide (CGRP), and substance P are released from local peptidergic fibers<sup>131,132</sup>. These mediator molecules are involved in the acute and prolonged development of hyperalgesia to, for instance, tactile and thermal stimuli as well as local inflammation, edema, and extravasation<sup>132-134</sup>. Many of these substances have also been directly or indirectly associated with itch signaling or itch sensitization or are found to be increased in clinical conditions characterized by acute or chronic itch<sup>38,135</sup>. In a number of chronic itch conditions also associated with inflammation such as AD, specific chemokines and interleukins (e.g., CCL17, IL-13 and IL-31) are known to directly engage or sensitize C-nociceptors<sup>136-138</sup>. For instance, IL-13 has been shown to induce upregulation of transient receptor potential ankyrin 1 (TRPA1) on pruriceptive C-fibers in rodents<sup>138</sup>. This interaction constitutes an example of a mechanism of peripheral itch sensitization. The inflammatory soup sparks activity in multiple intra-cellular signaling pathways in nociceptive neurons leading to increased phosphorylation and transcription of, e.g., transduction molecules characteristic for nociceptors, such as transient receptor potential channels (TRPs) and sodium channels (Na<sub>v</sub>1.8 and 1.9), ultimately leading to increased excitability of nociceptive and pruriceptive A $\delta$ - and C-fibers<sup>132,139</sup>.

2) *Central sensitization* affects neurons in the central nervous system and is mechanistically related to synaptic plasticity, activation of glial cells, spinal disinhibition and decreased endogenous modulation<sup>54,123,140,141</sup>. Excitatory synaptic communication between first-order neurons and spinal cord neurons can be facilitated, e.g., by the neurotransmitter glutamate and modulated by factors such as CGRP and brain-derived neurotrophic factor. Accordingly, post-synaptic glutamatergic receptors, are important for tuning synaptic transmission following persistent nociceptive activity<sup>123,141</sup>. Spinal changes include long-term potentiation of synapses as well as an increase in glial activity and hyper-responsiveness of nociceptive spinal dorsal horn neurons, leading to overall increased sensitivity to

noxious stimuli<sup>123,141</sup>. Both spinal microgliosis and astrocytosis have been extensively demonstrated in relation to pain and recent studies show that itch cause similar glial activation patterns<sup>142,143</sup>. Lastly, a large body of evidence also describes profound alterations in brain connectivity and even brain morphology associated with chronic pain<sup>144–147</sup> and to a lesser extent chronic itch<sup>148,149</sup>, thought to contribute to aberrant or amplified sensory perception.

### 1.3.1. ASSESSMENT OF ITCH SENSITIZATION

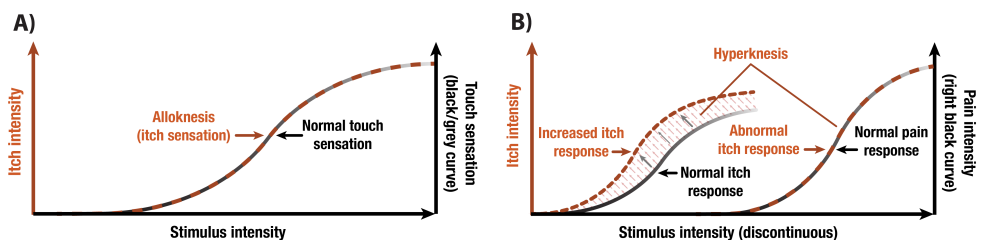
One of the sensory end results of the molecular processes mentioned above is a leftward shift of the stimulus-response curve to nociceptive (or pruriceptive) stimuli, i.e., responsiveness to previously subthreshold stimuli and increased responsiveness to suprathreshold stimulation<sup>13,118,150</sup>. In parallel, stimuli that are incapable of activating pruriceptive or nociceptive units under normal conditions (e.g., a light brush stroke) can be centrally rerouted in the sensitization process, allowing those stimuli to cause itch ('alloknesis') or pain ('allodynia')<sup>71,72,106</sup>. Various psychophysical tests have been developed to probe somatosensory sensitization<sup>151</sup>. Electrophysiological recording directly from peripheral nociceptive afferents in humans using microneurography was initially developed in the 1960s but still remains a clinically unfeasible option. However, microneurography can be used to measure sensitization of primary afferents in certain experimental designs, usually in healthy subjects following acute sensitization models<sup>86,152</sup>. Recording from central nociceptive or pruriceptive circuitry is not possible in humans. Thus, psychophysical means of characterizing itch sensitization, typically using chemical, mechanical or thermal stimulations, are considered the mainstay for human experimental and clinical research<sup>106,151</sup>. By far the most common way of assessing sensitization for itch is by applying chemical itch provocations or dynamic and punctuate mechanical stimuli (see *section 2.3.3* and *4.3.2*).

### 1.3.2. MECHANICAL ITCH DYSESTHESIAS AS PROXIES OF SENSITIZATION

Punctuate cutaneous mechanical stimuli delivered by a weighted needle or a thin filament induces a pricking sensation often associated with a delayed mild itch or tickling<sup>71,73,153</sup>. If the nature of the stimulus is dynamic and of lower intensity, such as drawing a hair across the skin surface, the resulting sensation will often be described as 'tickling' but the associated motor response is consistently scratching

or rubbing and thus in accordance with the definition of itch. This observation is not new; von Frey suggested in his last review on cutaneous sensations that superficial tickling of this kind might be a mixture of tactile and itch impulses<sup>63</sup>. In the 1930s Pritchard studied mechanically induced itching and tickling in patients with neuropathic lesions. Notably, reductions of itch were not observed in peripheral neuropathy patients with selective touch hypoesthesia and examinations of patients with lesions of the central nervous system revealed that itch and pain were always altered (e.g., abolished) in parallel, independently of touch sensation<sup>64,154,155</sup>.

Lewis' studies on cutaneous hyperalgesia<sup>156</sup> and the observation of altered sensitivity to light stroking next to an itching gnat bite led Bickford to investigate mechanically evoked itch in a skin area after an initial chemical itch provocation with histamine<sup>70</sup>. A preceding itch provocation was found to consistently produce a surrounding area of skin, termed 'itchy skin', in which light tactile stimuli elicited itch. These experiments were revisited and extended on by LaMotte *et al.* (1988 and 1991) when a more comprehensive understanding of somatosensory neurophysiology had developed<sup>70-72</sup> and the more precise terms 'alloknesis' and 'hyperknesis' was proposed instead of 'itchy skin'. *Alloknesis* describes the state in which an innocuous for instance tactile stimulus evokes itch, while *hyperknesis* principally acts as an umbrella term also encompassing the state in which there is enhanced itch to normally itch- or pain-provoking stimuli or simply lowered itch threshold to a given stimulus<sup>71-73,157</sup> (Fig. 3A and B). Thus, the terms alloknesis and hyperknesis are completely parallel to *allodynia* and *hyperalgesia*, for pain respectively<sup>158</sup>. Allo- and hyperknesis are referred to as being 'primary' if they occur within lesional skin or, e.g., within a skin area provoked with a pruritogen, and 'secondary' if they occur perifocally to such a lesion or provocation<sup>79,159</sup>.



**Figure 3. Concept illustrations of alloknesis and hyperknesis.** *Alloknesis (A)* comprises a switch in perception of a normally innocuous stimulus such as light stroking of the skin, which additionally or alternatively becomes itch evoking. *Hyperknesis (B)* represents a leftward shift in the stimulus-response curve for a normally itching stimulus or a somatosensory modality-switch phenomenon in which a typically pain-predominant stimulus is perceived as itching. The stimulus scale (marked with \*) on the x-axis of plot **B** is discontinuous and not all stimulus modalities can evoke both itch and pain. Moreover, hyperknesis occurring in response to a painful stimulus may not be elicited along the whole stimulus-response curve.

Currently, this terminology is used almost exclusively when referring to sensitization probed by mechanical stimuli. However, it was noted early in the literature that, e.g., hyperknesis could as well refer to increased itch in response to a chemical itch provocation<sup>160</sup>. These dysesthetic states may last for a couple of minutes to hours after an itch provocation or can be a persistent feature in for instance patients with chronic itch due to AD<sup>13,14,161</sup>. The methods by which itch dysesthesias can be quantified as well as their purported underlying mechanisms are summarized in *section 2.3.3*.

## 1.4. AIMS OF PHD PROJECT

The aim of this PhD project was to explore the applicability and utility of using histaminergic and non-histaminergic models of itch and itch sensitization in humans for three purposes:

1. To perform a basic mechanistic studies on itch and itch sensitization in healthy controls
2. To evaluate the antipruritic effects of novel or off-label pharmaceutical interventions
3. To explore potential pathway-selective sensitization for itch in patients with chronic itch

To this end the thesis is based on four peer-review papers: a literature study and three experimental studies (each addressing one of the bullet points above).

### 1.4.1. PAPERS AND DISSERTATION OVERVIEW

**Study I: Andersen HH**, Elberling J, Arendt-Nielsen L. Human Surrogate Models of histaminergic and non-histaminergic Itch. *Acta Derm Venereol.* 2015; 95: 771-777. (Review)

**Study II: Andersen HH**, Elberling J, Lo Vecchio S, Arendt-Nielsen L. Topography of itch: evidence of distinct coding for pruriception in the trigeminal nerve. *Itch.* 2016; 1: 1-10.

**Study III: Andersen HH**, Marker JB, Hoeck EA, Elberling J, Arendt-Nielsen L. Antipruritic effect of pretreatment with topical capsaicin 8% on histamine- and cowhage-evoked itch in healthy volunteers: a randomized,

vehicle-controlled, proof-of-concept trial. *Br J Dermatol.* 2017; 177: 42-49.

**Study IV: Andersen HH**, Elberling J, Sølvsten H, Yosipovitch G, Arendt-Nielsen L. Non-histaminergic and mechanical itch sensitization in atopic dermatitis. *Pain.* 2017; 158: 1780-1791.

These papers will from hereon be referred to as named above (Study I to IV). Options of itch models were extracted from the literature (Study I). The two selected models were utilized in a mechanistic study also presenting an easily applicable method for assessment of mechanical itch sensitization (Study II). In turn methodology from Study II was applied in the both Studies III and IV. Study II is a basic mechanistic study in healthy controls with the specific aim of addressing topographic sensitivity to itch and itch sensitization probing. Study III is a pharmaceutical PoC study on the antipruritic effect of 8% topical capsaicin based on initial bedside observations. Study IV is a comprehensive assessment of sensory sensitization in patients with chronic itch due to AD.

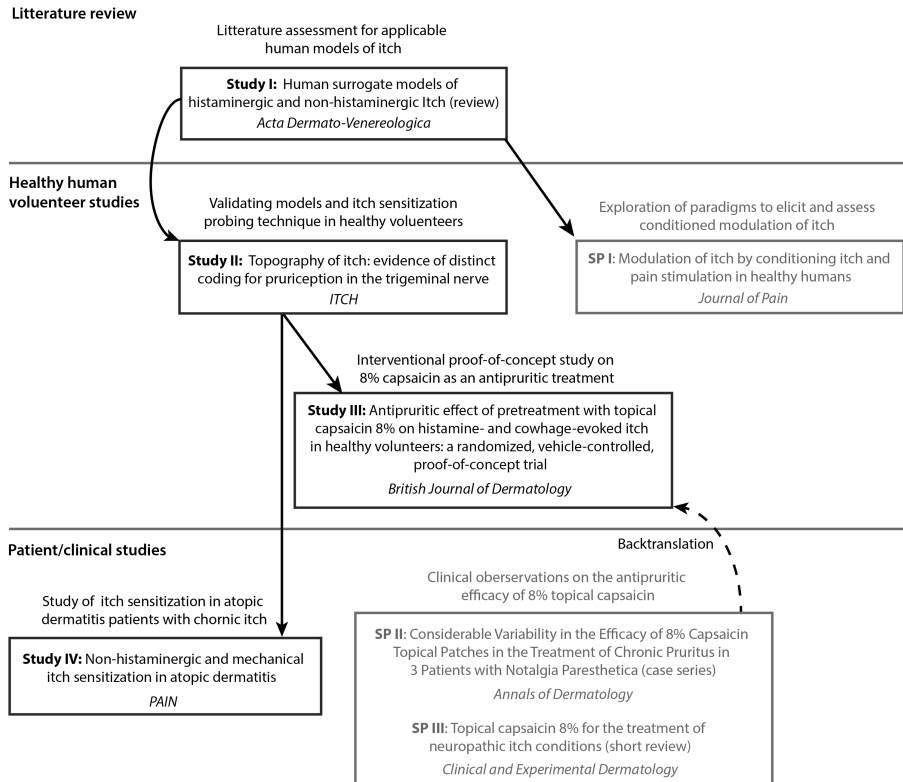
In addition, context and data is derived from four *supplementary papers*, hereafter referred to as SP I-III:

**SP I: Andersen HH**, van Laarhoven AIM, Elberling J, Arendt-Nielsen L. Modulation of itch by conditioning itch and pain stimulation in healthy humans. *J Pain.* 2017 [Epub ahead of print].

**SP II: Andersen HH**, Sand C, Elberling J. Considerable Variability in the Efficacy of 8% capsaicin topical patches in the treatment of chronic pruritus in 3 patients with notalgia paresthetica. *Ann Dermatol.* 2016; 28: 86-89. (*Case series*)

**SP III: Andersen HH**, L. Arendt-Nielsen, J. Elberling. Topical capsaicin 8% for the treatment of neuropathic itch conditions. *Clin and Exp Derm.* 2017; 42: 596-598. (*Short Review*)

The illustration below (Fig. 4) provides an overview of the studies, the basic research ideas and the relationship between the studies.



**Figure 4.** A schematic overview of the dissertation studies and associated supplementary papers. Based on a literature review (Study I), two itch models were applied in a basic study (Study II) and a pharmaceutical proof-of-concept study (Study III), of which the tested intervention was derived from clinical observations (Supplementary papers II and III). Subsequently, itch sensitivity was tested in AD patients with chronic itch (Study IV) using the methods initially applied in Study II and III. Lastly, results from Study IV, led to a study conducted to develop a psychophysical paradigm for assessment of endogenous descending itch inhibition (SP I).

# CHAPTER 2. HUMAN SURROGATE MODELS OF ACUTE ITCH

## 2.1. HUMAN SURROGATE MODELS OF SENSORY SYMPTOMATOLOGY

The purpose of somatosensory human surrogate models is to reversibly reproduce a specific symptom or set of symptoms associated with a particular disease<sup>123,162-165</sup>. Models are usually designed to evoke, e.g., pain, itch and/or sensitization through a particular mechanism, but models can also be used to study sensory symptoms not immediately associated with pain<sup>123,162-164,166</sup>. Cutaneous pain and hyperalgesia can be achieved by chemical, thermal, electrical or mechanical provocations as well as a combination of such stimuli<sup>162,164</sup>. Before, during and after the evoked pain or hyperalgesia is established, the somatosensory and, in particular, the nociceptive system can be probed, and mechanistic evaluations can be undertaken. Solely for the purpose of mimicking, for instance, neuropathic pain symptoms, an extensive list of human surrogate models exists<sup>164,167,168</sup>, many of which have been used in the profiling of analgesic compounds<sup>169</sup>.

Broadly speaking human surrogate models can serve three distinct purposes: 1) basic mechanistic studies in healthy volunteers (e.g., Study II and SPI), 2) pharmacological proof-of-concept studies to evaluate the efficacy of novel and existing compounds (e.g., Study III), 3) clinical studies where the models are used as mechanistic assessment tools or for diagnostic, prognostic or monitoring purposes (e.g., Study IV).

A good surrogate model is reliable and disease-relevant to the extent possible. A valid human model must temporarily induce the aspects of the symptomatology associated with the actual disease, preferably through a similar or the same mechanism that causes the symptom in the given disease state<sup>123,162-165</sup>. For instance, topical capsaicin specifically evokes cutaneous heat and pinprick hyperalgesia commonly observed in post-herpetic neuralgia, while the high-concentration L-menthol model specifically evokes cold allodynia, often observed in chemotherapy-induced peripheral neuropathy<sup>122,170-173</sup>. Similarly, a cutaneous pain model may not be a relevant way to study visceral pain mechanisms, and the ultraviolet B-model of cutaneous hyperalgesia may not be a relevant way to mimic neuropathic dysesthesias (normally not associated with significant cutaneous inflammation)<sup>162,164,165,174</sup>. For obvious ethical reasons, human models of both pain

and itch symptomatology are acute or subacute and do not usually involve prolonged skin inflammation, as is the case for the most prevalent chronic itch conditions<sup>162,164</sup>. As a consequence, the degree to which such models can mechanistically mimic the processes involved in chronic itch is a matter of ongoing contention<sup>174</sup>. To ameliorate this discordancy, it has been proposed to combine models of cutaneous neuronal sensitization, skin inflammation and/or barrier integrity deficiency with commonly used acute itch models to more adequately account for conditions present in common inflammatory dermatoses<sup>130,175,176</sup>, which constitute the largest fraction of chronic itch conditions.

## 2.2. METHODS OF ITCH PROVOCATION IN HUMANS

Study I narratively covers methods commonly used to induce itch in humans. For itch the vast majority of effective models rely on chemical provocations although itch can also be elicited with mechanical and electrical stimulation<sup>105,106</sup>. Based on a literature survey the two most commonly used human models of itch are application of histamine (skin prick test puncture, intradermal injection or iontophoretic delivery) and application of mucunain using spicules from the cowhage plant (*Mucuna var. pruriens*)<sup>105,106</sup>. These models are not only amongst the most effective described but the mechanisms by which they induce itch are also well explored (see *section 1.2.1*)<sup>42,57,103,106</sup>. In Studies II, III and IV both of these models of itch are used in parallel. There are several preceding examples of these human surrogate models of itch being used for both basic mechanistic studies<sup>57,177–179</sup> and pharmacological proof-of-concept studies<sup>35,180,181</sup> as well as explorative clinical studies<sup>14,126,182,183</sup>. Application of 1% histamine in the present studies was performed with skin prick test lancets because this delivery method has previously been found to evoke more itch and have higher reproducibility than applying histamine with iontophoresis or intradermal injection<sup>184</sup>.

As discussed for human surrogate models of pain, external validity is also crucial for models of itch. This relates particularly to the notion of histaminergic vs. non-histaminergic models<sup>185</sup>. If one aims to conduct a proof-of-concept study to test the potential efficacy of a potential antipruritic drug with the aim of later utilizing it to treat AD or psoriasis, using a histaminergic model does not constitute a reasonable way to do so<sup>42,56,57,105,106</sup>. The drug may very well be highly effective and reduce histaminergic itch, but since histamine is not a key factor for itch in, for instance, AD or psoriasis, the study would have no predictive power on the potential clinical effect of the drug, and thus there is a lack of meaningful generalizability



<sup>26,37,38,126,129,186</sup>. Instead non-histaminergic models, e.g., relying on PAR-2 signaling, which has been directly implicated in itch in AD, would be preferable and presumably give a more accurate impression of the clinical potential of the investigated compound <sup>42,128,181,187</sup>. Most chronic itch conditions are refractory to antihistamines or have a very modest response to antihistaminergic therapy <sup>26,37,38,126</sup>. Notable exceptions hereto are urticaria and allergic conjunctivitis, which are both thought to be largely histamine-driven <sup>37,38,188,189</sup>. As such, histaminergic models may be used in pharmaceutical proof-of-concept studies where the end-goal is to evaluate the antipruritic efficacy of compounds with these conditions in mind. Lastly, itch provocations using histamine have in many ways become the ‘gold standard’ of experimentally induced itch and as such they are frequently used simply as a predictable comparator condition to the less well-established non-histaminergic models <sup>180,181,187</sup>.

## ITCH PSYCHOPHYSICS

Three main aspects of itch psychophysics are important to address following itch provocations: 1) quantification, i.e., how much itch is evoked? 2) quality, i.e., what is the nature of the evoked itch, particularly whether pain is present? 3) dysesthesias, i.e., are (mechanical) dysesthesias established? <sup>106,190</sup>.

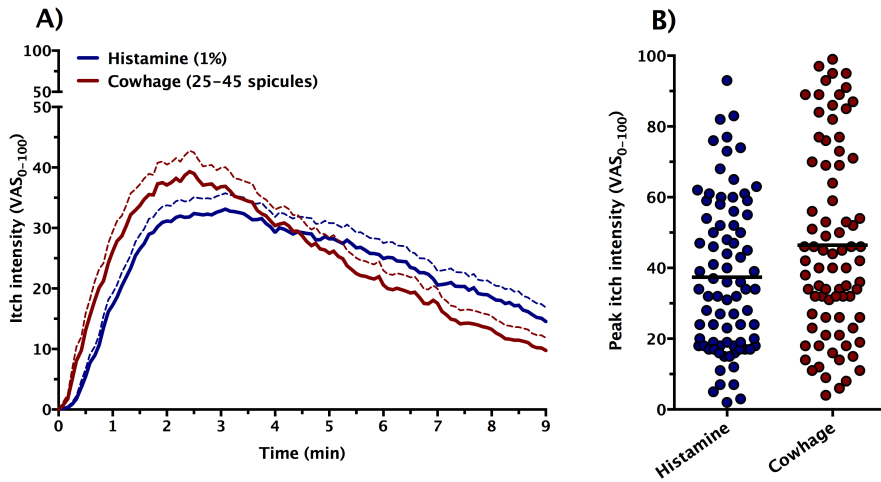
### 2.2.1. ITCH INTENSITY

As for pain in experimental and clinical pain studies, evoked and spontaneous itch can be rated using a variety of approaches <sup>191,192</sup>. The visual analogue scale (VAS<sub>0-10</sub> or VAS<sub>0-100</sub>) is ubiquitous and has been thoroughly validated for itch but previous studies have also used numerical rating scales (NRS), itch perception thresholds, perceptual matching techniques and the generalized labeled magnitude scale (GLMS) <sup>193-196</sup>. The VAS and NRS scales typically uses similar outer labels such as “no itch” and “worst imaginable itch” while the GLMS uses 7 quasi-logarithmically placed labels: from “no sensation” to “strongest imaginable sensation” <sup>55,194,197,198</sup>. For itch specifically, it has been suggested to provide additional anchors to modify the normal VAS, e.g., by adding a label which represents a scratch threshold such as “*first urge to scratch*” (typically at 30% of the scale) <sup>178,179,194,199</sup> or by adding an anchor that relates to the experienced itch intensity of an average mosquito bite <sup>42,57</sup>. These approaches comes with their own set of problems: from a semantic standpoint

any rating below an “*urge to scratch*” is not truly itch, and individuals may have very different reactions to mosquito bites. While the VAS and NRS are the most commonly used approaches, very recent evidence suggests that the GLMS might be more reliable for itch rating than a traditional or modified VAS<sup>194</sup>.

Rating itch and validation of itch rating tools does pose an unusual twofold problem, not present for parallel studies of pain. Firstly, while almost everyone has had episodes of severe, agonizing pain in their lifetime this is not necessarily the case for itch<sup>200–202</sup>. This conceivably makes it difficult for some subjects to relate to the extreme anchor of a VAS (commonly with the label: “worst imaginable itch”). Secondly, the VAS as a tool for pain measurement has been extensively validated in patients and healthy controls<sup>203–205</sup>. However, in experimental pain studies, the stimulation can be successfully scaled to match the entire theoretical stimulus-response curve, which is in stark contrast to itch stimulation. In other words, one can easily and reliably in a sample of healthy volunteers induce pain equal to  $VAS_{0-10} \approx 2, 4, 6, 8$  and 10, for instance by applying heat stimuli in the 40–52 °C range, but this is not possible for itch<sup>203,205,206</sup> (as discussed in SP I). For itch, all effective models are chemical, and there is no method that consistently induces peak itch above  $VAS_{0-10} \approx 5$  in healthy controls regardless of which modality is being used or even with attempts to induce preceding cutaneous sensitization<sup>74,106,130,175,176</sup>. This is both a methodological problem and scientifically enigmatic. Usually if the intensity of a pruritic stimulation is increased, e.g., higher concentration of histamine or higher miliampere in an electrical itch elicitation paradigm, the itch sensation only increases to about  $VAS_{0-10} \approx 3-5$ , after which pain concomitantly emerge and eclipses the itch<sup>62,74,82,102</sup>.

The itch intensity is typically rated in 1–30 second intervals until a clear decline of evoked itch intensity is evident. In a normal sample of subjects, a chemical itch provocation elicits a peak within 1–3 minutes, after which the itch starts to decline, usually subsiding completely within 8–15 minutes<sup>106</sup>. Different pruritogens have slightly different temporal profiles likely related to delivery methodology, local clearance rates, chemical characteristics of the pruritogen and potential differences in fiber tachyphylaxis. VAS measurements of itch intensity in healthy controls in response to histamine and cowhage are shown in Fig. 5A and B, which contain pooled data from Studies II, III and IV. With very few exceptions, studies across different laboratories with histamine and cowhage provocations in healthy controls find average peak itch intensities between 3–5 ( $VAS_{0-10}$ ) and moderate-to-high test-retest reliability<sup>57,184,194,207</sup>. For cowhage, this is quite remarkable considering that it is an entirely uncontrolled, plant-derived material often stored for prolonged periods and which is very difficult to apply in a standardized manner.

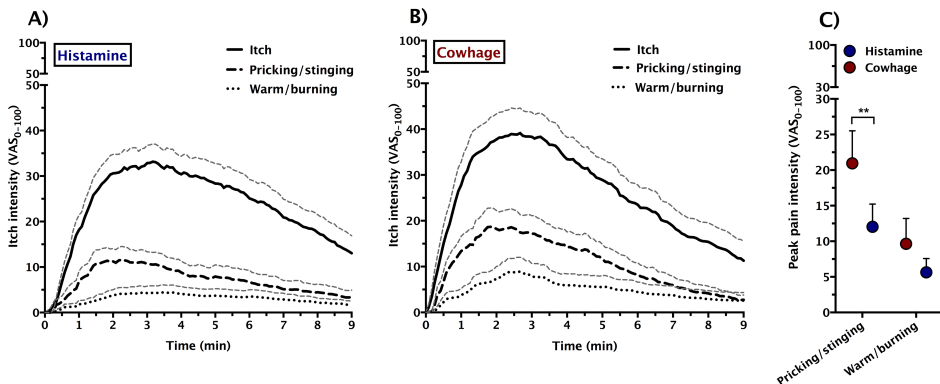


**Figure 5.** Itch evoked by cowhage and histamine in healthy controls. **A)** 0-9 minute temporal profiles of itch intensity from forearm sites (volar or dorsal) evoked by 1% histamine or 25-45 spicules ( $N = 55$  itch provocations, Studies II and III). Notice that cowhage induced itch peaks and declines more rapidly than that evoked by histamine. **B)** Individual peak itch intensities (horizontal bar denote mean) from  $N = 80$  cowhage and histamine provocations (Studies II, III and IV). Mean and standard error of mean (bars and dashed lines) depicted.

## 2.2.2. ITCH QUALITY

The quality of itch both in experimental and clinical studies is usually assessed using a modified version of the McGill Pain Questionnaire (and short form questionnaire). These questionnaires has been extensively used and validated<sup>208-211</sup>. The survey was altered to accommodate itch patients independently by two different groups, resulting in the *Eppendorfer itch questionnaire* and the “Yosipovitch” questionnaire<sup>8,51,212</sup>. In experimental studies where itch provocations are applied, it is common to utilize only a subset of selected descriptors particularly if a temporal intensity profile is desired<sup>55,178,182,198</sup>. Often, the nociceptive descriptors “pricking/stinging” and “warm/hot/burning” are quantified in addition to itch. More simplistically, some investigators ask the subjects just to rate the itch and pain on similar VAS scales<sup>14,82,161,178</sup>. The ratio between itch intensity and pain intensity provides information about the relative purity of the evoked sensations. Data from Study II show the nociceptive sensations associated with cowhage and histamine provocations (Fig. 6A, B and C). Of note, studies indicate that the itch-to-pain ratio is significantly skewed toward itch in response to application of algogens in chronic

itch patients and skewed towards pain in response to application of pruritogens in chronic pain patients<sup>14,161,213,214</sup>. Thus it appears that modality shifts in the perception of chemical itch and pain provocations are tightly associated with the nature of the preceding acute or chronic sensory input.



**Figure 6.** The quality of pain associated with pruritic provocations (Study II). Most itch models, including histamine (A) and in particular cowhage (B), give rise to mild cutaneous pain sensations commonly described as either pricking/stinging and/or warm/burning. The pricking/stinging component is significantly more intense for cowhage than for histamine (C). Subjects were told to disregard the mild initial pain immediately associated with insertion of spicules and skin pricks. Mean and standard error of mean (bars and dashed lines) depicted, \*\* =  $P \leq 0.01$ .

### 2.2.3. ITCH SENSITIZATION FEATURES

As described in section 1.3.2, acute and chronic itch are associated with sensitization processes running parallel to those observed for pain (i.e., allodynia and hyperalgesia, first and foremost to mechanical stimuli). Pruriceptive signaling can be facilitated in the peripheral and/or central nervous system, leading to itch-associated dysesthesias often tested by mechanical stimuli.

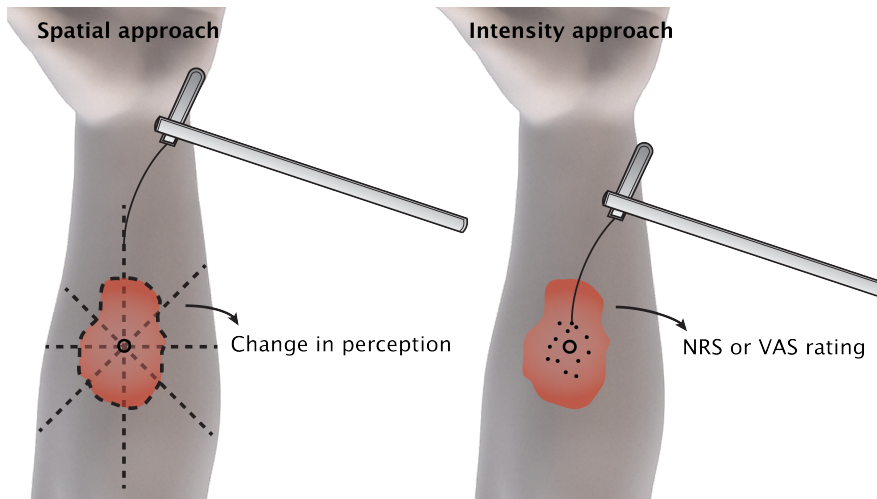
#### 2.2.3.1 Mechanisms of Itch sensitization to mechanical stimuli

In non-human primates, histamine injections have been shown to result in a small number of pruriceptive spinothalamic tract neurons exhibiting increased responses to stroking (alloknesis) or to a punctate skin stimulus (hyperknesis) which evokes pricking pain sometimes followed by itch in humans<sup>81,94</sup>. Since the primary afferent substrate for brush strokes is A $\beta$ -fibers and this type of stimulation never results in itch under normal conditions, itch provoked by brush strokes conceivably always

represents a central sensitization phenomenon of wide dynamic range neurons resulting from an initial PmC- or CMi-mediated barrage. The mechanism for hyperknesis is less clear, and it remains unknown which type of afferents mediates the mild itch sometimes associated with punctuate stimuli<sup>82,215</sup>. When occurring next to an itch provocation or an actively itchy skin lesion, hyperknesis is potentially mediated by type-I A $\delta$ -fibers through a central mechanism, as is the case for secondary pinprick hyperalgesia. On the other hand, itch evoked by pricking stimuli is usually reported with a 0.5-2 second delay<sup>14,216</sup>, indicating PmC-fibers as the peripheral substrate. When pinprick hyperknesis occurs inside an active skin lesion, a peripheral contribution or main mechanism is conceivable<sup>13,14,161</sup>. In the case of an inflammatory perturbation, mechanically insensitive C-afferents can develop *de novo* mechanosensitivity and PmC-fibers become more responsive to suprathreshold stimulation<sup>217</sup>. A recent rodent study suggested that mechanically induced itch rely on low-threshold mechano-receptors, i.e. not nociceptors, which are, under normal circumstances subject to strong lateral inhibition, and thus rarely occur in response to natural stimuli<sup>120</sup>. The presence of profound pinprick-evoked hyperknesis in chronically itchy AD lesions and to lesser extent beyond the lesions indicates concomitant peripheral and central contributions in AD neuronal sensitization<sup>13,14,218</sup>.

### 2.2.3.2 Assessing itch sensitization to mechanical stimuli

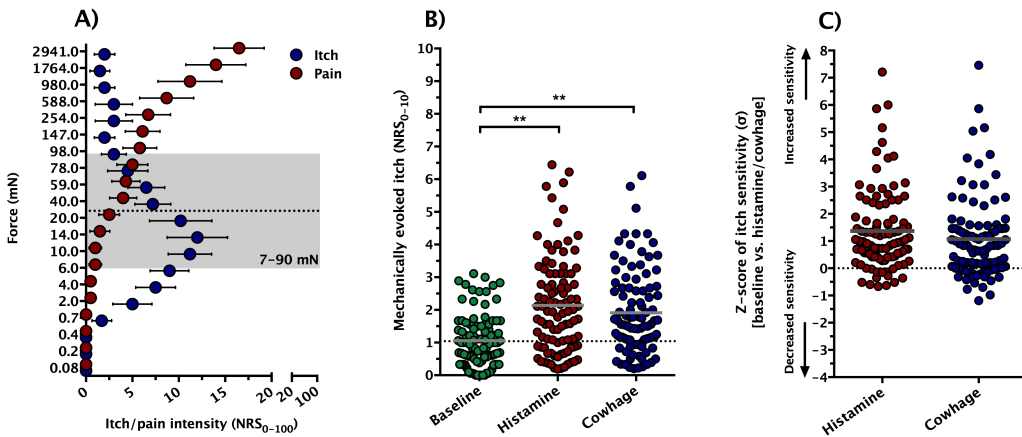
Itch sensitization to mechanical stimuli has been assessed using animal models (rodents and primates), human experimental models and chronic itch populations<sup>14,71,219,220</sup>. For human studies two different approaches are in use. Firstly, the spatial extent of allo- and hyperknesis can be mapped by stimulating the skin surrounding the pruritogen application site or in a lesional/peri-lesional skin area<sup>73,82,176,221,222</sup>. In humans, alloknesis is commonly assessed with a light brush, while hyperknesis is often assessed with a pinprick stimulators or filaments<sup>82,176</sup>. Stimuli are typically conducted in small increments following multiple vectors centripetally (see Fig. 7). The subjects report to the investigator when the stimuli turn from pure innocuous tactile sensations into itch (alloknesis) or turn from a pricking/slightly itchy to evoking noticeably more itch (hyperknesis)<sup>73</sup>. The disadvantages of the method relate to the fact that it is time consuming, relies on the presence of itch with a known locus, and is vulnerable to false positives.



**Figure 7. Commonly used methods for mechanical itch dysesthesia quantification.** Two methods for dysesthesia assessments are in use: a planimetric mapping of the spatial extent of the allo-/hyperknesis typically surrounding an itch provocation (left) or an assessment of the intensity of the dysesthesia conducted within the suspected dysesthetic area (right). The circle indicates the pruritogen application area. NRS = numerical rating scale, VAS = visual analogue scale.

The other approach relies on quantifying the intensity of the allo- and/or hyperknesis, usually assessed in the immediate vicinity to an itch provocation or in lesional/non-lesional skin of patients<sup>161,176,218,220</sup>. Here, the stimulation is conducted multiple times, often with different stimulus intensities, and the subject rates the presence and/or the intensity of the mechanically evoked itch<sup>13,129,161</sup>. The intensity or simply the presence of alloknesis can be quantified in response to brush strokes or von Frey filaments, and the intensity of hyperknesis in response to von Frey filaments, pinprick stimuli or weighted needles<sup>161,220</sup>. The disadvantage of this method is that it does not detect the spatial outline of the assessed, assumed dysesthesias and relies on the subject providing a magnitude rating rather than simply a perception shift. However, this method can readily be used on itch occurring from lesional, peri-lesional and in particular non-lesional skin in patients<sup>13,14,218,223</sup> without any prior itch provocation. No studies on the reliability of various methods of mechanical itch sensitization assessment have yet been published. In Study II, the relative within session test-retest reliability assessed by the intra-class correlation coefficient between averaged stimuli on the forearms was = 0.81, usually interpreted as ‘good’ or ‘excellent’<sup>224</sup>.

In Study 2 we showed that the optimal force range for evoking mild itch at baseline (and thus detecting hyperknesis) to von Frey stimuli is clearly below the perceptual mechanical pain threshold and around the known average mechanical threshold for human PmC-fibers (see Fig. 8A). This is well aligned with previous evidence derived from modified monofilaments<sup>52,176</sup>. By using von Frey stimuli in this force range, pain is rarely evoked, but mild itch is elicited in the majority of healthy subjects immediately following an initial prickly sensation. In Fig. 8B and C data from Studies II, III and IV show  $N = 100$  tests using von Frey filaments (averaged from 2,475 individual stimuli) in normal skin and following histamine and cowhage provocations. It is clear that the majority of healthy controls consistently develop mild hyperknesis following both histamine and cowhage provocation. Particularly when such mechanical stimulations are performed in patients with inflammatory skin disorders, barrier alterations have to be considered as potential biasing factors completely unrelated to cutaneous neuronal sensitivity. For instance, pinprick perception or mechanical detection thresholds might be altered in lichenified skin<sup>13</sup>, responses to chemical provocations delivered by iontophoresis might be exaggerated in excoriated areas with reduced barrier integrity and the temporal profile of evoked itch might be affected by increased or reduced vasomotor reactions that alter local tissue clearance of pruritogens<sup>129,220</sup>.



**Figure 8. Mechanical itch sensitization assessed with von Frey filaments.** *A)* Itch and pain ratings to 20 weight calibrated von Frey monofilaments ranging from 0.08 to 2941 mN. The grey box represents upper, median (dashed line), and lower ranges for the physiological mechano-responsiveness of human polymodal C-fibers assessed by microneurography<sup>86</sup>. Notice that despite optimization, the maximal average rating is  $\approx 12$  (NRS<sub>0-100</sub>). *B)* Mechanically evoked itch at baseline and following sensitization evoked by histamine and cowhage (grey bars show the mean). *C)* Z-transformed change of mechanically evoked itch sensitivity following sensitization achieved by histamine and cowhage provocation (individual baseline data and overall standard deviation). Data (*B* and *C*) pooled from Study II (spinally innervated areas), Study III (vehicle area) and Study IV (healthy controls). Mean and standard error of mean depicted. \*\* =  $P \leq 0.01$ .

### 2.3. VASOMOTOR RESPONSES TO ITCH PROVOCATIONS

Two types of local cutaneous vasomotor responses are commonly observed in response to chemical irritants and in particular pruritogens: wheals and neurogenic inflammation<sup>69,106,225,226</sup>. A wheal reaction is a small, pale, slightly raised and circumscribed dermal oedema immediately associated with the provoked skin area. The reaction extends a few millimeters beyond a skin prick puncture or an area where histamine has been iontophoretically delivered. The reaction is evoked by histamine acting on capillary receptors, causing a microvascular leakage subsequently leading to acute protein extravasation in the vascularized dermis<sup>106,184,227–230</sup>. A wheal reaction lasts 1-2 hours and remains completely uninterrupted when local cutaneous nerves are anaesthetized or ablated, signifying that it is entirely non-neuronal<sup>182,228</sup>. Thus a wheal reaction is a telltale sign as to whether a given reaction is associated with histaminergic signaling and how responsive the capillaries are to the histamine release (or exogenous introduction of histamine). For this very reason, wheal reactions are used diagnostically to assess allergic sensitization by testing reactivity to suspected allergens in relation to the positive control histamine reaction<sup>106,231,232</sup>. While more advanced techniques have become available, wheals can be measured with a simple ruler with typical assessments being either the longest diameter or the longest diameter and the orthogonal one (the latter being common practice for clinical purposes)<sup>227,233</sup>.

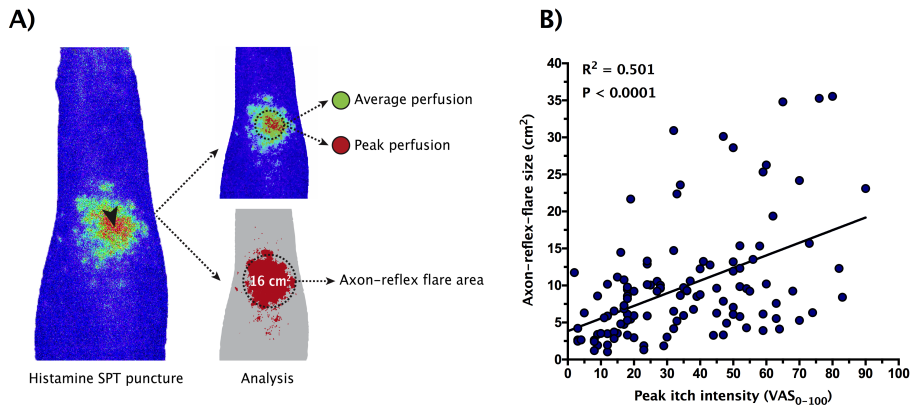
Neurogenic inflammation or neurogenic flare is a brief increase in superficial blood perfusion caused by retrograde signaling from activated dermo-epidermal peptidergic nerve fibers<sup>69,226,234</sup>. It occurs within as well as immediately surrounding the application area of a chemical irritant. When appearing in an otherwise unprovoked surrounding area, it is often referred to as ‘secondary’ neurogenic inflammation or as an *axon-reflex flare*<sup>90,235,236</sup>. The reaction is mechanistically well studied. As opposed to the wheal reaction, neurogenic flare depends on intact functioning of peptidergic dermo-epidermal fibers and can be almost entirely inhibited by local infiltration with anesthetics or capsaicin-induced termini ablation. In contrast, a proximal nerve anesthetization has no impact on the extent or severity of an evoked neurogenic inflammatory response, signifying that it is exclusively a peripheral occurrence<sup>235</sup>. The reaction is predominantly mediated by peptidergic C-fibers. While PmC-fibers likely have a minor contribution in homotopic flare generation, they cannot account for the axon-reflex phenomenon in part because their terminal arborizations are not sufficiently extensive. Instead, robust neurogenic inflammation and in particular secondary flare is thought to represent an efferent function of CMi-fibers<sup>90,106,237</sup>, which have extensive terminal branching and thus suitable morphology for releasing vasodilatory neuropeptides far



beyond the point of activation. The primary mediators of vasodilation are thought to be CGRP and substance P, with the latter proposed to play a less crucial role in the process<sup>229,234,237</sup>.

The neurogenic inflammatory response can be visually observed as a discontinuous erythematic area around the provocation site. Hence, it can be quantified planimetrically, for instance by marking the estimated circumference on a transparent sheet. However, accurate quantification using this approach is not trivial as the reaction is highly irregular, tends to fade centrifugally and appears differently depending on skin tone. Moreover, this type of quantification only concerns the spatial extent of the reaction but not its intensity. Instead, perfusion-imaging techniques such as Doppler flowmetry, speckle contrast imaging/full-field laser perfusion imaging (FLPI), spectrophotometry or infrared thermography can be applied<sup>42,238,239</sup>. These techniques, FLPI in particular, allow for more advanced analysis of the reaction (Fig. 9A). FLPI is a recent technique that works by illuminating a skin area with a preset laser light pattern in the wavelength of around 750 nm. This is just above the wavelength of visible light and within the reflectance spectrum of hemoglobin<sup>240-242</sup>. The reflection of the laser light from the investigated surface produces a contrast laser pattern known as a ‘speckle pattern’ that can be evaluated in close to real-time. When increased cutaneous blood flow occurs in an area the speckle pattern will exhibit lowered contrast<sup>241,243</sup>. Although the reliability of FLPI for assessment of evoked neurogenic inflammation has not yet been assessed, good reliability estimates are reported from clinical blood flow monitoring studies using FLPI<sup>242,244,245</sup>. Moreover, test-retest reliability of older, less sensitive methods of blood perfusion measurement such as laser Doppler flowmetry has been asserted in the context of cutaneous inflammation<sup>239,246</sup>. Because secondary neurogenic inflammation is almost exclusively mediated by CMi-fibers, assessment of the reaction provides a proxy measurement CMi-fiber activation (and thus indirectly the activated C-fiber subtype). In other words, the absence of a robust secondary neurogenic inflammatory response precludes CMi-fiber involvement, while a weak homotopic flare suggests PmC-fiber activation under normal circumstances. For histamine-induced itch, which is associated with a very robust neurogenic inflammatory response, several studies have found a significant positive correlation between itch intensity and axon-reflex flare size, hinting that the efferent reaction of CMi-fibers is tightly associated with the intensity of the itch perception that arises<sup>55,57,184,198,220</sup>. In Fig. 9B, this correlation is reproduced with data from N = 122 histamine provocations (Studies II and III). An itch provocation exclusively acting on PmC-fibers would be expected to produce only a very modest and restricted inflammatory response, as is the case for instance in response to cowhage (Study II). In some papers, it is claimed that cowhage never

evokes any distinguishable flare reactions<sup>177,247</sup>; however, this is probably inaccurate and rather represents that insufficiently sensitive assessment techniques were applied<sup>62,220</sup>. In our studies, we have occasionally observed visibly detectable micro-erythematic reactions and sometimes micro-wheals immediately within the area where the spicules were inserted<sup>220</sup>. In some cases, very mild vasomotor reactions are only visible by high-resolution FLPI.



**Figure 9. Histamine-induced neurogenic inflammation and its correlation with itch (Studies II and III).** *A*) A typical Full-field Laser Perfusion Imaging (FLPI) readout following a skin prick test lancet introduction of 1% histamine (black arrowhead). Two basic quantification techniques are illustrated; extraction of peak and mean values (top) and quantification of flare area about a chosen cut-off relative to a size reference area (bottom). *B*) Significant positive correlation between the size of the axon-reflex-flare and the reported peak itch intensity. Data derived from  $N = 122$  histamine 1% provocations performed with SPT lancets under various experimental conditions pooled between Studies II and III.

The relative C-fiber subtype selectivity of histamine and cowhage provocation is more the exception than the rule as most chemical irritants, pruritogens and allergens alike evoke some degree of activation of both C-fiber subtypes<sup>93,248,249</sup>. This can also explain why flare characteristics and itch intensity in response to histamine are correlated when the same is not the case for capsaicin-induced flare and pain intensity<sup>55,250</sup>. For histamine, the same activated units (CMi-fibers) selectively cause both the sensory (afferent) and the vascular (efferent) event. In the case of capsaicin, transient receptor potential vanilloid 1 (TRPV1) receptors are much more ubiquitously distributed on nociceptive units than for histamine (H1/4) receptors<sup>93,251,252</sup>. Accordingly, the sensory event in response to capsaicin is orchestrated by PmC- (perhaps predominantly), A $\delta$ - and CMi-fibers, while the vascular event is mediated by the CMi-fibers, which only constitute a minor fraction of the activated fiber pool<sup>92,93,253</sup>. Lastly, it should be kept in mind that the proxy

reaction is neurovascularly mediated. Beyond the sensory rating component and potential measurement error, considerable variability is probably also introduced by intra- and inter-individual differences in CGRP and substance P release capacity and vascular responsiveness to these substances.

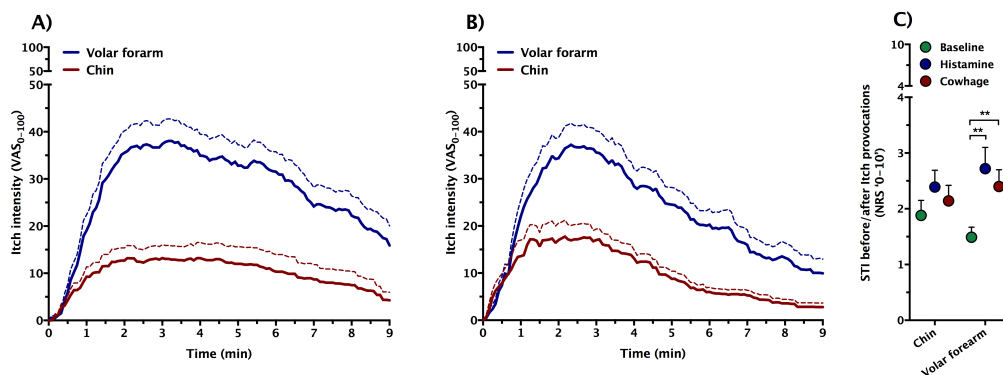
## 2.4. TOPOGRAPHICAL CONSIDERATIONS

The vast majority of human studies on itch have been conducted on the volar aspect of the forearm. This location is easily accessible, relatively homogenous, usually not too hairy and has long been a favored location for cutaneous pain studies. The first assessments of the topographical distribution and spatial acuity for touch across the body were published more than a century ago<sup>254</sup>, and that topography and regional acuity of the cutaneous nociceptive system have been extensively explored in recent decades<sup>255,256</sup>.

Differences in topography of mechanical and histaminergic itch sensation were recently shown between spinal and trigeminal innervated areas<sup>215</sup>, and are of relevance for several reasons. First, knowledge of the basic properties of the pruriceptive system across the body could be important to develop and optimize antipruritic treatments<sup>37,106</sup>. Second, in rodents, assessment of itch relies entirely on counting the scratch bouts that occur following a given provocation or in a chronic model<sup>257</sup>. The most common sites for pruritogen injections are the rostral back and the cheek. The cheek has more recently been introduced as an area that allows for discrimination between itch and pain behavior (scratching with the hind paw or wiping with the fore paw, respectively)<sup>258</sup>. Oppositely, the majority of human studies applying itch provocations in healthy volunteers or patients have done so on the forearms<sup>106</sup>. The extent to which potential anatomical differences influence the comparability and translatability between human and animal studies itch studies is unknown<sup>106</sup>. Thirdly, lesions in dermatological itch conditions often occur in distinct anatomical patterns. Psoriasis lesions are typically bilateral and occur very rarely in the trigeminal region, which is not uncommon for neuropathic pruritus conditions<sup>18,28</sup>. Prurigo nodularis and dermatitis herpetiformis occur frequently on extensor surfaces of the extremities<sup>259,260</sup>. AD lesions are overrepresented in the creases of the elbows and knees, while urticaria manifests frequently on the trunk and proximal extremities<sup>261</sup>. Such spatial patterns of skin lesions in diseases and associated sensations of itch have typically been attributed to differences in skin biology and barrier integrity<sup>50,262–265</sup>. However, potential neuroanatomical

differences in the receptiveness or coding of itch at different body sites have only been marginally investigated.

As clearly shown in Study 2 (Fig. 10A, B and C), there appears to be a noticeable discrepancy between human sensitivity to common pruritogens (histamine and cowhage) vs. what is observed in rodents. In humans, robust experimentally induced chemical itch cannot be elicited in the facial area and this area exhibits a distinct neurogenic inflammatory pattern in response to histamine<sup>215</sup>. In parallel, mechanical sensitization for itch or ‘hyperknesis’ is not established in the facial area following provocations with cowhage and histamine, but the sensitivity to mechanical itch stimuli is significantly higher at baseline compared to spinally innervated areas. This is aligned with a recent human psychophysical study suggesting that a specific mechanical pathway for itch exists in the trigeminal area while sensitivity to histamine provocations is greatly reduced there<sup>215</sup>. In combination, these findings highlight the complexity associated with translating findings from preclinical studies. In rodents chemically induced itch can easily be elicited in trigeminally innervated areas and the perhaps most frequently used skin area for itch provocations is the rostral back – paradoxically corresponding to an anatomical site which humans cannot readily scratch<sup>105,219,266</sup>.



**Figure 10. Differences in chemical and mechanical itch sensitivity between the volar forearm and the chin (Study II).** Temporal profiles of itch evoked by histamine (A) and cowhage (B) at the volar forearm versus the chin. C) Sensitivity to touch-evoked itch (STI) before (baseline) and after itch provocation (histamine or cowhage) at the volar forearm versus the chin. Notice that chemically induced itch (A and B) cannot be robustly elicited when provocation are conducted in the chin. Mean and standard error of mean (bars and dashed lines) depicted. \*\* =  $P \leq 0.01$ .

Lastly, to show that higher baseline blood perfusion in the facial area did not simply cause faster clearance of introduced pruritogens thus resulting in lower itch, an experiment with a vasoconstrictor was performed. When using a topical  $\alpha_2$ -adrenergic agonist (used clinically to induce vasoconstriction in facial skin), baseline perfusion was reduced to that of the volar forearm. For a subsequent itch provocation with histamine, there was no significant effect on itch evoked in the chin, although an insignificant trend towards higher area under the curve (AUC) of itch was observed. In the same sub-experiment, however, it was noted that pre-administration of a vasoconstrictor significantly increased the AUC of itch for a histaminergic provocation on the volar forearm without changing the peak itch intensity. Thus the effect of the  $\alpha_2$ -adrenergic agonist is mediated by altering the slope of decline of the itch, likely by hampering local clearance of histamine normally associated with increased superficial perfusion of the area. This approach could be used to evoke very prolonged itch in basic mechanistic studies. Moreover, the observation has potential implications for the interpretation of a number of experimental studies conducted in patients with AD (including Study IV). When assessing the temporal profiles of evoked itch in patients versus healthy controls, a commonly noted difference is a less steep slope of decline in of itch in patients with AD<sup>129,182,247</sup>. Coinciding with this observation is the frequent finding that patients with AD exhibit smaller and less intense neurogenic inflammatory reactions in non-lesional skin<sup>129,182,186,267</sup> (also observed in Study IV). Hence a flatter slope of decline, e.g., for histamine-induced itch in AD could be related to differences in cutaneous capillary perfusion.



# CHAPTER 3. TOPICAL CAPSAICIN-INDUCED SENSORY DESENSITIZATION

## 3.1. TOPICAL CAPSAICIN-INDUCED DESENSITIZATION

Capsaicin, the pungent compound of chili peppers, exerts its somatosensory effect by activating the TRPV1-receptor located predominately (in the context of sensory neurons) on subsets of C- and A $\delta$ -nociceptors<sup>249,268,269</sup>. This receptor was initially discovered 20 years ago<sup>268,270–272</sup>, and its role in somatosensation is still very actively investigated to date. Notably, the ‘natural’ activation of TRPV1 is normally much more transient than that which can be generated by a potent and stable exogenous agonist such as capsaicin or resiniferatoxin. Accordingly, after high-concentration topical transdermal administration or an intra-dermal injection of 10–100  $\mu$ g capsaicin, dose-dependent burning and stinging pain occurs, reflecting acute activation of aforementioned units<sup>168,273,274</sup>. Consequently, primary heat hyperalgesia is established, reflecting the lowered threshold of TRPV1-expressing nociceptors, and primary as well as secondary mechanical hyperalgesia is evoked, reflecting central sensitization to input from mechano-receptive fibers<sup>275–279</sup>. After the initial pain and hyperalgesia has subsided, the relevant skin area exhibits altered sensory sensitivity, particularly reduced sensitivity to warmth, painful heat and mechanical pain stimuli as well as decreased neurogenic inflammatory responses<sup>280–282</sup>. This defunctionalization is reversible, and a similar desensitizing effect can also be achieved with less initial pain by repeatedly applying a low-concentration cream to a skin area (usually for several days or weeks)<sup>280,283</sup>. Capsaicin-induced desensitization is thought to selectively affect TRPV1-expressing heat-sensitive C- and A $\delta$ -fibers, but discrepancies exist with regards to the degree to which mechanical pain desensitization also occurs<sup>280,281,284</sup>. The effect is restricted to the administration area, and should not be confused with the acute anesthesia typically occurring within minutes at the injection bleb following i.d. administration<sup>276</sup>, which is related to distinct mechanisms particularly relevant for PmC-fibers<sup>249</sup>.

Pharmacodynamically, the prolonged defunctionalizing effect has been proposed to rely on multiple parallel mechanisms<sup>269,285–287</sup>. First, robust increases in intracellular calcium caused by the opening of TRPV1 on both the cell membrane but also on the endoplasmic reticulum which stores Ca<sup>2+</sup>, could overpower cellular calcium sequestration mechanisms<sup>288–290</sup>. This would lead to osmotic changes and activate

calcium-dependent enzymes, including proteases, which subsequently could compromise cytoskeletal components, e.g., microtubules and lead to dysfunctional axoplasmic transport<sup>291-293</sup>. Second, capsaicin inhibits mitochondrial function independently of TRPV1 at a moment where cellular energy expenditure is high. This renders affected nerve terminals unable to maintain plasma membrane integrity, and they degenerate to the depth where the exposure to capsaicin was not sufficient to robustly inhibit mitochondrial function<sup>269,294,295</sup>. Often such reversible retraction occurs well into the dermis<sup>280,296</sup>. The extent to which the terminal fiber degeneration is *per se* the cause of functional desensitization is unclear, partly because most psychophysical testing is invariably affected by the increased depth of fiber terminals that follows a capsaicin ablation<sup>269,281</sup>.

A persistent but largely unsupported hypothesis on the mechanism of action of capsaicin-induced defunctionalization relates to purported peripheral substance P depletion. This emerged from early observations of reduced substance P concentration in the skin following capsaicin treatment at a time where this mediator was considered crucial in pain transmission<sup>297,298</sup>. Later substance P antagonists failed unequivocally as analgesics, and it became apparent that the terminal degenerative aspect of capsaicin would result in a peripheral reduction of all neuropeptides or nerve-related molecules<sup>299,300</sup>. Lastly, cutaneous substance P provocations in humans in the physiological concentration range have shown no or very limited sensory effects<sup>301</sup>. A potential role of substance P depletion, however, could be relevant on a central level.

### 3.2. TOPICAL CAPSAICIN AND ITCH

As described in detail in *section 1.2*, the peripheral pathways for itch and pain are mechanistically entwined with no clear-cut separation between the encoding of itch and pain arising from activity in C-nociceptors. The majority of human C-nociceptors express TRPV1, and micro-neurography studies indicate that essentially all PmC and most CMi-fibers are also responsive to capsaicin provocations although they do exhibit marked differences in their response patterns<sup>93,249</sup>. Moreover, itch can be induced directly in response to topical capsaicin if administered, for instance, on inactivated cowhage spicules<sup>55,178,198</sup>. Hence, effective capsaicin-induced desensitization of cutaneous C-fibers should be capable of profoundly inhibiting both histaminergic and non-histaminergic itch transmission.

While the neurophysiological basis for this assertion has been greatly expanded in recent years, the notion of capsaicin as a potential antipruritic is not new<sup>298,302</sup>. Low

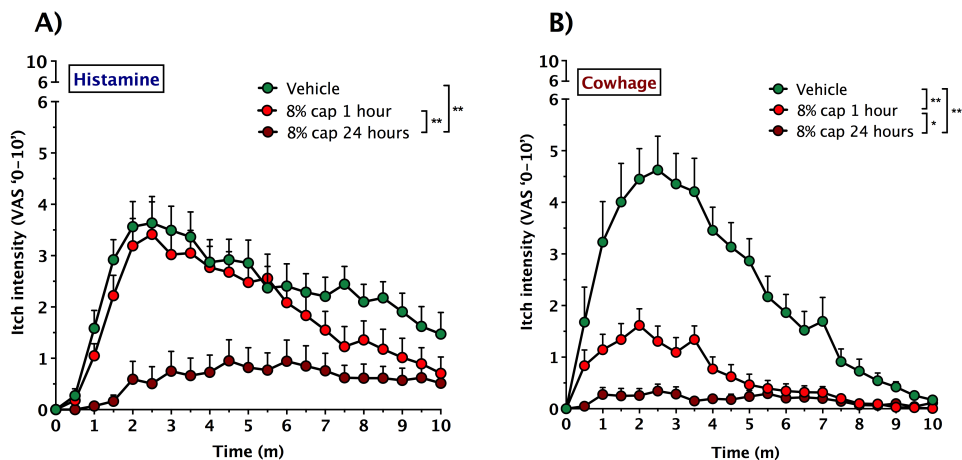


concentration topical capsaicin creams ( $\leq 0.075\%$ ) have been used clinically for both itch and pain for decades. However, two caveats have largely hindered effective clinical usage. 1) The low concentration capsaicin options are not very effective. 2) They require administration several times daily for weeks, which is often associated with mild pain and accordingly with poor adherence<sup>281,302</sup>. This is true also for neuropathic pain indications, where the usage of low-concentration capsaicin creams has been largely abandoned, as well as for itch where usage is marginal<sup>37,38,303,304</sup>. In a meta-analysis from 2010 summarizing trials on the effect of low-concentration capsaicin in itch conditions, the authors unambiguously concluded that: “*at present, there is no convincing evidence for the use of capsaicin to treat pruritus in any medical condition*”. Furthermore, it was noted that most trials on the antipruritic effect of low-concentration capsaicin had insignificant findings, clinically inapt effect sizes, were inadequately placebo-controlled or had other methodological shortcomings<sup>302</sup>. In human surrogate models of histaminergic itch low concentration topical capsaicin has previously yielded very contradictory results, with numerous studies reporting no significant antipruritic effects<sup>103,298</sup>.

### 3.3. ANTIPRURITIC POTENTIAL OF 8% TOPICAL CAPSAICIN

More recently, 8% capsaicin patches have become available and appear to have prolonged analgesic effects in some patients with peripheral neuropathic pain conditions such as post-herpetic neuralgia<sup>305–308</sup>. These patches contains  $\approx 825$  times as much capsaicin as the low-concentration capsaicin creams previously used in clinical trials and treatment of pruritus<sup>302</sup>. Because of this very high concentration and the transdermal delivery matrix, the clinical guidelines states that the patch should be applied for 1 hour per treatment session, and application can then be repeated every 3 months<sup>309,310</sup>. Beyond prolonged effects on warmth detection thresholds signifying desensitization of warm C-fibers<sup>307</sup>, the pain defunctionalization, e.g., decreased heat pain sensitivity, assessed by quantitative sensory testing (QST) following a single 1-hour treatment is short-lived<sup>281,311</sup>. Sensory function appears to normalize within a couple of weeks or even faster<sup>311</sup>, paralleled by a delayed recovery of epidermal nerve fiber density typically assessed by Protein gene product 9.5 immunostaining<sup>312</sup>. This hints towards a mismatch between the prolonged analgesic and antipruritic effect observed in some patients and the sensory desensitization effect observable with QST and neurogenic inflammatory provocations<sup>311</sup>. Very recently, prolonged application of 8% capsaicin patches, e.g., up to 44 hours (2 x 22 hours) has been used to achieve a profound inhibition of TRPV1-positive cutaneous nociceptors<sup>313</sup>. While 8%

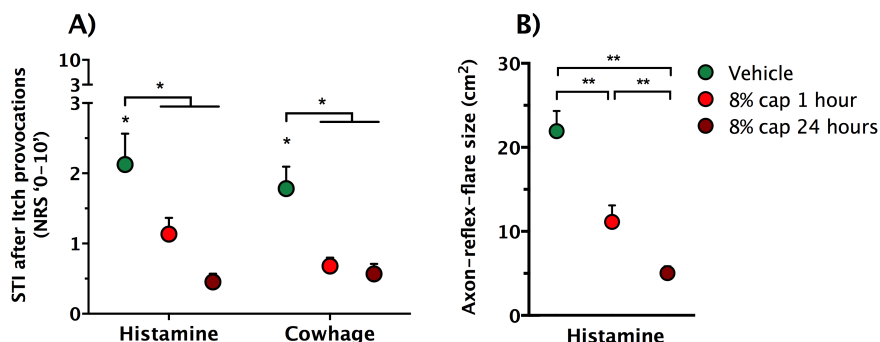
capsaicin is widely used to treat peripheral neuropathic pain (most treatment guidelines list it as a second-line option<sup>304,314</sup>), it has not been tested in relation to itch. However, indicative of a potential antipruritic effect of 8% topical capsaicin are several neuropathic itch case studies in which prolonged relieve of itch has been observed<sup>309,315</sup>. For instance, in SP II and III, the antipruritic effect of 8% topical capsaicin was described in case reports of patients with neuropathic itch.



**Figure 11. Itch responses to histamine (A) and cowhage (B) following vehicle, 1-hour and 24-hour 8% capsaicin pretreatment (Study III).** Temporal profiles of the evoked itch intensity from 0-10 minutes after each provocation. Notice that 1-hour capsaicin pretreatment profoundly inhibits cowhage- but not histamine-evoked itch. Mean and standard error of mean depicted. \* =  $P \leq 0.05$ , \*\* =  $P \leq 0.01$ .

In Study III, the normal clinical regimen of a 1-hour treatment was compared to the more vigorous ablation approach using a 24-hour administration schedule. Healthy volunteers underwent two identical 8% capsaicin pre-treatments on each forearm (vehicle patch, 1-hour 8% capsaicin and 24-hour 8% capsaicin) and subsequently the treated skin areas were probed for itch sensitivity using the histamine and cowhage itch models. Sensitivity to mechanically evoked itch and development of hyperknesis as well as neurogenic inflammatory responses to histamine were also assessed. The 24-hour topical capsaicin pretreatment significantly reduced itch evoked by histamine as well as cowhage (by ~75%) and reduced hyperknesis in both models (Fig. 11 and 12A, respectively). The shorter 1-hour capsaicin pretreatment only decreased cowhage-induced itch (by ~64%) but did not significantly reduce histaminergic itch. Neurogenic inflammation evoked by histamine was dose-dependently reduced by 8% capsaicin pretreatment (Fig. 12B). The antipruritic effects were considerably stronger than those observed in previous

studies using low-concentration capsaicin ointments in patients with chronic itch<sup>316-318</sup> and in studies on experimentally established histaminergic itch<sup>221,319</sup>. Of note, several such studies have been unable to detect any significant antipruritic effect of low-concentration capsaicin for both clinically occurring as well as experimentally evoked itch<sup>57,284,302,320</sup>.



**Figure 12. Effects of 8% capsaicin on hyperknesis and neurogenic inflammation.** Dose-dependent reductions in hyperknesis (A) and axon-reflex flare (B) after histamine and cowhage provocations in the skin areas pretreated with vehicle, 1-hour and 24-hour 8% capsaicin (Study III). STI = Sensitivity to touch-evoked itch. Mean and standard error of mean depicted. \* =  $P \leq 0.05$ , \*\* =  $P \leq 0.01$ .

### 3.3.1. PRURICEPTIVE FIBERS AFFECTED BY CAPSAICIN

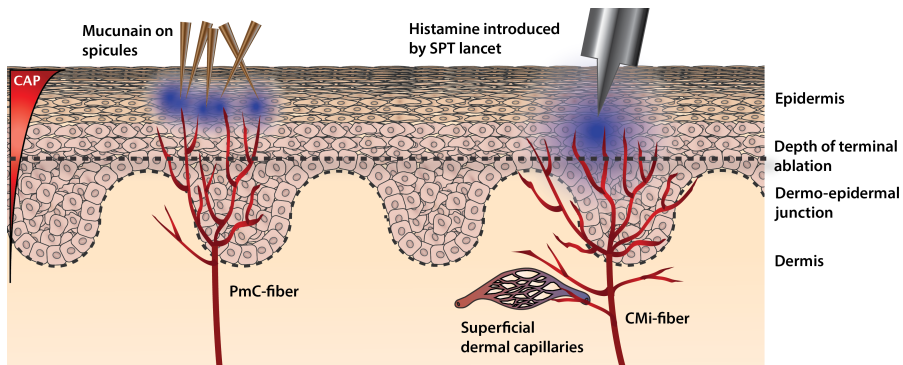
The 1-hour 8% capsaicin pretreatment resulted in significantly decreased itch in response to cowhage but did not reduce histaminergic itch, and the 24-hour pretreatment nearly abolished both cowhage- and histamine-induced itch. Several studies (and numerous reviews<sup>78,321</sup>) have asserted the mechanistic dogma that histaminergic itch relies on a functional coupling of H1R and TRPV1 (on CMi-fibers), while cowhage-induced itch transduction relies on a similar coupling between PAR-2/4 and TRPA1 (on PmC-fibers)<sup>41,79,321</sup>. In rodent DRGs, most TRPA1-positive neurons also appear to co-express TRPV1<sup>322,252</sup> although a recent study showed a substantial subpopulation of non-peptidergic TRPA1-positive neurons not characterized by TRPV1-expression<sup>251</sup> and an *in vitro* study showed a functional TRPV1/TRPA1 overlap of only 20% in mice DRG neurons<sup>114</sup>. Taken together these observations form the theoretical basis for why topical capsaicin pretreatment is capable of abolishing itch sensitivity for these two most extensively studied types of human itch provocations. The degree to which TRPA1-signaling is dependent on TRPV1 co-activation is currently unclear<sup>323,324</sup>. A recent study has

shown that formation of functional TRPV1/TRPA1 heteromers occurs and suggested that TRPA1-induced hyperalgesia relies entirely on TRPV1-expression<sup>323</sup>, contradicting the idea of functional independence of the receptors<sup>322,325</sup>. All these mechanistic studies have exclusively been performed in rodents, and well-established differences are present between rodents and primates in relation to somatosensory transduction of both itch and pain<sup>78,107</sup>. Hence, it would be relevant to confirm in a human model whether TRPA1-induced itch, pain and inflammation are inducible in skin where TRPV1-positive nociceptors have been defunctionalized, e.g., by pre-treatment with high-concentration capsaicin<sup>216,313</sup>. Moreover, rodent and non-human primate studies indicate that a subset of non-peptidergic, TRPV1-negative C-fibers expressing the Mas-related G-protein coupled receptor D (MRGPRD) exists and that these constitute a potential non-peptidergic third pruriceptive pathway<sup>326,327</sup>. Accordingly, activation of these fibers in humans by  $\beta$ -alanine injections (a MRGPRD ligand) causes significant itch<sup>326</sup>. MRGPRD-positive neurons have recently been found to be highly sensitized to mechanical and thermal stimuli in an animal model of contact dermatitis<sup>328</sup>. A future study using 8% capsaicin-induced desensitization could determine whether such sensory effects of  $\beta$ -alanine in humans do indeed act through a functionally significant population of TRPV1-negative non-peptidergic pruriceptive C-fibers.

### **3.3.2. PREFERENTIAL NON-HISTAMINERGIC ANTIPRURITIC EFFICACY OF 8% CAPSAICIN**

While the 1-hour pretreatment with 8% capsaicin significantly decreased itch in response to cowhage, histaminergic itch was unaffected by this intervention (Fig. 11). Three possibly overlapping hypotheses could explain this differential antipruritic potency: 1) CMi-fibers may be physiologically less prone to the desensitization effects of capsaicin than PmC-fibers are, and hence the short application time and resulting limited penetration were insufficient to adequately affect the histamine-responsive CMi-fibers. Evidence in favor of this stems from human micro-neurography of capsaicin injections, where a robust acute homotopic desensitizing effect of capsaicin occurs selectively for PmC-fibers but not CMi-fibers<sup>249</sup>. An underlying cause of reduced desensitization proneness of the CMi-fibers could be simply the fact that their large terminal arborizations would mean that, relative to the PmC-fibers, much less complete stimulation of their receptive field occurs in the present design (where only 2 x 2 cm areas were pretreated). 2) The endings of CMi-fibers could terminate lower in the epidermis and upper dermis than the PmC-fibers. In the literature CMi-fibers are often alluded to as branching

and terminating deeper in the skin<sup>57,103,321,329</sup> and anecdotal observations indicate that cowhage cannot evoke itch if the epidermis is experimentally removed<sup>102,330</sup>. Thus cowhage-sensitive termini must reside rather superficially. There is presently no firm evidence to support differential depth of PmC vs. CMI-fibers due to a lack of specific markers, but the nature of histamine-evoked axon-reflex flare indirectly suggests that this fiber type must be branching into the vascularized dermis<sup>90</sup>. The concentration gradient produced by topical capsaicin would result in more profound defunctionalization of the most superficial TRPV1-positive fibers (see Fig. 13, for illustration). 3) The active pruritogen in cowhage is an enzyme, which weighs ~36 kDa, and was administered using spicules. Histamine is a much smaller molecule of ~0.11 kDa and was applied with a skin prick lancet. This could have led to histamine being effectively delivered somewhat deeper in the epidermis thus reaching nerves less effectively desensitized by the 1-hour capsaicin treatment<sup>106</sup>.



**Figure 13. Potential mechanisms for the differential desensitizing efficacy of capsaicin on C-mechano-insensitive (CMI) versus polymodal C-fibers (PmC) observed in Study III.** Topical capsaicin penetrates the skin creating a concentration gradient. Dashed grey line (top) marks hypothetical depth to which C-fiber defunctionalization occurs. Because PmC-fiber terminals transmitting itch presumably reside more superficially than those of CMI-fibers those are desensitized by less capsaicin exposure. Additionally, mucunain on spicules may be introduced more superficially than histamine administered by a skin prick test (SPT) lancet. When cutaneous pruriceptive units are sensitized and probably spontaneously active (causing itch), localized defunctionalization of capsaicin-responsive terminals in the epidermis can greatly reduce the itch (SP II and III<sup>13</sup>). CMI-fibers, as opposed to PmC-fibers, interact closely with dermal capillaries to induce neurogenic inflammation.

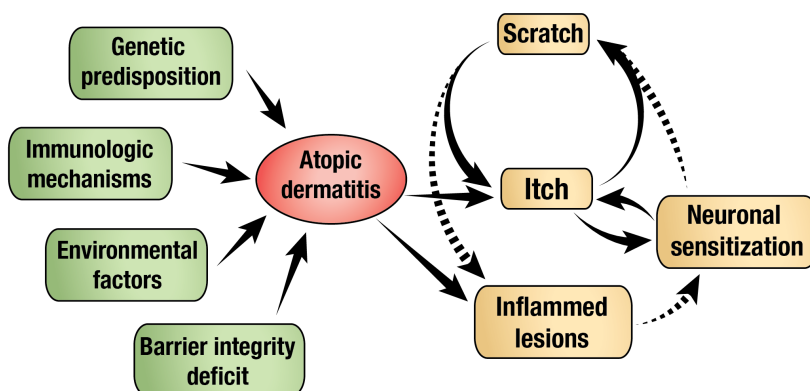
In summary, this study for the first time demonstrated pronounced antipruritic effects of high-concentration capsaicin pretreatment, particularly towards cowhage-induced itch, which is thought to mimic itch related to inflammatory dermatoses<sup>247</sup>. The 8% topical capsaicin treatment is currently approved for non-diabetic neuropathic pain with a contraindication being lesional skin. Thus, investigations of the antipruritic efficacy, duration and tolerability in other sensory models potentially involving compromised skin and eventually in focal itch conditions are needed to establish the potential clinical relevance of 8% capsaicin for treatment of chronic itch.

# CHAPTER 4. ITCH SENSITIZATION IN PATIENTS WITH ATOPIC DERMATITIS

## 4.1. ATOPIC DERMATITIS

Atopic dermatitis is a chronic pruritic inflammatory skin condition that is particularly active in children and adolescents. It is one of the most itchy skin dermatoses and affects millions of patients worldwide<sup>26,262,331,135</sup>. The severity and prevalence of the condition tends to decrease with age although a significant fraction of patients will suffer from recurrent AD throughout adulthood<sup>34,33233,34</sup>. Some studies estimate that the disease will clear in adolescence in approximately 60% of childhood AD cases. The disease is characterized by lesions with erythema, exudation, excoriations, lichenification, and xerosis as well as chronic or episodic itch and cutaneous pain<sup>8,10,26,262,331</sup>. Lesions are particularly common in the creases of the knees and elbows (flexor areas) but they often tend to change location throughout the course of the disease<sup>8,16</sup>.

Within the dermatological disease category, AD is the most significant contributor to YLD accumulation and can in severe cases be devastating for affected patients<sup>25</sup>. As described in *section 1.1.* for chronic itch in general, AD can significantly decrease quality of life and is associated with a substantial socioeconomic burden<sup>31,34,333,334</sup>. The majority of AD patients point to itch as being the single most bothersome disease component<sup>335,336</sup>. Cutaneous pain has previously been reported as occurring in the majority of patients with active AD, but the pain intensity has not been addressed. In Study IV we found that a sample of 25 AD patients with chronic itch above >3 on an NRS had moderate daily pain (39.7; VAS<sub>0-100</sub>) associated with their itch (60.7; VAS<sub>0-100</sub>). The condition and the related itch in particular is often difficult to treat adequately; antihistamines are ineffective as antipruritics in AD, and while corticosteroids decrease episodic inflammation and to a moderate extent the sensory symptoms, they are associated with significant side effects and can potentially exacerbate aspects of the diseases following prolonged usage<sup>26,37,40,337,338</sup>. Pathoetiologically, AD is associated with genetic, immunological, environmental and skin barrier factors<sup>262,339</sup>, and the relative role of each of these contributive factors has been comprehensively studied. Oppositely, the potential efferent and afferent roles of cutaneous sensory nerves in AD is much more sporadically explored<sup>14,126,340</sup>. Based on prior literature on etiology and sensory aberrations in AD as well as results from Study IV, a conceptual model of how itch sensitization ties into the symptomatology of AD can be created (Fig. 14).

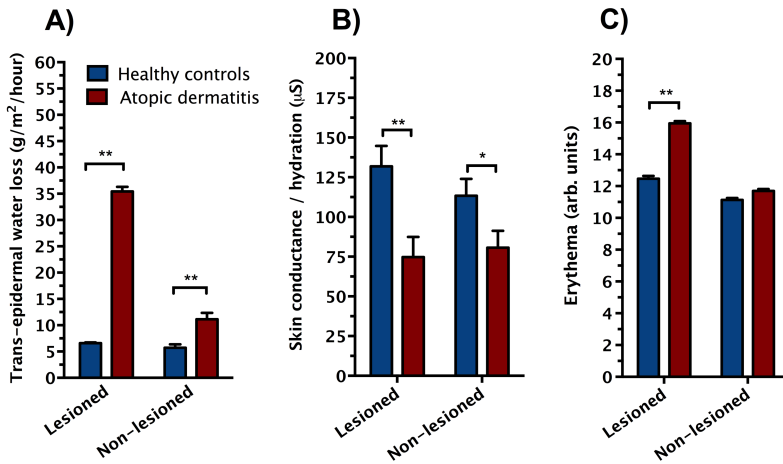


*Figure 14. Conceptual model of the contribution of itch sensitization in atopic dermatitis (AD). Green boxes represent known disease mechanisms involved in the generation and maintenance of AD. When a flare-up occurs itch and inflamed lesions are maintained by excessive scratching (itch-scratch-itch cycle). The severe ongoing itch, and presumably the local inflammation, contributes to peripheral and central neuronal sensitization, which can be measured psychophysically and causes increased itch and scratching.*

#### 4.1.1. SKIN ALTERATIONS IN ATOPIC DERMATITIS

In Study IV, skin abnormalities commonly associated with AD (xerosis, erythema and reduced barrier integrity) were objectively detected and quantified in lesional and non-lesional skin of AD patients (Fig. 15A, B and C) compared to homologous areas in the healthy controls. These skin abnormalities were measured by skin conductance (xerosis), spectrophotometry (erythema) and trans-epidermal water loss (barrier integrity). A scoring atopic dermatitis (SCORAD) evaluation was also undertaken to assess the overall severity of AD in each patient. Notably, modest but significant extra-lesional barrier alterations (xerosis and trans-epidermal water loss) in the AD patients paralleled their psychophysically assessed increased itch and pinprick pain sensitivity. The skin parameters; conductance, spectrophotometry, trans-epidermal water loss as well as the SCORAD system have been thoroughly assessed for validity and reliability<sup>239,341–348</sup>.





**Figure 15. Skin barrier parameters in lesional and non-lesional areas of patients with AD and homologous areas of healthy controls (Study IV).** A) Epidermal barrier integrity measured by trans-epidermal water loss. B) Skin hydration assessed by electrical skin conductance. C) Erythema assessed by spectrophotometry. Mean and standard error of mean depicted. \* =  $P \leq 0.05$ , \*\* =  $P \leq 0.01$ .

#### 4.1.2. ITCH IN ATOPIC DERMATITIS

Despite extensive research effort, the mechanism of itch in AD is still unknown and it is likely that multiple parallel mechanisms are at play<sup>26,135,349,350</sup>. Itch is a sensation that arises from signaling in the peripheral and central somatosensory system and ultimately the brain. However, in the genesis of chronic itch in AD patients, it is evident that several neuronal and non-neuronal structures are interacting with no singular signaling pathway of itch mediation. Rather, itch in AD is likely caused by a complex interplay between the epidermal skin barrier, cutaneous nerve fibers, endogenous as well as exogenous pruritogenic molecules, aberrant immune signaling and the central nervous systems<sup>14,26,128,262,135</sup>. These structures interact, often in positive feedback loop-like manners, to create not only itch but also inflammatory skin lesions. The itch prompts scratching of the affected skin area, leading to mechanical damage of the lesions and perpetuating the disease – a phenomenon often referred to as the ‘itch/scratch’-cycle<sup>26,349</sup>. Even when scratching is avoided during the day, nocturnal itch often leads to intense scratching and resultant poor sleep quality<sup>350,351</sup>. The suspected peripheral neuronal pathways involved in itch processing are described in *section 1.2*. On a molecular level, a vast array of receptors (e.g., TRPV1, TRPA1, PAR-2/4 and MRGPRs), locally secreted

signaling molecules (e.g., tryptase, histamine, NGF and substance P), and cytokines/chemokines (chemokine ligands 1/11, thymic stromal lymphopoietin, and interleukins; IL-4, -13, and -31) have all been implicated in the mediation of itch and itch sensitization in AD or rodent models of persistent itch<sup>78,79,150,352–354</sup>. Hence, successful ‘catch-all’ anti-inflammatory antipruritics for AD and other chronic inflammatory itch conditions would perhaps have to elicit their effect relatively high upstream in the relevant signaling cascades<sup>135,355</sup>. On the other hand, improved understanding of the neurophysiology and molecular characteristics of primary afferents C-fibers involved in itch processing may yield targets that can be inhibited to block pruriceptive transduction or transmission.

#### 4.1.3. EMERGING DRUGS FOR ATOPIC DERMATITIS

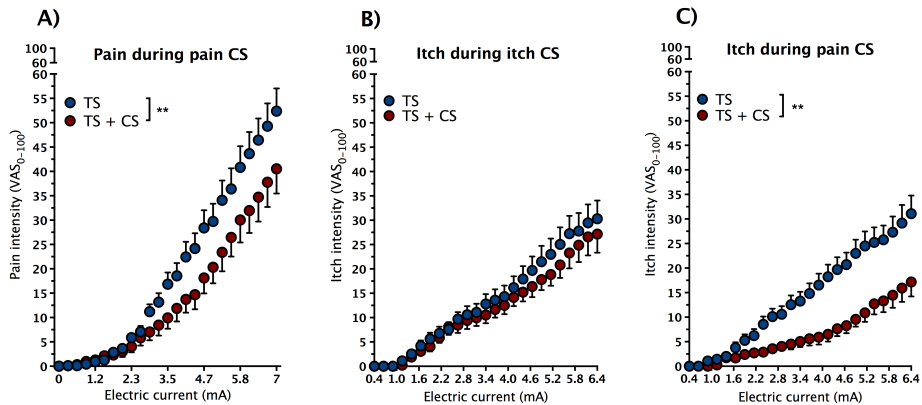
Seemingly effective systemic biological therapeutics are underway or have recently been approved. In March 2017, the FDA approved the IL-4/IL-13 inhibitor Dupilumab (Sanofi Pharma S/A)<sup>356</sup>. Dupilumab was approved following a series of clinical trials showing very high anti-inflammatory and antipruritic efficacy in patients with moderate to severe AD<sup>49,357</sup>. Another promising biologic currently in a phase IIb trial is the monoclonal antibody Nemolizumab (Galderma Pharma S/A), which works by inhibiting IL-31 receptor-A. A recently published phase-IIa study of Nemolizumab showed that a 0.5 mg/kg subcutaneous dose administered every 4 weeks provided rapid and substantial itch relief with an average itch reduction of 59.8% achieved over the 12-week treatment course<sup>358</sup>. Following the recent market approval of Dupilumab, Sanofi announced a US list price of Dupilumab treatment at \$37000/year. Thus, potential tools to predict which severely affected AD patients that will benefit the most from the treatment are warranted. Finally, pharmaceutical development of antipruritics in general is rapidly picking up speed. A patent watch report from September 2017 noted that despite numerous new patents and emerging drugs, the area is still disproportionately under-researched when considering the size of the potential market<sup>359</sup>.

## 4.2. SENSITIZATION IN PATIENTS WITH PAIN

Aberrant somatosensory sensitivity to various types of mechanical, thermal, electrical and chemical stimuli is well documented in chronic pain conditions<sup>151,360,361</sup>. In patients with peripheral neuropathic pain, large standardized QST

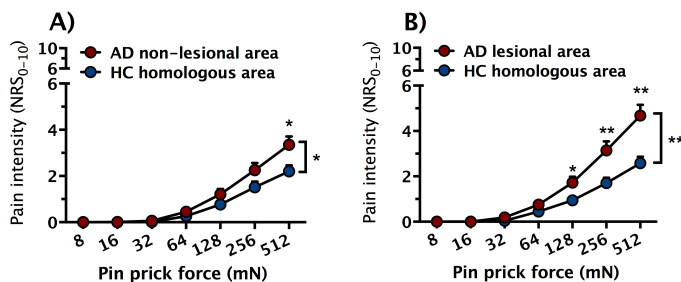
studies have shown profound sensory gain- and loss-of-function compared to healthy controls<sup>122,362</sup>. Neuropathic pain patients generally exhibit one of three overall sensory phenotypes; sensory loss, thermal hyperalgesia and mechanical hyperalgesia<sup>362</sup>. While some diagnoses are predominantly associated with one sensory subgroup, these phenotypes encompass patients with widely different clinical neuropathic pain etiologies and thus represent a sensory mechanistic evaluation<sup>314,363,364</sup>. Large QST studies have also been conducted in musculoskeletal pain conditions such as low-back pain and knee osteoarthritis where profound sensory sensitization is also evident, particularly to stimulation of musculoskeletal tissues<sup>364-366</sup>. In patients with chronic ocular pain, headache, visceral pain etc. similar observations have repeatedly been made<sup>367-370</sup>. As described in *section 1.3*, such sensory aberrations likely often involve both central and peripheral sensitization mechanisms.

Pain sensitization does not only manifest to simple controlled sensory tests such as mechanical or thermal threshold assessments. More advanced sensory paradigms measuring central mechanisms, such as conditioned pain modulation (diffuse noxious inhibitory control) and temporal summation, also frequently detect abnormalities in patients with chronic pain, e.g., reduced endogenous pain inhibition and increased pain facilitation<sup>364,365,371,372</sup>. Such paradigms have only just begun to be translated and introduced in chronic itch patients<sup>223,373</sup>, and methodological studies establishing the validity of these paradigms are needed<sup>206</sup>. SP I represents an initial attempt to investigate and establish psychophysical paradigms for assessment of endogenous descending itch inhibition in humans<sup>206</sup>. The main finding of this study was that itch is an insufficient conditioning stimulus for the elicitation of conditioned descending modulation of itch whereas a conditioning pain stimulus is highly effective (Fig. 16A, B and C). Recently, several interventional studies have proposed that QST profiling in pain patients not only allows researchers to make mechanistic inferences about the studied disease but can also be used to predict treatment responsiveness to various analgesics and even surgical interventions (e.g., to identify patients at risk for developing postoperative pain)<sup>307,366,372,374</sup>. For instance, by subgrouping neuropathic pain patients using QST, Demant *et al.* (2014) showed significantly improved pain relief in response to treatment with oxcarbazepine in patients with so-called “irritable-nociceptor” characteristics and achieved superior numbers needed to treat than current first-line drugs for neuropathic pain treatment<sup>304,375</sup>. Hence, because QST profiling essentially probes the status of the nociceptive system, it may be used as a tool to select the most optimal treatment based on the specific mechanism driving pain in a given patient<sup>314,362</sup>.



**Figure 16. Experiments on conditioning modulation of itch by contralateral pain and itch stimuli (SP I).** Healthy volunteers ( $N = 26$ ) were presented with an initial electrical itch or pain stimulus (test stimulus = TS) followed by the same stimulation during either a conditioning pain or itch stimulation (conditioning stimulus = CS). **A)** The intensity of a pain stimulus is significantly reduced when contralateral pain is present, known as ‘conditioned pain modulation’ (CPM)-effect. **B)** The intensity of an itch TS not significantly reduced during conditioning itch stimulation. **C)** The intensity of an itch TS is, however, significantly decreased during conditioning pain stimulation suggesting that the descending pain inhibition modulates itch processing. Mean and standard error of mean depicted. \*\* =  $P \leq 0.01$ .

As opposed to the extensive evidence showing pain sensitization (and other sensory abnormalities) in chronic pain disorders comparatively little research has been conducted into whether conditions associated with chronic itch impose parallel phenomena<sup>6,106</sup>. Of the sensory tests applied in studies of patients with AD, most have been directly adapted from clinical pain research, which conceivably could limit their sensitivity. In Study IV, we found significantly increased pain in response to controlled suprathreshold pinprick stimulation in patients with atopic dermatitis both in lesional skin and to a lesser extent in non-lesional skin, indicative of PmC-fiber sensitization (Fig. 17A and B). Notably, patients with AD did not exhibit altered thermal sensory sensitivity as compared to the control group. With itch-specific sensory tests using von Frey and chemical provocations, more profound differences were observed. Patients with AD exhibited intra-lesional and extra-lesional itch sensitization selectively to cowhage provocations. Moreover, patients with AD had exaggerated responses to itch-evoking mechanical stimuli both intra-lesionally as well as extra-lesionally and developed increased hyperknesis following itch provocations.



**Figure 17. Mechanical hyperalgesia to suprathreshold pain stimuli in patients with atopic dermatitis (AD) (Study IV).** Mechanical pain sensitivity to pinprick stimuli from 8 to 512 mN, in non-lesional (A) and lesional skin (B) of AD patients compared to homologous sites in healthy controls.

### 4.3. THE SENSORY CORRELATES OF ITCH SENSITIZATION IN ATOPIC DERMATITIS

As opposed to the various molecular mechanisms suspected to be involved in itch sensitization, which are outlined in *section 1.3*, this paragraph deals with the accompanying somatosensory changes previously documented and extends on prior findings with data from Study IV. The standardized QST tests applied in Study IV have been extensively tested for test-retest reliability, yielding generally good results<sup>376-378</sup>. Notably, almost all studies conducting somatosensory testing in chronic itch patients have been performed in AD. In addition to the high prevalence of AD with severe itch, this is probably related to three common clinical observations that indicate altered sensory processing in AD: 1) patients frequently report allodynia associated with, e.g., certain fabrics<sup>8,126,350</sup>, 2) patients often report itch exacerbations when feeling warm or associated with perspiration<sup>12,379,380</sup>, and 3) patients commonly describe abnormal cutaneous sensations such as burning, tingling and pricking associated with their lesions<sup>8,10,12</sup>. The sections below outline quantitative sensory findings in patients with AD.

### 4.3.1. THERMOSENSORY CHANGES

Standardized thermal QSTs have only been sparsely assessed in patients with AD. One previous study demonstrated minor ( $< 1^{\circ}\text{C}$ ) but significant impairments in warmth and cold detection thresholds in patients with AD<sup>381</sup> whereas a recent study failed to detect similar differences in thermal sensitivity despite very comparable assessment methodology<sup>382</sup>. Accordingly, in Study IV we did not observe any significant alterations in thermal sensitivity in either lesional or non-lesional skin. These observations suggest that alterations in simple thermal detection and pain thresholds are likely not a key feature in AD as is the case, for instance, in certain musculoskeletal and neuropathic pain conditions<sup>362,383–385</sup>. Oppositely, both lesional and non-lesional skin yielded mean thermal thresholds comparable to those found in the normative QST data sets<sup>122,151</sup>. It should be noted that thermal detection and pain thresholds might be a suboptimal way to assess aberrations of thermal sensory processing in AD. Clearly, warmth is a very commonly reported aggravating factor<sup>8,51</sup> (in agreement with results from Study IV). Conceivably, itch sensitization to thermal stimuli could take the form of a sensory modality-shift, i.e., the abnormal induction of itch following an innocuous or noxious warmth stimulation.

The strongest evidence in favor of this type of sensory phenomenon comes from a previous study that found that noxious suprathreshold heat stimuli evoke itch in AD whereas the same stimulus exclusively evokes pain in healthy controls<sup>14</sup>. This conceptually corresponds to heat hyperknesis because pruriceptive units are presumably also activated during normal heat stimulation<sup>42,86,88,251</sup>. In agreement with this finding, several of the participating patients in Study IV spontaneously reported that the repeated heat stimuli associated with the heat pain threshold assessment provoked itch when performed in lesional skin, but this was not systematically recorded. Lastly, in a subacute human model of contact dermatitis elicited by squaric acid dibutyl ester, profound itch can be provoked by heat stimuli even though such stimuli are purely perceived as eliciting warmth sensation or burning pain in control skin<sup>176</sup>. Tests designed to detect ‘heat hyperknesis’, i.e., a modality-shift in thermal sensation, are conceivably more specific for the assessment of itch sensitization in patients with AD than standardized thermal QSTs.

### 4.3.2. MECHANICAL ITCH DYSESTHESIAS

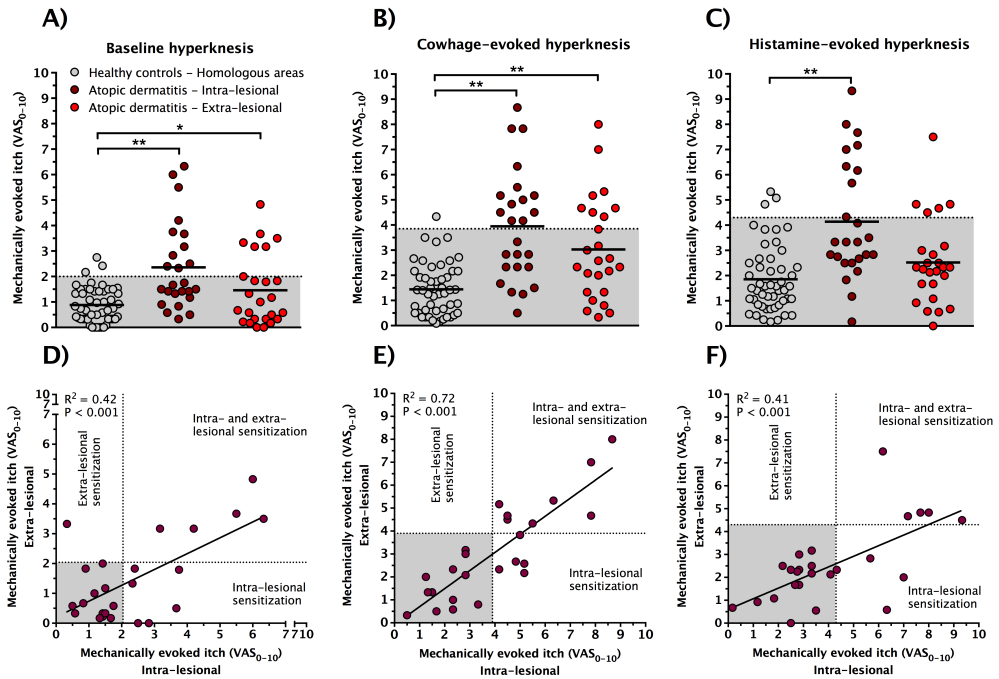
Eight studies have assessed mechanical itch dysesthesias in patients with AD utilizing very diverse assessment methodology. The diversity is mainly related to whether lesional or non-lesional skin is tested, which devices that are used in the tests, how the subjects are instructed to rate and whether an initial itch provocation is conducted. This has yielded a relatively inconsistent pattern of results from clinical studies of alloknesis and hyperknesis (Table 1). Two studies have demonstrated alloknesis occurring restricted to lesional and peri-lesional skin areas<sup>126,161</sup>, and the phenomenon is likely more or less dependent on ongoing spontaneous itch nearby<sup>73,176</sup>. These baseline itch sensitization abnormalities align with patient self-reported symptoms and the observation that certain fabrics are capable of evoking robust itch in AD<sup>126</sup>. However, when quantifying the spatial extent of alloknesis or hyperknesis developed following an itch provocation in non-lesional skin, AD patients do not seem to develop significantly larger areas of mechanical dysesthesias than healthy controls<sup>82,221,340,386</sup> (although trends towards sensitization have been observed<sup>82</sup>). These results however have mostly been obtained with histamine as the only itch provocation, and limited assessment of baseline differences in response to the mechanical stimuli have been performed.

Study	Assessment methods	Studied mechanical itch dysesthesia	
		Lesional	Non-lesional
Wahlgren et al. (1990) <sup>126</sup>	Wool fibers (Intensity method)	<u>Spontaneous</u> : ↑Hyperknesis, likely both lesional and extra-lesional	
Heyer et al. (1995) <sup>340</sup>	Sensory brush (Spatial mapping method <sup>1</sup> )	N/A	<u>Evoked</u> : ↓Alloknesis
Weisshaar et al. (1998) <sup>221</sup>	Sensory brush (Spatial mapping method <sup>1</sup> )	N/A	<u>Evoked</u> : ↓Alloknesis
Ikoma et al. (2004) <sup>14</sup>	Weighted needle stimulators (Intensity method)	<u>Spontaneous</u> : ↑Hyperknesis	<u>Spontaneous</u> : ↑Hyperknesis (intra and peri-lesional)
Ikoma et al. (2005) <sup>82</sup>	Sensory brush / pin prick (Spatial mapping method <sup>2</sup> )	N/A	<u>Evoked</u> : → Alloknesis <u>Evoked</u> : → Hyperknesis <sup>5</sup>
Hosogi et al. (2006) <sup>161</sup>	Sensory brush (Intensity method)	<u>Spontaneous</u> : ↑Alloknesis	<u>Spontaneous</u> : →Alloknesis
Laarhoven et al. (2007) <sup>218</sup>	Von Frey stimulators (Intensity method)	<u>Spontaneous</u> : ↑Hyperknesis <sup>3</sup>	<u>Spontaneous</u> : ↑Hyperknesis <sup>4</sup>
Andersen et al. (2017) <sup>13</sup>	Von Frey stimulators (Intensity method)	<u>Spontaneous and evoked</u> : ↑Hyperknesis	<u>Spontaneous and evoked</u> : ↑Hyperknesis

**Table 1 – Findings from studies on mechanical itch dysesthesias in patients with atopic dermatitis (AD) versus healthy controls.** The table lists studies assessing alloknesis and/or hyperknesis as well as the methods applied in each study. <sup>1</sup> = after an iontophoretic histamine provocation, <sup>2</sup> = after electrically induced itch, <sup>3</sup> = predominantly lesional, <sup>4</sup> = predominantly non-lesional, <sup>5</sup> = trend toward more hyperknesis in AD patients was observed. **Arrows**: sensitivity in patients vs. controls: ↑ = significantly increased responses in patients ↓ = significantly decreased responses in patients, → no significant differences. “Spontaneous” refers to allo/hyperknesis without any preceding itch provocation while “evoked” refers to assessment of the itch dysesthesia(s) following an itch provocation.

Oppositely, it is evident that robust hyperknesis is present in lesional AD skin, when the method of quantifying itch intensity ratings in response to controlled punctuate mechanical stimuli without prior itch provocations, is used<sup>13,14,161,218</sup>. Good evidence is currently lacking from all other chronic itch conditions, but occasionally similar findings or trends have been described in psoriasis, chronic post-burn itch and on a case basis in neuropathic itch patients, where it occurs within, and perifocally to, the itching skin area<sup>15,161,309</sup>. When it comes to extra-lesional hyperknesis in AD, the evidence is somewhat unclear. Ikoma *et al.* (2004) found significant hyperknesis almost entirely confined to lesional skin in response to weighted needle stimulation (significant hyperknesis was detected 1 cm outside of lesions). Oppositely, both Laarhoven *et al.* (2007) and Study IV documented significant hyperknesis in non-lesional skin probed using von Frey stimulators (see Table 1). In Study IV, we also combined itch intensity ratings in response to punctuate stimuli with proceeding itch provocations (histamine and cowhage) and observed increased aggravation of itch sensitization in the AD patients following itch provocation (based on methodology applied in Study II and III). This has not been explored before in chronic itch patients, but in a subacute model of contact dermatitis induced in healthy volunteers very similar results were found in peri-lesional skin<sup>176</sup>. High inter-variability in the severity of hyperknesis seems evident amongst patients with AD (although this is not well-documented). This could indicate the existence of sensory phenotypes, e.g., high versus low mechanical itch sensitization (see Fig. 18A, B and C, based on data from Study IV). Moreover, extra-lesional hyperknesis appears to almost exclusively occur in patients also displaying hyperknesis in lesional skin (Fig. 18D, F and G)<sup>13</sup>.



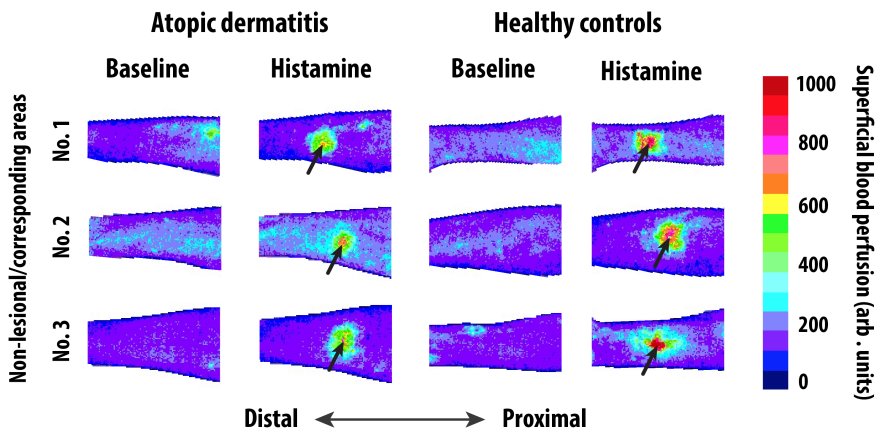


**Figure 18. The variability of mechanical hyperknesis in atopic dermatitis (AD) compared to healthy controls (Study IV).** *A*) Mean and individual hyperknesis data assessed at baseline in lesional (dark red) and non-lesional (bright red) skin of patients with AD ( $n = 25$ ) compared to healthy controls ( $n = 25$ ). Data from homologous healthy control areas is pooled (50 data points). Marked grey areas indicate the healthy control average  $+1.96$  standard deviations (SD), thus constituting a limit at which hyperknesis on an individual level can be detected. *B* and *C*) As in (*A*), but hyperknesis was assessed after itch from cowhage or histamine had subsided. **Bottom row**; intra-lesional responses to mechanical itch provocations correlated with the responses to extra-lesional provocations at baseline (*D*), following cowhage (*E*), and following histamine (*F*). Note that not all patients displayed exaggerated responses and that patients either have sensitization restricted to their lesions or affecting both their lesional and non-lesional skin.

### 4.3.3. SENSITIVITY TO PRURITOGENS

Sensitivity to chemical itch provocations is the most investigated aspect of the somatosensory status of AD patients (Table 2), perhaps because chemical itch provocations constitute the only very robust yet simple method of itch elicitation in healthy controls. The most commonly applied pruritogen by far is histamine, which has repeatedly been used in both lesional and non-lesional skin of patients with AD versus healthy controls to assess itch sensitization (Table 2). Results from such studies are relatively well aligned; in AD lesions, histamine usually evoke the same or moderately higher itch intensity than in homologous skin areas of healthy

controls. This finding is in accordance with the results of Study IV. In non-lesional AD skin areas, histamine provocations are generally found to evoke itch at the same or a moderately lower intensity as compared to healthy controls, which is also aligned with the results of Study IV. This suggests that CMi-fiber responses to histamine are robustly anomalous neither in non-lesional nor lesional AD skin – an observation that corresponds with the fact that antihistamines are not effective in AD. Oppositely, a majority of studies have found significantly reduced neurogenic flare in non-lesional skin of patients with AD, indicating decreased reactivity to histamine<sup>129,182,186,267</sup>. A similar reduction in axon-reflex-flare size and intensity was observed in Study IV (Fig. 19) and has been observed in response to several other pruritogens such as substance P and serotonin<sup>267,387</sup>. The mechanisms behind this decreased vasoreactivity are unknown, but the most conceivable are depletion of vasoactive neuropeptides, desensitization/tolerance of vasculature to said neuropeptides or secondary skin changes associated with AD or AD therapy.



**Figure 19. Flare reaction in atopic dermatitis (AD).** A series of Full-Field Laser Perfusion (FLPI) images recorded at baseline as well as following histamine in non-lesional skin of patients with atopic dermatitis and homologous skin of healthy controls (Study IV). Note the blunted flare response to histamine in non-lesional skin of AD patients.

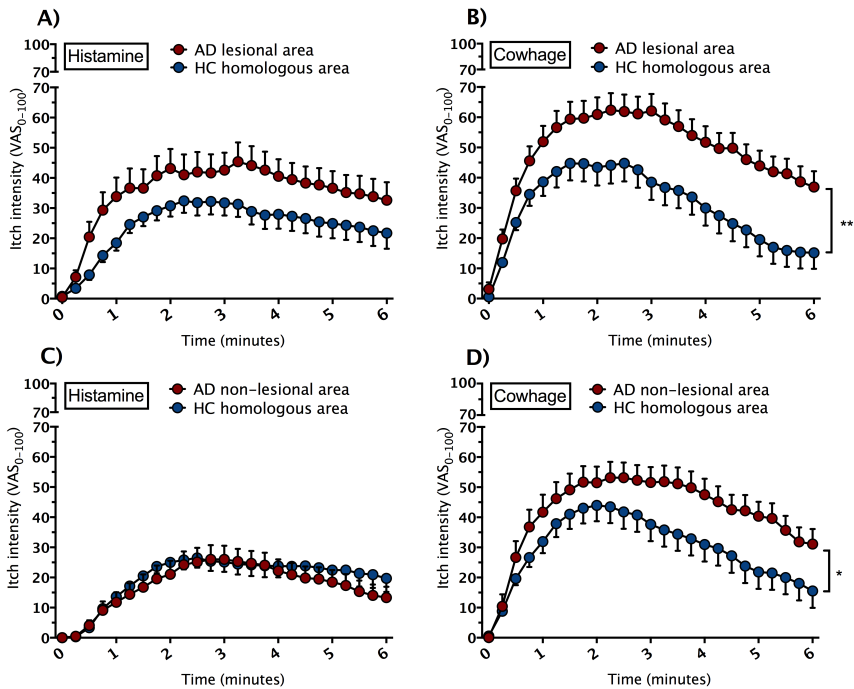
More than a dozen pruritogenic or algogenic substances, including acetylcholine, bradykinin, citrate buffer (low pH-solution), compound 48/80, IL-31, SLIGKV, substance P, vasoactive intestinal peptide (VIP), serotonin, mustard oil and capsaicin, have been applied in a single or occasionally a couple studies including patients with AD (lesional and/or non-lesional skin) versus healthy controls<sup>13,126,128,129,161,182,247,267,388–393</sup>. Most of these studies have either yielded negative results or their results have not been unambiguously reproduced. A couple of findings stand out: 1) Acetylcholine has been found to evoke more itch and less pain in non-lesional skin of patients with AD<sup>392,394</sup> with the combined sensory intensity

being unchanged between groups indicating a profound modality-shift. 2) A study has shown that a microdialysis infusion of citrate buffer (pH=3) at 4  $\mu$ L/minute causes significant itch in both lesional and non-lesional AD skin areas but only mild pain in healthy controls <sup>14</sup>. A low pH provocation could elicit itch through several parallel mechanisms and does as such not provide particularly specific information about the nature of the sensitization. However, because acid-evoked itch can be mimicked in healthy skin by preceding histamine conditioning and because a recent study suggested that acidosis (known to occur under inflammatory conditions) counteracts tachyphylaxis in itch fibers, these findings are worth revisiting <sup>14,395</sup>.

Psychophysical outcome	Chemical provocation	Skin area in patients		References
		Lesional	Non-lesional	
Peak itch intensity	Serotonin	N/A	→	267
	Compound 48/80	N/A	→→	126,230
	Cowhage	↑	→→↗	13,182,247
	Histamine	→→→↑↑	→→→→→→↓	13,126,129,161,182,267,388-390
	IL-31	N/A	→	396
Itch area under the curve or mean intensity	Serotonin	→	→	161,267
	Acetylcholine	N/A	↑↑	391-393
	Bradykinin	↑	→	161
	Citrate buffer	↑	↑	14
	Compound 48/80	N/A	→	126
	Cowhage	↑	→↑↑	13,182,247
	Histamine	→→↑↑	→→→→→→→↓	13,126,129,161,182,267,388-390
	IL-31	N/A	→	396
	Substance P	→	→	161,387
	SLIGKV	↑	↗	128
	VIP	N/A	→↓	391,392
	VIP and Acetylcholine	N/A	→↑	391,392
Itch duration	Serotonin	N/A	→	267
	Acetylcholine	N/A	↑↑	393,394
	Bradykinin	↑	→	161
	Compound 48/80	N/A	→	126
	Cowhage	N/A	↑	182
	Histamine	↑	→→→→	126,182,267,389
	IL-31	N/A	→	396

**Table 2. Studies conducted in atopic dermatitis (AD) patients and healthy controls comparing sensory sensitivity to pruritogenic/algogenic chemical provocations.** Lesional/non-lesional columns represent a comparison between AD skin and homologous healthy control skin. Each arrow represents a finding from an individual study. **Arrows:** → = No significant itch sensitivity difference; ↑ = Increased itch sensitivity in AD patients; ↓ = Decreased itch sensitivity in AD patients; ↗ = trend ( $P = 0.05-0.1$ ) towards increased itch sensitivity in AD patients; N/A = Not assessed. IL-31 = Interleukin 31, VIP = vasoactive intestinal peptide

Finally, three studies including cowhage provocations in patients with AD and healthy controls have been conducted after cowhage was ‘rediscovered’ as an itch model a decade ago<sup>13,182,247</sup>. In line with the results of Study IV, a recent study testing only non-lesional skin found increased itch area under the curve (but not peak itch intensity) in response to cowhage in patients with AD<sup>182</sup>. Oppositely, an earlier, smaller study in 15 AD patients and 15 healthy controls found no differences between the groups. However the cowhage-evoked itch was unusually strong in the healthy controls so a ceiling effect could have been present<sup>247</sup>. In a single study, the tethered PAR-2 ligand SLIGKV (presumably eliciting itch through the same mechanism as mucunain) was intradermally injected and gave rise to higher itch ratings in lesional AD skin compared to controls and as well as a trend towards higher ratings in non-lesional AD skin<sup>128</sup>. Study IV is the first study to investigate sensitivity to cowhage in lesional AD skin. We found that patients display robustly increased cowhage-induced itch intra-lesionally but also to lesser extent in non-lesional skin (Fig. 20A-D). In summary, the prominent itch responses specifically to non-histaminergic chemical pruritic stimulation in AD suggest that the itch sensitization implicated in the sensory symptomatology appears to be pathway-specific and extent beyond lesional skin.



**Figure 20.** Itch sensitization to cowhage in atopic dermatitis (AD). Temporal profiles of itch intensity evoked by histamine (A and C) and cowhage (B and D) provocations in lesional (A and B) and non-lesional (C and D) skin as well as in respective control skin (Study IV). Histamine-induced itch was not significantly increased in lesional AD skin (A), but a tendency was observed ( $P = 0.07$ , after multiplicity correction). Robust sensitization to cowhage-evoked itch in AD is evident (B and D). AD = Atopic dermatitis; HC = Healthy controls. Mean and standard error of mean depicted. \* =  $P \leq 0.05$ , \*\* =  $P \leq 0.01$ .

#### 4.4. POTENTIAL CLINICAL UTILITY OF PSYCHOPHYSICAL TESTS FOR ITCH SENSITIZATION

As has been shown in patients with chronic pain, sensory phenotyping by QST developed specifically for probing sensory aberrations associated with itch could perhaps be used to guide diagnosis and optimize pharmacotherapy in patients with chronic itch. In a recent paper by Hawro *et al.* (2016), itch and cutaneous vasomotor reactions to cowhage and histamine were proposed as potential diagnostic markers of AD of particular value in atypical or mild cases<sup>182</sup>. Similarly, tools to predict responsiveness to novel drugs could be valuable as new and very expensive biologics becomes available. This is true not only for AD but also for conditions

such as psoriasis and prurigo. While these types of studies have yet to be undertaken in patients with chronic itch, it is clear that centrally acting antipruritics can be of use in otherwise treatment-refractory patients <sup>127,397</sup>. Antipruritic therapy should, whenever possible be focused on reducing local lesions and skin inflammation as well as targeting the underlying cause of itch when identifiable <sup>40,129,350</sup>. However, tentatively, it could be suggested that, for instance, AD patients with no signs of itch sensitization, e.g., no allo-/hyperknesis nor increased responses to chemical provocation in non-lesional skin, would respond well to peripherally acting anti-inflammatory drugs. On the other hand, patients with significant intra- and extra-lesional itch sensitization would conceivably benefit from additional antipruritics inhibiting central itch processing as well as sensitization <sup>129</sup>. Thus psychophysical assessments of cutaneous sensitivity could potentially improve selection of the most ideal treatment approach in chronic itch patients – although much more groundwork is needed to adequately appraise this notion.

## CHAPTER 5. CONCLUSION

The present PhD project applied human surrogate models of itch and itch sensitization for basic, translational and clinical research purposes. Results from Study II showed that there are considerable differences between chemical and mechanically evoked itch sensitivity in spinal versus trigeminal innervated areas and that von Frey monofilaments below the mechanical pain threshold can be used to assess experimentally evoked hyperknesis. Results from Study III demonstrated profound antipruritic effects of high-concentration topical capsaicin treatment towards the two most commonly applied models of itch. This result underlines how pruriception in human skin is largely dependent on capsaicin-sensitive cutaneous fibers and indicates that high-concentration capsaicin-induced desensitization might be of clinical value as an antipruritic therapeutic option. Finally, Study IV, conducted in AD patients with chronic itch and healthy controls, revealed pathway-specific non-histaminergic itch sensitization as well as mechano-nociceptive sensitization occurring both intra- and extra-lesionally in patients with AD. The study thus demonstrated that AD patients, beyond having spontaneous itch, display considerable cutaneous somatosensory aberrations and lends mechanistic support to the observation that antihistamines are ineffective as antipruritics in AD. Finally, it can be inferred that PAR-2/TRPA1-mediated itch conveyed by PmC-fibers appears to be a promising potential target for future itch-relieving drugs in AD.

In summary, the studies presented within this dissertation have hopefully contributed to an improved understanding of itch and itch sensitization in healthy humans and in patients with AD as well as demonstrated that surrogate models of itch are applicable tools to probe the human pruriceptive system for a versatile range of purposes.

### 5.1 FUTURE PERSPECTIVES

In spite of a steep increase in studies investigating rodent and human itch processing in the last decade the area is still disproportionately small relative to the clinical impact of chronic itch<sup>398-400</sup>. Numerous pioneering mechanistic studies on itch conducted in rodents have recently been published<sup>43,45-48,108,113,120,401-404</sup>, but when considering the known inter-species dissimilarities, considerable and challenging research remains to be conducted to translate and validate these preclinical findings in the context of human neurobiology. Particularly, new and more standardizable

non-histaminergic human surrogate models of pathophysiologically relevant itch; improved and more thoroughly tested methods to assess itch sensitization; a better understanding of human peripheral itch transduction mechanisms, itch encoding and sensitization processes, and more knowledge on segmental and descending endogenous itch inhibition is needed. Once this is accomplished, further research can more effectively be directed towards basic studies of the pruriceptive system as well as testing of novel antipruritic drug candidates or non-pharmaceutical interventions with the aim of identifying new and improved options for itch relief.



# LITERATURE LIST

- 1 Ständer S, Schäfer I, Phan NQ, *et al.* Prevalence of Chronic Pruritus in Germany: Results of a Cross-Sectional Study in a Sample Working Population of 11,730. *Dermatology* 2010; **221**:229–35.
- 2 Dalgard F, Svensson Å, Holm JØ, Sundby J. Self-Reported Skin Morbidity among Adults: Associations with Quality of Life and General Health in a Norwegian Survey. *J Investig Dermatol Symp Proc* 2004; **9**:120–5.
- 3 Matteredne U, Apfelbacher CJ, Loerbroks A, *et al.* Prevalence, correlates and characteristics of chronic pruritus: a population-based cross-sectional study. *Acta Derm Venereol* 2011; **91**:674–9.
- 4 Rea JN, Newhouse ML, Halil T. Skin disease in Lambeth. A community study of prevalence and use of medical care. *Br J Prev Soc Med* 1976; **30**:107–14.
- 5 Ständer S, Weisshaar E, Mettang T, *et al.* Clinical Classification of Itch: a Position Paper of the International Forum for the Study of Itch. *Acta Derm Venereol* 2007; **87**:291–4.
- 6 Ikoma A, Steinhoff M, Ständer S, *et al.* The neurobiology of itch. *Nat Rev Neurosci* 2006; **7**:535–47.
- 7 Yosipovitch G, Greaves MW, Schmelz M. Itch. *Lancet* 2003; **361**:690–4.
- 8 Dawn A, Papoiu ADP, Chan YH, *et al.* Itch characteristics in atopic dermatitis: Results of a web-based questionnaire. *Br J Dermatol* 2009; **160**:642–4.
- 9 Yosipovitch G, Ansari N, Goon A, *et al.* Clinical characteristics of pruritus in chronic idiopathic urticaria. *Br J Dermatol* 2002; **147**:32–6.
- 10 Brenaut E, Garlandezec R, Talour K, Misery L. Itch characteristics in five dermatoses: Non-atopic eczema, atopic dermatitis, urticaria, psoriasis and scabies. *Acta Derm Venereol* 2013; **93**:573–4.
- 11 Goon ATJ, Yosipovitch G, Chan YH, Goh CL. Clinical characteristics of generalized idiopathic pruritus in patients from a tertiary referral center in Singapore. *Int J Dermatol* 2007; **46**:1023–6.
- 12 O'Neill JL, Chan YH, Rapp SR, Yosipovitch G. Differences in itch characteristics between psoriasis and atopic dermatitis patients: Results of a web-based questionnaire. *Acta Derm Venereol* 2011; **91**:537–40.
- 13 Andersen HH, Elberling J, Sølvsten H, *et al.* Nonhistaminergic and mechanical itch sensitization in atopic dermatitis. *Pain* 2017; **158**:1780–91.
- 14 Ikoma A, Fartasch M, Heyer G, *et al.* Painful stimuli evoke itch in patients with chronic pruritus: central sensitization for itch. *Neurology* 2004; **62**:212–7.
- 15 Andersen HH, Sand C, Elberling J. Considerable Variability in the Efficacy of 8% Capsaicin Topical Patches in the Treatment of Chronic Pruritus in 3 Patients with Notalgia Paresthetica. *Ann Dermatol* 2016; **28**:86–9.
- 16 Beltrani VS. The clinical spectrum of atopic dermatitis. *J Allergy Clin Immunol* 1999; **104**:S87–98.
- 17 Hong J, Buddenkotte J, Berger TG, Steinhoff M. Management of Itch in Atopic Dermatitis. *Semin Cutan Med Surg* 2011; **30**:71–86.
- 18 Oaklander AL, Cohen SP, Raju SVY. Intractable postherpetic itch and cutaneous deafferentation after facial shingles. *Pain* 2002; **96**:9–12.
- 19 Kini SP, DeLong LK, Veledar E, *et al.* The impact of pruritus on quality of life: the skin equivalent of pain. *Arch Dermatol* 2011; **147**:1153–6.
- 20 Yosipovitch G, Goon A, Wee J, *et al.* The prevalence and clinical characteristics of pruritus among patients with extensive psoriasis. *Br J Dermatol* 2000; **143**:969–73.
- 21 Gupta MA, Gupta AK. Depression modulates pruritus perception. A study of pruritus in psoriasis, atopic dermatitis and chronic idiopathic urticaria. *Ann N Y Acad Sci* 1999; **885**:394–5.
- 22 Sheehan-Dare R a, Henderson MJ, Cotterill J a. Anxiety and depression in patients with chronic urticaria and generalized pruritus. *Br J Dermatol* 1990; **123**:769–74.
- 23 Picardi A, Lega I, Tarolla E. Suicide risk in skin disorders. *Clin Dermatol* 2013; **31**:47–56.
- 24 Halvorsen JA, Dalgard F, Thoresen M, *et al.* Itch and pain in adolescents are associated with suicidal ideation: A population-based cross-sectional study. *Acta Derm Venereol* 2012; **92**:543–6.

- 25 Vos T, Allen C, Arora M, *et al.* Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet* 2016; **388**:1545–602.
- 26 Wahlgren CF. Itch and atopic dermatitis: An overview. *J Dermatol* 1999; **26**:770–9.
- 27 Szepietowski JC, Reich A. Pruritus in psoriasis: An update. *Eur J Pain* 2016; **20**:41–6.
- 28 Oaklander AL. Common neuropathic itch syndromes. *Acta Derm Venereol* 2012; **92**:118–25.
- 29 Frese T, Herrmann K, Sandholzer H. Pruritus as reason for encounter in general practice. *J Clin Med Res* 2011; **3**:223–9.
- 30 Stander S, Stumpf A, Osada N, *et al.* Gender differences in chronic pruritus: women present different morbidity, more scratch lesions and higher burden. *Br J Dermatol* 2013; **168**:1273–80.
- 31 Ellis CN, Drake LA, Prendergast MM, *et al.* Cost of atopic dermatitis and eczema in the United States. *J Am Acad Dermatol* 2002; **46**:361–70.
- 32 Mancini AJ, Kaulback K, Chamlin SL. The socioeconomic impact of atopic dermatitis in the United States: A systematic review. *Pediatr Dermatol* 2008; **25**:1–6.
- 33 Flohr C, Williams HCG. Epidemiology of Atopic Dermatitis. *Harper's Textb Pediatr Dermatology Third Ed* 2011; **1**:1–15.
- 34 Carroll CL, Balkrishnan R, Feldman SR, *et al.* The burden of atopic dermatitis: Impact on the patient, family, and society. *Pediatr Dermatol* 2005; **22**:192–9.
- 35 Wahlgren CF, Hägermark O, Bergström R. The antipruritic effect of a sedative and a non-sedative antihistamine in atopic dermatitis. *Br J Dermatol* 1990; **122**:545–51.
- 36 Wahlgren CF. Itch and atopic dermatitis: clinical and experimental studies. *Acta Derm Venereol* 1991; **165**:1–53.
- 37 Patel T, Yosipovitch G. Therapy of Pruritus. *Expert Opin Pharmacother* 2010; **11**:1673–82.
- 38 Yosipovitch G, Bernhard JD. Chronic Pruritus. *N Engl J Med* 2013; **368**:1625–34.
- 39 Arkwright PD, Motala C, Subramanian H, *et al.* Management of difficult-to-treat atopic dermatitis. *J Allergy Clin Immunol Pract* 2013; **1**:142–51.
- 40 Weisshaar E, Szepietowski JC, Darsow U, *et al.* European guideline on chronic pruritus: In cooperation with the European dermatology forum (EDF) and the European academy of dermatology and venereology (EADV). *Acta Derm Venereol* 2012; **92**:563–81.
- 41 Handwerker HO. Chapter 1 - Itch Hypotheses. In: *Itch Mechanisms and Treatment*, 1st ed. , CRC Press/Taylor & Francis, Boca Raton (FL), US, 2014.
- 42 Namer B, Carr R, Johaneck LM, *et al.* Separate Peripheral Pathways for Pruritus in Man. *J Neurophysiol* 2008; **100**:2062–9.
- 43 Wilson SR, Nelson AM, Batia L, *et al.* The ion channel TRPA1 is required for chronic itch. *J Neurosci* 2013; **33**:9283–94.
- 44 Miller G. Biomedicine. Grasping for clues to the biology of itch. *Science* 2007; **318**:188–9.
- 45 Wilson SR, Gerhold KA, Bifolck-Fisher A, *et al.* TRPA1 is required for histamine-independent, Mas-related G protein-coupled receptor-mediated itch. *Nat Neurosci* 2011; **14**:595–602.
- 46 Sun Y-G, Chen Z-F. A gastrin-releasing peptide receptor mediates the itch sensation in the spinal cord. *Nature* 2007; **448**:700–3.
- 47 Liu Q, Tang Z, Surdenikova L, *et al.* Sensory neuron-specific GPCR Mrgprs are itch receptors mediating chloroquine-induced pruritus. *Cell* 2009; **139**:1353–65.
- 48 Sun Y-G, Zhao Z-Q, Meng X-L, *et al.* Cellular basis of itch sensation. *Science* 2009; **325**:1531–4.
- 49 Simpson EL, Bieber T, Guttman-Yassky E, *et al.* Two Phase 3 Trials of Dupilumab versus Placebo in Atopic Dermatitis. *N Engl J Med* 2016; **375**:2335–48.
- 50 Malajian D, Guttman-Yassky E. New pathogenic and therapeutic paradigms in atopic dermatitis. *Cytokine* 2015; **73**:311–8.
- 51 Darsow U, Scharein E, Simon D, *et al.* New aspects of itch pathophysiology: Component analysis of atopic itch using the 'Eppendorf Itch Questionnaire'. *Int Arch Allergy Immunol* 2001; **124**:326–31.

## LITERATURE LIST

- 52 van Laarhoven AIM, Kraaijaat FW, Wilder-Smith OH, *et al.* Sensitivity to itch and pain in patients with psoriasis and rheumatoid arthritis. *Exp Dermatol* 2013; **22**:530–4.
- 53 Arendt-Nielsen L, Nie H, Laursen MB, *et al.* Sensitization in patients with painful knee osteoarthritis. *Pain* 2010; **149**:573–81.
- 54 Woolf CJ. Central sensitization: Implications for the diagnosis and treatment of pain. *Pain* 2011; **152**:S2–15.
- 55 Sikand P, Shimada SG, Green BG, LaMotte RH. Similar itch and nociceptive sensations evoked by punctate cutaneous application of capsaicin, histamine and cowhage. *Pain* 2009; **144**:66–75.
- 56 Sikand P, Dong X, LaMotte RH. BAM8-22 Peptide Produces Itch and Nociceptive Sensations in Humans Independent of Histamine Release. *J Neurosci* 2011; **31**:7563–7.
- 57 Johaneck LM, Meyer R a, Hartke T, *et al.* Psychophysical and Physiological Evidence for Parallel Afferent Pathways Mediating the Sensation of Itch. *J Neurosci* 2007; **27**:7490–7.
- 58 Reddy VB, Iuga AO, Shimada SG, *et al.* Cowhage-Evoked Itch Is Mediated by a Novel Cysteine Protease: A Ligand of Protease-Activated Receptors. *J Neurosci* 2008; **28**:4331–5.
- 59 Hägermark O. Influence of antihistamines, sedatives, and aspirin on experimental itch. *Acta Derm Venereol* 1973; **53**:363–8.
- 60 Ständer S, Schmelz M. Chronic itch and pain--similarities and differences. *Eur J Pain* 2006; **10**:473–8.
- 61 Ross SE. Pain and itch: insights into the neural circuits of aversive somatosensation in health and disease. *Curr Opin Neurobiol* 2011; **21**:880–7.
- 62 LaMotte RH, Shimada SG, Green BG, Zeltman D. Pruritic and Nociceptive Sensations and Dysesthesias From a Spicule of Cowhage. *J Neurophysiol* 2009; **101**:1430–43.
- 63 Von Frey M. Zur physiologie der juckempfindung. *Arch Neerl Physiol* 1922; **7**:142–5.
- 64 Rothman S. Physiology of itching. *Physiol Rev* 1941; **21**:357–81.
- 65 Von Frey M. Untersuchungen über die sinnesfunctionen der menschlichen haut: 1. abhandlung: Druckempfindung und schmerz. Michigan, S. Hirzel, 1896.
- 66 Dallenbach KM. Pain: History and Present Status. *Am J Psychol* 1939; **52**:331.
- 67 Sinclair DC. Cutaneous sensation and the doctrine of specific nerve energies. *Brain* 1955; **78**:584–614.
- 68 Dale HH, Laidlaw PP. The physiological action of beta-aminazolyethylamine. *J Physiol* 1910; **41**:318–44.
- 69 Lewis T. The blood vessels of the human skin. *Br Med J* 1926; **2**:61–2.
- 70 Bickford RGL. Experiments relating to the itch sensation, its peripheral mechanism, and central pathways. *Clin Sci* 1938; **3**:377–86.
- 71 LaMotte RH. Psychophysical and neurophysiological studies of chemically induced cutaneous pain and itch. In: *Progress in Brain Research*. , 1988; 331–5.
- 72 Lamotte RH. Subpopulations of ‘Nocifensor Neurons’ Contributing to Pain and Allodynia, Itch and Alloknesis. *Am Pain Soc J* 1992; **1**:115–26.
- 73 Simone D a, Alreja M, LaMotte RH. Psychophysical studies of the itch sensation and itchy skin (‘alloknesis’) produced by intracutaneous injection of histamine. *Somatosens Mot Res* 1991; **8**:271–9.
- 74 Cormia FE. Experimental Histamine Pruritus - I: Influence of Physical and Psychological Factors on Threshold Reactivity. *J Invest Dermatol* 1952; **19**:21–34.
- 75 Cormia FE, Kuykendall V. Experimental Histamine Pruritus - II: Nature; Physical and Environmental Factors Influencing Development and Severity. *J Invest Dermatol* 1953; **20**:429–46.
- 76 Keele CA, Armstrong D. Substances Producing Pain and Itch. In: *Monographs of the Physiological Society*, 1st ed. London, Edward Arnold, 1964.
- 77 Armstrong D, Dry RML, Keele CA, Markham JW. Observations on chemical excitants of cutaneous pain in man. *J Physiol* 1953; **120**:326–51.
- 78 Bautista DM, Wilson SR, Hoon M a. Why we scratch an itch: the molecules, cells and circuits of itch. *Nat Neurosci* 2014; **17**:175–82.

- 79 LaMotte RH, Dong X, Ringkamp M. Sensory neurons and circuits mediating itch. *Nat Rev Neurosci* 2014; **15**:19–31.
- 80 Wall PD, Cronly-Dillon JR. Pain, itch, and vibration. *Arch Neurol* 1960; **2**:365–75.
- 81 Davidson S, Zhang X, Khasabov SG, *et al.* Pruriceptive spinothalamic tract neurons: physiological properties and projection targets in the primate. *J Neurophysiol* 2012; **108**:1711–23.
- 82 Ikoma A, Handwerker H, Miyachi Y, Schmelz M. Electrically evoked itch in humans. *Pain* 2005; **113**:148–54.
- 83 Michaelis M, Häbler HJ, Jänig W, *et al.* Silent afferents: A separate class of primary afferents? *Clin Exp Pharmacol Physiol* 1996; **23**:99–105.
- 84 Meyer R a, Davis KD, Cohen RH, *et al.* Mechanically insensitive afferents (MIAs) in cutaneous nerves of monkey. *Brain Res* 1991; **561**:252–61.
- 85 Davis KD, Meyer R a, Campbell JN. Chemosensitivity and sensitization of nociceptive afferents that innervate the hairy skin of monkey. *J Neurophysiol* 1993; **69**:1071–81.
- 86 Weidner C, Schmelz M, Schmidt R, *et al.* Functional attributes discriminating mechano-insensitive and mechano-responsive C nociceptors in human skin. *J Neurosci* 1999; **19**:10184–90.
- 87 Georgopoulos a P. Functional properties of primary afferent units probably related to pain mechanisms in primate glabrous skin. *J Neurophysiol* 1976; **39**:71–83.
- 88 Schmidt R, Schmelz M, Forster C, *et al.* Novel classes of responsive and unresponsive C nociceptors in human skin. *J Neurosci* 1995; **15**:333–41.
- 89 Schmidt R, Schmelz M, Ringkamp M, *et al.* Innervation territories of mechanically activated C nociceptor units in human skin. *J Neurophysiol* 1997; **78**:2641–8.
- 90 Schmelz M, Michael K, Weidner C, *et al.* Which nerve fibers mediate the axon reflex flare in human skin? *Neuroreport* 2000; **11**:645–8.
- 91 Andrew D, Craig a D. Spinothalamic lamina I neurons selectively sensitive to histamine: a central neural pathway for itch. *Nat Neurosci* 2001; **4**:72–7.
- 92 Schmelz M, Schmidt R, Bickel a, *et al.* Specific C-receptors for itch in human skin. *J Neurosci* 1997; **17**:8003–8.
- 93 Schmelz M. Chemical Response Pattern of Different Classes of C-Nociceptors to Pruritogens and Algogens. *J Neurophysiol* 2003; **89**:2441–8.
- 94 Simone DA. Comparison of Responses of Primate Spinothalamic Tract Neurons to Pruritic and Algogenic Stimuli. *J Neurophysiol* 2003; **91**:213–22.
- 95 Handwerker HO. Microneurography of pruritus. *Neurosci Lett* 2010; **470**:193–6.
- 96 Handwerker HO, Schmelz M. Pain: itch without pain-a labeled line for itch sensation? *Nat Rev Neurol* 2009; **5**:640–1.
- 97 McMahon SB, Koltzenburg M. Itching for an explanation. *Trends Neurosci* 1992; **15**:497–501.
- 98 Twycross R. Itch: scratching more than the surface. *QJM* 2003; **96**:7–26.
- 99 Berth-Jones J, Graham-Brown RA. Failure of terfenadine in relieving the pruritus of atopic dermatitis. *Br J Dermatol* 1989; **121**:635–7.
- 100 Shelley WB, Arthur R. Studies on cowhage (*Mucuna pruriens*) and its pruritogenic proteinase, mucunain. *AMA Arch Derm* 1955; **72**:399–406.
- 101 Shelley WB, Arthur RP. Mucunain, the active pruritogenic proteinase of cowhage. *Science* 1955; **122**:469–70.
- 102 Shelley WB, Arthur R. The neurohistology and neurophysiology of the itch sensation in man. *AMA Arch Derm* 1957; **76**:296–323.
- 103 Johaneck LM, Meyer RA, Friedman RM, *et al.* A Role for Polymodal C-Fiber Afferents in Nonhistaminergic Itch. *J Neurosci* 2008; **28**:7659–69.
- 104 Davidson S, Zhang X, Yoon CH, *et al.* The Itch-Producing Agents Histamine and Cowhage Activate Separate Populations of Primate Spinothalamic Tract Neurons. *J Neurosci* 2007; **27**:10007–14.
- 105 Hoeck EA, Marker JB, Gazerani P, *et al.* Preclinical and human surrogate models of itch. *Exp Dermatol* 2016; **25**:750–7.

## LITERATURE LIST

- 106 Andersen HH, Elberling J, Arendt-Nielsen L. Human Surrogate Models of Histaminergic and Non-histaminergic Itch. *Acta Derm Venereol* 2015; **95**:771–7.
- 107 Ma C, Nie H, Gu Q, *et al.* In vivo responses of cutaneous C-mechanosensitive neurons in mouse to punctate chemical stimuli that elicit itch and nociceptive sensations in humans. *J Neurophysiol* 2012; **107**:357–63.
- 108 Sun S, Xu Q, Guo C, *et al.* Leaky Gate Model: Intensity-Dependent Coding of Pain and Itch in the Spinal Cord. *Neuron* 2017; **93**:840–53.
- 109 Pereira PJS, Lerner EA. Gate Control Theory Springs a Leak. *Neuron* 2017; **93**:723–4.
- 110 Namer B, Reeh P. Scratching an itch. *Nat Neurosci* 2013; **16**:117–8.
- 111 Schmelz M. Itch and pain. *Neurosci Biobehav Rev* 2010; **34**:171–6.
- 112 Greaves MW, Wall PD. Pathophysiology of itching. *Lancet* 1996; **348**:938–40.
- 113 Han L, Ma C, Liu Q, *et al.* A subpopulation of nociceptors specifically linked to itch. *Nat Neurosci* 2013; **16**:174–82.
- 114 Than JY-XLXL, Li L, Hasan R, Zhang X. Excitation and Modulation of TRPA1, TRPV1, and TRPM8 Channel-expressing Sensory Neurons by the Pruritogen Chloroquine. *J Biol Chem* 2013; **288**:12818–27.
- 115 Kremer A, Stengel M, Reeh P, *et al.* LPA induces itch and pain in humans depending on the mode of application. *Acta Dermato-Venereologica, Suppl* 2015; **95**:892.
- 116 Hoeck E, Marker J, Andersen H, *et al.* Positive correlations between itch- and pain-evoked dysesthesiae: an observational study implying similar mechanisms. *World Congr Pain* 2016; :PW0406.
- 117 Schmelz M. Neurophysiology and Itch Pathways. In: *Pharmacology of Itch* (Cowan A, Yosipovitch G, eds), 1st ed. Berlin, Heidelberg, Springer Berlin Heidelberg, 2015; 39–56.
- 118 Schmelz M. Itch and pain differences and commonalities. *Handb Exp Pharmacol* 2015; **227**:286–301.
- 119 Ross SE, Mardinly AR, McCord AE, *et al.* Loss of Inhibitory Interneurons in the Dorsal Spinal Cord and Elevated Itch in Bhlhb5 Mutant Mice. *Neuron* 2010; **65**:886–98.
- 120 Bourane S, Duan B, Koch SC, *et al.* Gate control of mechanical itch by a subpopulation of spinal cord interneurons. *Science* 2015; **350**:550–4.
- 121 Latremoliere A, Woolf CJ. Central Sensitization: A Generator of Pain Hypersensitivity by Central Neural Plasticity. *J Pain* 2009; **10**:895–926.
- 122 Maier C, Baron R, Tölle TR, *et al.* Quantitative sensory testing in the German Research Network on Neuropathic Pain (DFNS): somatosensory abnormalities in 1236 patients with different neuropathic pain syndromes. *Pain* 2010; **150**:439–50.
- 123 Sandkühler J. Models and mechanisms of hyperalgesia and allodynia. *Physiol Rev* 2009; **89**:707–58.
- 124 Liang YF, Haake B, Reeh PW. Sustained sensitization and recruitment of rat cutaneous nociceptors by bradykinin and a novel theory of its excitatory action. *J Physiol* 2001; **532**:229–39.
- 125 Jiang Y-M, Huang C, Peng Z, *et al.* Acidosis counteracts itch tachyphylaxis to consecutive pruritogen exposure dependent on acid-sensing ion channel 3. *Mol Pain* 2017; **13**:174480691772111.
- 126 Wahlgren CF, Hagermark O, Bergstrom R. Patients' perception of itch induced by histamine, compound 48/80 and wool fibres in atopic dermatitis. *Acta Derm Venereol* 1990; **71**:488–94.
- 127 Pongcharoen P, Fleischer ABB. An evidence-based review of systemic treatments for itch. *Eur J Pain* 2016; **20**:24–31.
- 128 Steinhoff M, Neisius U, Ikoma A, *et al.* Proteinase-activated receptor-2 mediates itch: a novel pathway for pruritus in human skin. *J Neurosci* 2003; **23**:6176–80.
- 129 Ikoma A, Rukwied R, Ständer S, *et al.* Neuronal sensitization for histamine-induced itch in lesional skin of patients with atopic dermatitis. *Arch Dermatol* 2003; **139**:1455–8.
- 130 Rukwied RR, Main M, Weinkauff B, Schmelz M. NGF sensitizes nociceptors for cowhage- but not histamine-induced itch in human skin. *J Invest Dermatol* 2013; **133**:268–70.
- 131 Ringkamp M, Raja SN, Campbell JN, Meyer RA. Peripheral Mechanisms of Cutaneous Nociception. In: *Textbook of Pain* (McMahon S, Koltzenburg M, Tracey I, Turk DC, eds), 5th ed. , Elsevier, 2015;

- 1–30.
- 132 Gold MS, Gebhart GF. Nociceptor sensitization in pain pathogenesis. *Nat Med* 2010; **16**:1248–57.
- 133 Gangadharan V, Kuner R. Pain hypersensitivity mechanisms at a glance. *Dis Model Mech* 2013; **6**:889–95.
- 134 Basbaum AI, Bautista DM, Scherrer G, Julius D. Cellular and molecular mechanisms of pain. *Cell* 2009; **139**:267–84.
- 135 Mollanazar NK, Smith PK, Yosipovitch G. Mediators of Chronic Pruritus in Atopic Dermatitis: Getting the Itch Out? *Clin Rev Allergy Immunol* 2016; **51**:263–92.
- 136 Sonkoly E, Muller A, Lauerma AI, *et al.* IL-31: a new link between T cells and pruritus in atopic skin inflammation. *J Allergy Clin Immunol* 2006; **117**:411–7.
- 137 Jahnz-Rozyk K, Targowski T, Paluchowska E, *et al.* Serum thymus and activation-regulated chemokine, macrophage-derived chemokine and eotaxin as markers of severity of atopic dermatitis. *Allergy Eur J Allergy Clin Immunol* 2005; **60**:685–8.
- 138 Oh M-HM-H, Oh SY, Lu J, *et al.* TRPA1-dependent pruritus in IL-13-induced chronic atopic dermatitis. *J Immunol* 2013; **191**:5371–82.
- 139 Julius D, Basbaum AI. Molecular mechanisms of nociception. *Nature* 2001; **413**:203–10.
- 140 Scholz J, Woolf CJ. The neuropathic pain triad: neurons, immune cells and glia. *Nat Neurosci* 2007; **10**:1361–8.
- 141 Ji R-R, Kohno T, Moore K a, Woolf CJ. Central sensitization and LTP: do pain and memory share similar mechanisms? *Trends Neurosci* 2003; **26**:696–705.
- 142 Zhang Y, Dun SL, Chen Y-H, *et al.* Scratching activates microglia in the mouse spinal cord. *J Neurosci Res* 2015; **93**:466–74.
- 143 Shiratori-Hayashi M, Koga K, Tozaki-Saitoh H, *et al.* STAT3-dependent reactive astrogliosis in the spinal dorsal horn underlies chronic itch. *Nat Med* 2015; **21**:1–8.
- 144 May A. Chronic pain may change the structure of the brain. *Pain* 2008; **137**:7–15.
- 145 Wager TD, Atlas LY, Lindquist M a, *et al.* An fMRI-based neurologic signature of physical pain. *N Engl J Med* 2013; **368**:1388–97.
- 146 Napadow V, LaCount L, Park K, *et al.* Intrinsic brain connectivity in fibromyalgia is associated with chronic pain intensity. *Arthritis Rheum* 2010; **62**:2545–55.
- 147 Giesecke T, Gracely RH, Grant MAB, *et al.* Evidence of Augmented Central Pain Processing in Idiopathic Chronic Low Back Pain. *Arthritis Rheum* 2004; **50**:613–23.
- 148 Ishiiji Y, Coghill RC, Patel TS, *et al.* Distinct patterns of brain activity evoked by histamine-induced itch reveal an association with itch intensity and disease severity in atopic dermatitis. *Br J Dermatol* 2009; **161**:1072–80.
- 149 Papoiu ADP, Emerson NM, Patel TS, *et al.* Voxel-based morphometry and arterial spin labeling fMRI reveal neuropathic and neuroplastic features of brain processing of itch in end-stage renal disease. *J Neurophysiol* 2014; **112**:1729–38.
- 150 Ikoma A. Updated neurophysiology of itch. *Biol Pharm Bull* 2013; **36**:1235–40.
- 151 Rolke R, Baron R, Maier C, *et al.* Quantitative sensory testing in the German Research Network on Neuropathic Pain (DFNS): Standardized protocol and reference values. *Pain* 2006; **123**:231–43.
- 152 Vallbo AB, Hagbarth K-EK-E, Wallin BG. Microneurography: how the technique developed and its role in the investigation of the sympathetic nervous system. *J Appl Physiol* 2004; **96**:1262–9.
- 153 Atanassoff PG, Brull SJ, Zhang J, *et al.* Enhancement of experimental pruritus and mechanically evoked dysesthesiae with local anesthesia. *Somatosens Mot Res* 1999; **16**:291–8.
- 154 Pritchard EB. The Clinical Significance of Variations in Tickle Sensibility. *Proc R Soc Med* 1933; **26**:697–704.
- 155 Graham DT, Goodell H, Wolff HG. Neural mechanisms involved in itch, itchy skin, and tickle sensations. *J Clin Invest* 1951; **30**:37–49.
- 156 Lewis T. Experiments relating to cutaneous hyperalgesia and its spread through somatic nerves. *Clin Sci* 1935; **2**:373–423.

## LITERATURE LIST

- 157 Brull SJ, Atanassoff PG, Silverman DG, *et al.* Attenuation of experimental pruritus and mechanically evoked dysesthesiae in an area of cutaneous allodynia. *Somatosens Mot Res* 1999; **16**:299–303.
- 158 IASP Taxonomy - Pain Terms [WWW Document]. Part III Pain Terms, A Curr List with Defin Notes Usage. 2011.URL <https://www.iasp-pain.org/Taxonomy> [accessed on 25 September 2017].
- 159 LaMotte RH. Encyclopedia of Pain - Allodynia and Alloknesis. Berlin, Heidelberg, Springer Berlin Heidelberg, 2013 doi:10.1007/978-3-642-28753-4.
- 160 Handwerker HO. Pain and allodynia, itch and alloknesis: An alternative hypothesis. *APS J* 1992; **1**:135–8.
- 161 Hosogi M, Schmelz M, Miyachi Y, Ikoma A. Bradykinin is a potent pruritogen in atopic dermatitis: a switch from pain to itch. *Pain* 2006; **126**:16–23.
- 162 Arendt-Nielsen L, Yarnitsky D. Experimental and clinical applications of quantitative sensory testing applied to skin, muscles and viscera. *J Pain* 2009; **10**:556–72.
- 163 Modir JG, Wallace MS. Human experimental pain models 3: heat/capsaicin sensitization and intradermal capsaicin models. *Methods Mol Biol* 2010; **617**:169–74.
- 164 Klein T, Magerl W, Rolke R, Treede R-D. Human surrogate models of neuropathic pain. *Pain* 2005; **115**:227–33.
- 165 Olesen AE, Andresen T, Staahl C, Drewes AM. Human experimental pain models for assessing the therapeutic efficacy of analgesic drugs. *Pharmacol Rev* 2012; **64**:722–79.
- 166 Magerl W, Treede RD. Secondary tactile hypoesthesia: A novel type of pain-induced somatosensory plasticity in human subjects. *Neurosci Lett* 2004; **361**:136–9.
- 167 Andersen HH, Olsen R V, Møller HG, *et al.* A review of topical high-concentration L-menthol as a translational model of cold allodynia and hyperalgesia. *Eur J Pain* 2014; **18**:315–25.
- 168 Simone D a., Baumann TK, LaMotte RH. Dose-dependent pain and mechanical hyperalgesia in humans after intradermal injection of capsaicin. *Pain* 1989; **38**:99–107.
- 169 Altis K, Schmidt A, Angioni C, *et al.* Analgesic efficacy of tramadol, pregabalin and ibuprofen in menthol-evoked cold hyperalgesia. *Pain* 2009; **147**:116–21.
- 170 Wasner G, Schattschneider J, Binder A, Baron R. Topical menthol—a human model for cold pain by activation and sensitization of C nociceptors. *Brain* 2004; **127**:1159–71.
- 171 Nassini R, Gees M, Harrison S, *et al.* Oxaliplatin elicits mechanical and cold allodynia in rodents via TRPA1 receptor stimulation. *Pain* 2011; **152**:1621–31.
- 172 Petersen KL, Fields HL, Brennum J, *et al.* Capsaicin evoked pain and allodynia in post-herpetic neuralgia. *Pain* 2000; **88**:125–33.
- 173 Andersen HHH, Poulsen JN, Uchida Y, *et al.* Cold and L-menthol-induced sensitization in healthy volunteers—a cold hypersensitivity analogue to the heat/capsaicin model. *Pain* 2015; **156**:880–9.
- 174 McGonigle P, Ruggeri B. Animal models of human disease: challenges in enabling translation. *Biochem Pharmacol* 2014; **87**:162–71.
- 175 Thomsen JS, Sonne M, Benfeldt E, *et al.* Experimental itch in sodium lauryl sulphate-inflamed and normal skin in humans: a randomized, double-blind, placebo-controlled study of histamine and other inducers of itch. *Br J Dermatol* 2002; **146**:792–800.
- 176 Pall PS, Hurwitz OE, King BA, LaMotte RH. Psychophysical measurements of itch and nociceptive sensations in an experimental model of allergic contact dermatitis. *J Pain* 2015; **16**:741–9.
- 177 Papoiu ADP, Coghill RC, Kraft RA, *et al.* A tale of two itches. Common features and notable differences in brain activation evoked by cowhage and histamine induced itch. *Neuroimage* 2012; **59**:3611–23.
- 178 Hartmann EM, Handwerker HO, Forster C. Gender differences in itch and pain-related sensations provoked by histamine, cowhage and capsaicin. *Acta Derm Venereol* 2015; **95**:25–30.
- 179 Kosteletzky F, Namer B, Forster C, Handwerker H. Impact of Scratching on Itch and Sympathetic Reflexes Induced by Cowhage (*Mucuna pruriens*) and Histamine. *Acta Derm Venereol* 2009; **89**:271–7.
- 180 Gibson R a., Robertson J, Mistry H, *et al.* A Randomised Trial Evaluating the Effects of the TRPV1 Antagonist SB705498 on Pruritus Induced by Histamine, and Cowhage Challenge in Healthy

- Volunteers. *PLoS One* 2014; **9**:e100610.
- 181 Papoiu A, Valdes-Rodriguez R, Nattkemper L, *et al.* A Novel Topical Formulation Containing Strontium Chloride Significantly Reduces the Intensity and Duration of Cowhage-Induced Itch. *Acta Derm Venereol* 2013; **93**:520–6.
- 182 Hawro T, Lehmann S, Altrichter S, *et al.* Skin provocation tests may help to diagnose atopic dermatitis. *Allergy Eur J Allergy Clin Immunol* 2016; **71**:1745–52.
- 183 Schmelz M, Hilliges M, Schmidt R, *et al.* Active ‘itch fibers’ in chronic pruritus. *Neurology* 2003; **61**:564–6.
- 184 Darsow U, Ring J, Scharein E, Bromm B. Correlations between histamine-induced wheal, flare and itch. *Arch Dermatol Res* 1996; **288**:436–41.
- 185 Rothwell PM. External validity of randomised controlled trials: ‘To whom do the results of this trial apply?’ *Lancet* 2005; **365**:82–93.
- 186 Heyer G, Koppert W, Martus P, Handwerker HO. Histamine and cutaneous nociception: histamine-induced responses in patients with atopic eczema, psoriasis and urticaria. *Acta Derm Venereol* 1998; **78**:123–6.
- 187 Hawro T, Fluhr JW, Mengeaud V, *et al.* Polidocanol inhibits cowhage - but not histamine-induced itch in humans. *Exp Dermatol* 2014; **23**:922–3.
- 188 Tharp MD. Chronic urticaria: pathophysiology and treatment approaches. *J Allergy Clin Immunol* 1996; **98**:325–30.
- 189 Leonardi A. Role of histamine in allergic conjunctivitis. *Acta Ophthalmol Scand Suppl* 2000; **78**:18–21.
- 190 Fostini AC, Girolomoni G. Experimental elicitation of itch: Evoking and evaluation techniques. *J Dermatol Sci* 2015; **80**:13–7.
- 191 Ohnhaus EE, Adler R. Methodological problems in the measurement of pain: A comparison between the verbal rating scale and the visual analogue scale. *Pain* 1975; **1**:379–84.
- 192 Hawker GA, Mian S, Kendzerska T, French M. Measures of adult pain: Visual Analog Scale for Pain (VAS Pain), Numeric Rating Scale for Pain (NRS Pain), McGill Pain Questionnaire (MPQ), Short-Form McGill Pain Questionnaire (SF-MPQ), Chronic Pain Grade Scale (CPGS), Short Form-36 Bodily Pain Scale. *Arthritis Care Res* 2011; **63**:240–52.
- 193 Kido-nakahara M, Katoh N, Saeki H, *et al.* Comparative Cut-off Value Setting of Pruritus Intensity in Visual Analogue Scale and Verbal Rating Scale. 2015; :345–6.
- 194 Jones O, Schindler I, Holle H. Assessing acute itch intensity: General labelled magnitude scale is more reliable than classic visual analogue scale. *Acta Derm Venereol* 2017; **97**:375–6.
- 195 Reich A, Heisig M, Phan NQ, *et al.* Visual analogue scale: evaluation of the instrument for the assessment of pruritus. *Acta Derm Venereol* 2012; **92**:497–501.
- 196 Phan NQ, Blome C, Fritz F, *et al.* Assessment of pruritus intensity: Prospective study on validity and reliability of the visual analogue scale, numerical rating scale and verbal rating scale in 471 patients with chronic pruritus. *Acta Derm Venereol* 2012; **92**:502–7.
- 197 Hayes JE, Allen AL, Bennett SM. Direct comparison of the generalized Visual Analog Scale (gVAS) and general Labeled Magnitude Scale (gLMS). *Food Qual Prefer* 2013; **28**:36–44.
- 198 Sikand P, Shimada SG, Green BG, LaMotte RH. Sensory responses to injection and punctate application of capsaicin and histamine to the skin. *Pain* 2011; **152**:2485–94.
- 199 Mochizuki H, Inui K, Yamashiro K, *et al.* Itching-related somatosensory evoked potentials. *Pain* 2008; **138**:598–603.
- 200 Crook J, Rideout E, Browne G. The prevalence of pain in a general population. *Pain* 1984; **18**:299–314.
- 201 Von Korff M, Dworkin SF, Le Resche L, Kruger A. An epidemiologic comparison of pain complaints. *Pain* 1988; **32**:173–83.
- 202 Bassols A, Bosch F, Campillo M, *et al.* An epidemiological comparison of pain complaints in the general population of Catalonia (Spain). *Pain* 1999; **83**:9–16.
- 203 Price DD, McGrath PA, Rafii A, Buckingham B. The validation of visual analogue scales as ratio



- scale measures for chronic and experimental pain. *Pain* 1983; **17**:45–56.
- 204 Carlsson AM. Assessment of chronic pain. I. Aspects of the reliability and validity of the visual analogue scale. *Pain* 1983; **16**:87–101.
- 205 Price DD, Bush FM, Long S, Harkins SW. A comparison of pain measurement characteristics of mechanical visual analogue and simple numerical rating scales. *Pain* 1994; **56**:217–26.
- 206 Andersen HH, van Laarhoven AIM, Elberling J, Arendt-Nielsen L. Modulation of Itch by Conditioning Itch and Pain Stimulation in Healthy Humans. *J Pain* 2017. doi:10.1016/j.jpain.2017.07.002.
- 207 Andersen HH, Sørensen A-KR, Nielsen GAR, *et al.* A Test-Retest Reliability Study of Human Experimental Models of Histaminergic and Non-histaminergic Itch. *Acta Derm Venereol* 2017; **97**:198–207.
- 208 Melzack R. The McGill Pain Questionnaire: Major properties and scoring methods. *Pain* 1975; **1**:277–99.
- 209 Melzack R. The short-form McGill pain questionnaire. *Pain* 1987; **30**:191–7.
- 210 Byrne M, Troy A, Bradley L a, *et al.* Cross-validation of the factor structure of the McGill Pain Questionnaire. *Pain* 1982; **13**:193–201.
- 211 Dworkin RH, Turk DC, Trudeau JJ, *et al.* Validation of the short-form McGill pain questionnaire-2 (SF-MPQ-2) in acute low back pain. *J Pain* 2015; **16**:357–66.
- 212 Yosipovitch G, Zucker I, Boner G, *et al.* A questionnaire for the assessment of pruritus: validation in uremic patients. *Acta Derm Venereol* 2001; **81**:108–11.
- 213 Baron R, Schwarz K, Kleinert A, *et al.* Histamine-induced itch converts into pain in neuropathic hyperalgesia. *Neuroreport* 2001; **12**:3475–8.
- 214 Birklein F, Claus D, Riedl B, *et al.* Effects of cutaneous histamine application in patients with sympathetic reflex dystrophy. *Muscle and Nerve* 1997; **20**:1389–95.
- 215 Fukuoka M, Miyachi Y, Ikoma A. Mechanically evoked itch in humans. *Pain* 2013; **154**:897–904.
- 216 Andersen HH, Marker JB, Hoeck EA, *et al.* Antipruritic effect of pretreatment with topical capsaicin 8% on histamine- and cowhage-evoked itch in healthy volunteers: a randomized, vehicle-controlled, proof-of-concept trial. *Br J Dermatol* 2017; **177**:107–16.
- 217 Meyer R a, Campbell JN. Myelinated nociceptive afferents account for the hyperalgesia that follows a burn to the hand. *Science* 1981; **213**:1527–9.
- 218 van Laarhoven AIM, Kraaimaat FW, Wilder-Smith OH, *et al.* Generalized and symptom-specific sensitization of chronic itch and pain. *J Eur Acad Dermatology Venereol* 2007; **21**:1187–92.
- 219 Akiyama T, Carstens MI, Ikoma A, *et al.* Mouse model of touch-evoked itch (alloknesis). *J Invest Dermatol* 2012; **132**:1886–91.
- 220 Andersen HH, Elberling J, Lo Vecchio S, Arendt-Nielsen L. Topography of itch: evidence of distinct coding for pruriception in the trigeminal nerve. *Itch* 2016; **1**:1–10.
- 221 Weisshaar E, Heyer G, Forster C, Handwerker HO. Effect of topical capsaicin on the cutaneous reactions and itching to histamine in atopic eczema compared to healthy skin. *Arch Dermatol Res* 1998; **290**:306–11.
- 222 Robert H. L. Secondary cutaneous dysaesthesiae. In: *Neurobiology of Nociceptors* (Belmonte C, Cervero F, eds), 1st ed. , Oxford University Press, 1996; 390–417.
- 223 van Laarhoven AIM, Ulrich DJO, Wilder-Smith OH, *et al.* Psychophysiological Processing of Itch in Patients with Chronic Post-burn Itch: An Exploratory Study. *Acta Derm Venereol* 2016; **96**:613–8.
- 224 Fleiss JL. Chapter 1. Reliability of Measurement. In: *The Design and Analysis of Clinical Experiments*, 1st ed. Hoboken, NJ, USA, John Wiley & Sons, Inc., 1999.
- 225 Forster C, Greiner T, Nischik M, *et al.* Neurogenic flare responses are heterogeneous in superficial and deep layers of human skin. *Neurosci Lett* 1995; **185**:33–6.
- 226 Schmelz M, Petersen LJ. Neurogenic inflammation in human and rodent skin. *Physiology* 2001; **16**:33–7.
- 227 Heinzerling L, Mari A, Bergmann K-C, *et al.* The skin prick test - European standards. *Clin Transl Allergy* 2013; **3**:3.

- 228 Bjerring P, Arendt-Nielsen L. A quantitative comparison of the effect of local analgesics on argon laser induced cutaneous pain and on histamine induced wheal, flare and itch. *Acta Derm Venereol* 1990; **70**:126–31.
- 229 Schmelz M, Luz O, Averbek B, Bickel a. Plasma extravasation and neuropeptide release in human skin as measured by intradermal microdialysis. *Neurosci Lett* 1997; **230**:117–20.
- 230 Rukwied R, Lischetzki G, McGlone F, *et al*. Mast cell mediators other than histamine induce pruritus in atopic dermatitis patients: a dermal microdialysis study. *Br J Dermatol* 2000; **142**:1114–20.
- 231 Dreborg S. Allergen skin prick test should be adjusted by the histamine reactivity. *Int Arch Allergy Immunol* 2015; **166**:77–80.
- 232 Andersen HH, Lundgaard AC, Petersen AS, *et al*. The Lancet Weight Determines Wheal Diameter in Response to Skin Prick Testing with Histamine. *PLoS One* 2016; **11**:e0156211.
- 233 Dreborg ES, Frew A, Frew A. Position paper: Allergen standardization and skin tests. The European Academy of Allergology and Clinical Immunology. *Allergy* 1993; **48**:48–82.
- 234 Steinhoff M, Ständer S, Seeliger S, *et al*. Modern aspects of cutaneous neurogenic inflammation. *Arch Dermatol* 2003; **139**:1479–88.
- 235 Groetzner P, Weidner C. The human vasodilator axon reflex - An exclusively peripheral phenomenon? *Pain* 2010; **149**:71–5.
- 236 Olsen RV, Andersen HH, Möller HG, *et al*. Somatosensory and vasomotor manifestations of individual and combined stimulation of TRPM8 and TRPA1 using topical L-menthol and trans-cinnamaldehyde in healthy volunteers. *Eur J Pain* 2014; **18**:1333–42.
- 237 Birklein F, Schmelz M. Neuropeptides, neurogenic inflammation and complex regional pain syndrome (CRPS). *Neurosci Lett* 2008; **437**:199–202.
- 238 Gazerani P, Pedersen NS, Drewes AM, Arendt-Nielsen L. Botulinum toxin type A reduces histamine-induced itch and vasomotor responses in human skin. *Br J Dermatol* 2009; **161**:737–45.
- 239 Mørch CD, Gazerani P, Nielsen TA, Arendt-Nielsen L. The UVB cutaneous inflammatory pain model: A reproducibility study in healthy volunteers. *Int J Physiol Pathophysiol Pharmacol* 2013; **5**:203–15.
- 240 Boas DA, Dunn AK. Laser speckle contrast imaging in biomedical optics. *J Biomed Opt* 2010; **15**:011109–12.
- 241 Eriksson S, Nilsson J, Stureson C. Non-invasive imaging of microcirculation: a technology review. *Med Devices* 2014; :445–52.
- 242 Lindahl F, Tesselaar E, Sjöberg F. Assessing paediatric scald injuries using laser speckle contrast imaging. *Burns* 2013; **39**:662–6.
- 243 Video capability revives interest in laser method [WWW Document]. *Opt Laser Eur Mag*. URL <http://optics.org/article/27263>.
- 244 Stewart CJ, Frank R, Forrester KR, *et al*. A comparison of two laser-based methods for determination of burn scar perfusion: Laser Doppler versus laser speckle imaging. *Burns* 2005; **31**:744–52.
- 245 Nomura S, Inoue T, Ishihara H, *et al*. Reliability of laser speckle flow imaging for intraoperative monitoring of cerebral blood flow during cerebrovascular surgery: Comparison with cerebral blood flow measurement by single photon emission computed tomography. *World Neurosurg* 2014; **82**:E753–7.
- 246 Nilsson GE, Tenland T, Öberg PA. Evaluation of a laser Doppler flowmeter for measurement of tissue blood flow. *IEEE Trans Biomed Eng* 1980; **27**:597–604.
- 247 Papoiu ADP, Tey HL, Coghill RC, *et al*. Cowhage-induced itch as an experimental model for pruritus. A comparative study with histamine-induced itch. *PLoS One* 2011; **6**:e17786.
- 248 Handwerker HO, Forster C, Kirchhoff C. Discharge patterns of human C-fibers induced by itching and burning stimuli. *J Neurophysiol* 1991; **66**:307–15.
- 249 Schmelz M, Schmid R, Handwerker HO, Torebjörk HE. Encoding of burning pain from capsaicin-treated human skin in two categories of unmyelinated nerve fibres. *Brain* 2000; **123**:560–71.
- 250 Ali Z, Meyer RA, Campbell JN. Secondary hyperalgesia to mechanical but not heat stimuli following a capsaicin injection in hairy skin. *Pain* 1996; **68**:401–11.

- 251 Usoskin D, Furlan A, Islam S, *et al.* Unbiased classification of sensory neuron types by large-scale single-cell RNA sequencing. *Nat Neurosci* 2015; **18**:145–53.
- 252 Kobayashi K, Fukuoka T, Obata K, *et al.* Distinct expression of TRPM8, TRPA1, and TRPV1 mRNAs in rat primary afferent neurons with A $\delta$ /C-fibers and colocalization with Trk receptors. *J Comp Neurol* 2005; **493**:596–606.
- 253 Gazerani P, Andersen OK, Arendt-Nielsen L. A human experimental capsaicin model for trigeminal sensitization. Gender-specific differences. *Pain* 2005; **118**:155–63.
- 254 Weber E. *De subtilitate tactus.*, 2nd ed. London, Academic Press, UK, 1834.
- 255 Mancini F, Bauleo A, Cole J, *et al.* Whole-body mapping of spatial acuity for pain and touch. *Ann Neurol* 2014; **75**:917–24.
- 256 Schlereth T, Magerl W, Treede RD. Spatial discrimination thresholds for pain and touch in human hairy skin. *Pain* 2001; **92**:187–94.
- 257 Shimada SG, LaMotte RH. Behavioral differentiation between itch and pain in mouse. *Pain* 2008; **139**:681–7.
- 258 Klein A, Carstens MI, Carstens E. Facial injections of pruritogens or algogens elicit distinct behavior responses in rats and excite overlapping populations of primary sensory and trigeminal subnucleus caudalis neurons. *J Neurophysiol* 2011; **106**:1078–88.
- 259 Vaidya DC, Schwartz RA. Prurigo nodularis: a benign dermatosis derived from a persistent pruritus. *Acta Dermatovenerol Croat* 2008; **16**:38–44.
- 260 Dieterich W, Laag E, Bruckner-Tuderman L, *et al.* Antibodies to tissue transglutaminase as serologic markers in patients with dermatitis herpetiformis. *J Invest Dermatol* 1999; **113**:133–6.
- 261 Sánchez-Borges M, Capriles-Hulett A, Caballero-Fonseca F. Demographic and clinical profiles in patients with acute urticaria. *Allergol Immunopathol* 2015; **43**:409–15.
- 262 Thyssen JP, Kezic S. Causes of epidermal filaggrin reduction and their role in the pathogenesis of atopic dermatitis. *J Allergy Clin Immunol* 2014; **134**:792–9.
- 263 Bos JD, De Rie MA. The pathogenesis of psoriasis: immunological facts and speculations. *Immunol Today* 1999; **20**:40–6.
- 264 Bos JD, de Rie MA, Teunissen MBM, Piskin G. Psoriasis: dysregulation of innate immunity. *Br J Dermatol* 2005; **152**:1098–107.
- 265 Fostini AC, Girolomoni G, Tessari G. Prurigo nodularis: an update on etiopathogenesis and therapy. *J Dermatolog Treat* 2013; **24**:458–62.
- 266 LaMotte RH, Shimada SG, Sikand P. Mouse models of acute, chemical itch and pain in humans. *Exp Dermatol* 2011; **20**:778–82.
- 267 Rasul A, Nordlind K, Wahlgren C-F. Pruritic and Vascular Responses Induced by Serotonin in Patients with Atopic Dermatitis and in Healthy Controls. *Acta Derm Venereol* 2013; **93**:277–80.
- 268 Caterina MJ, Rosen T a, Tominaga M, *et al.* A capsaicin-receptor homologue with a high threshold for noxious heat. *Nature* 1999; **398**:436–41.
- 269 Anand P, Bley K. Topical capsaicin for pain management: therapeutic potential and mechanisms of action of the new high-concentration capsaicin 8% patch. *Br J Anaesth* 2011; **107**:490–502.
- 270 Caterina MJ, Schumacher M a, Tominaga M, *et al.* The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature* 1997; **389**:816–24.
- 271 Davis JB, Gray J, Gunthorpe MJ, *et al.* Vanilloid receptor-1 is essential for inflammatory thermal hyperalgesia. *Nature* 2000; **405**:183–7.
- 272 Tominaga M, Caterina MJ, Malmberg AB, *et al.* The cloned capsaicin receptor integrates multiple pain-producing stimuli. *Neuron* 1998; **21**:531–43.
- 273 Baumann TK, Simone DA, Shain CN, LaMotte RH. Neurogenic hyperalgesia: the search for the primary cutaneous afferent fibers that contribute to capsaicin-induced pain and hyperalgesia. *J Neurophysiol* 1991; **66**:212–27.
- 274 Andersen HH, Elberling J, Sharma N, *et al.* Histaminergic and non-histaminergic elicited itch is attenuated in capsaicin-evoked areas of allodynia and hyperalgesia: A healthy volunteer study. *Eur J Pain* 2017; **21**:1098–109.

- 275 Simone DA, Sorkin LS, Oh U, *et al.* Neurogenic hyperalgesia: Central neural correlates in responses of spinothalamic tract neurons. *J Neurophysiol* 1991; **66**:228–46.
- 276 LaMotte RH, Tsai EFP, Shain CN, *et al.* Neurogenic hyperalgesia: psychophysical studies of underlying mechanisms. *J Neurophysiol* 1991; **66**:190–211.
- 277 Klede M. Central Origin of Secondary Mechanical Hyperalgesia. *J Neurophysiol* 2003; **90**:353–9.
- 278 Koltzenburg M, Lundberg LE, Torebjörk HE. Dynamic and static components of mechanical hyperalgesia in human hairy skin. *Pain* 1992; **51**:207–19.
- 279 Magerl W, Fuchs PN, Meyer RA, Treede R-D. Roles of capsaicin-insensitive nociceptors in cutaneous pain and secondary hyperalgesia. *Brain* 2001; **124**:1754–64.
- 280 Nolano M, Simone DA, Wendelschafer-Crabb G, *et al.* Topical capsaicin in humans: Parallel loss of epidermal nerve fibers and pain sensation. *Pain* 1999; **81**:135–45.
- 281 Malmberg AB, Mizisin AP, Calcutt NA, *et al.* Reduced heat sensitivity and epidermal nerve fiber immunostaining following single applications of a high-concentration capsaicin patch. *Pain* 2004; **111**:360–7.
- 282 Webster LR, Nunez M, Tark MD, *et al.* Tolerability of NGX-4010, a capsaicin 8% dermal patch, following pretreatment with lidocaine 2.5%/prilocaine 2.5% cream in patients with post-herpetic neuralgia. *BMC Anesthesiol* 2011; **11**:1–8.
- 283 Bjerring P, Arendt-Nielsen L. Inhibition of histamine skin flare reaction following repeated topical applications of capsaicin. *Allergy* 1990; **45**:121–5.
- 284 Simone DA, Ochoa J. Early and late effects of prolonged topical capsaicin on cutaneous sensibility and neurogenic vasodilatation in humans. *Pain* 1991; **47**:285–94.
- 285 Üçeyler N, Sommer C. High-Dose Capsaicin for the Treatment of Neuropathic Pain: What We Know and What We Need to Know. *Pain Ther* 2014; **3**:73–84.
- 286 Crimi N, Polosa R, Maccarrone C, *et al.* Effect of topical administration with capsaicin on skin responses to bradykinin and histamine in man. *Clin Exp Allergy* 1992; **22**:933–9.
- 287 O’Neill J, Brock C, Olesen AE, *et al.* Unravelling the mystery of capsaicin: a tool to understand and treat pain. *Pharmacol Rev* 2012; **64**:939–71.
- 288 Chung M-K, Güler AD, Caterina MJ. TRPV1 shows dynamic ionic selectivity during agonist stimulation. *Nat Neurosci* 2008; **11**:555–64.
- 289 Gallego-Sandín S, Rodríguez-García A, Alonso MT, García-Sancho J. The endoplasmic reticulum of dorsal root ganglion neurons contains functional TRPV1 channels. *J Biol Chem* 2009; **284**:32591–601.
- 290 Huang W, Wang H, Galligan JJ, Wang DH. Transient receptor potential vanilloid subtype 1 channel mediated neuropeptide secretion and depressor effects: role of endoplasmic reticulum associated Ca<sup>2+</sup> release receptors in rat dorsal root ganglion neurons. *J Hypertens* 2008; **26**:1966–75.
- 291 Chard PS, Bleakman D, Savidge JR, Miller RJ. Capsaicin-induced neurotoxicity in cultured dorsal root ganglion neurons: Involvement of calcium-activated proteases. *Neuroscience* 1995; **65**:1099–108.
- 292 Han P, McDonald HA, Bianchi BR, *et al.* Capsaicin causes protein synthesis inhibition and microtubule disassembly through TRPV1 activities both on the plasma membrane and intracellular membranes. *Biochem Pharmacol* 2007; **73**:1635–45.
- 293 Goswami C, Schmidt H, Hucho F. TRPV1 at nerve endings regulates growth cone morphology and movement through cytoskeleton reorganization. *FEBS J* 2007; **274**:760–72.
- 294 Shimomura Y, Kawada T, Suzuki M. Capsaicin and its analogs inhibit the activity of NADH-coenzyme Q oxidoreductase of the mitochondrial respiratory chain. *Arch Biochem Biophys* 1989; **270**:573–7.
- 295 Athanasiou A, Smith PA, Vakilpour S, *et al.* Vanilloid receptor agonists and antagonists are mitochondrial inhibitors: How vanilloids cause non-vanilloid receptor mediated cell death. *Biochem Biophys Res Commun* 2007; **354**:50–5.
- 296 Polydefkis M, Hauer P, Sheth S, *et al.* The time course of epidermal nerve fibre regeneration: Studies in normal controls and in people with diabetes, with and without neuropathy. *Brain* 2004; **127**:1606–15.

## LITERATURE LIST

- 297 Anand P, Bloom SR, McGregor GP. Topical capsaicin pretreatment inhibits axon reflex vasodilatation caused by somatostatin and vasoactive intestinal polypeptide in human skin. *Br J Pharmacol* 1983; **78**:665–9.
- 298 Bernstein JE, Swift RM, Soltani K, Lorincz a L. Inhibition of axon reflex vasodilatation by topically applied capsaicin. *J Invest Dermatol* 1981; **76**:394–5.
- 299 Hill R. NK1 (substance P) receptor antagonists - Why are they not analgesic in humans? *Trends Pharmacol Sci.* 2000; **21**:244–6.
- 300 Herbert MK, Holzer P. Why are substance P(NK1)-receptor antagonists ineffective in pain treatment? *Anaesthetist* 2002; **51**:308–19.
- 301 Weidner C, Klede M, Rukwied R, *et al.* Acute effects of substance P and calcitonin gene-related peptide in human skin--a microdialysis study. *J Invest Dermatol* 2000; **115**:1015–20.
- 302 Gooding SMD, Canter PH, Coelho HF, *et al.* Systematic review of topical capsaicin in the treatment of pruritus. *Int J Dermatol* 2010; **49**:858–65.
- 303 Dworkin RH, O'Connor AB, Backonja M, *et al.* Pharmacologic management of neuropathic pain: evidence-based recommendations. *Pain* 2007; **132**:237–51.
- 304 Finnerup NB, Attal N, Haroutounian S, *et al.* Pharmacotherapy for neuropathic pain in adults: a systematic review and meta-analysis. *Lancet Neurol* 2015; **14**:162–73.
- 305 Jensen TS, Høye K, Fricová J, *et al.* Tolerability of the capsaicin 8% patch following pretreatment with lidocaine or tramadol in patients with peripheral neuropathic pain: A multicentre, randomized, assessor-blinded study. *Eur J Pain* 2014; **18**:1240–7.
- 306 Mou J, Paillard F, Turnbull B, *et al.* Efficacy of Qutenza® (capsaicin) 8% patch for neuropathic pain: A meta-analysis of the qutenza clinical trials database. *Pain* 2013; **154**:1632–9.
- 307 Mainka T, Malewicz NM, Baron R, *et al.* Presence of hyperalgesia predicts analgesic efficacy of topically applied capsaicin 8% in patients with peripheral neuropathic pain. *Eur J Pain* 2016; **20**:116–29.
- 308 McCormack PL. Capsaicin dermal patch: in non-diabetic peripheral neuropathic pain. *Drugs* 2010; **70**:1831–42.
- 309 Andersen HH, Arendt-Nielsen L, Elberling J. Topical capsaicin 8% for the treatment of neuropathic itch conditions. *Clin Exp Dermatol* 2017; **42**:596–8.
- 310 Jones VM, Moore K a, Peterson DM. Capsaicin 8% topical patch (Qutenza)--a review of the evidence. *J Pain Palliat Care Pharmacother* 2011; **25**:32–41.
- 311 Landmann G, Lustenberger C, Schleinker W, *et al.* Short lasting transient effects of a capsaicin 8% patch on nociceptor activation in humans. *Eur J Pain* 2016; **20**:1443–53.
- 312 Kennedy WR, Vanhove GF, Lu S ping, *et al.* A Randomized, Controlled, Open-Label Study of the Long-Term Effects of NGX-4010, a High-Concentration Capsaicin Patch, on Epidermal Nerve Fiber Density and Sensory Function in Healthy Volunteers. *J Pain* 2010; **11**:579–87.
- 313 Henrich F, Magerl W, Klein T, *et al.* Capsaicin-sensitive C- and A-fibre nociceptors control long-term potentiation-like pain amplification in humans. *Brain* 2015; **138**:2505–20.
- 314 Colloca L, Ludman T, Bouhassira D, *et al.* Neuropathic pain. *Nat Rev Dis Prim* 2017; **3**:17002.
- 315 Misery L, Erfan N, Castela E, *et al.* Successful Treatment of Refractory Neuropathic Pruritus with Capsaicin 8% Patch: A Bicentric Retrospective Study with Long-term Follow-up. *Acta Derm Venereol* 2014; **95**:0.
- 316 Ständer S, Luger T, Metzke D. Treatment of prurigo nodularis with topical capsaicin. *J Am Acad Dermatol* 2001; **44**:471–8.
- 317 Lysy J, Sistiery-Ittah M, Israelit Y, *et al.* Topical capsaicin--a novel and effective treatment for idiopathic intractable pruritus ani: a randomised, placebo controlled, crossover study. *Gut* 2003; **52**:1323–6.
- 318 Wallengren J, Klinker M. Successful treatment of notalgia paresthetica with topical capsaicin: vehicle-controlled, double-blind, crossover study. *J Am Acad Dermatol* 1995; **32**:287–9.
- 319 Imai S. Effect of topically applied capsaicin on the cutaneous reaction to histamine. *Arch Dermatol Res* 1991; **283**:414–6.

- 320 Wallengren. Brachioradial pruritus: A recurrent solar dermatopathy. *J Am Acad Dermatol* 1999; **41**:657–8.
- 321 Davidson S, Giesler GJ. The multiple pathways for itch and their interactions with pain. *Trends Neurosci* 2010; **33**:550–8.
- 322 Bautista DM, Jordt SE, Nikai T, *et al*. TRPA1 Mediates the Inflammatory Actions of Environmental Irritants and Proalgesic Agents. *Cell* 2006; **124**:1269–82.
- 323 Everaerts W, Gees M, Alpizar YA, *et al*. The capsaicin receptor TRPV1 is a crucial mediator of the noxious effects of mustard oil. *Curr Biol* 2011; **21**:316–21.
- 324 Staruschenko A, Jeske NA, Akopian AN. Contribution of TRPV1-TRPA1 interaction to the single channel properties of the TRPA1 channel. *J Biol Chem* 2010; **285**:15167–77.
- 325 Jordt S-E, Bautista DM, Chuang H, *et al*. Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. *Nature* 2004; **427**:260–5.
- 326 Liu Q, Sikand P, Ma C, *et al*. Mechanisms of Itch Evoked by beta-Alanine. *J Neurosci* 2012; **32**:14532–7.
- 327 Wooten M, Weng H-J, Hartke T V, *et al*. Three functionally distinct classes of C-fibre nociceptors in primates. *Nat Commun* 2014; **5**:4122.
- 328 Qu L, Fan N, Ma C, *et al*. Enhanced excitability of MRGPRA3- and MRGPRD-positive nociceptors in a model of inflammatory itch and pain. *Brain* 2014; **137**:1039–50.
- 329 Ringkamp M, Schepers RJ, Shimada SG, *et al*. A Role for Nociceptive, Myelinated Nerve Fibers in Itch Sensation. *J Neurosci* 2011; **31**:14841–9.
- 330 Broadbent JL. Observations on itching produced by cowhage, and on the part played by histamine as a mediator of the itch sensation. *Br J Pharmacol Chemother* 1953; **8**:263–70.
- 331 Hanifin JM, Chan S. Biochemical and immunologic mechanisms in atopic dermatitis: new targets for emerging therapies. *J Am Acad Dermatol* 1999; **41**:72–7.
- 332 Mortz CG, Andersen KE. Allergic contact dermatitis in children and adolescents. *Contact Dermatitis* 1999; **41**:121–30.
- 333 Seiffert K, Hilbert E, Schaechinger H, *et al*. Psychophysiological reactivity under mental stress in atopic dermatitis. *Dermatology* 2005; **210**:286–93.
- 334 Zachariae R, Lei U, Haedersdal M, Zachariae C. Itch severity and quality of life in patients with pruritus: preliminary validity of a Danish adaptation of the itch severity scale. *Acta Derm Venereol* 2012; **92**:508–14.
- 335 Weisshaar E, Diepgen TL, Bruckner T, *et al*. Itch intensity evaluated in the German Atopic Dermatitis Intervention Study (GADIS): Correlations with quality of life, coping behaviour and SCORAD severity in 823 children. *Acta Derm Venereol* 2008; **88**:234–9.
- 336 Pfeifer-Ott B, Godfrey J. Patients report many different signs and symptoms as indicators of impending flare in atopic eczema. *Abstr 12th EADV Congr Barcelona, Spain, 15-18 October, 2003* 2003; **17**:165–426.
- 337 Rapaport MJ, Lebowitz M. Corticosteroid addiction and withdrawal in the atopic: the red burning skin syndrome. *Clin Dermatol* 2003; **21**:201–14.
- 338 Abraham A, Roga G. Topical steroid-damaged skin. *Indian J Dermatol* 2014; **59**:456.
- 339 Hanifin JM. Basic and clinical aspects of atopic dermatitis. *Ann Allergy* 1984; **52**:386–95.
- 340 Heyer G, Ulmer FJ, Schmitz J, Handwerker HO. Histamine-induced itch and allodynia (itchy skin) in atopic eczema patients and controls. *Acta Derm Venereol* 1995; **75**:348–52.
- 341 Pinnagoda J, Tupker RA, Smit JA, *et al*. The intra- and inter-individual variability and reliability of transepidermal water loss measurements. *Contact Dermatitis* 1989; **21**:255–9.
- 342 Angelova-Fischer I, Bauer A, Hipler UC, *et al*. The Objective Severity Assessment of Atopic Dermatitis (OSAAD) score: Validity, reliability and sensitivity in adult patients with atopic dermatitis. *Br J Dermatol* 2005; **153**:767–73.
- 343 Holm EA, Wulf HC, Thomassen L, Jemec GBE. Assessment of atopic eczema: clinical scoring and noninvasive measurements. *Br J Dermatol* 2007; **157**:674–80.
- 344 Andersen PH, Maibach HI. Skin irritation in man: a comparative bioengineering study using

- improved reflectance spectroscopy. *Contact Dermatitis* 1995; **33**:315–22.
- 345 Schmitt J, Langan S, Williams HC. What are the best outcome measurements for atopic eczema? A systematic review. *J Allergy Clin Immunol* 2007; **120**:1389–98.
- 346 Clarys P, Clijsen R, Taeymans J, Barel AO. Hydration measurements of the stratum corneum: Comparison between the capacitance method (digital version of the Corneometer CM 825®) and the impedance method (Skicon-200EX®). *Ski Res Technol* 2012; **18**:316–23.
- 347 Sugarman J, Fluhr J, Fowler A, *et al.* The Objective Severity Assessment of Atopic Dermatitis Score. *Arch Dermatol* 2003; **139**:1417–22.
- 348 Heinrich U, Koop U, Leneveu-Duchemin MC, *et al.* Multicentre comparison of skin hydration in terms of physical-, physiological- and product-dependent parameters by the capacitive method (Corneometer CM 825). *Int J Cosmet Sci* 2003; **25**:45–53.
- 349 Yosipovitch G, Papoiu ADP. What causes itch in atopic dermatitis? *Curr Allergy Asthma Rep* 2008; **8**:306–11.
- 350 Yarbrough KB, Neuhaus KJ, Simpson EL. The effects of treatment on itch in atopic dermatitis. *Dermatol Ther* 2013; **26**:110–9.
- 351 Patel T, Ishiujii Y, Yosipovitch G. Nocturnal itch: Why do we itch at night? *Acta Derm Venereol* 2007; **87**:295–8.
- 352 Wilson S, Bautista D. Itching for relief. *Nat Neurosci* 2013; **16**:775–7.
- 353 Jeffrey J, Kim S, Chen Z-F. Itch Signaling in the Nervous System. *Physiology* 2011; **26**:286–92.
- 354 Homey B, Steinhoff M, Ruzicka T, Leung DYM. Cytokines and chemokines orchestrate atopic skin inflammation. *J Allergy Clin Immunol* 2006; **118**:178–89.
- 355 Oetjen LK, Mack MR, Feng J, *et al.* Sensory Neurons Co-opt Classical Immune Signaling Pathways to Mediate Chronic Itch. *Cell* 2017; **129**:742–51.
- 356 FDA approves new eczema drug Dupixent [WWW Document]. FDA News Release. 2017.URL <https://www.fda.gov/newsevents/newsroom/pressannouncements/ucm549078> [accessed on 11 September 2017].
- 357 Beck L a, Thaci D, Hamilton JD, *et al.* Dupilumab treatment in adults with moderate-to-severe atopic dermatitis. *N Engl J Med* 2014; **371**:130–9.
- 358 Ruzicka T, Hanifin JM, Furue M, *et al.* Anti-Interleukin-31 Receptor A Antibody for Atopic Dermatitis. *N Engl J Med* 2017; **376**:826–35.
- 359 Leung B, Lowery D. Patent watch: The evolving intellectual property landscape for pruritus therapies. *Nat Rev Drug Discov* 2017. doi:10.1038/nrd.2017.161.
- 360 Hansson P, Backonja M, Bouhassira D. Usefulness and limitations of quantitative sensory testing: Clinical and research application in neuropathic pain states. *Pain* 2007; **129**:256–9.
- 361 Yarnitsky D, Granot M. Quantitative sensory testing. *Handb Clin Neurol* 2006; **81**:397–409.
- 362 Baron R, Maier C, Attal N, *et al.* Peripheral neuropathic pain: a mechanism-related organizing principle based on sensory profiles. 2017; **158**.
- 363 Baron R, Förster M, Binder A. Subgrouping of patients with neuropathic pain according to pain-related sensory abnormalities: A first step to a stratified treatment approach. *Lancet Neurol* 2012; **11**:999–1005.
- 364 Vaegter HB, Graven-Nielsen T. Pain modulatory phenotypes differentiate subgroups with different clinical and experimental pain sensitivity. *Pain* 2016; **157**:1480–8.
- 365 Graven-Nielsen T, Vaegter HB, Finocchietti S, *et al.* Assessment of musculoskeletal pain sensitivity and temporal summation by cuff pressure algometry. *Pain* 2015; **156**:2193–202.
- 366 Petersen KK, Arendt-Nielsen L, Simonsen O, *et al.* Presurgical assessment of temporal summation of pain predicts the development of chronic postoperative pain 12 months after total knee replacement. *Pain* 2015; **156**:55–61.
- 367 Galor A, Levitt RC, Felix ER, *et al.* Neuropathic ocular pain: an important yet undervalued feature of dry eye. *Eye (Lond)* 2015; **29**:301–12.
- 368 Andersen HH, Yosipovitch G, Galor A. Neuropathic symptoms of the ocular surface: Dryness, pain, and itch. *Curr Opin Allergy Clin Immunol* 2017; **17**. doi:10.1097/ACI.0000000000000389.

- 369 Lipton RB, Bigal ME, Ashina S, *et al.* Cutaneous allodynia in the migraine population. *Ann Neurol* 2008; **63**:148–58.
- 370 King CD, Wong F, Currie T, *et al.* Deficiency in endogenous modulation of prolonged heat pain in patients with Irritable Bowel Syndrome and Temporomandibular Disorder. *Pain* 2009; **143**:172–8.
- 371 Skou ST, Graven-Nielsen T, Rasmussen S, *et al.* Widespread sensitization in patients with chronic pain after revision total knee arthroplasty. *Pain* 2013; **154**:1588–94.
- 372 Yarnitsky D, Granot M, Nahman-Averbuch H, *et al.* Conditioned pain modulation predicts duloxetine efficacy in painful diabetic neuropathy. *Pain* 2012; **153**:1193–8.
- 373 van Laarhoven AIM, Kraaijmaat FW, Wilder-Smith OH, *et al.* Heterotopic pruritic conditioning and itch--analogous to DNIC in pain? *Pain* 2010; **149**:332–7.
- 374 Demant DT, Lund K, Vollert J, *et al.* The effect of oxcarbazepine in peripheral neuropathic pain depends on pain phenotype: a randomised, double-blind, placebo-controlled phenotype-stratified study. *Pain* 2014; **155**:2263–73.
- 375 Demant DT, Lund K, Vollert J, *et al.* The effect of oxcarbazepine in peripheral neuropathic pain depends on pain phenotype: A randomised, double-blind, placebo-controlled phenotype-stratified study. *Pain* 2014; **155**:2263–73.
- 376 Wylde V, Palmer S, Learmonth ID, Dieppe P. Test-retest reliability of Quantitative Sensory Testing in knee osteoarthritis and healthy participants. *Osteoarthritis Cartilage* 2011; **19**:655–8.
- 377 Geber C, Klein T, Azad S, *et al.* Test-retest and interobserver reliability of quantitative sensory testing according to the protocol of the German Research Network on Neuropathic Pain (DFNS): A multi-centre study. *Pain* 2011; **152**:548–56.
- 378 Moloney N a., Hall TM, Doody CM. Reliability of thermal quantitative sensory testing: A systematic review. *J Rehabil Res Dev* 2012; **49**:191.
- 379 Nattkemper LA, Lee HG, Valdes-Rodriguez R, *et al.* Cholinergic induction of perspiration attenuates nonhistaminergic pruritus in the skin of patients with atopic dermatitis and healthy controls. *Br J Dermatol* 2015; **173**:282–4.
- 380 Yosipovitch G, Goon ATJ, Wee J, *et al.* Itch characteristics in Chinese patients with atopic dermatitis using a new questionnaire for the assessment of pruritus. *Int J Dermatol* 2002; **41**:212–6.
- 381 Yudina MM, Toropina GG, Lvov AN, Gieler U. Innovative neurophysiological methods in itch research: Longlatency evoked potentials after electrical and thermal stimulation in patients with atopic dermatitis. *Acta Derm Venereol* 2011; **91**:656–9.
- 382 Pereira M, Lotts T, Dreyer T, *et al.* Somatosensory Dysfunctions in Patients with Chronic Pruritus. *Abstr Eur Pain Fed* 2015; :P060.
- 383 Baron R, Binder A, Wasner G. Neuropathic pain: diagnosis, pathophysiological mechanisms, and treatment. *Lancet Neurol* 2010; **9**:807–19.
- 384 Fernández-De-Las-Peñas C, Galán-Del-Río F, Ortega-Santiago R, *et al.* Bilateral thermal hyperalgesia in trigeminal and extra-trigeminal regions in patients with myofascial temporomandibular disorders. *Exp Brain Res* 2010; **202**:171–9.
- 385 Vaegter HB, Palsson TS, Graven-Nielsen T. Facilitated Pronociceptive Pain Mechanisms in Radiating Back Pain Compared With Localized Back Pain. *J Pain* 2017; **18**:973–83.
- 386 Weisshaar E, Dunker N, Gollnick H. Topical capsaicin therapy in humans with hemodialysis-related pruritus. *Neurosci Lett* 2003; **345**:192–4.
- 387 Heyer G, Hornstein OP, Handwerker HO. Reactions to intradermally injected substance P and topically applied mustard oil in atopic dermatitis patients. *Acta Derm Venereol* 1991; **71**:291–5.
- 388 Ishiiji Y, Coghill RC, Patel TS, *et al.* Repetitive scratching and noxious heat do not inhibit histamine-induced itch in atopic dermatitis. *Br J Dermatol* 2008; **158**:78–83.
- 389 Schneider G, Ständer S, Burgmer M, *et al.* Significant differences in central imaging of histamine-induced itch between atopic dermatitis and healthy subjects. *Eur J Pain* 2008; **12**:834–41.
- 390 Wahlgren CF, Ekblom A. Two-point discrimination of itch in patients with atopic dermatitis and healthy subjects. *Acta Derm Venereol* 1996; **76**:48–51.
- 391 Rukwied R, Heyer G. Administration of acetylcholine and vasoactive intestinal polypeptide to atopic eczema patients. *Exp Dermatol* 1999; **8**:39–45.



LITERATURE LIST

- 392 Rukwied R, Heyer G. Cutaneous reactions and sensations after intracutaneous injection of vasoactive intestinal polypeptide and acetylcholine in atopic eczema patients and healthy controls. *Arch Dermatol Res* 1998; **290**:198–204.
- 393 Vogelsang M, Heyer G, Hornstein OP. Acetylcholine induces different cutaneous sensations in atopic and non-atopic subjects. *Acta Derm Venereol* 1995; **75**:434–6.
- 394 Heyer G, Vogelgsang M, Hornstein OP. Acetylcholine is an inducer of itching in patients with atopic eczema. *J Dermatol* 1997; **24**:621–5.
- 395 Jiang Y-M, Huang C, Peng Z, *et al.* Acidosis counteracts itch tachyphylaxis to consecutive pruritogen exposure dependent on acid-sensing ion channel 3. *Mol Pain* 2017; **13**:1–8.
- 396 Hawro T, Saluja R, Weller K, *et al.* Interleukin-31 does not induce immediate itch in atopic dermatitis patients and healthy controls after skin challenge. *Allergy Eur J Allergy Clin Immunol* 2014; **69**:113–7.
- 397 Cowan A, Kehner GB, Inan S. Targeting Itch with Ligands Selective for  $\kappa$  Opioid Receptors. *Handb Exp Pharmacol* 2015; **226**:291–314.
- 398 Chen Z-F. Center for the Study of Itch opens [WWW Document]. URL [http://csi.wustl.edu/news/center\\_for\\_the\\_study\\_of\\_itch\\_opens](http://csi.wustl.edu/news/center_for_the_study_of_itch_opens) [accessed on 17 September 2017].
- 399 Yosipovitch G, Kwatra SG. Living with Itch: A Patient’s Guide, 1st ed. Baltimore, The Johns Hopkins University Press, 2013.
- 400 Szepietowski J, Weisshaar E. Itch - Management in Clinical Practice, 1st ed. Basel, Karger Medical and Scientific Publishers, 2016.
- 401 Liu Y, Abdel Samad O, Zhang L, *et al.* VGLUT2-Dependent Glutamate Release from Nociceptors Is Required to Sense Pain and Suppress Itch. *Neuron* 2010; **68**:543–56.
- 402 Liu T, Xu Z-Z, Park C-K, *et al.* Toll-like receptor 7 mediates pruritus. *Nat Neurosci* 2010; **13**:1460–2.
- 403 Liu Q, Weng H-J, Patel KN, *et al.* The distinct roles of two GPCRs, MrgprC11 and PAR2, in itch and hyperalgesia. *Sci Signal* 2011; **4**:ra45.
- 404 Mu D, Deng J, Liu K-F, *et al.* A central neural circuit for itch sensation. *Science* 2017; **357**:695–9.

ISSN (online): 2246-1302  
ISBN (online): 978-87-7210-083-8

AALBORG UNIVERSITY PRESS