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STUDIES ON ITCH AND SENSITIZATION FOR ITCH IN HUMANS

BY
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PHD THESIS

by

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Dissertation submitted

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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 almindige 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 ødelægende 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-sensiblisering 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 området.

Resultater fra det andet studie demonstrerede en markant antipruritisk effekt af høj-koncentrations topisk capsaicinbehandling og viste, at de to hyppigst anvendte humane kløe-provokationersmodeller begge virker via capsaicin-sensitive kutane nervefibre. Afslutningsvist afdækkede det tredje studie signalbane-specifik ikke-histaminergic kløe-sensiblisering 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 sensibiliserings-modeller er alsidige og nyttige forskningsmetoder inden for human-eksperimentel og klinisk forskning i forhold til at opnå en bedre forståelse for de mekanismer, som forårsager akut og kronisk kløe.

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- 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

$H_{1/4}$ – 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_2

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

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 [1–4], who suffer from chronic itch this is far from the case [5]. 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 [5–7].

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 [8–14]. For example, in AD, 57.3-87% of the patients report pain from the lesional skin areas [8,10]. 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 [15], a patient with AD who scratches until drawing blood every day [16,17], 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 [18]. Despite sparse research, chronic itch is consistently found to significantly reduce quality of life, for instance related to disturbed sleep, attention and sexual function [9,11,16,19,20]. Itch is also consistently linked to increased rates of anxiety, depression and even suicidal ideation [21–24].

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) [25]. 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

[25]). These are three conditions that include itch as a major symptom, and where itch is very often the primary complaint of the patients [10,26,27]. Additionally, a myriad of non-dermatological disorders, such as systemic and neuropathic diseases are associated with significant itch [5,28–30]. 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 billon at that time, and since then the prevalence of the disease has increased significantly [31–33]. This cost is to a large extent driven by a high disease prevalence and a poor efficacy of the available therapeutic options [31,32,34].

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 [35–39]. As of 2017, no 'general use' antipruritics are available, and the development of efficacious pharmaceutical treatments has proven arduous and ineffective [37,38,40]. This is perhaps because the human neurobiology and pathophysiology of itch is relatively poorly elucidated despite numerous recent advancements, particularly within the last decade [37,41–48]. 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 [38,49,50]. However, it is frequently observed, for instance in AD, that even significant remission of the lesions does not necessarily relieve the associated itch [16,26,36,51]. In fact, in the case of AD, the objective lesional evaluation and the subjective severity of itch are surprisingly poorly correlated [51]. This observation, amongst others, has lead 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 [13,14,52–54]. 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 [37,38,40]. Lastly, the fact that an effective non-histaminergic model of itch has only recently been established and mechanistically explored [42,55–58] 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 [59].

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*.) [60,61]. 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 [55,57,62]. 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 [37,60] 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 [63]. His suggestion that itch results from mildly painful stimuli, known as the *intensity hypothesis*, (Fig. 1) considers itch to constitutes 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 [64]. 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 [65–67].

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

itch [69], while Bickford was the first to describe "itchy skin", the perceptual correlate of spinal itch sensitization [70] (later dubbed alloknesis and hyperknesis [71–73]). In the 1950s, Cormia and Kuykendall observed itch in response to heat stimuli in partly anesthetized human skin [74,75]. 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 [74,75]. 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 [76,77]. 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 [60]; and patients often report severe itch exacerbations which are not associated with a transition to pain [6,38,78,79].

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) [80]. Notably, this idea of a characteristic temporal discharge pattern being a key differentiation feature of itch was proposed as early as 1941 [64] and rekindled by a recent study in non-human primates [81]. However, this pattern hypothesis is unsupported by psychophysical data [82], and the potential central filtering mechanisms are largely unaccounted for.

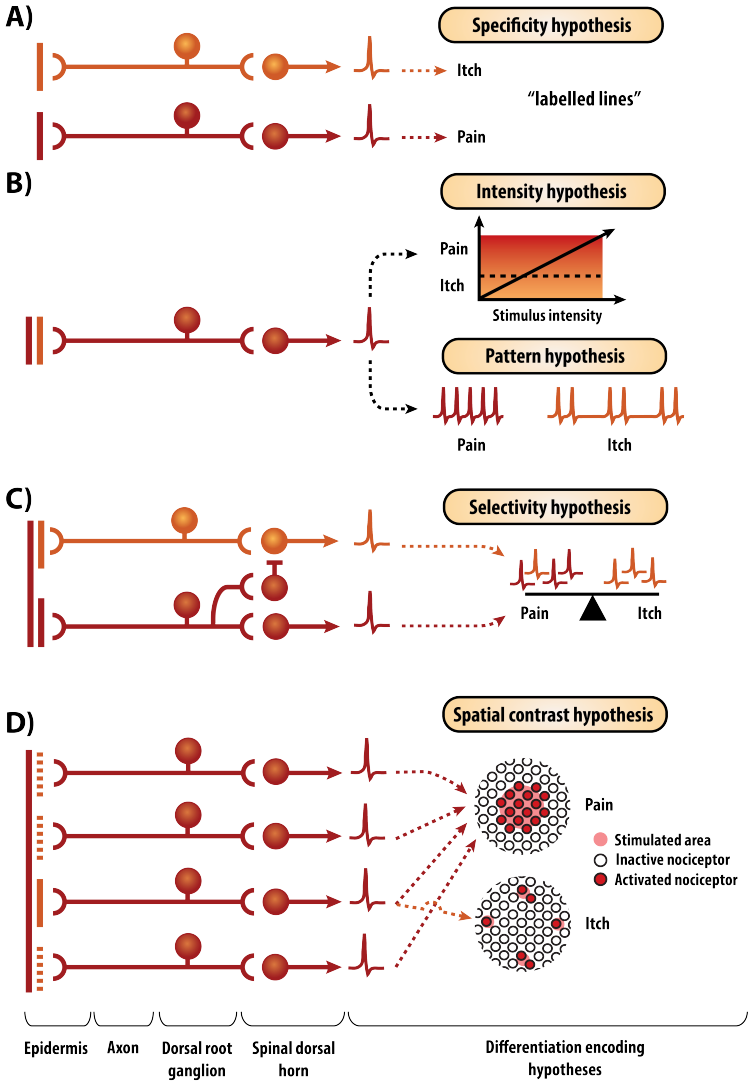

Figure 1. Proposed neuronal encoding hypotheses to explain differentiation between itch and pain sensations. *Red = nociceptive signaling, orange = pruriceptive signaling. Vertical bars left of the receptive fields represent noxious (red) and pruritic (orange) stimuli.* ***A)*** *Two separate 'labeled 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) [83–88]. 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 [86,89,90]. 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 [91,92]. 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 algogens subjectively causing pain [93,94]. 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 [93,95,96]. 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) [97]. For histaminergic human itch transduction, this remains the status quo; CMi-fibers expressing histamine-receptors 1 and 4 ($H_{1/4}$), 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 [26,37,38,98]. 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 [26,28,35,36,99]. Less than a decade ago, neuroscientists began to reappraise the properties of cowhage as an itch inducer [42,55–58]. 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 [100–102]. 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 [100,101], which in 2008 was shown to work by engaging the proteinase-activated receptors 2/4 (PAR-2/4) expressed on epidermal C-fibers [58]. Within the last decade, comparative microneurographic, psychophysical and vasomotor imaging studies on itch induced by cowhage and histamine provocations have been conducted [42,55,57,62,103]. 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 [42,57,103]. 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 [56,57]. Thus a notion of two distinct peripheral subpathways for itch has emerged, provisionally referred to as 'histaminergic' and 'nonhistaminergic' itch [57,104,105] (Fig. 2A and B). This *ad hoc* taxonomy is somewhat suboptimal when, as frequently done, used in reference to the neuronal pathways *per se* [38,106] – '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 [107], highlighting that notable species differences exist for neurophysiology of itch and pain and underlining the need for translation of mechanisms elucidated in rodents [41]. Because microneurography experiments have consistently shown that essentially all PmC-fibers respond to various pain-evoking stimuli as well as cowhage [42,81,103], the explanatory encoding models mentioned above have recently been rendered insufficient, and the discussion of the differentiation encoding of itch and pain has resurfaced [96,108–110].

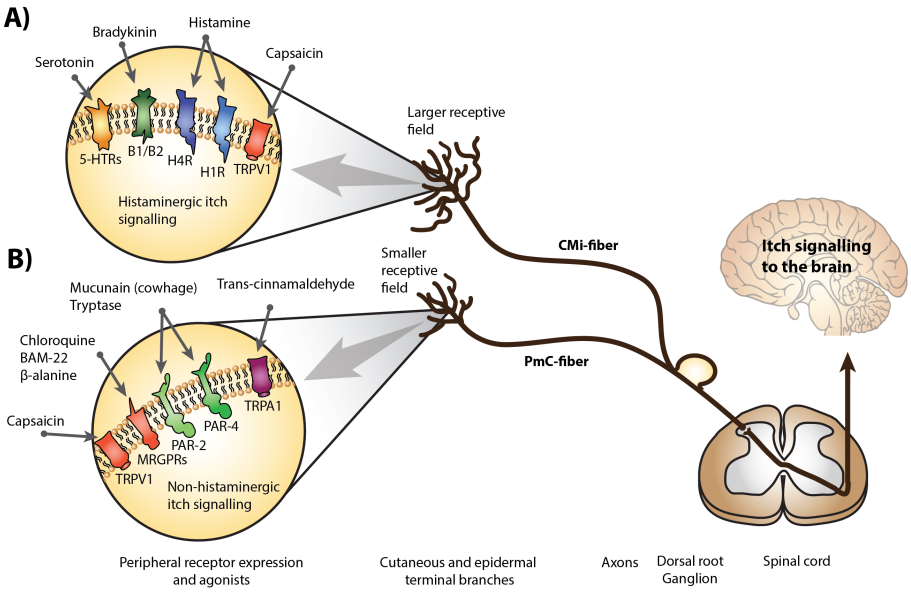

Figure 2. An overview of peripheral induction of itch by activation of receptors on mechano-insensitive (CMi) and polymodal (PmC) C-nociceptors. *Only agonists applied as pruritogens in humans are shown. Pruriceptive CMi-fibers (**A**) express e.g. 5-HTRs, B1/B2, H1/4R and TRPV1 and conduct histaminergic itch, while subgroups of PmC-fibers (**B**) expresses 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* [105].

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 [42,110,111]. 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 [42]. The hypothesis, tentatively articulated in the 1990s [97,112], 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 [42,110]. 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 [110]. 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 (MRGPRA1) constitute 5% of the entire C-fiber population, and chloroquine-responsive fibers represent 12.8% of the total DRG population in mice [92,93,113,114]. 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 [115,116].

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 [110,117]. 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 [28,118]. 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 [108,119,120].

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 [53,121–123]. The International Association for the Study of Pain defines sensitization in the context of pain as: *"increased responsiveness of nociceptive neurons"* [123]. 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 [54,123]. 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 [111,124,125]. Secondly, the patterns of pain- and itch-evoked dysesthesias are highly similar in terms of spatiotemporal properties [71,73]. Lastly, several lines of indirect evidence, e.g., poor correlation between lesional severity and itch [16,51], itch in response to normally innocuous mechanical stimuli (such as certain fabrics) [14,126], examples of significant antipruritic effect of centrally acting GABAnergic, serotonergic and noradrenergic drugs [127], and altered expression of molecular transducers on epidermal C-fibers [128], 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 [54,121,123] 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 [129,130]. 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 [131]. In addition neuropeptides such as calcitonin gene-related peptide (CGRP), and substance P are released from local peptidergic fibers [131,132]. 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 [132–134]. 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 [38,135]. 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 [136–138]. For instance, IL-13 has been shown to induce upregulation of transient receptor potential ankyrin 1 (TRPA1) on pruriceptive C-fibers in rodents [138]. 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 ($Na_v1.8$ and 1.9), ultimately leading to increased excitability of nociceptive and pruriceptive Aδ- and C-fibers [132,139].

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 [54,123,140,141]. 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 [123,141]. 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 [123,141]. Both spinal microgliosis and astrocytosis have been extensively demonstrated in relation to pain and recent studies show that itch cause similar glial activation patterns [142,143]. Lastly, a large body of evidence also describes profound alterations in brain connectivity and even brain morphology associated with chronic pain [144–147] and to a lesser extent chronic itch [148,149], 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 [13,118,150]. 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') [71,72,106]. Various psychophysical tests have been developed to probe somatosensory sensitization [151]. 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 [86,152]. 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 [106,151]. 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 [71,73,153]. 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 [63]. 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 [64,154,155].

Lewis' studies on cutaneous hyperalgesia [156] 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 [70]. 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 [70–72] 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 [71–73,157] (Fig. 3A and B). Thus, the terms alloknesis and hyperknesis are completely parallel to *allodynia* and *hyperalgesia*, for pain respectively [158]. 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 [79,159].

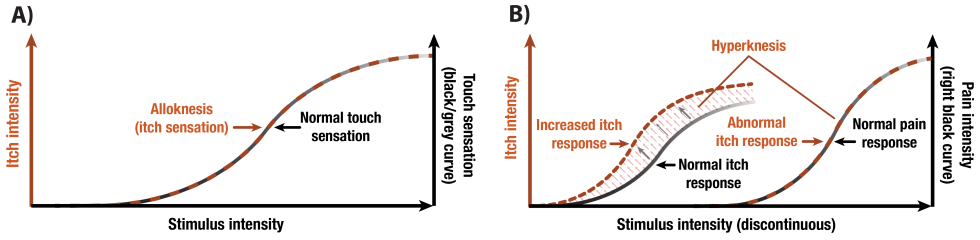

Figure 3. Concept illustrations of alloknesis and hyperknesis. *Alloknesis (**A**) comprises a switch in perception of a normally innocuous stimulus such as light stroking of the skin, which additionally or alternatively becomes itch evoking. Hyperknesis (**B**) represents a leftward shift in the stimulus-response 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 [160]. 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 [13,14,161]. 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.

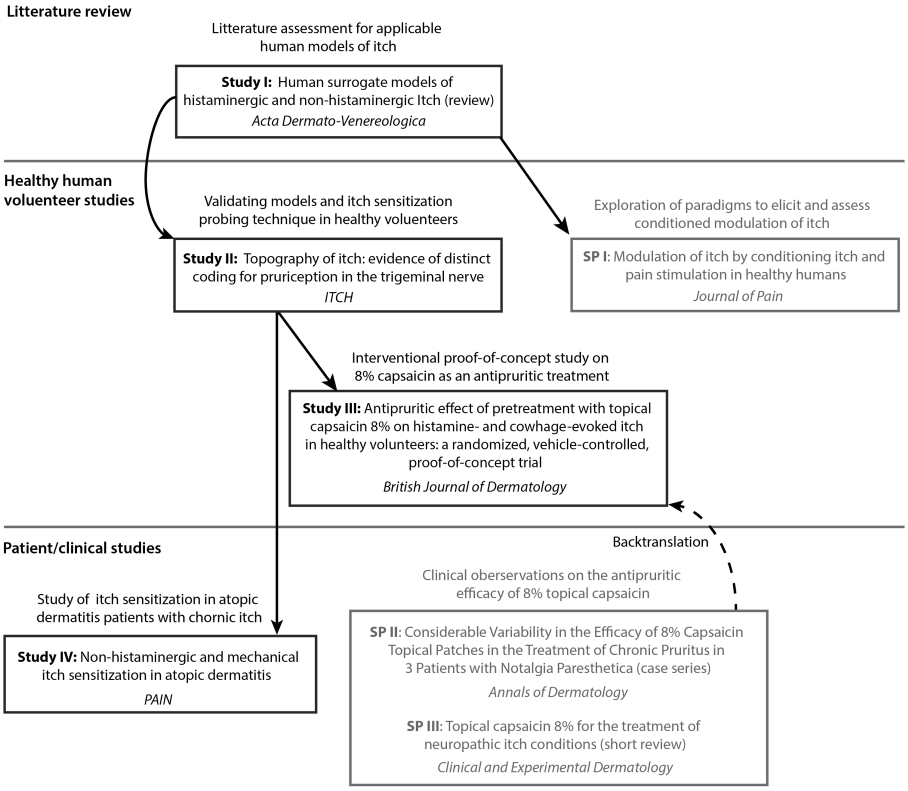

Figure 4. A schematic overview of the dissertation studies and associated supplementary papers. *Based on a literature review (Study I), two itch models were applied in a basic study (Study II) and a pharmaceutical proof-of-concept study (Study III), of which the tested intervention was derived from 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 [123,162–165]. 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 [123,162–164,166]. Cutaneous pain and hyperalgesia can be achieved by chemical, thermal, electrical or mechanical provocations as well as a combination of such stimuli [162,164]. 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 [164,167,168], many of which have been used in the profiling of analgesic compounds [169].

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 [123,162–165]. 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 [122,170–173]. 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) [162,164,165,174]. 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 [162,164]. As a consequence, the degree to which such models can mechanistically mimic the processes involved in chronic itch is a matter of ongoing contention [174]. 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 [130,175,176], 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 [105,106]. 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*) [105,106]. 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*) [42,57,103,106]. 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 [57,177–179] and pharmacological proof-of-concept studies [35,180,181] as well as explorative clinical studies [14,126,182,183]. 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 [184].

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 [185]. 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 treat AD or psoriasis, using a histaminergic model does not constitute a reasonable way to do so [42,56,57,105,106]. 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

[26,37,38,126,129,186]. 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 [42,128,181,187]. Most chronic itch conditions are refractory to antihistamines or have a very modest response to antihistaminergic therapy [26,37,38,126]. Notable exceptions hereto are urticaria and allergic conjunctivitis, which are both thought to be largely histamine-driven [37,38,188,189]. 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 [180,181,187].

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? [106,190].

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 [191,192]. The visual analogue scale (VAS_{0-10} or VAS_{0-100}) 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) [193–196]. 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" [55,194,197,198]. 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) [178,179,194,199] or by adding an anchor that relates to the experienced itch intensity of an average mosquito bite [42,57]. 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 [194].

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 [200–202]. 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 [203–205]. 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 $VAS_{0\text{-}10} \approx$ 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 [203,205,206] (as discussed in SP I). For itch, all effective models are chemical, and there is no method that consistently induces peak itch above $VAS_{0\text{-}10} \approx 5$ in healthy controls regardless of which modality is being used or even with attempts to induce preceding cutaneous sensitization [74,106,130,175,176]. 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 $VAS_{0\text{-}10} \approx$ 3-5, after which pain concomitantly emerge and eclipses the itch [62,74,82,102].

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 [106]. 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 ($VAS_{0\text{-}10}$) and moderate-to-high test-retest reliability [57,184,194,207]. 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.

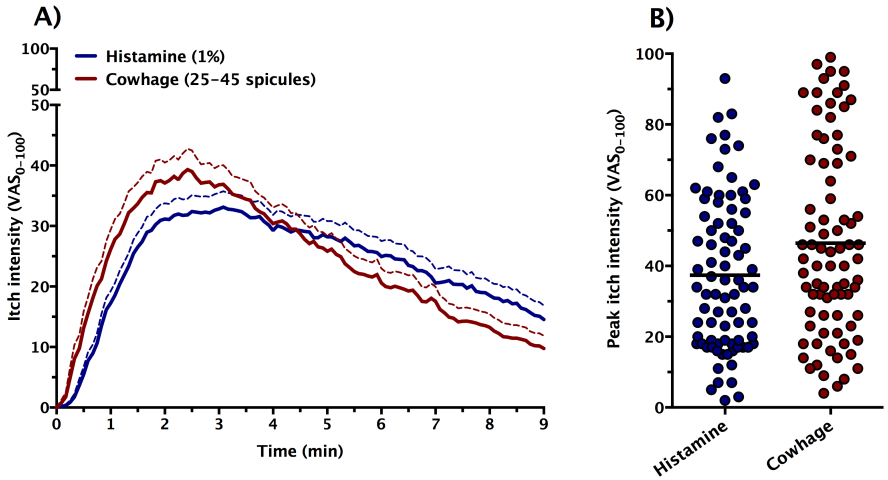

Figure 5. Itch evoked by cowhage and histamine in healthy controls. *A) 0-9 minute temporal profiles of itch intensity from forearm sites (volar or dorsal) evoked by 1% histamine or 25-45 spicules (N = 55 itch provocations, Studies II and III). Notice that cowhage induced itch peaks and declines more 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 [208–211]. The survey was altered to accommodate itch patients independently by two different groups, resulting in the *Eppendorfer itch questionnaire* and the "*Yosipovitch*" questionnaire [8,51,212]. 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 [55,178,182,198]. 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 [14,82,161,178]. 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 [14,161,213,214]. 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.

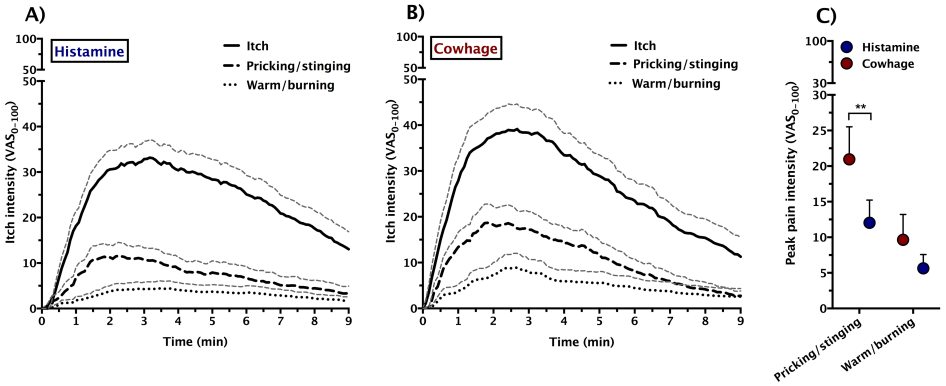

Figure 6. The quality of pain associated with pruritic provocations (Study II). *Most itch models, including histamine (**A**) and in particular cowhage (**B**), give rise to mild cutaneous pain sensations commonly described as either pricking/stinging and/or warm/burning. The pricking/stinging component 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 [81,94]. Since the primary afferent substrate for brush strokes is Aβ-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 [82,215]. When occurring next to an itch provocation or an actively itchy skin lesion, hyperknesis is potentially mediated by type-I Aδ-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 [14,216], indicating PmC-fibers as the peripheral substrate. When pinprick hyperknesis occurs inside an active skin lesion, a peripheral contribution or main mechanism is conceivable [13,14,161]. 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 [217]. 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 [120]. 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 [13,14,218].

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 [14,71,219,220]. 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 [73,82,176,221,222]. In humans, alloknesis is commonly assessed with a light brush, while hyperknesis is often assessed with a pinprick stimulators or filaments [82,176]. 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) [73]. 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.

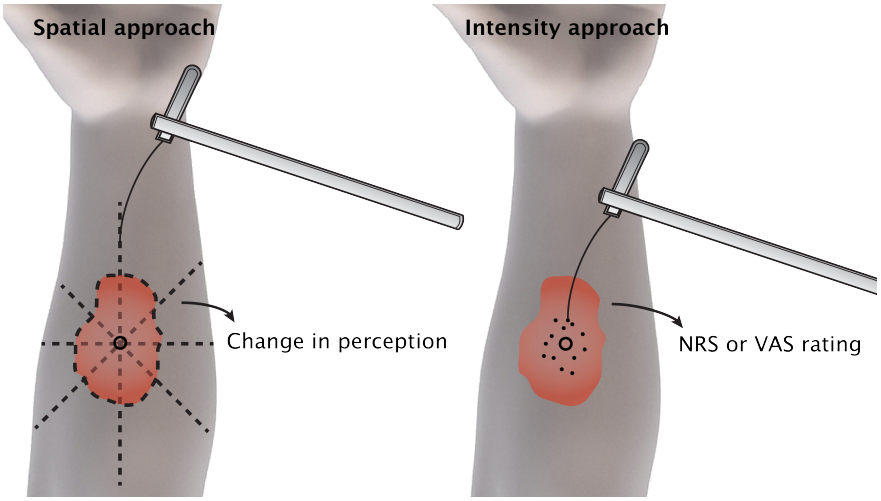

Figure 7. Commonly used methods for mechanical itch dysesthesia quantification. *Two methods for dysesthesia assessments are in use: a planimetric mapping of the spatial extent of the allo-/hyperknesis typically surrounding an itch provocation (left) or an assessment of the intensity of the dysesthesia 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 [161,176,218,220]. 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 [13,129,161]. 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 [161,220]. 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 [13,14,218,223] 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’ [224].

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 [52,176]. 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 [13], 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 [129,220].

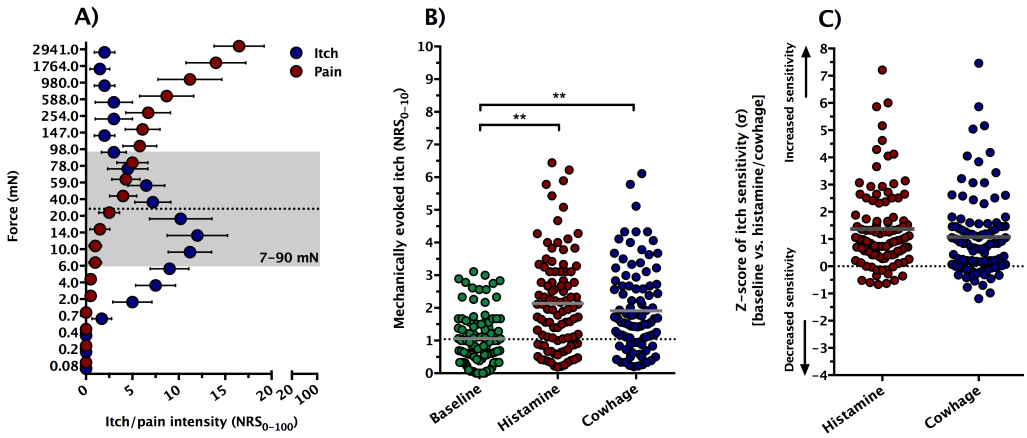

Figure 8. Mechanical itch sensitization assessed with von Frey filaments. ***A)*** *Itch and pain ratings to 20 weight calibrated von Frey monofilaments ranging from 0.08 to 2941 mN. The grey box represents upper, median (dashed line), and lower ranges for the physiological mechano-responsiveness of human polymodal C-fibers assessed by microneurography [86]. Notice that despite optimization, the maximal average rating is ≈ 12 (NRS_{0-100}).* ***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 [69,106,225,226]. 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 [106,184,227–230]. 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 [182,228]. 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 [106,231,232]. 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) [227,233].

Neurogenic inflammation or neurogenic flare is a brief increase in superficial blood perfusion caused by retrograde signaling from activated dermo-epidermal peptidergic nerve fibers [69,226,234]. 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* [90,235,236]. 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 [235]. 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 [90,106,237], 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 [229,234,237].

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 [42,238,239]. 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 [240–242]. 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 [241,243]. 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 [242,244,245]. 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 [239,246]. 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 [55,57,184,198,220]. 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 [177,247]; however, this is probably inaccurate and rather represents that insufficiently sensitive assessment techniques were applied [62,220]. 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 [220]. In some cases, very mild vasomotor reactions are only visible by high-resolution FLPI.

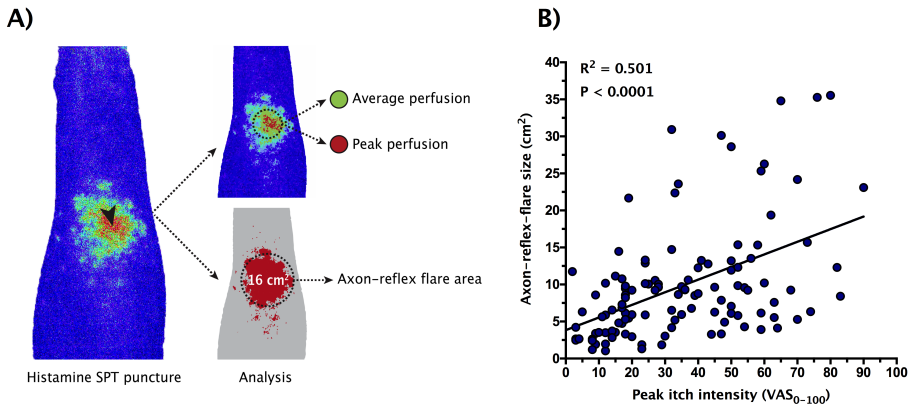

Figure 9. Histamine-induced neurogenic inflammation and its correlation with itch (Studies II and III). ***A)*** *A typical Full-field Laser Perfusion Imaging (FLPI) readout following a skin prick test lancet introduction of 1% histamine (black arrowhead). Two basic quantification techniques are illustrated; 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 algogens alike evoke some degree of activation of both C-fiber subtypes [93,248,249]. 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 [55,250]. 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 [93,251,252]. Accordingly, the sensory event in response to capsaicin is orchestrated by PmC- (perhaps predominantly), Aδ- and CMi-fibers, while the vascular event is mediated by the CMi-fibers, which only constitute a minor fraction of the activated fiber pool [92,93,253]. 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 [254], and that topography and regional acuity of the cutaneous nociceptive system have been extensively explored in recent decades [255,256].

Differences in topography of mechanical and histaminergic itch sensation were recently shown between spinal and trigeminal innervated areas [215], 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 [37,106]. Second, in rodents, assessment of itch relies entirely on counting the scratch bouts that occur following a given provocation or in a chronic model [257]. 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) [258]. Oppositely, the majority of human studies applying itch provocations in healthy volunteers or patients have done so on the forearms [106]. The extent to which potential anatomical differences influence the comparability and translatability between human and animal studies itch studies is unknown [106]. 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 [18,28]. Prurigo nodularis and dermatitis herpetiformis occur frequently on extensor surfaces of the extremities [259,260]. AD lesions are overrepresented in the creases of the elbows and knees, while urticaria manifests frequently on the trunk and proximal extremities [261]. 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 [50,262–265]. 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 [215]. 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 [215]. 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 [105,219,266].

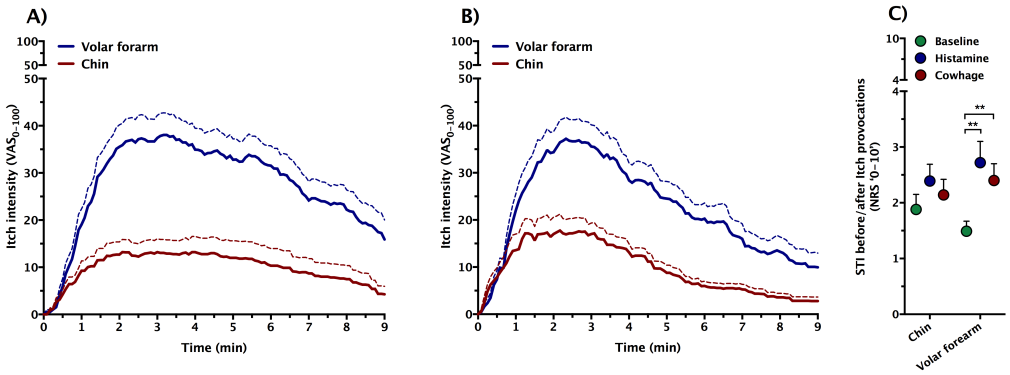

Figure 10. Differences in chemical and mechanical itch sensitivity between the volar forearm and the chin (Study II). *Temporal profiles of itch evoked by histamine (**A**) and cowhage (**B**) at the volar forearm versus the chin. **C**) Sensitivity to touch-evoked itch (STI) before (baseline) and after 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 ≤ 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 α_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 α_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 [129,182,247]. Coinciding with this observation is the frequent finding that patients with AD exhibit smaller and less intense neurogenic inflammatory reactions in non-lesional skin [129,182,186,267] (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 Aδ–nociceptors [249,268,269]. This receptor was initially discovered 20 years ago [268,270–272], 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 μg capsaicin, dose-dependent burning and stinging pain occurs, reflecting acute activation of aforementioned units [168,273,274]. 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 [275–279]. 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 [280–282]. 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) [280,283]. Capsaicin-induced desensitization is thought to selectively affect TRPV1-expressing heat-sensitive C- and Aδ-fibers, but discrepancies exist with regards to the degree to which mechanical pain desensitization also occurs [280,281,284]. 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 [276], which is related to distinct mechanisms particularly relevant for PmC-fibers [249].

Pharmacodynamically, the prolonged defunctionalizing effect has been proposed to rely on multiple parallel mechanisms [269,285–287]. 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^{2+}, could overpower cellular calcium sequestration mechanisms [288–290]. 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 [291–293]. 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 [269,294,295]. Often such reversible retraction occurs well into the dermis [280,296]. 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 [269,281].

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 [297,298]. 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 [299,300]. Lastly, cutaneous substance P provocations in humans in the physiological concentration range have shown no or very limited sensory effects [301]. 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 [93,249]. Moreover, itch can be induced directly in response to topical capsaicin if administered, for instance, on inactivated cowhage spicules [55,178,198]. 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 [298,302]. Low

concentration topical capsaicin creams (≤ 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 [281,302]. 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 [37,38,303,304]. 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 [302]. 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 [103,298].

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 [305–308]. These patches contains ≈ 825 times as much capsaicin as the low-concentration capsaicin creams previously used in clinical trials and treatment of pruritus [302]. 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 [309,310]. Beyond prolonged effects on warmth detection thresholds signifying desensitization of warm C-fibers [307], the pain defunctionalization, e.g., decreased heat pain sensitivity, assessed by quantitative sensory testing (QST) following a single 1-hour treatment is short-lived [281,311]. Sensory function appears to normalize within a couple of weeks or even faster [311], paralleled by a delayed recovery of epidermal nerve fiber density typically assessed by Protein gene product 9.5 immunostaining [312]. 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 [311]. 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 [313]. While 8%

capsaicin is widely used to treat peripheral neuropathic pain (most treatment guidelines list it as a second-line option [304,314]), 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 [309,315]. For instance, in SP II and III, the antipruritic effect of 8% topical capsaicin was described in case reports of patients with neuropathic itch.

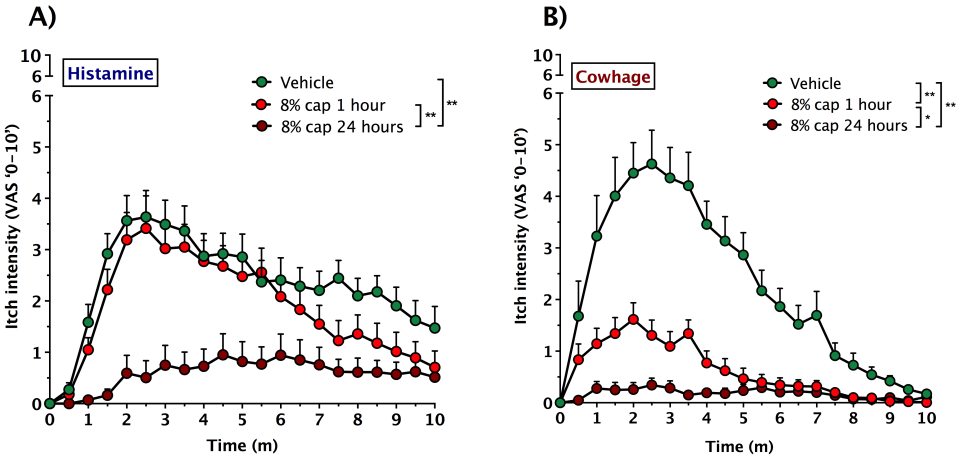

Figure 11. Itch responses to histamine (A) and cowhage (B) following vehicle, 1-hour and 24-hour 8% capsaicin pretreatment (Study III). *Temporal profiles of the evoked itch intensity from 0-10 minutes after each provocation. Notice that 1-hour capsaicin pretreatment profoundly inhibits cowhage- but not histamine-evoked itch. Mean and standard error of mean depicted. * = P ≤ 0.05, ** = P ≤ 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 [316–318] and in studies on experimentally established histaminergic itch [221,319]. 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 [57,284,302,320].

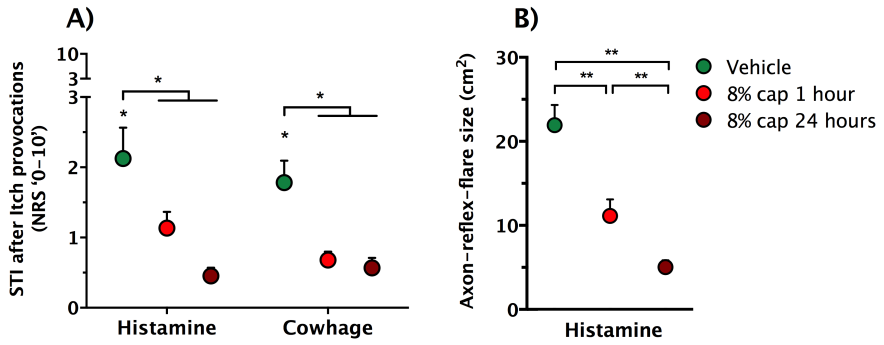

Figure 12. Effects of 8% capsaicin on hyperknesis and neurogenic inflammation. *Dose-dependent reductions in hyperknesis (**A**) and axon-reflex flare (**B**) after histamine and cowhage provocations in the skin areas pretreated with vehicle, 1-hour and 24-hour 8% capsaicin (Study III). STI = Sensitivity to touch-evoked itch. Mean and standard error of mean depicted. * = P ≤ 0.05, ** = P ≤ 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 [78,321]) 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) [41,79,321]. In rodent DRGs, most TRPA1-positive neurons also appear to co-express TRPV1 [322,252] although a recent study showed a substantial subpopulation of non-peptidergic TRPA1-postive neurons not characterized by TRPV1-expression [251] and an *in vitro* study showed a functional TRPV1/TRPA1 overlap of only 20% in mice DRG neurons [114]. 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 [323,324]. A recent study has

shown that formation of functional TRPV1/TRPA1 heteromers occurs and suggested that TRPA1-induced hyperalgesia relies entirely on TPRV1-expression [323], contradicting the idea of functional independence of the receptors [322,325]. 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 [78,107]. 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 [216,313]. 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 [326,327]. Accordingly, activation of these fibers in humans by β-alanine injections (a MRGPRD ligand) causes significant itch [326]. MRGPRD-positive neurons have recently been found to be highly sensitized to mechanical and thermal stimuli in an animal model of contact dermatitis [328]. A future study using 8% capsaicin-induced desensitization could determine whether such sensory effects of β-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 [249]. 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 [57,103,321,329] and anecdotal observations indicate that cowhage cannot evoke itch if the epidermis is experimentally removed [102,330]. 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 [90]. 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 [106].

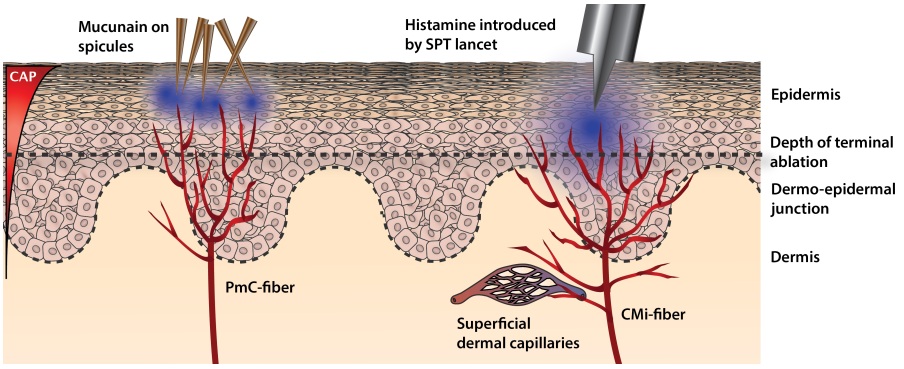

Figure 13. Potential mechanisms for the differential desensitizing efficacy of capsaicin on C-mechano-insensitive (CMi) versus polymodal C-fibers (PmC) observed in Study III. *Topical capsaicin penetrates the skin creating a concentration gradient. Dashed grey line (top) marks hypothetical depth to which 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 [15]). 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 [247]. 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 [26,262,331,135]. 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 [34,33233,34]. 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 [8,10,26,262,331]. 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 [8,16].

Within the dermatological disease category, AD is the most significant contributor to YLD accumulation and can in severe cases be devastating for affected patients [25]. 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 [31,34,333,334]. The majority of AD patients point to itch as being the single most bothersome disease component [335,336]. 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; $VAS_{0\text{-}100}$) associated with their itch (60.7; $VAS_{0\text{-}100}$). 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 [26,37,40,337,338]. Pathoetiologically, AD is associated with genetic, immunological, environmental and skin barrier factors [262,339], 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 [14,126,340]. 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).

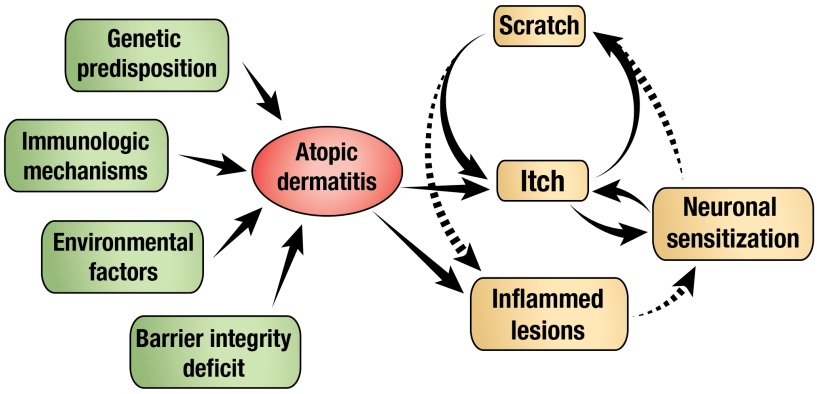

Figure 14. Conceptual model of the contribution of itch sensitization in atopic dermatitis (AD). *Green boxes represent known disease mechanisms involved in the generation and maintenance of AD. When a flare-up occurs itch and inflamed lesions are maintained by excessive scratching (itch-scratch-itch 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 [239,341–348].

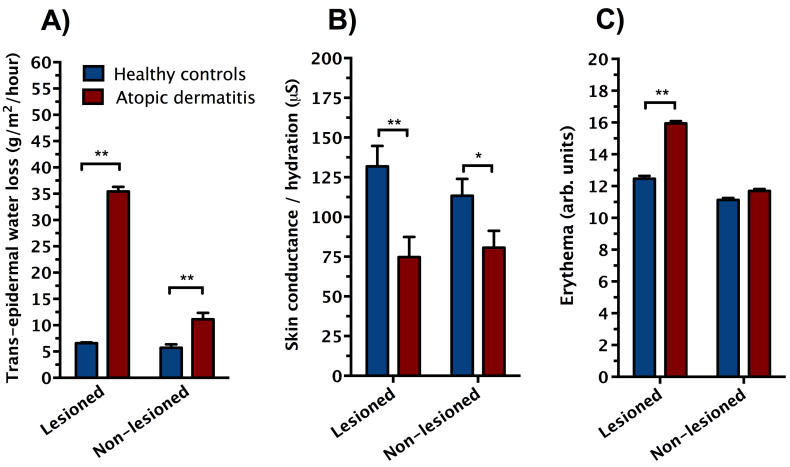

Figure 15. Skin barrier parameters in lesional and non-lesional areas of patients with AD and homologous areas of healthy controls (Study IV). ***A)*** *Epidermal barrier integrity measured by trans-epidermal water loss.* ***B)*** *Skin hydration assessed by electrical skin conductance.* ***C)*** *Erythema 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 [26,135,349,350]. 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 [14,26,128,262,135]. 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 [26,349]. Even when scratching is avoided during the day, nocturnal itch often leads to intense scratching and resultant poor sleep quality [350,351]. 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 [78,79,150,352–354]. 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 [135,355]. 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) [356]. Dupilumab was approved following a series of clinical trials showing very high anti-inflammatory and antipruritic efficacy in patients with moderate to severe AD [49,357]. 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 [358]. 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 disproportionally under-researched when considering the size of the potential market [359].

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 [151,360,361]. In patients with peripheral neuropathic pain, large standardized QST

studies have shown profound sensory gain- and loss-of-function compared to healthy controls [122,362]. Neuropathic pain patients generally exhibit one of three overall sensory phenotypes; sensory loss, thermal hyperalgesia and mechanical hyperalgesia [362]. 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 [314,363,364]. 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 [364–366]. In patients with chronic ocular pain, headache, visceral pain etc. similar observations have repeatedly been made [367–370]. 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 [364,365,371,372]. Such paradigms have only just begun to be translated and introduced in chronic itch patients [223,373], and methodological studies establishing the validity of these paradigms are needed [206]. SP I represents an initial attempt to investigate and establish psychophysical paradigms for assessment of endogenous descending itch inhibition in humans [206]. 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) [307,366,372,374]. 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 [304,375]. 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 [314,362].

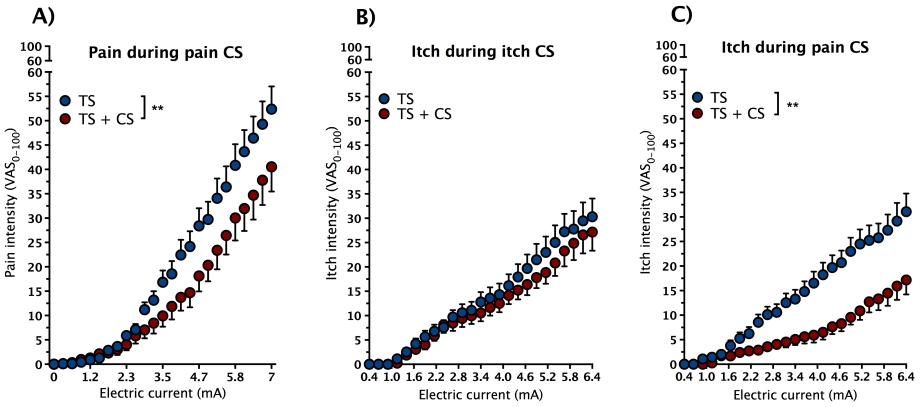

Figure 16. Experiments on conditioning modulation of itch by contralateral pain and itch stimuli (SP I). *Healthy volunteers (N = 26) were presented with an initial electrical itch or pain stimulus (test stimulus = TS) followed by the same stimulation during either a conditioning pain or itch stimulation (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 ≤ 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 [6,106]. 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.

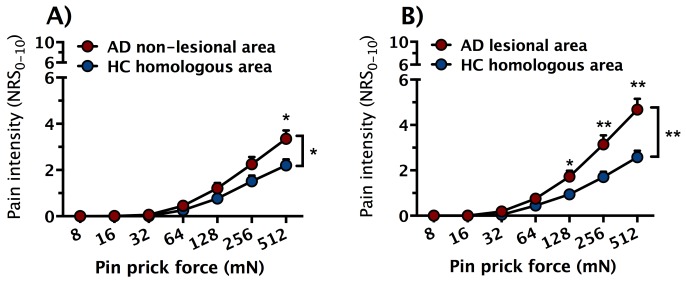

Figure 17. Mechanical hyperalgesia to suprathreshold pain stimuli in patients with atopic dermatitis (AD) (Study IV). *Mechanical pain sensitivity to pinprick stimuli from 8 to 512 mN, in non-lesional (**A**) and lesional skin (**B**) of AD patients compared to homologous sites in healthy controls.*

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 [376–378]. 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 alloknesis associated with, e.g., certain fabrics [8,126,350], 2) patients often report itch exacerbations when feeling warm or associated with perspiration [12,379,380], and 3) patients commonly describe abnormal cutaneous sensations such as burning, tingling and pricking associated with their lesions [8,10,12]. 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°C) but significant impairments in warmth and cold detection thresholds in patients with AD [381] whereas a recent study failed to detect similar differences in thermal sensitivity despite very comparable assessment methodology [382]. 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 [362,383–385]. Oppositely, both lesional and non-lesional skin yielded mean thermal thresholds comparable to those found in the normative QST data sets [122,151]. 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 [8,51] (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 [14]. This conceptually corresponds to heat hyperknesis because pruriceptive units are presumably also activated during normal heat stimulation [42,86,88,251]. 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 [176]. 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 [126,161], and the phenomenon is likely more or less dependent on ongoing spontaneous itch nearby [73,176]. 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 [126]. 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 [82,221,340,386] (although trends towards sensitization have been observed [82]). 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) [126]	Wool fibers (Intensity method)	Spontaneous: ↑Hyperknesis, likely both lesional and extra-lesional	
Heyer et al. (1995) [340]	Sensory brush (Spatial mapping method [1])	N/A	Evoked: ↓Alloknesis
Weisshaar et al. (1998) [221]	Sensory brush (Spatial mapping method [1])	N/A	Evoked: ↓Alloknesis
Ikoma et al. (2004) [14]	Weighted needle stimulators (Intensity method)	Spontaneous: ↑Hyperknesis	Spontaneous: ↑Hyperknesis (intra and peri-lesional)
Ikoma et al. (2005) [82]	Sensory brush / pin prick (Spatial mapping method [2])	N/A	Evoked: → Alloknesis Evoked: → Hyperknesis[5]
Hosogi et al. (2006) [161]	Sensory brush (Intensity method)	Spontaneous: ↑Alloknesis	Spontaneous: →Alloknesis
Laarhoven et al. (2007) [218]	Von Frey stimulators (Intensity method)	Spontaneous: ↑Hyperknesis[3]	Spontaneous: ↑Hyperknesis[4]
Andersen et al. (2017) [13]	Von Frey stimulators (Intensity method)	Spontaneous and evoked: ↑Hyperknesis	Spontaneous and evoked: ↑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. [1] = after an iontophoretic histamine provocation, [2] = after electrically induced itch, [3] = predominantly lesional, [4] = predominantly non-lesional, [5] = 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 [13,14,161,218]. 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 peri-focally to, the itching skin area [15,161,309]. 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 [176]. 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) [13].

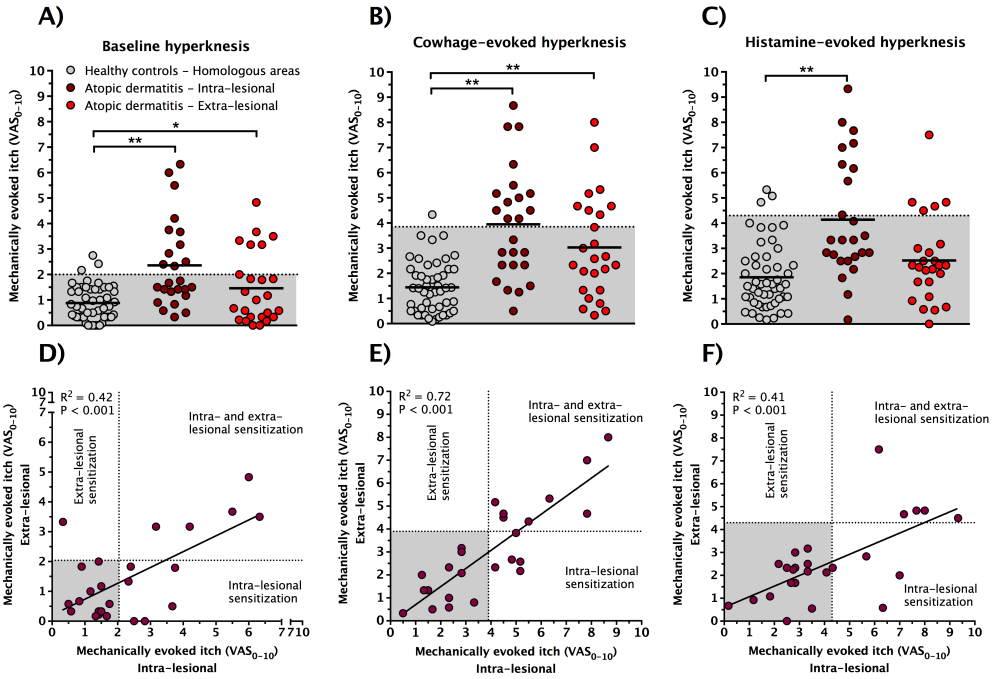

Figure 18. The variability of mechanical hyperknesis in atopic dermatitis (AD) compared to healthy controls (Study IV). ***A****) Mean and individual hyperknesis data assessed at baseline in lesional (dark red) and non-lesional (bright red) skin of patients with AD (n = 25) compared to healthy controls (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 [129,182,186,267]. 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 [267,387]. 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.

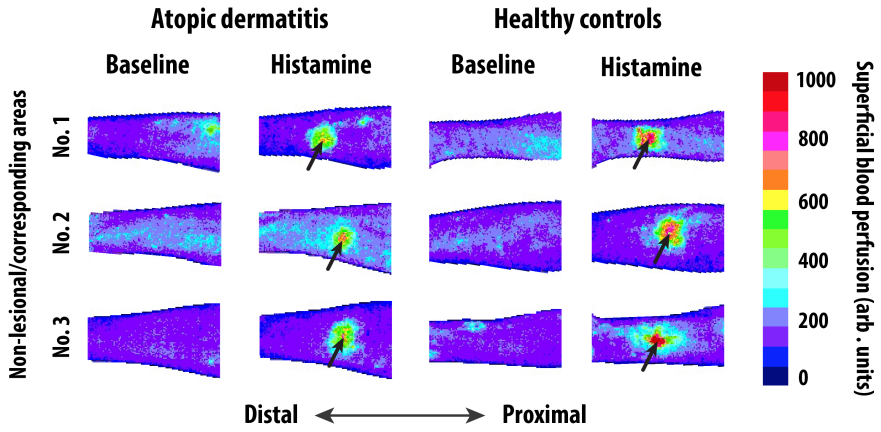

Figure 19. Flare reaction in atopic dermatitis (AD). *A series of Full-Field Laser Perfusion (FLPI) images recorded at baseline as well as following histamine in non-lesional skin of patients with atopic dermatitis and homologous skin of healthy controls (Study IV). Note the blunted flare response to 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 [13,126,128,129,161,182,247,267,388–393]. 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 [392,394] 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 µL/minute causes significant itch in both lesional and non-lesional AD skin areas but only mild pain in healthy controls [14]. 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 [14,395].

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 [13,182,247]. 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 [182]. 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 [247]. 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 [128]. 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.

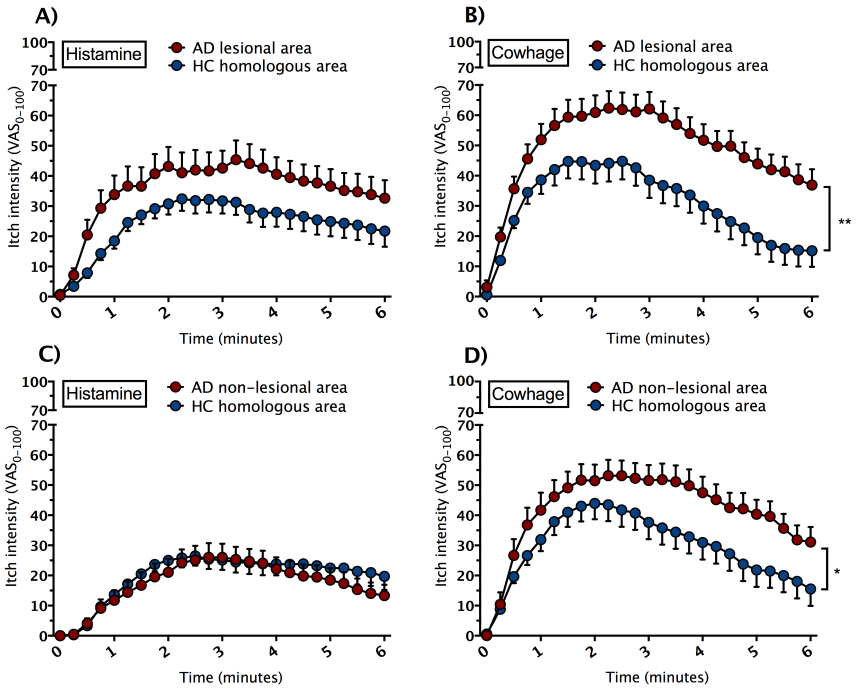

Figure 20. Itch sensitization to cowhage in atopic dermatitis (AD). *Temporal profiles of itch intensity evoked by histamine (**A** and **C**) and cowhage (**B** and **D**) provocations in lesional (**A** and **B**) and non-lesional (**C** and **D**) skin as well as in respective control skin (Study 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 ≤ 0.05, ** = P ≤ 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 [182]. 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 [127,397]. 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 [40,129,350]. 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 [129]. 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 disproportionally small relative to the clinical impact of chronic itch [398–400]. Numerous pioneering mechanistic studies on itch conducted in rodents have recently been published [43,45–48,108,113,120,401–404], 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.

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