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Itch

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TITLE PAGE

i. Title

The many challenges of human experimental itch research

ii. Summary/abstract (1-2 paragraphs)

Itch has long been a neglected sense within somatosensory research, and with good reason: acute itch, although relatively easy to trigger, is notoriously difficult to control experimentally. Its time course and behaviour cannot easily be predicted and participants find it difficult to quantify (and indeed qualify) the sensation. Even scratch behaviour in response to itch is weakly correlated to the amount of itch someone is experiencing. This chapter will focus on the three main methods of acute, experimental itch induction: chemically evoked itch (through histamine and cowhage), mechanically or electrically evoked itch, and psychologically evoked itch (through visual and auditory means). The basic materials and experimental designs will be described along with our personal experiences of trying to study itch using these methods. Itch research is not for the faint-hearted; there are more failures along the road than successes. We do it because itch remains one of the most elusive and fascinating areas of somatosensory research. It can give excruciating pain or intense pleasure with just a single scratch and unlocking its mysteries will help the countless thousands who experience debilitating pruritic skin conditions such as eczema, atopic dermatitis and psoriasis.

iii. Keywords (5-10 keywords)

acute itch, auditory evoked itch (AEI), chemically evoked itch, cowhage, electrical/mechanically evoked itch, experimental induction, histamine, pruritus, psychologically evoked itch, visually evoked itch (VEI)

1. INTRODUCTION

Itch is one of our most basic bodily sensations and serves a vital protective function. Even those who lose their sense of touch through large fibre neuropathy still have the ability to feel itch [1]. The first published studies on human experimental itch research date back to the beginning of the 20th Century [2]. Although relatively easy to trigger (using itching powder - *mucuna pruriens* - or mechanical stimulation with wool), little was known about the underlying mechanisms at the time. Historically, itch was conceived as a form of pain. As early as 1922, von Frey wrote a short paper devoted to the 'problem of pruritus' [3]. In it, he postulated that itch and pain resulted from the same stimulus and were served by the same nerve network. The weaker stimulus led to itch while the stronger stimulus led to pain, and this could be manipulated in a linear way such that increasing the pressure on the stimulus – a plant bristle – led to increasing intensity of sensation up to prickling and even burning. The average delay period between application of the stimulus and itching to occur was 10s, and this delay was necessary for the release of chemicals to trigger the sensation. Scratching or rubbing relieved itch by diluting or removing the stimulating substance released in the skin. While von Frey's original intensity hypothesis has since been replaced by more complex interactive accounts [4], his succinct summary not only provides a description of itch that we would recognise today, but he clearly identifies one of the main challenges of human experimental itch research: namely, the slow time course of the itch sensation to develop from delivery of the stimulus.

In this Chapter we will focus on the many challenges associated with experimentally inducing itch and the variety of different methods for eliciting itch. We focus on those we have had some first-hand experience of including chemically evoked itch (using histamine and cowhage), mechanically evoked itch (using electrical stimuli) and psychologically evoked itch (using visual and/or auditory stimuli). We will discuss some of the best ways to

assess itch and the key methodological challenges of studying acute itch.

2. MATERIALS

2.1 Chemically evoked itch

INSERT TABLE 1 ABOUT HERE

Researchers wishing to study acute itch in humans can choose between a variety of chemical itch induction methods, each with their own set of advantages and disadvantages (see Table 1). A well-established substance in this context is histamine, often employed in the form of 1% histamine dihydrochloride in aqueous solution. Since histamine cannot cross the intact skin barrier, several methods have been developed to deliver histamine to its site of action at the junction of epidermis and dermis, where the terminals of itch-related C-fibres are located [7, 8].

One such method is the histamine prick test, which is well established both as a research tool and as a control stimulus in routine allergy diagnosis. In a histamine prick test, a drop of histamine solution is placed on the target site and the skin is then pricked through the drop using the tip of a sterile lancet. With this method, tiny amounts (1-2µl maximum; [9]) of the histamine solution are delivered into the upper layers of the skin. Due to the construction of the lancet (a 1mm tip followed by a broad shoulder), penetration depth is very limited. After a latency of about 35s, a mosquito-bite like itch sensation starts to develop, peaking around 120s after the onset of the skin prick [10] followed by a slow decay **(Note 4.1)**. Due to the vasodilatory effect of histamine, the area surrounding the skin prick is raised (wheal), and is encircled by an area of reddened skin (flare). Itch, wheal and flare tend to completely subside 30 to 60 minutes after the histamine prick. The

intensity of the itch sensation varies as a function of the histamine dose up to a limit [7] **(Note 4.2)**.

Another method is application of histamine by iontophoresis using an electric current. This typically involves creating a gel by dissolving histamine dihydrochloride in methylcellulose [5, 11]. The gel is then placed into a delivery electrode (the anode) and a larger reference cathode is fixed nearby. Subsequently, a small electrical current is applied which causes the positively charged histamine ions to be driven into the skin by repulsion of the charges from the anode. As with the histamine prick test, itch intensity varies in a dose-dependent manner during iontophoresis by manipulating either the strength of the histamine solution or aspects of the electrical stimulation (i.e., current and length of stimulation; [12]).

Finally, there are some slightly more invasive methods to ensure transepidermal delivery of histamine for an induction of itch. Van de Sand and colleagues [13] aimed for an intense, long-lasting itch stimulus for their research. To achieve this, they slightly abraded the target skin site and then covered these pre-treated sites with a histamine gel. In the past (e.g., [14]), researchers have also injected histamine. However, this approach has been discontinued, partly because injection of histamine tends to elicit a mixture of pain and itch, rather than a pure itch sensation [7].

In addition to the histamine itch pathway, there is also a separate histamine independent itch pathway that has been increasingly better understood during the past decade [15]. This alternative pathway is, for example, stimulated by the tropical plant cowhage, in particular when the tiny hair-like spicules covering the seed pods of the plant become lodged in the skin. Mucunain, the itch-inducing agent of the cowhage spicules, binds to proteinase activated receptors 2/4 (PAR 2/4) in the epidermis [16] and induces itch that

differs from histamine in terms of its local skin reaction (little or no flare for cowhage; [17, 6]), the type of nociceptive C-fibres involved (cowhage: mechanosensitive, histamine: mechanoinsensitive; [18, 19]), and the quality of the itch response (cowhage: more pricking, stinging and burning; [20, 21, 6]). Some authors have argued that PAR2/PAR4-mediated itch may be a more appropriate model for understanding pathological itch than models based on histamine, since antihistamines have limited effectiveness in treating chronic itch [22].

Since mucunain is not yet widely available as a synthesized compound, itch researchers have so far relied on manual insertion of the cowhage spicules to activate the PAR2/PAR4 pathway **(Note 4.3)**. In one technique, a small number of individual spicules are glued to an applicator (e.g., a small cotton stick) so that the spicules protrude from it perpendicularly [20, 21]. The spicules are then inserted at a 30º angle into the skin so that approximately 0.2mm of the tip of the spicule enters the skin. Other research groups place a small number of spicules on the skin and then rub for a period of 30 to 45 seconds. This results in some of the spicules becoming lodged in the skin [23, 22]. Both approaches yield an itch sensation peaking around 1-2 minutes after beginning of the stimulation, followed by a slow decay.

2.2 Electrical/mechanically evoked itch

Electrical or mechanical stimulation has been shown to produce controlled levels of itching in early studies (e.g., [24, 8, 25]). Constant monophasic pulsations of direct positive current at 50Hz and 10ms on/off cycles were passed through non-invasive electrodes placed on the skin and a linear response of itch intensity (as measured by mean response times to detect itching at differing levels of stimulation) was found (e.g., [24]). However, the reproducibility and intensity of the itch sensation was not high. More recent studies have

used highly innovative methods for delivering electrical or mechanical stimulation to produce a more definitive and reproducible itch sensation (see below).

2.2.1 How to create an electrically evoked itch stimulus

Transcutaneous electrical stimulation is typically delivered through a stainless-steel wire attached to the skin controlled by a programmable stimulator such as Digitimer. Saline soaked gauze pads act as the reference electrode (anode). Constant current stimulation of different durations and frequencies are then applied. For example, [26] used durations ranging between 0.08 – 8ms and frequencies between 2 – 200Hz with stimulation applied to the left wrist.

We have also tried transcutaneous electrical nerve stimulation (TENS) to deliver an itch-inducing stimulus. TENS is a method of pain relief involving the use of a mild electric current (frequency 50–100 Hz, pulse width 50–200μs). It is delivered through a battery-operated device that has leads connected to electrode pads through which current is transmitted directly to the skin layers activating A-beta fibres. When the machine is switched on, small electrical impulses are delivered to the affected area of the body, which is often felt as a tingling sensation. It was our hope that we could modify this tingling sensation by increasing the electric current to elicit the sensation of itch in a controlled way; however, this was not the case, and we were unable to produce a reliable itch sensation in our participants who mainly reported just tingling sensations (although previous studies have produced a reliable itch sensation with increasing current, e.g., [25]).

2.2.2 How to create a mechanically evoked itch stimulus

A gentler stimulus is used to create the sensation of itch using mechanical rather than

electrical stimulation. For example, in a study by Fukuoka et al [27] a probe for mechanical stimulation was created. One end of the 30cm probe had an electrically controlled piezoelectric actuator that horizontally vibrated in the range of 0° to 0.2° at a frequency of 1 to 50Hz. This led to the horizontal vibration of a stainless-steel wire loop with amplitude 0 to 1mm at the other end of the probe. The wire loop could touch and vibrate only vellus hairs (i.e., the barely noticeable hair that develops on most of a person's body during childhood) and not the skin surface. The frequency and amplitude were fixed at 8Hz and 1mm for most testing sessions.

2.3 Psychologically evoked itch

In addition to itches that arise due to mechanical or chemical stimulation of the skin it is possible for a non-tactile stimulus to induce or increase itch sensations through psychological suggestion, i.e., with no physical stimulation of the skin. These effects can be created or manipulated in an experimental setting using visual or auditory stimuli **(Note 4.4)**. For example, people typically feel itch sensations, which they want to scratch, when presented with videos, static pictures or sounds of itch-related stimuli like insects or other people scratching. In contrast to chemically or mechanically evoked itch, this type of itch inducing stimulation is non-invasive and no skin manipulation is needed making it the ideal stimulus for investigating experimentally induced itch in both skin healthy controls and those with a pruritic skin condition where stimulation of the skin might worsen their condition.

2.3.1 How to create an itch inducing stimulus set for visually evoked itch (VEI)

We have typically used static images to trigger visually evoked itch in the laboratory. An itch evoking event can take many forms and some events may act as a more potent trigger for some people than others. It therefore helps to first generate a reasonably large set of

well-matched itch and non-itch images and categorise the content of the images as much as possible. Itch content was first considered in [28], which used three image categories: insects (context), insects touching the skin (skin contact), and people scratching the skin (skin response). Skin response was subsequently divided into two separate categories to differentiate between the action of scratching and the result of skin irritation [29], resulting in four image categories:

(i) skin contact: images of itch-related vs. non–itch-related objects in contact with the skin (e.g., insects crawling on the hand vs. marbles touching the skin);
(ii) skin response: images of human responses to itch (i.e., scratching) or non–itch-related touching of the skin (i.e., washing the hands);
(iii) context: images where itch or non-itch stimuli were seen in the environment but not on the body (e.g., ants crawling on the ground or butterflies flying); and
(iv) skin condition: images of hives or freckles

As well as itch and non-itch, our images were also balanced by body part [30]. We have previously used arms and hands, legs and feet, head and neck, and torso [29, 31]. Their inclusion allows for the systematic investigation of body location differences in VEI.

Next, it's important to match the itch and non-itch images as much as possible on attributes such as appearance and composition. For example, brightness and colour saturation should be as similar as possible so that one image is not simply more 'attention grabbing' than another. Similarly, the position of the models, the angle of view and the exact body locations should be as closely matched as possible, and the contents cover approximately the same proportion of both image areas **(Note 4.5)**. As a final step it can be useful to carry out a test survey to ensure itch and non-itch images are matched in

every aspect except how itchy they make people feel [31]. Previously we had simply asked people to rate how stimulating the image appeared to them, using a seven-point scale illustrated with Self-Assessment Manikin pictograms [32], a non-verbal pictorial assessment technique that directly measures the pleasure, arousal, and dominance associated with a person's affective reaction to a wide variety of stimuli, to assist with the interpretation of stimulating.

2.3.2 How to create an itch inducing stimulus set for auditory evoked itch (AEI)

Contagious itch has also been investigated in the auditory modality by [33] who found that scratching sounds, particularly at higher frequencies, led to increased itching in both psoriatic and skin healthy participants compared to rubbing sounds. A particular challenge in auditory evoked itch is to obtain good quality scratching recordings. Due to the very limited loudness of a scratching sound, recordings often have a poor signal to noise ratio. We recommend using professional recording equipment to minimize the influence of background noise.

Similar to the considerations already made for VEI, the auditory experimental items and control items should only differ on the variable of interest (e.g., scratch vs. rubbing as a control sound), but be identical in all other respects. Particularly helpful tools in this respect are Audacity (www.audacityteam.org) for editing and Praat (http://www.fon.hum.uva.nl/praat/) for advanced processing. Both packages are available free of charge. For example, Praat allows matching stimuli for overall loudness, or decomposing sounds into frequency bands [33]. When presenting scratch sounds to the participants, care must be taken to maintain the natural faint loudness of scratching, as too high a volume can lead to a hyper-realistic impression of scratching, reducing the overall validity of the experiment.

2.4 How can we assess itch?

The assessment of itch can take place on at least four different levels: (1) via self-reports, (2) through the observation of behaviour, (3) via physiological correlates in the peripheral nervous system and (4) through measuring neural correlates in the central nervous system.

2.4.1. Self-report measures

Self-report measures have a long-standing tradition in human itch research. For quantitative itch measurements (e.g., of acute itch intensity), there are a variety of available rating scales including classic visual analogue scales, verbal rating scales and hybrid forms (i.e., analogue scales with additional verbal anchor points, [21, 12], **Note 4.6**). There are also instruments available to assess the qualitative aspect of acute itch, such as the adapted version of the Eppendorf Itch Questionnaire [20].

2.4.2. Behavioural observation

Given that itch is usually defined as an unpleasant behaviour associated with the urge to scratch, one possibility to assess the level of itch an organism is currently experiencing is to count the number of spontaneous scratching movements they show in response to pruritic stimulation. While such observational measurements are widely used in non-human itch research, they have only recently found more widespread adoption in studies involving human participants [e.g., 30, 28, 29]. Analysis of the frequency of spontaneous scratching can provide insights into the intensity of the itch experience but can also be used to determine which aspects of an observed scratching behaviour are spontaneously produced by a participant [34, 35]. It is important, though, that researchers make clear *a priori* coding rules about which behaviour to code (e.g., to distinguish scratching from

habitual self-touches). It is also highly desirable to make video recordings of the behaviour, as opposed to on-the-fly coding of behaviour, to enable analysis of inter-rater agreement and demonstrate reliability of coding decisions [36].

2.4.3. Physiological correlates

Studying physiological correlates of itch in the peripheral nervous system has been a particularly productive approach in understanding the neural fibres involved in itch processing. While most of this work has been carried out with animals, some methods are also applicable for research involving human participants. One of the most direct ways to quantify the neural signals causally contributing to itch in humans is to obtain extracellular recordings of single C-fibres via microneurography [37]. In this approach, thin tungsten needle electrodes are placed inside nerve fascicles, which enable the recording of a single-unit discharge from myelinated and unmyelinated fibres. This has allowed researchers to demonstrate separate peripheral pathways for cowhage and histamine induced itch in humans [19].

The skin reactions following histamine application (either via skin prick or iontophoresis) provide another physiological correlate of itch in the peripheral nervous system. Skin reactions to histamine are known as a triple response: First, an initial and faint localized transient skin reddening (i.e., initial local vasodilation), followed by a wheal surrounded by a flare. A wheal is a vascular leakage response to histamine, observed as a raised, often pale and circumscribed dermal oedema whereas a flare is an area of reddened skin, reflecting increased superficial perfusion following an axon reflex [38]. The size of wheal and flare can easily be quantified, and serve as an objective indicator that itch induction has been successful. Flare size shows a moderate correlation with subjective itch intensity, at least when histamine is applied via the prick test [7]. The changes in blood

perfusion following histamine application can also be continuously monitored using laser doppler flowmetry [39]. The application of cowhage, in contrast, does not result in an axon flare reflex [6]; therefore, no wheal or flares are measurable for this type of itch.

2.4.4. Central nervous system correlates

Finally, itch can be assessed through measuring neural correlates in the central nervous system. The brain network involved in the processing of acute itch (for review, see [40, 41]) consists of contralateral somatosensory cortices (S1 and S2), bilateral supplementary motor area, insula and anterior cingulate cortices (ACC) as well as ipsilateral inferior frontal gyrus (IFG). It has been argued that functional specialization exists within this cortical network, with a sensory-discriminative role for somatosensory cortices, in particular S1. Consistent with this idea, neural activation in this area varies as a function of histamine concentration [42] and perceived itch intensity is reduced when inhibitory brain stimulation is applied to S1 [40, 43] **(Note 4.7.)**.

3. METHODS

3.1 Chemically evoked itch

The focus here will be on those chemical induction methods for which we have the most experience, that is, the histamine prick test and the cowhage rubbing method.

3.1.1 Histamine Prick Test

3.1.1.1 Preparation

The histamine vials should be stored in a fridge when not in use and be regularly checked for expiration dates. On the day of testing, they should be taken into the testing room well in advance of the test so that the solution is warmed up to room temperature.

3.1.1.2 Induction

It is helpful to show the lancet to the participant before the actual prick test, in particular, to point out that due to the construction of the lancet (small tip, followed by a broad shoulder), it is impossible to penetrate deep into the skin. In our studies, we have used the volar aspect of the forearm as the target site, about 3cm proximal to the wrist crease. The target site does not require any preparation, but should be clean and free from wounds, rashes or other impairments of the skin barrier. Participants should be instructed to keep their arms still and should aim to keep their arm sufficiently supinated so that that the target area is level (to avoid the solution running off to the side). Following this, the applicator is taken out of the vial, ensuring that no air bubbles are contained. The experimenter then places a single drop of the solution on the forearm of the participant. It is important to not touch the forearm of the participant with the tip of the applicator while doing so, otherwise the solution in the vial will no longer be sterile. Further advice on standards and safety guidelines for the histamine prick are given in [44]. The experimenter then removes a new sterile lancet from the packaging, taking care to not touch the tip of the lancet. They then prick the skin through the drop in one swift but gentle motion. A small red dot at the end of the trial at the application site (indicating capillary bleeding) usually indicates that too much force was used. Presence of a wheal and flare can be used as visual confirmation that the prick test has been successful. The used lancet should be disposed of immediately into a sharp’s disposal container, and the drop of histamine wiped off.

3.1.1.3 Data collection

Following the prick test data collection can be begin, for example, by asking participants for itch intensity ratings. While the individual time course of a histamine trial can vary greatly from one person to the next, most participants experience at least 5 minutes of itch following histamine prick. If the skin reaction is mapped, this should occur at a fixed

interval (e.g., 10 minutes after skin prick onset).

3.1.1.4 Tips & Tricks for the histamine prick test

The histamine prick test is a good way to get started with chemically induced itch research since it does not require preparation of substances. The fact that wheal and flare give visual feedback about whether a trial has been successful is very useful for novice experimenters, as it allows one to repeat failed trials (when no wheal or flare are visible). It should be noted though that flares can be difficult to demarcate in darker skin types. When multiple pricks are performed in a single experimental session, great care should be taken to leave sufficient time (at least 30 minutes) between trials to minimize carry-over effects. The prick test can be administered in a double-blind fashion by having an external person tape a random code over the labels of vials with either histamine or control solution.

3.1.2 Cowhage

3.1.2.1 Preparation

Before the experimental session, the researcher needs to count out a dose of spicules (40 – 50) using tweezers and, if required, a magnifying glass. In our lab, we have placed these doses into small, folded pieces of paper, which are then secured with a Biro clip in advance of testing.

3.1.2.2 Induction

The target site (volar aspect of forearm) is first demarcated by creating a rectangular shape using cellulose tape, with an edge length of 4cm. The experimenter then places a dose of cowhage into the centre of the target area. Subsequently, the experimenter rubs the cowhage spicules into the skin of the participant in small circular movements for 45s. Throughout the induction period, it is important to remind the participant to keep the

forearm sufficiently supinated to avoid the spicules falling off to the side.

3.1.2.3 Data collection

After the end of the induction period, data collection can begin, for example, in the form of repeated ratings (see histamine). At the end of the data collection period, any spicules can be removed from the arm using scotch tape.

3.1.2.4 Tips & Tricks

We have good experience with wiping the target area using a plain cotton cloth at the end of the trial, which helps to soothe irritated skin. As is the case with histamine, it is important to leave enough time between individual cowhage trials (at least 30 minutes) to avoid carry-over effects. A placebo control for cowhage can be achieved by autoclaving the spicules, which inactivates the itch eliciting protease mucunain. If one does not have access to an autoclave device, the same effect can also be achieved by steaming the spicules in a pressure cooker. Since a single stray spicule can elicit itch, great care must be taken in the lab to remove all stray cowhage spicules at the end of each experimental session.

3.2 Electrical or mechanically evoked itch

3.2.1 Basic paradigms for electrically evoked itch

Historically, electrical stimulation was seen as by far the most controllable means of eliciting itch. Electrical stimulation offered the promise of a terminable sensation of itch linearly related to the amount of stimulation, unlike histamine or other chemical inducers, which can be highly variable. Electrically evoked itch has been delivered by insertion of a copper wire into the skin, or via wires or electric plates placed on the skin (using standard metal electrocardiography plate electrodes; [24, 8, 25]). However, the findings of these

studies were difficult to replicate, likely because of the differing methods used, and the itch sensation produced could last anywhere between 1 and 12 minutes, preventing multiple trials.

More recently, Ikoma and colleagues [26] have systematically studied electrically evoked itch using transcutaneous electrical stimulation, which allowed multiple repetitions of trials. For example, in their basic experiment, 50 pulses with 2ms duration at a frequency of 50Hz were applied to the left wrist over 30s. This produced a pure itch sensation in 80% of those tested with a delay between stimulation and sensation of 1s. Electrical stimulation was shown to be most effective for pulse durations of more than 2ms and frequencies of more than 50Hz. They then demonstrated that itch intensity increased in a linear fashion with increasing pulse duration or frequency. In one experiment, pulse durations varied between 0.08 and 8ms (0.08, 2, 4, and 8ms) with a fixed frequency of 50Hz, whilst in another experiment, stimulus frequencies varied between 2 and 200Hz (2, 10, 20, 50, 100 and 200Hz) with a fixed pulse duration of 2ms. These permutations were applied in a random order at intervals of 30s. Each test stimulus was compared with a reference stimulus (50Hz, 2ms pulses) and participants rated itch intensity relative to the initial reference stimulus on a 10-point numerical rating scale (NRS). In addition to relative itch intensity, the extent of alloknesis (i.e., itch evoked by light brushing) and hyperknesis (i.e., itch evoked by pricking) were tested by application of a cotton bud or pinprick to the tested area of skin, respectively.

3.2.2 Basic paradigms for mechanically evoked itch

In the study by Fukuoka et al [27] mechanical stimulation was applied to the vellus hairs of the face (chin, cheek and forehead) and the arm (midpoint between the wrist and elbow on the volar aspect of the forearm). Light touching with a cotton swab acted as the control.

Itch intensity on a 10-point NRS was measured (using the fingers if the face was the stimulation site) and the maximum intensity of itch during a 90s period was assessed. Participants were also asked if they wanted to scratch the site and whether it had the following characteristics: crawling, tickling, stinging, burning, stabbing and pricking.

3.3 Psychologically evoked itch

One of the earliest methods for inducing itch through psychological means was via a short lecture on itch [45]. Unsurprisingly, the audience scratched significantly more during the itch lecture than during a subsequent lecture on relaxation, and itch ratings were highest after the itch lecture for both participants with healthy skin and those with self-reported skin conditions. More recent studies have sought to separate the relative contributions of vision and sound on psychologically evoked itch (see below). The effectiveness of psychological methods of itch induction can be further enhanced via attentional and expectancy-based manipulations [46].

3.3.1 Basic paradigms for VEI

Itch can be elicited by purely visual means using either static images or video presentation of moving images. Stimulus presentation followed by an itch rating is the most basic paradigm, to which further measures can be added.

3.3.1.1 Static images

In our previous studies we have typically grouped stimuli into blocks of 4 trials that are either all itch or all non-itch images, with image content and body part represented equally. Blocks, and the trials within those blocks, are then presented randomly. Practice trials can be used to familiarise participants with the task followed by 64 experimental trials with no repetition [e.g., 31]. Each image is on screen for 8s, after which participants are presented

with a simple VAS scale showing a horizontal line running left to right from 1 (not itchy at all) to 9 (very itchy) on which participants rate how itchy they feel *at that moment*. Although we tend to use a mouse button to move a cursor along the scale, you can also use the left/right arrow keys on the keyboard or simply type the number from 1 – 9 to record the rating. A further adaptation to the basic paradigm is to determine the location of any itches felt. A subsequent screen can be used to display two body outlines (based on the McGill Pain Questionnaire) showing the front and back to enable participants to click on the body where they feel itchy. These are marked with a red circle after each click and then categorised by body location (arm, leg, head, torso) along with the trial in which they occurred.

The itch rating forms one part of the outcome measure for VEI paradigms. The second part is formed by the observation and recording of participants' scratching behaviour. This is much trickier, and the method for identifying what constitutes a scratch can vary across different studies, so it's important to have a systematic approach and be consistent. We have previously used the following criteria to determine what actions are recorded as a scratch: Scraping the skin surface with fingernails and/or rubbing either the skin directly or clothing against the skin in a way that causes friction. Actions not recorded as a scratch include rubbing that would simply move the skin or massage the underlying tissue and tucking back hair or adjusting clothing. If only the sum total of scratches is being recorded (i.e., with no details about the location or duration of the scratch) then we have typically had the experimenter record these in real time during the experiment. The experimenter is in the room with the participant sitting at a distance and out of the participant's eye-line but still able to clearly see if the participant is scratching. If the location and/or duration of the scratch is being measured, then it makes sense to record scratch behaviour using a webcam positioned so that the participant cannot see it directly (as it's important not to

disrupt the participant's natural scratch behaviour) but the experimenter can still see all the participant's body. Understandably, this is a very tricky thing to control. For the purposes of ethical approval, you need to be explicit that you will be monitoring people's behaviour either directly or through recording their scratch behaviour on video, as they need to consent to this. However, in doing so you also draw attention to the fact that their scratch behaviour is being monitored, and this may cause some people to suppress or amplify their normal scratch behaviour **(Note 4.7.2)**.

In addition to recording how itchy the participant feels, where on the body they feel itchy and the number and location of any scratches, as a final outcome measure, we have previously asked participants to rate how itchy they thought the person in the picture felt [28, 29]. These results correlated with one another, indicating that empathy with another person's itch may be influential in creating the VEI effect.

3.3.1.2. Moving images

Further evidence that VEI may be an empathic experience based on simulating the bodily experience of others comes from [30]. In the first study to use functional magnetic resonance imaging (fMRI) to identify the neural correlates of contagious itch, participants were shown 20s video clips of bodily scratching or a control condition (Fig 1). The bodily scratching movement consisted of continuous scraping of the left forearm, left upper arm, chest, right forearm or right upper arm, using four curled fingers of one hand. The control condition was continuous tapping of one of these locations. One male and one female model were filmed with only the waist to the neck visible. The stimulus set consisted of 20 videos in total (2 conditions, scratch vs. no scratch x 5 body locations x 2 models). The experiment used a blocked design. Each block consisted of one 20s video, followed by a fixation cross presented for 3.3s. Next, participants were asked to rate the intensity of

itchiness induced by the video using a button press on a scale from 0 (not at all) to 7 (extremely). An additional verbal label ‘moderately’ was placed at the mid-point of the scale. This screen was followed by the fixation cross again before the start of the next block. One experimental run consisted of 20 blocks, and participants completed 4 experimental runs during the fMRI part of the study.

INSERT FIGURE 1 ABOUT HERE

Viewing scratching activated the ‘itch matrix’ in a way similar to when people experience actual bodily itch sensations. The itch matrix includes the anterior insula, primary somatosensory, prefrontal and premotor cortices. These areas are associated with mirroring and simulation of actions (e.g., premotor cortex), sensory aspects of itch (e.g., S1) and top-down predictions of interoceptive signals, which may enable simulation of the feeling of itching (e.g., anterior insula).

Other researchers have also used videos of people scratching to induce itch in participants with atopic dermatitis (AD, e.g., [47]). Interestingly, they found activation of the supplementary motor area, left ventral striatum and right orbitofrontal cortex, areas of the frontostriatal circuit, which is associated with the urge to scratch. Participants would have to strongly suppress scratch behaviour whilst in the MRI scanner to prevent distortion of the image from movement artefacts. This is a particular problem for MRI studies of itch and especially those studies in populations with pruritic skin conditions. Previous studies have demonstrated that a combination of watching videos of people scratching while participants received either histamine or saline administration caused self-reported itch intensity to increase in all participants. This resulted in a doubling of spontaneous scratching episodes in those with AD, who also appeared to scratch a more widespread

area for longer [22].

3.3.2 Basic paradigms for AEI

3.3.2.1 Auditory only

Auditory evoked itch can be investigated using either a categorical (e.g., scratch sounds vs. control sounds) or a parametric manipulation (e.g., effect of linear increase of scratch loudness). The study by [33] realized both experimental manipulations by presenting scratching sounds (as experimental items) in addition to rubbing sounds, which served as a control condition. Furthermore, the amplitude of high frequency sounds (i.e., frequencies above 1000Hz) were either attenuated, enhanced or unchanged. The effect of this amplitude manipulation was that the sounds either had an edge (in the case of enhanced high frequency amplitude) or a slightly muffled quality (in the case of attenuated high frequency amplitude). These sounds were presented to a group of patients with psoriasis as well as a group of healthy controls who were asked to rate the amount of induced itch. Results showed that, in healthy controls, scratching sounds induced greater itch than rubbing sounds. Additionally, the magnitude of itch was found to vary as a function of the high frequency amplitude. Finally, there was a group by amplitude interaction, with patients showing greater vulnerability to the high frequency enhanced sounds relative to controls.

In summary, we hope to have laid bare some of the challenges of conducting itch research but it was not our intention to dissuade anyone from undertaking this type of research! If basic science is to be of any benefit to society it needs scientists to take up the challenge and translate the findings from studies of acute experimental itch into effective clinical interventions to provide a better understanding of pruritic disease states and how best to treat them.

4. NOTES

4.1. Slowness of itch

Probably the biggest methodological challenge, at least when chemical itch-induction methods are used, is the inherent slowness of itch. The sluggish nature of the itch response, where one trial lasts at least 5 minutes followed by at least 20 minutes of waiting before another trial can commence, has a number of adverse consequences for researchers. First, the length of time makes it impossible to determine individual sensory thresholds for itch, as is standard procedure in other sensory research domains (e.g., pain – see Valentini et al., this volume). Because the pruritic dose cannot be adjusted to a person's threshold, the same dose (e.g., 1% histamine) elicits an itch response with considerable inter-individual variation, with some participants experiencing only a very mild itch that has completely subsided after 3 minutes, whereas others experience an intense itch lasting 15 minutes or longer. A second direct consequence of the slowness is that it severely limits the number of data points available for analysis. The combination of both factors results in considerable random unexplained variation in each itch study, which severely limits the power of the statistical analysis.

4.2. When itch becomes pain

Drezga et al [42] used different concentrations of histamine solution to determine the relationship between dose concentration and itch intensity. Up to a level of 1% concentration, they observed a linear and positive relationship between dose and itch intensity. However, at very high concentrations, or when injected directly into the skin, other authors have reported that histamine tends to induce pain rather than itch (e.g., [7]).

4.3. Dose variability of cowhage

Induction methods based on cowhage face an additional random source of variation since

a recombinant version of the itch-inducing protease mucunain is not yet commercially available. The amount of mucunain in a cowhage spicule varies depending on the origin of the plant [48]; furthermore, only a fraction of the number of spicules applied to the skin become lodged into the skin and subsequently start to induce itch [34]. Thus, unlike in the histamine prick test, where the researcher can have a relatively good estimate of how much of the pruritogen went beyond the skin barrier [9], this is unknown for cowhage. Development of a commercially available recombinant mucunain would be big step forward here [48].

4.4. Technical aspects of producing auditory and visual itch stimuli

From our experience of auditory itch, it can be a technical challenge to make good sound recordings of scratching sounds and there were some surprising insights into which sounds are most itch inducing. Similarly, it is also very challenging to produce a well-controlled stimulus set for VEI and several factors such as the novelty, arousing, or unpleasant nature of the images needs to be accounted for.

4.5. Controlling the visual stimulus

You may also have to pixelate the model's face to remove cues to emotional reactions.

4.6. Prior itch experience and choice of rating scales

For studies that rely on itch intensity ratings as a major outcome variable, there are some important considerations regarding prior itch experience of participants as well as the choice and implementation of the rating scale to be used. Itch ratings rely on verbal anchor points to guide participants in their use of the rating space. For example, many itch studies use a visual analogue scale where the top end of the scale is labelled 'worst itch imaginable'. However, healthy volunteers coming into the lab to take part in a study may

not have had previous intense itch experiences. For example, we have found in pilot studies that many UK students have never experienced a mosquito bite. This lack of previous experiences makes it impossible to use an itch rating scale in a meaningful way because the verbal anchor points cannot serve their purpose as a reference point. To mitigate this problem, we recommend additional time be spent at the beginning of a session to familiarize the participant with the rating scale, including rating of familiar events (e.g., itchy scalp, mosquito bite, wound itch). If the paradigm involves chemically induced itch, it is useful to have participants experience and rate an initial familiarization stimulus before data collection begins [49, 50]. For studies focussing on chemically induced itch, we recommend hybrid scales, such as the General Labelled Magnitude Scale [21], since they provide more reliable itch intensity estimates than classic visual analogue scales [49]. If participants are asked to make repeated ratings (e.g., over the course of a whole itch trial) then care should be taken to ensure that the ratings are independent of each other. In particular, participants should not be able to see previous ratings as this increases the risk that participants only provide ordinal level data (e.g., has itch intensity increased or has it decreased relative to the last rating provided?) as opposed to the intended ratio level data. This is an issue, for example, when continuous rating devices are used (e.g., continuous computerized visual analogue scales, CoVAS).

4.7. Other factors

There may be several other factors that contribute to how a person experiences itch:

4.7.1. External environment

For example, testing in a cold room dulls the sensation whereas heat increases it. Ideally, the temperature and humidity of the testing room should be recorded.

4.7.2. Demand characteristics

Unfortunately, simply asking people about itch can often make them itchy by focussing attention on sub-perceptual sensations and thereby bringing them into conscious awareness. This may be particularly important in those with an on-going pruritic skin condition [29], although there is evidence to suggest individual differences in a non-pruritic person's capacity to experience itch (the so-called 'itchish' person; [51]). Tomasch and colleagues [51] showed an increased ratio of small C-fibres to large A-fibres (3-12 times as many) in those people who rate itch more intensely. Obviously, evidence of an existing pruritic skin condition should be checked during recruitment. Finally, it is sometimes difficult to differentiate self-touch from scratch in video recordings, and participants may produce more self-touch because they feel uncomfortable rather than a genuine itch sensation that prompts the behaviour.

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FIGURE CAPTIONS

Fig 1. A selection of images taken from [30]. A: Upper arm scratch (left) and control touch (right). B: Chest scratch (left) and control touch (right). C: Lower arm scratch (left) and control touch (right)

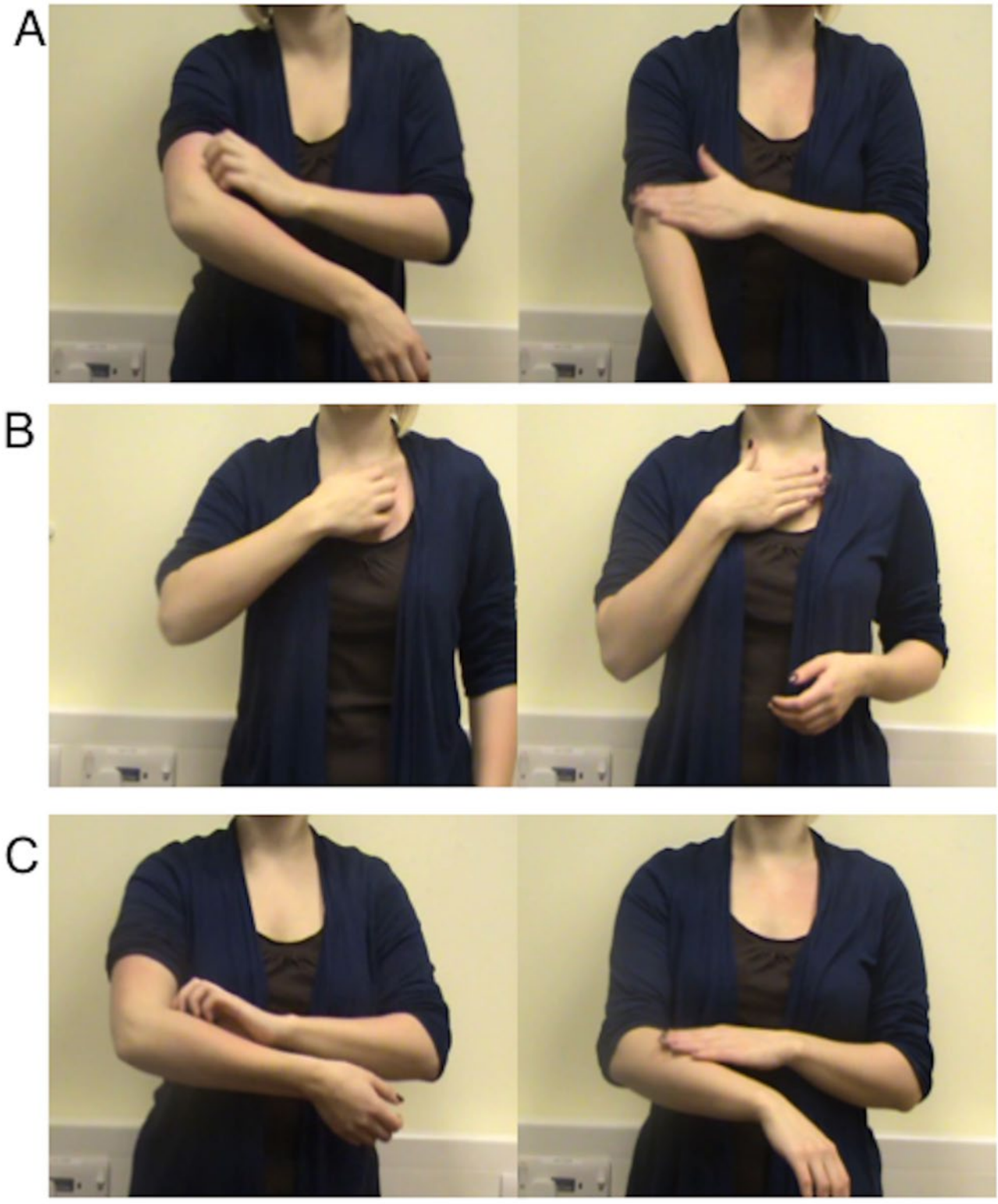
A
B
C

TABLE CAPTIONS

Table 1: Overview of materials required for induction and assessment of chemically induced itch and their respective advantages and disadvantages

Method	Required materials	Advantages (+) and Disadvantages (-)
Histamine prick test	• Histamine vials (concentrations of 1%, 0.1%, and 0.01%) • Control vials (saline solution) • Skin prick lancets ○ (all available from Allergy Therapeutics, Worthing, UK)	+ simple, does not require any special equipment + histamine solutions can be bought ready-made + allows assessment of skin response - limited options for manipulating dose intensity
Histamine iontophoresis	• Histamine gel (e.g., 1% histamine dissolved in 2.5% methylcellulose), needs to be created on site either by a lab or a pharmacist • Iontophoresis device • Delivery and dispersive electrodes (both device and electrodes are available, for example from Perimed Instruments, Järfälla, Sweden)	+ more degrees of freedom (dose, infusion time, current) for manipulating dose intensity than prick test method + allows assessment of skin response - requires special equipment - solutions need to be prepared on site
Cowhage (rubbing method)	• Cowhage spicules (available upon request from Dr Ethan Lerner, Massachusetts General Hospital, or from Zandu Pharmaceuticals,	+ simple method to stimulate non-histaminergic pathway + no special equipment required

	Mumbai, India) • Scotch tape to demarcate target area • Cotton cloth (to remove stray spicules and reduce skin irritation)	- limited control over dose intensity - cowhage-induced itch does not produce measurable skin reactions or changes in blood perfusion
Cowhage (spicule insertion method)	• Cowhage spicules (see above) • Cotton tab (as applicator) • Glue (to attach spicules to applicator)	+ allows more control over dose intensity than rubbing method + no special equipment required - cowhage-induced itch does not produce measurable skin reactions or changes in blood perfusion
Assessment of skin reaction (wheal & flare)	• Translucent paper and pen [5] • Or alternatively, use kajal eyeliner to draw outline directly on skin, follow by digital photograph (with ruler added for scale, see [6])	
Assessment of blood perfusion	• Laser Doppler Flowmetry (available, for example from Moor Instruments)	