Exploring the Process of Itch and its Dimensionality: Investigations Using Transcranial Magnetic Stimulation

Olivia Hollie Jones

PhD

University of Hull
Department of Psychology
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Abstract

This thesis explored three main areas of acute itch: firstly, how to reliably measure it; secondly, whether it is of a multi-dimensional nature, and lastly, which brain regions are crucial in the process. Chapter 2 reports an experiment that directly compared the re-test reliability of three commonly used measurement scales (pVAS, tVAS and gLMS). The general Labelled Magnitude Scale (gLMS) generated the least variance in itch intensity ratings between testing sessions and was therefore taken as the most reliable and administered for the following experiments. Chapter 3 and 4 explored the dimensionality of histamine and cowhage induced itch. The aims of these chapters were, (1) to explore any changes in the time-course and peak of itch intensity/unpleasantness, induced by varying stimuli doses, (2) to examine any dissociation between itch intensity and unpleasantness, which would indicate that they are dissociable dimensions. The results demonstrated that there was a significant linear trend for both intensity and unpleasantness, however there was no significant difference between the dimensions. Based on these results, it was decided that only the intensity should be measured in the following transcranial magnetic stimulation (TMS) experiment, as the unpleasantness dimension did not appear to add any additional information. Chapter 5 describes a TMS study, investigating which brain areas have a necessary function in the process of histamine and cowhage induced itch. The aim was to explore any differences in the perceived itch intensity, after brain stimulation to the somatosensory cortices (S1 and S2) and the inferor frontal gyrus (IFG), in comparison to the control area (superior parietal lobe; SPL). The results demonstrated that only TMS to S1 significantly reduced the itch intensity when administered via the histamine prick test. There was also a significant reduction of the wheal induced in the S1 and IFG condition. There was however, no significant reduction of the flare for any condition. There was also no significant difference in itch intensity or skin response, for any of the brain regions stimulated when cowhage was administered. In summary, the results indicate that S1 has a crucial role in the processing of itch intensity, and that the histamine prick test and TMS are ideal for exploring this. More investigation is necessary however, to explore the role of S2 and the IFG in itch perception.

Contents

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Declaration

This thesis comprises the candidate's own original work and has not, whether in the same or different form, been submitted to this or any other University for a degree. Selected aspects of the research described in this thesis have been presented elsewhere.

Chapter 1 Literature Review

1.1 What is Itch?

Itch is a complex and largely subjective experience which was defined by the German physician Samuel Hafenreffer more than 340 years ago, as 'an unpleasant sensation provoking the desire to scratch' (Rost, 1953). It is an effective sensation used to protect the skin from various potential hazards. For instance, scratching an insect bite enables the victim to remove the ant or mosquito before it can leave further toxins.

It was originally argued that itch was solely induced by low-intensity stimulation of nociceptors which mediate pain sensation when the intensity is increased (Bishop, 1943; Lewis & Zotterman, 1927; Von Frey, 1922). This therefore promoted the long-held belief that itch was simply a milder form of pain and that if the pain intensity was reduced enough, it would become an itch sensation. This in turn held back the progress of itch research.

It was not until 1997 that researchers discovered the first itch-specific nerve fibres, which responded preferentially to histamine and were not sensitive to pain (Schmelz, Schmidt, Bickel, Handwerker, & Torebjörk, 1997). They discovered this using a microneurography technique, which does not rely on mechanical excitation of nerve endings. This technique detects C-units through electrical search stimuli, and their activation via histamine, heat, mechanical, or other types of stimulation (Schmelz et al., 1995). Out of the 56 units that were tested, only 8 responded to histamine iontophoresis (1 mA, 20 sec) which induced itch sensations which lasted several minutes. They therefore concluded that these C-fibres represented a new class of afferent nerve fibres with particularly thin axons but excessive terminal branching. These fibres have exceptionally low conduction velocities and are also insensitive to mechanical stimuli, which potentially explains why they have been missed in the past. It is therefore only within the past 20 years that this original theory of itch has been rejected due to numerous contradictory findings and itch has been viewed as a sensation which should be studied in its own right.

1.1.1 Definition: Acute Versus Chronic Itch

Despite the fact that acute itch is a daily experience generally relieved by briefly scratching the area, in its chronic form (pruritus lasting six weeks or more; Ständer et al., 2007) it can be debilitating (Davidson & Giesler, 2010). Acute itch is elicited by injury or inflammation of body tissue and activation of pruriceptive fibres at the site of local tissue damage (Yosipovitch, Greaves, Fleischer, & McGlone, 2004). Individuals can still suffer from a skin disorder such as eczema but it does not necessarily mean it is chronic, as the itch sensation

can stop long before healing has completed. Chronic itch is of a multidimensional nature involving various components with regards to the processing of sensory, cognitive and motivational information, as will be explained below. This makes the experience of itch difficult to quantify. However, in comparison to acute itch, chronic itch differs in that therapies which provide immediate itch relief do not resolve the underlying pathological process and the itch will remain post-treatment**.**

1.2 Why is Itch a Relevant Area of Study?

The topic of itch despite its importance has been relatively neglected, especially in comparison to pain research. With regards to sensory information, it is claimed that 'pain is king, and itch is the court jester' (Sanders & Guip, 2008, p.16). Itch research has therefore significantly lagged behind, leaving many gaps in the literature and patients suffering with a range of conditions which doctors are unable to treat. In addition to this, research has demonstrated that there is a low concordance in diagnoses between general practitioners (GPs) and dermatologists across a whole spectrum of skin conditions, which indicates that GPs are often undertrained within the area (Moreno, Tran, Chia, Lim, & Shumack, 2007). This in turn results in misdiagnoses and a lack of adequate treatment. This in turn has a great impact upon sufferers, as there is an estimated 17 million chronic itch patients in America (Sanders & Guip, 2008) and one in four people will experience chronic itch during their lifetime (Matterne, Apfelbacher, Vogelgsang, Loerbroks, & Weisshaar, 2013).

Chronic disorders of the skin involving intense itching, such as atopic eczema, have become more common in recent decades, affecting up to 20% of children (Kalliomäki et al., 2001) and 17% of adults (Ständer & Luger, 2010). It is estimated by a range of research groups that between 83 - 87% of patients with atopic dermatitis (AD) reported daily itch (Chrostowska-Plak, Salomon, Reich, & Szepietowski, 2009; Yosipovitch et al., 2002), along with 64 - 85% of psoriasis sufferers (Prignano, Ricceri, Pescitelli, & Lotti, 2009; Sampogna et al., 2004; Yosipovitch, Goon, Wee, Chan, & Goh, 2000).

Disorders of this nature profoundly impact quality of life and the long term discomfort caused can be debilitating. Conditions such as this where itch (pruritus) is a dominating symptom can have adverse consequences, including sleep deprivation, and significant contributions to psychosocial morbidity such as depression, anxiety, poor self-esteem and difficulty concentrating (Yosipovitch & Papoiu, 2008). It can also leave sufferers in physical pain if the individual continues to scratch the area causing excoriation (graze or tear of the skin surface).

There are also a range of conditions which many do not spontaneously associate with itch. It is estimated for instance, that 73% of burn victims suffer pruritus two years post-injury and that 87% of burn survivors experience itch on a daily basis (Parnell, Nedelec, Rachelska, & LaSalle, 2012). Other conditions such as liver failure, multiple sclerosis, HIV and late-state cancer often result in itch symptoms and although painkillers are crucial for many patients they often replace pain with severe additional itch. This reality therefore highlights the importance of the progression of research into pruritus not only for victims of skin disorders, but a range of diseases and conditions.

It is also necessary to acknowledge the strain that the lack of chronic itch treatments has financially on the economy, as although the exact costs of chronic itch has not been yet been estimated, we can think about the estimated cost of $3> billion per year as a result of atopic dermatitis alone (National Institute of Arthritis and Musculoskeletal and Skin Diseased; NIAMS) and reliably assume that the economic cost of chronic itch is likely to be much higher due to the incidence of chronic itch under many different conditions (Akiyama & Carstens, 2013).

The progression of itch research therefore holds great significance for a range of individuals and their quality of life depends on it. While relatively good progress has been made in identifying itch-specific receptors and peripheral pathways (Schmelz, 2002), surprisingly little is known about the processing of itch in the central nervous system (for review, see Ikoma et al., 2006). There is currently no universally accepted gold standard therapy for itch (Patel & Yosipovitch, 2010) and despite the fact that there are topical and systemic antipruritic drugs available, the fact still remains that adequate treatment is limited due to the current lack of knowledge about the mechanisms underlying itch.

1.3 How Do We Reliably Measure Itch?

The accurate measurement of itch intensity is key to evaluating its severity and in turn, the therapeutic outcome of chronic itch patients (Furue et al., 2013). The development of a reliable pruritus measurement however is difficult due to its subjective nature and currently there is no single method recognised as gold standard. It is recommended that at least two independent methods (e.g., visual analogue scales and questionnaires) are necessary in order to reliably assess itch intensity in clinical studies (Reich & Szepietowski, 2013).

According to Furue et al. (2013), members of the Japanese Society Dermatoallergology and Contact Dermatitis (JSDACD) discussed core items for evaluating pruritus in clinical

settings. Nine items associated with chronic pruritus (e.g., sleep disturbance, itch frequency and itch site) were proposed and evaluated with regards to their importance. The itch intensity was the highest ranked. Despite the increasing recognition of its importance however, there is still no absolute consensus of which measurement tools should be employed and validation of these instruments in chronic pruritus is still ongoing (Phan et al., 2012).

1.3.1 The Effective Use of Visual Analogue Scales

Various types of rating scales have been administered and evaluated in the study of clinical itch. One of the most popular measurement scales used is the Visual Analogue Scale (VAS), as it provides a simple and rapid estimation of itch (Phan et al., 2012; Reich et al., 2012). The pure VAS (pVAS) ranges from 0 (no itch) to 100 (the most intense itch imaginable) and the participant is asked to place a mark on the line that corresponds to the intensity of the pain he or she is experiencing.

Other researchers however, add an additional 'Scratch Threshold' set at 33% of the scale (tVAS; Darsow, Ring, Scharein, & Bromm, 1996; Magerl & Handwerker, 1988) and participants are instructed that above this threshold they would feel the impulse to scratch.

The VAS was originally developed to assess pain and evaluations of the scale within the field have been mixed. Bijur, Silver, & Gallagher (2001) claimed that the pVAS is sufficiently reliable as an assessment of acute pain, whereas other researchers have argued that the lack of verbal anchors creates ambiguity, because individuals are unsure where exactly they should place their mark (González-Fernández et al., 2014; Kersten, Küçükdeveci, & Tennant, 2012).

It has also been found that patients are more prone to making mistakes when using it, in comparison to other scales. For example, in a study by Peters, Patijn, & Lamé (2007) patients were asked to assess their weakest, strongest and average pain in the last week. If the scale is interpreted correctly, it is expected that the strongest pain would be rated as stronger than (or at least equal to) average pain, and average pain as stronger than (or equal to) their weakest pain. This however, was not always the case and it was concluded that patients using the pVAS were more susceptible to making these types of mistakes, which indicated ordinal misunderstanding of the scale.

Phan et al. (2012) also analysed and compared the reliability of the VAS, along with the numerical rating scale (NRS) and the verbal rating scale (VRS). The NRS usually consists of a series of numbers ranging from, for example, 0 to 10 and the ends of the scale are labelled to indicate "no pain" and the "worst pain possible." The participant chooses the number that

best corresponds to the level of pain he or she is experiencing. The VRS however, consists of a series of words commonly used to describe pain (e.g., no pain, mild pain, moderate pain, severe pain). The participant reads the words and chooses the one that best describes the pain they are experiencing. A score (e.g., from 0–3) that is assigned to each word is then used to measure pain levels. The results demonstrated a high test re-test reliability of all three scale types (r = 0.74 - 0.80), when the rating interval duration was one hour. Similarly, a study by Reich et al. (2012), who also completed a test-retest comparison of the aforementioned three scales but administering a 3-hour interval, found that all scales showed very good reproducibility and concluded that the VAS is a valuable method of pruritus measurement (r = 0.87). They also found no significant differences between the horizontal and vertical presentation format of the VAS.

Despite supporting the value of using the VAS to objectively measure itch, Yosipovitch (2003) also documented potential issues when administering it, including the occurrence of missing values due to the scale not being self-explanatory or participants being unfamiliar with the scale. Yosipovitch also emphasised that one commonly reported flaw of the VAS appears to be its verbal labels, which may not represent the same intensities to all participants. However, despite the possibility that the VAS is not an optimal tool, Reich et al. (2013) maintained that it cannot be dispensed with until a more reliable procedure is developed.

It is arguable that some of these aforementioned issues, such as lack of verbal information and ordinal misunderstanding, could potentially be avoided by using a rating scale which includes additional verbal indictors (see below) and the employment of a training session prior to the experiment. One commonly used measurement scale which is considered by some as more self-explanatory, is the general version of the Labelled Magnitude Scale (gLMS; Bartoshuk et al., 2004; Green et al., 1996; LaMotte, Shimada, Green, & Zelterman, 2009). Participants judge the magnitude of itch on a vertical line which includes semantic labels of “no sensation” at 0, “barely detectable” at 1, “weak” at 6, “moderate” at 17, “strong” at 35, “very strong” at 53 and “strongest imaginable sensation of any kind” at 100. The gLMS also enables the category labels to be positioned according to their semantic magnitudes.

Green et al. (1996) argued that the strength of sensations is more often communicated with words, such as a 'strong' smell, or a 'weak' cup of coffee, as opposed to using numbers. Therefore scales requiring responses based on words may be more natural and direct.

Additionally, it could be argued that it is a hybrid scale in that it combines the best of both worlds, by providing participants with more opportunity to communicate the intensity of itch using words, while still retaining the fine-grained 0 – 100 range of the VAS. However, it could also be argued that individuals may not interpret the verbal anchors in the same way, for instance, what one person may define as a strong itch another may not.

In summary, there are a variety of items to consider to ensure a reliable measurement of acute itch. Although various research has documented the reliability of different rating scales for assessing chronic itch intensity research (Elman et al., 2010; Phan et al., 2012; Reich & Szepietowski, 2013), a systematic analysis and comparison of rating scales for the assessment of acute experimental itch is currently lacking.

1.4 Methods of Artificial Itch Induction

Acute itch can be artificially induced in healthy participants in the laboratory using a variety of techniques, such as, the administration of histamine (e.g., via the histamine prick test/iontophoresis), or the insertion of spicules from the Mucuna Pruriens plant. Methods such as these enable us to explore its process and learn more about its mechanisms. There is however, no gold-standard method of eliciting and evaluating itch as of yet.

1.4.1 Histamine

Histamine is one of the best-evaluated pruritogen and is the gold standard stimuli for investigating the process of itch in laboratory settings. Histamine receptor 1 (H1) is a major receptor implicated in itch sensation, located on C fibres that are mechanically insensitive. Itch is evoked after a latency of up to one minute and is usually accompanied by the appearance of a wheal (a small raised lump) and a flare (redness around the wheal; Lewis & Zotterman, 1927). It is possible to quantify the magnitude of these skin reactions by measuring the diameter and correlating it with the subjective itch. Darsow, Ring, Scharein, & Bromm, 1996) for example, have demonstrated that the histamine skin prick induced flare reactions which correlated with the intensity of reported itch ($r = 0.56$; $P < 0.01$; see Figure 1.1 for an example of prick test and skin reactions). The induced itch and skin reactions can generally be largely diminished or inhibited by antihistamines (Kremer, Feramisco, Reeh, Beuers, & Oude Elferink, 2014; Ringkamp & Meyer, 2014). In the laboratory the experimenter has substantial control over the itch elicited, as histamine shows a clear dose-response relationship (Drzezga et al., 2001; Mochizuki et al., 2003). This means it is an ideal stimuli for exploring any slight differences in the itch intensity response and particularly

helpful in learning more about brain processes involved (Drzezga et al., 2001; Kleyn, McKie, Ross, Elliott, & Griffiths, 2012; Mochizuki et al., 2003; Walter et al., 2005).

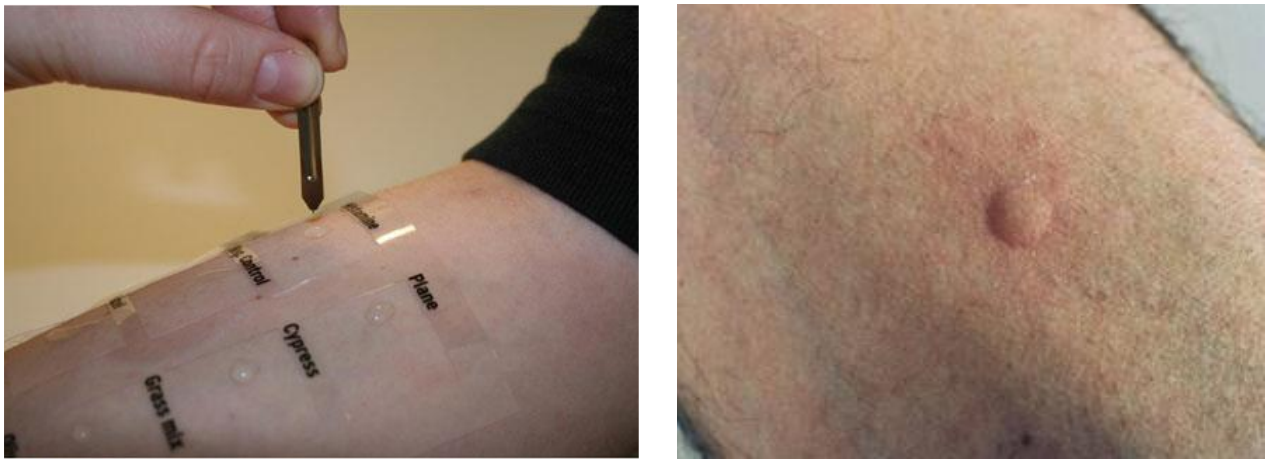


Figure 1.1 Left: An example of how a histamine skin prick test is performed in clinical practice. After a drop of solution has been placed onto the skin, the relevant area is pricked with a special lancet. Reprinted from Heinzerling et al. (2013). Right: A raised wheal and red flare after a histamine prick test. Reprinted from 'Dermatopedia | A Dermatology guide and database for Patients'.

1.4.2 Cowhage

Another more recent method of inducing itch is using cowhage spicules from the bean plant, Mucuna Pruriens (also known as velvet bean), which has long been known to induce itch when inserted into the skin (Shelley & Arthur, 1957). Mucuna Pruriens grows wild in the tropics, including Africa and India and has various uses including the medicinal treatment of a variety of diseases and conditions such as anxiety, arthritis, parasitic infections and Parkinson's disease (Gourie-Devi, Ramu, & Venkataram, 1991). It is also used for joint and muscle pain, as fodder plant for animals and to treat snakebites. Cowhage, however, is known for the strong itch it causes when the hairs (spicules) covering the seed pods come into contact with the skin (see Figure 1.2). The name cowhage originates from the Hindi term 'kiwach' meaning 'bad rubbing' (Namer et al., 2008).

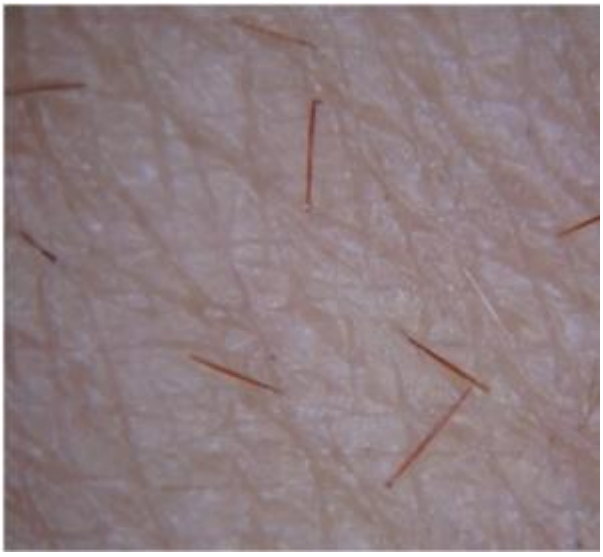


Figure 1.2 Left: A photograph of the Mucuna Pruriens pods which are covered by spicules. Reprinted from 'Medicinal Uses of Cowhage,"Horse-eye Bean","Gonca","Kauncha","Kavach","Kapikachu", "Atmagupta,"Herbal Medicinal Plant,Herbal Medicos - Herbal Medicine Plants'. Right: An image of spicules inserted into the skin by gentle rubbing, under polarising light at 56 magnification. Reprinted from Papoiu, Tey, Coghill, Wang, & Yosipovitch (2011).

When a spicule is inserted in the skin it will provoke a sensation of itch, along with the nociceptive sensation of pricking and burning (Sikand, Shimada, Green, & LaMotte, 2009). The active pruritogen in cowhage spicules was identified by Arthur & Shelley (1955) as a proteinase and named mucunain. This novel cysteine proteinase was found to activate the proteinase activated receptors-2 and 4 (PAR2/PAR4; Reddy, Iuga, Shimada, LaMotte, & Lerner, 2008). The PAR2 receptor has been implicated in the itch associated with atopic dermatitis (Steinhoff et al., 2003). This discovery has enabled the recent development of an intervention for treating pruritus related conditions by blocking the PAR2 pathway (MacDonald et al., 2013). These methods involve the administration of an antibody or antigen-binding fragment which specifically binds human PAR2. This intervention, however, is still in its trial phase.

Cowhage evokes a histamine-independent itch with little or no accompanying flare (Johanek et al., 2007; Sikand et al., 2009). Antihistamines are therefore ineffective in reducing cowhage-induced itch (Johanek et al., 2007). Evidence so far therefore indicates that cowhage and histamine induced itch are mediated through two different afferent pathways in the peripheral nervous system (see Figure 1.3). Various researchers have demonstrated that cowhage is a suitable experimental itch model and it has been recently used in a variety of studies exploring a histamine-independent pathway of itch (LaMotte et al., 2009; Papoiu et al., 2011; Rukwied, Main, Weinkauf, & Schmelz, 2013).

1.4.3 The multiple pathways for itch and their interactions with pain

The skin senses serve a discriminative function which allows us to not only manipulate objects and detect touch and temperature, but also detect threats which are likely to damage the skin, such as substances which induce itch or pain (Lloyd et al., 2015). Exploring the pathways stimulated by the skin receptors during these processes is therefore crucial in understanding how and why the body reacts in a certain way. It also informs treatments, such as, painkillers and antihistamines, when we want to reduce the body's natural reaction (particularly in chronic cases when the reaction goes beyond protecting the body).

Itch and pain appear to be independent sensations because nociceptive and pruriceptive stimuli each provoke unique behavioural responses (Shimada & LaMotte, 2008). The skin contains some nerve cells which solely respond to *itch* and others that respond only to *pain, however despite this, itch has notable i*nteractions with pain and it is this crossover which is key to understanding and explaining various aspects of itch. Itch is also related to pain because it can be reduced by nociceptive counter-stimuli (e.g. scratching) and analgesic opioids often have the adverse side-effect of producing itch (Szarvas et al., 2003). Itch and pain also have similarities in the respect that itch-producing agents activate nociceptive primary afferent fibres and can generate pruritic and nociceptive sensations simultaneously (Sikand et al. 2009). The relationship and interactions between itch and pain are therefore not as straight-forward as it was once thought and there is still much to be explored and understood.

Typically, pruritogens stimulate skin receptors and activate the peripheral pathway of itch. This provokes a signalling cascade and action potentials in at least two types of C-fibres (mechanically insensitive and Polymodal). These nerve fibres conduct the action potential to the dorsal horn of the spinal cord (Fig. 1.3). Despite the fact that identifying pathways of itch has progressed in recent years, there is still an ongoing debate with regards to the encoding of itch in the afferent pathway. Similar to theories on pain, the opposing positions are the specificity theory (labelled lines theory) and the pattern theory. Those in support of the specificity theory argue that there are modality-specific receptors and peripheral nerves that constitute a 'labelled line' from the skin to the brain, which was originally outlined by Muller (1826). Others however, who support the pattern theory, claim that somatic sensations like itch are generated by receptors and peripheral nerves that are not specific to the stimulus but deliver a series of signals modulated and decoded centrally (Craig, 2003).

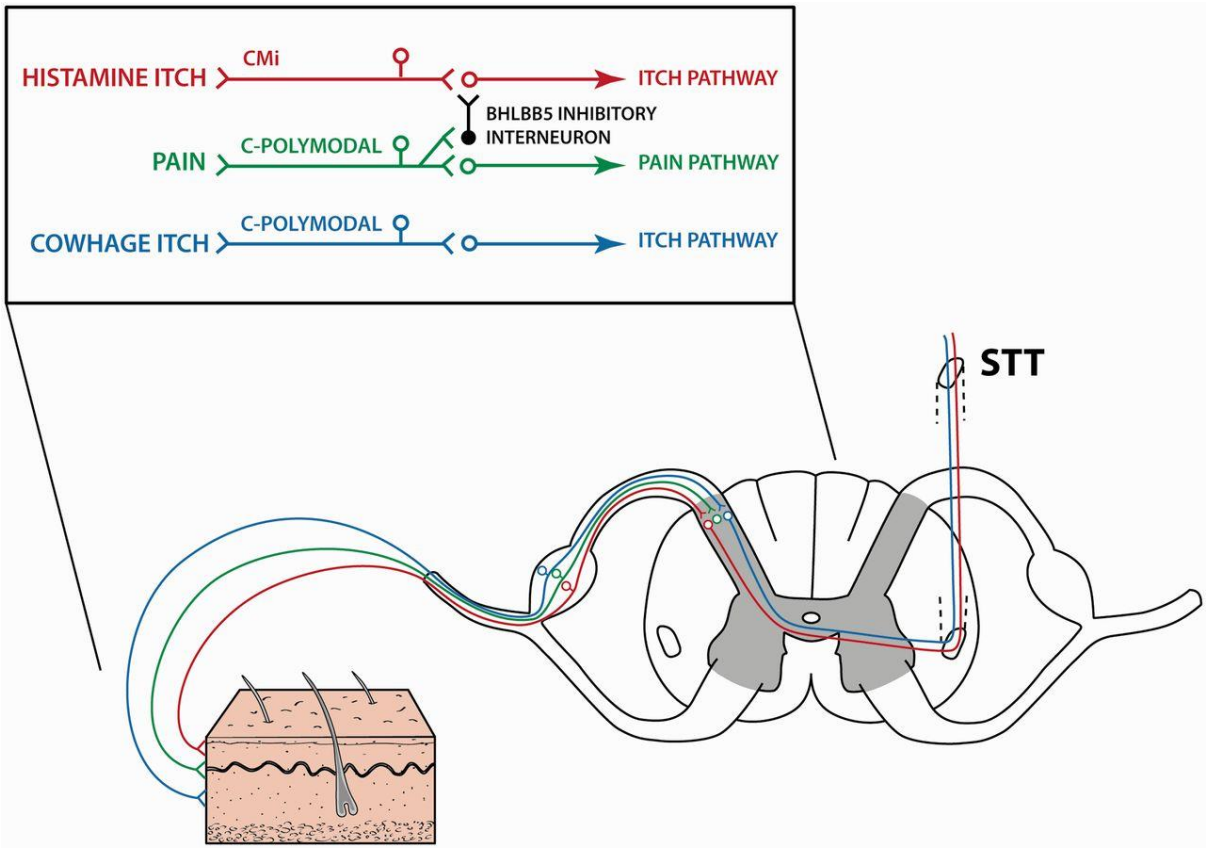


Figure 1.3 How the pain (green) and itch (histamine in blue and cowhage in red) pathways are activated via histamine and cowhage skin receptors in the epidermis and dermis, respectively. Impulses are transmitted primarily through mechanically insensitive C-fibres (CMi) and Polymodal C-fibres, to secondary neurons in the dorsal horn. One means of modulation by the pain pathway (depicted only partially) is through a Bhlbb5 interneuron. STT = spinothalamic tract; Bhlbb5 = transcription factor protein; CMi = mechanically insensitive C-fibres; C-polymodal = polymodal C-fibres. Reprinted from Dhand & Aminoff (2013).

The labelled-line theory however, does not explain at least two experimental occurrences. First, the itch pathway may be activated by pain-producing stimuli and secondly, different pruritogens may activate other peripheral pathways. This debate is ongoing however and many support the mechanism that includes both labelled lines and pattern decoding, which is coined the 'population-coding theory' which suggests that pain and itch labelled lines interconnect through excitatory and inhibitory interneurons that modulate the activity of each other, usually in the spinal cord. If pain and itch fibres are activated together, the sensation of pain alone may then emerge because inhibition from interneurons and central descending pathways masks the itch sensation. For example, certain spinal interneurons (Bhlhb5) inhibit itch pathways within the dorsal horn which may represent mediators between noxious and pruritic pathways, and therefore allow scratch to inhibit itch (Fig. 1.3). However, in chronic

form, failed crosstalk of these lines can generate 'pro-pain' and 'pro-itch' pathways irrespective of the stimulus (Ma, 2012).

1.4.4 Evaluating Methods: Histamine Versus Cowhage

Papoiu et al. (2011) compared the use of cowhage and histamine-induced itch as an experimental model for pruritus. The aim of the study was to test the validity of cowhage to induce itch in comparison to the established histamine paradigm. They also tested the combined effect of the two substances. The results showed that the mean and peak intensity of the itch were higher after the application of cowhage in comparison to histamine. They also found the combined application of cowhage and histamine created greater mean and peak intensity, compared to when histamine was applied on its own. There was however, no significant difference between the combined application of cowhage and histamine, compared to cowhage alone. Their findings therefore indicate that cowhage induces a more intense itch sensation to that of histamine and that it is the dominating factor in itch perception when both pathways are stimulated simultaneously.

Clinical pruritus is commonly accompanied by pain or nociceptive sensations such as stinging or burning (Binder, Koroschetz, & Baron, 2008). Chronic neuropathic itch, for instance, can occur after injuries or other dysfunctions of somatic afferent pathways (Brewer, Lee, Downs, Oaklander, & Yezierski, 2008; Oaklander, Bowsher, Galer, Haanpää, & Jensen, 2003). It could therefore be argued that cowhage-induced itch has an advantage over histamine, because of its additional nociceptive sensations. LaMotte et al. (2009) explored this idea by examining the sensations of a single cowhage spicule. They discovered that itch was typically accompanied by nociceptive sensations of pricking/stinging and burning, and therefore concluded that itch evoked by cowhage can co-exist with nociceptive sensations and cutaneous dysesthesias (an unpleasant abnormal sense of touch) as occurs in clinical pruritus.

In addition to this, Kosteletzky, Namer, Forster, & Handwerker (2009) completed a questionnaire which included 24 attributes of itch on a 4-point scale. They found that there were no significant differences between the ratings of histamine and cowhage found in 21 of these. The only three items which showed significant differences were “stinging”, “sharp” and “prickly”, which cowhage obtained higher ratings in comparison to histamine. This therefore provides further evidence that cowhage is an ideal experimental model for investigating chronic itch conditions within the laboratory.

However, although it is possible to explore the process of itch in a laboratory it is important to again point out the fact that acute and chronic conditions are very different, particularly with regards to the duration and the affective dimension. For example, chronic conditions are long in duration and can have a strong negative impact on an individual's quality of life, particularly emotionally, which does not occur in acute conditions. This means research using a clinical population (e.g. chronic itch patients) is vital in understanding conditions. Despite this however, exploring symptoms of chronic conditions (e.g. itch intensity) can be challenging due to the fact that severity can vary rapidly. A huge advantage therefore of techniques using experimentally induced symptoms, such as itch, is the fact that the researcher has more control over the stimuli which is not possible in clinical conditions.

1.5 Is Acute Itch Multidimensional?

On a day-to-day basis, we experience numerous senses and feelings that we have to interpret. One of the ways in which we do this involves processing the sensory and affective information, which does not always go hand in hand. In taste for example, we can experience something as very intense and at the same time it can be very pleasant or unpleasant (e.g. a fresh lemon is known to have an intense sour taste, which some people enjoy and others do not). People therefore can experience the sensory and affective dimensions of varying items or situations differently.

It is widely accepted that chronic itch is a multidimensional sensation, encompassing both sensory and affective components. The sensory dimension represents the quantitative aspect (i.e. how strong or intense the itch feels and its location), whilst the affective dimension refers to the qualitative element of the itch (how unpleasant, disturbing or uncomfortable it is; Price, McGrath, Rafii, & Buckingham, 1983). The importance of this dissociation is evident in research on chronic itch patients which highlights the fact that it is not the intensity per se, but the amount of unpleasantness that damages quality of life (Zachariae, Zachariae, Lei, & Pedersen, 2008). The majority of laboratory-based studies exploring acute itch however, adopt methodologies which view it as a unidimensional sensation, selectively measuring the intensity.

A critical question is therefore whether acute and chronic itch differ in their dimensionality. It could be the case that laboratory-based itch is in fact unidimensional, which would suggest that the measurement of intensity is an adequate proxy to capture the sensation. However, if it is multidimensional, then continuing to selectively measure intensity and ignoring the

affective factors may be problematic and it could be argued that current models of acute itch are not well suited to understand chronic itch.

Relatively limited research has been completed to dissociate the dimensions of experimentally induced itch, however there is a vast amount which has successfully done so in both clinical and experimental pain (Beecher, 1959; Derbyshire et al., 1997; Melzack & Casey, 1968; Price, Harkins, & Baker, 1987; Price, 2000; Rainville, Feine, Bushnell, & Duncan, 1992; Tölle et al., 1999; Vogt, Derbyshire, & Jones, 1996). If this dissociation can be repeated in acute itch, then successful treatment strategies for reducing pain unpleasantness could potentially be adapted for chronic itch patients. After an initial review from the related domain of pain, subsequent sections will therefore evaluate both behavioural and neural evidence which would suggest that acute itch has dissociable dimensions, which should be selectively measured.

1.5.1 Evaluation of Behavioural Evidence for the Dimensionality of Pain

It is the consensus that pain is of a multidimensional nature, supported by the official definition by the International Association for the Study of Pain (IASP). This definition incorporates three core elements of pain including: the association with and threat of injury; the unpleasant and emotional experience; and its subjectivity (Chen, 2001). However, despite the fact that pain is officially considered and defined as a subjective emotional experience, it has only been within the past recent 15 years that the importance of the affective aspect of pain has become salient in research (Chapman et al., 2001).

There are two subcomponents of the affective dimension of pain. The first is the "primary" affect which is the moment-by-moment unpleasantness, encompassing emotional and contextual feelings relating to the present or short-term future (e.g., distress, fear or anticipation; Price, 2000). The other is the "secondary" affect, which involves emotional feelings toward long-term implications and consequences of being in pain, such as suffering. The present review will focus on and compare the sensory (intensity) and primary affective dimensions of pain. The secondary affect, despite its importance, is not directly relevant to the current thesis which attempts to disentangle the primary dimensions of acute sensations (such as itch and pain) as opposed to secondary more long-term effects, which should be viewed as a topic in its own right.

1.5.1.1 Clinical Pain

The experience of pain as a sensory, cognitive and affective-motivational phenomena is of great importance. Some argue that pain cannot be realistically defined or accurately measured

one-dimensionally (Beecher, 1959; Melzack & Casey, 1968). Various studies have explored and distinguished the sensory (intensity) and affective (unpleasantness) dimensions and provided interesting findings in support of their independent measurement, in both chronic and acute pain.

It has been proposed by Price and colleagues that individuals who suffered pain associated with a serious threat to health or life would rate their pain significantly greater on an affective visual analogue scale (VAS) than those whose pain was not as threatening, despite the fact that both individuals would provide exactly the same intensity ratings (Price et al., 1984). An appropriate example of this is a claim by Melzack (1984) who argued that the intensity of pain associated with childbirth is greater than cancer or chronic back pain, yet only a fraction of the affective unpleasantness generated by most chronic pain conditions.

Price et al. (1987) later went on to test this theory through attempting to characterise the magnitudes of intensive and affective dimensions of varying types of clinical pain. Price et al. gathered data from a range of pain patients including labour, cancer and chronic back pain and measured their perceived pain experience. The findings demonstrated that the affective dimension of clinical pain can be selectively amplified by context (e.g., perceived degree of threat to health or life). Chronic pain and cancer patients, for instance, gave higher affective ratings in comparison to their sensory ratings of clinical pain. The women giving birth and those who received short-term experimental pain, however, perceived the affective dimension as lower in comparison to the sensory dimension. Price et al. also discovered that focusing on the impending birth of the child was associated with much less pain unpleasantness than when the patient focused on pain or avoiding pain. These findings indicate that cognitive factors (e.g., anticipation and expectation) can influence pain affect.

The study therefore provides empirical evidence that different sensory and affective intensity relationships characterise varying types of pain. This therefore supports the theory that cognitive and contextual factors selectively influence the affective-motivational dimension of pain. This particular research holds great relevance as it contributes to more accurately defining the influence of etiological and psychological factors, which have the potential to selectively enhance or reduce the affective and the sensory dimension of pain.

1.5.1.2 Acute Experimentally Induced Pain

As previously outlined, the sensory and affective dimensions of clinical pain, such as childbirth or chronic pain, can be successfully dissociated. This raises the question whether

this is also the case for acute laboratory-based pain. This was considered by (Rainville et al., 1992) who compared sensory and affective responses to four types of experimental noxious stimuli including contact heat, electric shock, muscle ischemia and cold-water immersion. Their aim was to discover whether various types of experimental stimuli evoke different affective and sensory aspects of pain perception.

The overall results demonstrated that the sensory-discriminative and affective elements of pain evoked by the phasic pain stimuli (contact heat and electric shock) were significantly different. The participants mean estimates of unpleasantness, for example, were significantly less than those of intensity, for both contact heat and electric shock. Participants were therefore able to discriminate among different magnitudes of pain evoked, either by evaluating perceived intensity or through estimating the degree of unpleasantness, associated with the varying levels of noxious stimulation.

There was however, no significant difference between the rated dimensions evoked by the tonic pain stimuli (muscle ischemia or cold-water immersion), as participants provided equivalent ratings in the intensity and affective dimensions. These differences in the perceived intensity and unpleasantness associated with tonic and phasic pain therefore are markedly different. Rainville et al. further explored this by regrouping the data as tonic vs phasic which confirmed that the phasic stimulus evoked significantly less unpleasantness in comparison to the tonic ($t = 3.38$, $p = 0.003$). This therefore may indicate that they differ in the way they are processed, as both the electric shock and the contact heat produce a brief phasic sensation of pain which is localised to a relatively small area, in comparison with the tonic stimulus which generates pain involving not only the skin but also deeper structures of the hand and forearm. Rainville et al. (1992) therefore concluded that the results indicate that sensory discriminative and affective aspects of phasic pain can be separated, both linguistically and conceptually.

Rainville and colleagues also completed a range of studies exploring the dimensions of pain which influenced much of the research that followed. In an early study Rainville, Duncan, Price, Carrier, & Bushnell (1997) successfully used hypnotic suggestion in order to increase/decrease pain unpleasantness selectively, without manipulating the perceived pain intensity. A more recent study further emphasised the independence and separation of sensory and affective aspects of pain perception, again through using affective-motivational strategies. Rainville, Carrier, Hofbauer, Bushnell, & Duncan (1999) demonstrated that the

modulation in pain unpleasantness ratings was largely independent of variations in perceived pain intensity. They found a significant correlation between stimulus-evoked heart rate increase and ratings of pain unpleasantness selectively, without an accompanying increase in intensity. This indicates a direct functional interaction between pain affect and autonomic activation. The overall results therefore show that hypnotic suggestions can be successfully used as a form of cognitive intervention, to selectively modulate the affective dimensions of pain. This enables the exploration and dissociation of the possible relationship between these psychological dimensions in pain and changes in physiological responses to the noxious stimuli.

Overall, despite the fact that the intensity and unpleasantness of pain are closely linked, there are various methods and factors that can influence the dimensions, independently of one another. For example, the fact that potential life or health threatening factors can selectively enhance the affective dimension over the sensory dimension. The affective dimension can also be selectively reduced when there is no such threat, for example, when pain has positive associations (e.g., birth of a child). These selective measurements therefore have good applicable significance and are vitally important in distinguishing whether treatments have analgesic effects and/or alter the affective dimension of pain through psychological factors. Using simple measures of the separate dimensions could potentially identify these factors in individual patients and within specific types of treatments.

1.5.2 Evaluation of Behavioural Evidence for the Dimensionality of Itch

When evaluating chronic itch it is becoming increasingly acknowledged that the affective dimension is crucial in its assessment and questionnaires have been constructed accordingly. Darsow, Mautner, Bromm, Scharein, & Ring (1997) developed the Eppendorf Itch Questionnaire (EIQ) as an instrument for assessing chronic clinical itch. They argued that a multidimensional itch questionnaire, such as the EIQ, may be more suited to fulfil the criteria of the complexity of itch perception in comparison to the usual visual analogue scales used to assess itch quantification. For instance, the emphasis on the psychosocial significance of emotional suffering from chronic itch, which they argued could lead to the intensity of the sensation increasing.

Kosteletzky, Namer, Forster, & Handwerker (2009) developed a shortened version of the EIQ (adapted from Darsow, Mautner, Bromm, Scharein, & Ring, 1997) more suited to experimental itch than clinical, including 24 out of the 80 sensory and affective descriptors.

Descriptors such as ‘cruel’ and ‘torturing’ where removed and descriptors like ‘unpleasant’ and ‘disturbing’ remained, in order to selectively capture the appropriate affective sensations, which could be successfully applied to acute itch. This adapted questionnaire enabled the affective aspect of itch to be subdivided into immediate/primary unpleasantness (e.g., how unpleasant/uncomfortable the itch is) and the secondary affect more related to the emotional feelings directed toward long-term implications of having an itch (e.g., suffering), similar to the distinction that has been made for pain. They specifically aimed to compare significant differences between the qualitative sensations of cowhage and histamine induced itch however, therefore a direct comparison of sensory versus affective aspects of experimental induced itch were not highlighted.

So far, there have only been a limited number research groups which have studied acute itch as a multidimensionsal sensation (Drzezga et al., 2001; Kleyn et al., 2012; Mochizuki et al., 2003; Walter et al., 2005). The earliest study which examined the affective dimension of acute itch (in addition to the sensory one) was by Drzezga et al. (2001) who completed repeated positron emission tomography regional cerebral blood flow (rCBF) measurements using O^{15}-labelled water on six participants. This technique measures physiological function through monitoring blood flow and therefore the aim of this was to further explore the functional anatomy of itch sensation. Drzezga et al. (2001) therefore used nine logarithmically increasing concentrations of histamine and saline as a control in skin prick tests. This was administered 2 minutes before each rCBF measurement. The behavioural results indicated that there was a difference between the reported ratings of mean intensity (24 ± 15 to 51 ± 31) and unpleasantness (19 ± 16 to 46 ± 35), however no statistical analyses were performed to discover if this difference was significant.

Mochizuki et al. (2003) and Walter et al. (2005) also measured both dimensions independently using the histamine prick test. However, neither of these studies tested whether there was a significant interaction between stimulus dose intensity and dimension (intensity vs. unpleasantness ratings). Such an interaction would have demonstrated evidence for the multidimensionality of acute itch. Despite the fact that there were no statistical analyses on the difference between the dimensions, however, Walter et al. (2005) reported a slight variation.

Overall, although it is well recognised that both the sensory and affective dimensions are vital when measuring and quantifying chronic itch and evaluating treatments, there is a vast lack

of application to acute itch laboratory-based studies. As previously mentioned, if it is in fact the case that acute itch is multidimensional, then selectively measuring the intensity alone could be damaging to the progression of research and in turn treatment trials.

1.5.3 Evaluation of Neural Evidence for the Dimensionality of Pain

To explore the neural basis of pain numerous researchers have applied a range of noxious stimuli to healthy participants, while monitoring the brain activity using various neuroimaging techniques. Through this research a selection of brain regions have been identified as the central network, consistently activated by pain. This network has been coined "the pain matrix". Despite the fact that these regions are highly interactive, research increasingly indicates a segregation of the sensory-discriminative (e.g., the quality, location, and intensity) and affective aspects of the pain experience. This suggests that pain subcomponents, such as intensity and unpleasantness, predominantly activate distinct specialised brain areas (Derbyshire et al., 1997; Tölle et al., 1999; Vogt et al., 1996).

Brain regions with selective links to the affective dimension of pain, include the anterior cingulate cortex (ACC; Rainville, 2002; Rainville et al., 1997; Tölle et al., 1999), the dorsolateral prefrontal cortex (Borckardt et al., 2011) and the insula (Melzack & Casey, 1968), whereas the sensory dimension has been found to predominately activate the primary and secondary somatosensory cortices (Bornhövd et al., 2002; Coghill, Sang, Maisog, & Iadarola, 1999; Frot, Magnin, Mauguière, & Garcia-Larrea, 2007; Grundmann et al., 2011; Iannetti, Zambreanu, Cruccu, & Tracey, 2005; Ikoma, Cevikbas, Kempkes, & Steinhoff, 2011; Kanda et al., 2003; Porro et al., 2007; Timmermann et al., 2001; Valmunen et al., 2009). Therefore it is evident that not only can pain be dissociated on a behavioural level, but there is increasing evidence to suggest that they also differ on a cortical level.

As previously mentioned, successful attempts of using hypnotic suggestions have been made to selectively increase or decrease the affective dimension of pain, while keeping the sensory dimension constant (Rainville et al., 1997; Tölle et al., 1999). Using positron emission tomography (PET), hypnotic suggestions which significantly increased the unpleasantness ratings resulted in much larger activity in the ACC, in comparison with the low unpleasantness condition, yet no differences were evident in primary somatosensory cortex (S1; Rainville et al., 1997).

A further PET study by Tölle et al. (1999) confirmed these results using noxious heat. The findings indicated that although several brain regions were activated during pain, only pain

unpleasantness activated the ACC. Interestingly, a similar study by Hofbauer, Rainville, Duncan, & Bushnell (2001) who used hypnotic suggestions but selectively manipulated the pain intensity, as opposed to the affective dimension, produced changes mainly in S1. These studies therefore suggest that the ACC but not somatosensory cortices, may be crucial for pain affect. These findings are also consistent with the impairment in pain sensation reported in an individual with a lesion in the right S1 and S2 as a result of a stroke (Ploner, Freund, & Schnitzler, 1999). An MRI performed 3 days after the stroke confirmed that the lesion was confined to the right hemisphere and there was no evidence of long-term damage to the left. The authors concluded that for the first time in humans, they were able to demonstrate a loss of pain sensation with preserved pain affect.

Additional support for the theory that the ACC is selectively involved in the affective component comes from a meta-analysis by Peyron, Laurent, & García-Larrea (2000). They concluded that the process of intensity coding in the ACC has not been supported by functional imaging research. A study by Tölle et al. (1999), for example, did not find any relationship between ACC blood flow and stimulus intensity. Also, in various pain studies ACC activation was reported to increase without any manipulation of stimulus intensity. For instance, Craig, Reiman, Evans, & Bushnell (1996) induced pain through a combination of two non-noxious stimuli using a 'thermal grill technique' which alternated warm and cool bars. The ACC was not activated by either stimulus in isolation, yet demonstrated enhanced rCBF when applied simultaneously. This therefore indicates that ACC changes could be a result of subjective pain sensation, as opposed to the actual stimulus intensity. Similarly, Peyron, Laurent, & García-Larrea (2000) reported increased ACC activation without any manipulation of stimulus intensity, during a 'distraction' experiment entailing decreased pain sensation. These studies would therefore suggest that there is a lack of literature in support of ACC activation in response to the intensity of stimuli.

Studies using brain stimulation techniques have also provided some interesting findings which again suggest a relative separation of cerebral regions that are involved more directly in sensory or affective aspects of the pain experience. Lockwood, Iannetti, & Haggard (2013) discovered that when TMS pulses were administered over the secondary somatosensory cortex (S2), the participants' judgement of the pain intensity was reduced, relative to the control stimulation (vertex). Valmunen et al. (2009) also delivered rTMS over S2 which resulted in increased heat pain thresholds on the face. These results therefore expand upon correlational findings which suggest S2 has a role in pain intensity (Frot et al., 2007) and

enables us to conclude that there is potentially a causal role for S2 in the encoding of pain intensity.

Another crucial method of investigating which cortical areas have a causal role in distinct dimensions of pain are lesion studies. For instance, damage to large parts of the insula has been found among patients with pain asymbolia (Berthier, Starkstein, & Leiguarda, 1988; Weinstein, Kahn, & Slote, 1955). Patients with this condition do not display behaviour indicative of threat or intrusion in response to painful stimuli, despite their ability to report the sensory qualities of the stimuli. Greenspan, Lee, & Lenz (1999) demonstrated that patients with lesions in the insula had normal thermal and mechanical pain thresholds but longer tolerance times when tested with painful ice-water (Dong et al., 1989). These studies therefore suggest that the insula has a primary role in processing the affective aspect of pain, which can be disrupted when damage occurs.

In summary, there has been a range of approaches to understanding the brain mechanisms underlying sensory and affective dimensions of pain. In particular, research combining brain imaging or stimulation with psychophysical methods has led to a greater understanding of how these dimensions are interrelated and can be modulated by cognitive factors (Price, 2002).

1.5.4 Evaluation of Neural Evidence for the Dimensionality of Itch

Similar to pain research, there have been attempts to isolate the cortical "itch matrix" incorporating a network of brain regions consistently activated in various neuroimaging studies. However, it has been argued by some that the search for a single "itch centre" in the brain will not lead to a conclusive result, as this concept does not take into account the multidimensionality of the sensation (Darsow et al., 2000; Kleyn et al., 2012). Unfortunately, as previously outlined, very few researchers have independently measured the sensory and affective dimensions of itch and even less have reported any differences in cortical activation.

One of these few studies is by Drzezga and colleagues, who aimed to associate the subcomponents of itch with specialised activated brain areas using correlation analysis. In their positron emission tomography (PET) study, Drzezga et al. (2001) used nine logarithmically increasing histamine concentrations (0.03 - 8%) and saline as a control, via skin prick tests to the right forearm. The results demonstrated that the itch induced a significant activation predominantly in the contralateral somatosensory cortex and in the ipsilateral and contralateral SMA, premotor cortex, primary motor cortex, prefrontal cortex

and the cingulate gyrus. There was however, no activation in the secondary somatosensory cortex, which the authors described as 'striking' and suggested that the functional significance of S2 is not predominantly known for pain either. This study however, was completed prior to more recent brain stimulation experiments which suggest a causal role of S2 in pain (Lockwood et al., 2013; Valmunen et al., 2009).

Using correlation analyses, activation of the following regions demonstrated a graded increase in rCBF with the logarithm of the histamine concentration: left supplementary motor area (SMA), motor cortex, premotor cortex, right inferior parietal and lateral prefrontal cortex. The subjective ratings of unpleasantness however, strongly correlated with the ACC and right SMA. Activation of the left dorsolateral prefrontal cortex (Brodmann areas 9, 10) and posterior insula, also correlated with the subjective itch unpleasantness. These findings are interesting, as they highlight the fact that not only do the dimensions of histamine induced itch appear to differ on a psychophysical, but also on a cortical level.

Other studies which have selectively measured the dimensions unfortunately have not examined the subcomponents using a correlation analysis. Mochizuki et al. (2003) and Walter et al. (2005), for example, used the histamine prick test to investigate brain activation during the course of a histamine reaction, but did not provide information on whether there was a significant difference between the two dimensions or whether specific brain regions were selectively activated in correlation with intensity or unpleasantness ratings. Another study by (Hsieh, Hagermark, et al., 1994) measured cerebral activation during itch induced by intracutaneous injections of histamine. Activation of several cortical structures, including the anterior cingulate, supplementary motor area (SMA), premotor area and inferior parietal lobule, was demonstrated, however this study also suffered from a lack of correlation analysis, to distinguish any differences evoked by the dimensions independently.

As previously highlighted, another way of discovering whether brain regions have a specialised role in itch perception is by selectively modulating a dimension and monitoring any difference in brain activity. Psychological factors, such as expectancy for instance, are known to significantly modulate ratings, along with activity in brain areas associated with the affective dimensions of both itch (Napadow et al., 2015) and pain (Kong et al., 2008; Tracey, 2010). For example, Scholz & Hermanns (1994) found that atopic dermatitis (AD) patients provided higher itch ratings when histamine delivery was accompanied by negative verbal suggestions, with regards to the skin response and itch perception. Napadow et al. (2015)

expanded upon these results in a recent fMRI study, which indicated that nocebo-induced itch produced greater itch sensation, in comparison to the control and interestingly, also resulted in an increased activity of the dorsolateral prefrontal cortex (dlPFC), which is greatly associated with the affective dimension of itch. This research is therefore crucial in the process of exploring and understanding the role of psychological factors involved in the manipulation of itch perception and indicating which brain mechanisms are selectively involved.

Neuroimaging studies have also clearly shown experimentally induced itch activates the ACC and insula in healthy subjects, which are areas known to be involved with emotional processing (Darsow et al., 2000; Drzezga et al., 2001; Hsieh et al., 1994; Leknes et al., 2007; Mochizuki et al., 2003; Walter et al., 2005). It is also known that scratching an itch may suppress the emotional components of pruritus and can even be a pleasurable experience (Yosipovitch et al., 2008). This therefore is another potential way of investigating the brain regions associated with the affective dimension of pain.

A study by Papoiu et al., (2013) aimed to assess the correlations between brain activation and psychophysical ratings of itch relief (pleasurability of scratching). The study compared the patterns of brain activity evoked by self-scratching with passive scratching (experimenter scratches itch). The results demonstrated that active scratching generated higher ratings of pleasurability and interestingly induced greater deactivation of both the ACC and insula. These findings therefore support their role in the process of reward by scratching, which is highly associated with feelings of pleasurability (and of course the case of unpleasantness).

Several additional studies reported that activity in the insula was positively correlated with the subjective itch sensation and particularly the unpleasantness (Bergeret et al., 2011; Herde, Forster, Strupf, & Handwerker, 2007; Leknes et al., 2007; Mochizuki et al., 2007; Papoiu, Coghill, Kraft, Wang, & Yosipovitch, 2012). It also has a particular role in empathy for pain (Bird et al., 2010; Lamm & Singer, 2010). Recent research indicates that the insula may also have a similar role with regards to empathy in itch perception. Studies investigating the neural mechanism underlying contagious itch using fMRI (Holle, Warne, Seth, Critchley, & Ward, 2012; Mochizuki & Kakigi, 2015) demonstrated that several brain regions including the insula, SMA, PM, and PFC were activated while viewing others experiencing itch. This would therefore indicate that the insula, amongst other areas, may in fact have a distinct role in the empathy of itch.

1.5.5 Evaluation Summary

In summary, although some attempts of considering acute itch as a multidimensional sensation have been made, reported results on potential dissociation between sensory and affective components both on a subjective and cortical level are sparse. If the cerebral processing of itch is similar to pain, then there could potentially be complex patterns of activated regions involved in the different subcomponents of the sensation. Various studies focusing on pain have shown that subcomponents such as pain intensity and unpleasantness activate distinct specialised brain areas (Derbyshire et al., 1997; Tölle et al., 1999; Vogt et al., 1996). This would indicate therefore that focusing solely on the intensity of itch could result in other critical aspects of the process being ignored, which could potentially be essential in the progression toward possible treatments of chronic itch. For example, the possibility of reducing unpleasantness by repeated TMS stimulation of the insula/ACC or using psychological interventions (e.g., relaxation training) to selectively manipulate the affective dimension of the itch (Bae et al., 2012; Ehlers, Stangier, & Gieler, 1995; Lavda, Webb, & Thompson, 2012; Schut et al., 2013).

1.6. Techniques used to Explore the Neural Basis of Itch

Various neuroimaging methods such as fMRI and PET have been used in the area of itch. These methods are extremely valuable, enabling experimenters to measure correlations between input stimulus (e.g., itch or pain), behaviour (e.g., intensity ratings) and providing both spatial and temporal information on activated brain regions. Despite this, there is one particularly well noted limitation. These approaches are only correlational as we can only observe a correlation between stimulation and activation, which does not necessarily indicate that the observed activations are causally related to the behavioural task (Pascual-Leone, Walsh, & Rothwell, 2000). Therefore, no matter how strong the experimental design is, neuroimaging methods alone can never be sufficient to infer causality. TMS on the other hand, can be used to experimentally manipulate cortical excitability as it directly interferes with neural activity in the stimulated area (Oberman, Edwards, Eldaief, & Pascual-Leone, 2011). TMS studies are therefore thought to provide stronger causal evidence than correlations observed in neuroimaging studies (Lockwood et al., 2013).

1.6.1 Introduction to Transcranial Magnetic Stimulation

TMS works through creating a virtual brain lesion, which can disrupt normal brain activity (Pascual-Leone, Bartres-Faz, & Keenan, 1999; Walsh & Rushworth, 1999). Through delivering stimulation and comparing the consequences of the temporary lesion to necessary

controls, (e.g., the vertex or sham stimulation), it is possible to investigate whether activity in a particular brain region is essential for a given task (Pascual-Leone et al., 1999). This method therefore aids the development of research on itch from correlational methods, such as neuroimaging, towards the ability to make causal inferences, as to which areas are necessary for the central nervous generation of itch.

In order to successfully and reliably administer TMS, the precise positioning of the coil above the anatomically or functionally defined target brain region for each individual participant is needed. This requires the use of an imaging-guided neuronavigation of the TMS coil (see procedure). When a current is passed through the coil, a magnetic pulse is generated. When this magnetic field induced in a coil is positioned near the scalp, it results in electrical currents in the underlying tissue (Kobayashi & Pascual-Leone, 2003; Wagner, Valero-Cabre, & Pascual-Leone, 2007). When TMS is applied at the appropriate intensity, the induced electrical current depolarizes the neurons (Pascual-Leone, Davey, Rothwell, Wasserman, & Puri, 2002). This in turn, induces a virtual lesion by adding noise to the normal neural firing pattern, with an estimated spatial resolution of 0.5 – 1.5 cm (Walsh & Rushworth, 1999).

Much of what we have learned about brain functions has developed from exploring its abnormalities, for example, the behavioural consequences of brain lesions. Although this has been invaluable, the use of TMS has a number of advantages over it (Pascual-Leone et al., 1999). One of the primary advantage is that it can be used in healthy participants, which controls for any potential confounds of general health issues the patient may have. The effect of this virtual lesion can be investigated immediately, therefore avoiding the possibility of functional reorganisation in patients with lesions. It can also be repeated within and between participants, allowing experiments to be conducted on groups of participants for direct comparisons.

The magnetic pulses delivered via TMS can be applied in a number of different ways, resulting in varying behavioural outcomes (Hallett, 2007). These effects can be either inhibitory or excitatory. In single pulse TMS just one pulse is delivered per trial. This allows the observation of the contribution of an area to a given task via varying the delivery time of the pulse and measuring a behavioural outcome. This method however, would not be ideal when attempting to explore the neural basis of itch due to its long time course (8-10 minutes). In order to create a longer lasting effect, multiple pulses can be delivered per trial, such as the triple pulse design (Schuhmann, Schiller, Goebel, & Sack, 2009). This is known as repetitive

TMS (rTMS), which involves low or high frequency trains of pulses delivered over a period of time before a given task (e.g., itch induction).

There are two main ways of administering rTMS, online or offline. During online rTMS participants perform a task and at a specific time prior to or during the task, a train of TMS pulses are administered. Generally, online rTMS with less than 1 Hz pulse delivery often leads to inhibitory effects and 5 Hz or more usually results in facilitatory effects (Hoogendam, Ramakers, & Di Lazzaro, 2010). In offline rTMS, brain stimulation is performed prior to starting a task, as the effects of rTMS can last beyond the stimulation time. Offline low-frequency stimulation rTMS therefore can be applied in order to induce a longer lasting suppression of neural activity (Ridding & Rothwell, 2007). This approach is advantageous, as rTMS is not required at the same time as task is performed, therefore eliminating many of the concurrent effects of online TMS, such as behavioural and attentional effects (Bolognini & Ro, 2010).

Theta Burst Stimulation (TBS) is a specific type of rTMS which is particularly useful in altering cortical excitability. TBS has been crucial in studies investigating cognitive functions and is also implemented in treatment interventions for a variety of neurological conditions (Oberman et al., 2011). Research suggests that this type of TMS can modulate behaviour for at least 20 minutes following 20 s of stimulation (Nyffeler et al., 2009). There are two commonly used patterns in TBS, continuous (cTBS) and intermittent (iTBS). In cTBS, bursts of 3 pulses at 50 Hz are applied at a frequency of 5 Hz for either 20 seconds (100 bursts) or 40 seconds (200 bursts; Fitzgerald et al., 2006). In iTBS, 20 2 s periods (10 bursts) of TBS are applied at a rate of 0.1 Hz. The excitatory and inhibitory effects of this type of stimulation can be altered either by the continuous (offline) or intermittent (online) delivery of these theta bursts over time. cTBS can induce inhibitory neural effects that outlast the duration of the stimulation (Huang et al., 2005). The current study administered 40 s of cTBS, to ensure the TMS effects outlasted the estimated 30 minutes it took to complete the experiment.

1.7 Background of Regions of Interest

There have been a variety of approaches to understand the brain mechanisms underlying the neural processing of pain. In particular, research combining brain imaging or stimulation with psychophysical methods is providing crucial information on these complex processes. Itch, however, is lagging behind in comparison to the field of pain and currently there are very limited published brain stimulation studies on acute itch. The evidence that is available so far indicates that the neural networks for itch and pain are largely overlapping (Ikoma,

Handwerker, Miyachi, & Schmelz, 2005), therefore when TMS to a brain region has been shown to inhibit pain, an assumption can be made that it will have a similar effect on itch. Thus, by reviewing findings from brain stimulation studies which have successfully manipulated acute pain, we can build a picture to inform the current TMS experiment.

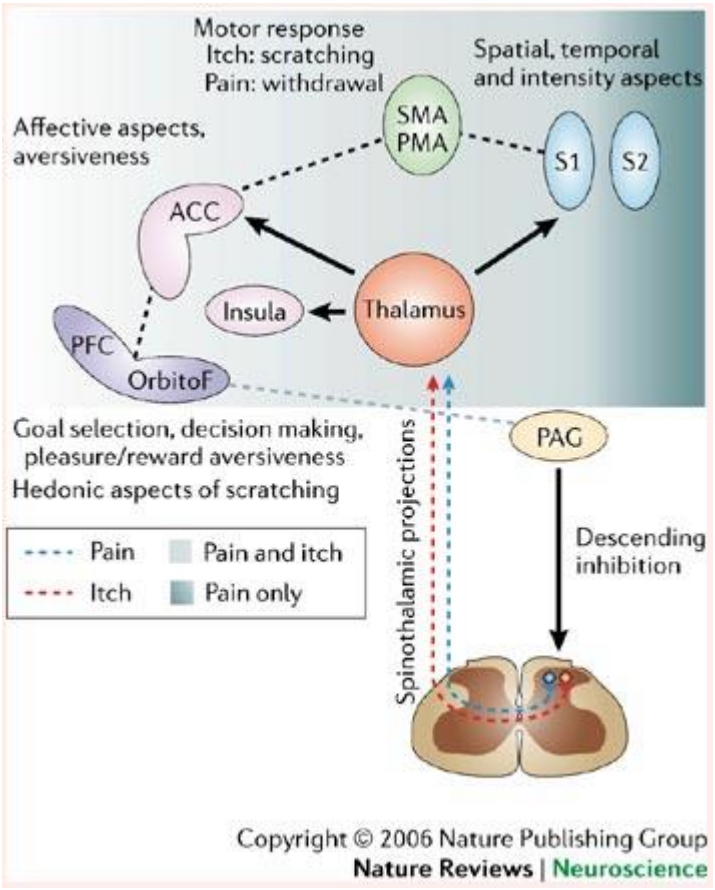


Figure 2.4 Areas activated by both pain and itch stimuli (light grey) include the thalamus, insular cortex (insula), anterior cingulated cortex (ACC), prefrontal cortex (PFC), orbitofrontal cortex (OrbitoF), supplementary motor area (SMA), premotor area (PMA), cerebellum and primary somatosensory cortex (S1). The effects of the activation of the particular areas common to pain and itch are indicated by black arrows. Differential responses (red, itch; blue, pain) are found mainly for the motor response and the lack of activation of the secondary somatosensory cortex (S2; dark grey). Activation of prefrontal areas and the orbitofrontal cortex can be associated with hedonic aspects of scratching. Activation of the periaqueductal grey (PAG) area is involved in the modulation of itch and pain. Reprinted from Ikoma et al. (2006).

1.7.1 The Role of the Somatosensory Cortices in the Pain Process

In order to explore the neural basis of pain, studies have used a range of noxious stimuli whilst observing the brain activity using various neuroimaging techniques. This has enabled the identification of a central network consistently activated by pain (see introduction). In summary, brain regions with a selective link to the affective dimension include the dorsolateral prefrontal cortex (dlPFC), ACC and insula, whereas the sensory aspect has been found to predominately activate the primary and secondary somatosensory cortices (See Fig. 1.4; Bornhövd et al., 2002; Coghill et al., 1999; Frot et al., 2007; Grundmann et al., 2011; Iannetti et al., 2005; Ikoma et al., 2011; Kanda et al., 2003; Porro et al., 2007; Timmermann et al., 2001; Valmunen et al., 2009).

Various experiments investigating the neural network of pain intensity discrimination have found evidence for the involvement of both S1 and S2 (Bornhövd et al., 2002; Coghill et al., 1999; Frot et al., 2007; Grundmann et al., 2011; Iannetti et al., 2005; Kanda et al., 2003; Porro et al., 2007; Timmermann et al., 2001; Valmunen et al., 2009). However, findings are not consistent, and there are contrasting hypotheses regarding their specific roles and contributions to the processing of pain, as outlined below.

Hofbauer et al. (2001) used hypnotic suggestions to selectively manipulate pain intensity, which produced changes primarily in S1, therefore suggesting that S1 may be crucial in the processing of pain intensity. These findings are consistent with the impairment in pain sensation reported in an individual with a lesion in the right S1 and S2 as a result of a stroke (Ploner, Freund, & Schnitzler, 1999; see introduction). Other studies have also found evidence for S1 involvement in pain intensity encoding (Coghill et al., 1999; Timmermann et al., 2001). Furthermore, Frot, Magnin, Mauguière, & Garcia-Larrea (2007) discovered that S2 responses correlated with the subjective ratings of pain intensity using evoked potentials from intracranial implanted electrodes in S2. Similarly, Bornhövd et al., (2002) documented that BOLD responses in S2 distinguished between varying intensities of noxious stimulation.

As previously highlighted, correlations between neural activity and perceived intensity does not infer causality, therefore if a region demonstrates a response which correlates with the stimulus intensity, this does not necessarily mean that the region is essential in intensity processing. Because TMS studies directly interfere with neural activity in the stimulated area, they provide stronger causal evidence than neuroimaging experiments. Recent studies which

stimulated S1 or S2, and assessed the effect of these 'virtual lesions' on location or intensity judgement of experimentally induced pain therefore provide very informative findings.

TMS and transcranial direct current stimulation (tDCS) have therefore been particularly insightful; however the results are not entirely consistent and can be difficult to interpret and compare due to the range of brain stimulation paradigms and noxious stimuli administered. Some studies have found evidence in support of the function of S1 in experimental pain perception, whereas others have not. Grundmann et al. (2011), for example, reported that cathodal tDCS at 1 mA current intensity to S1 reduced sensitivity to painful stimulation. Porro et al. (2007) discovered that TMS (trains of three TMS pulses, 40 msec apart) over S1 significantly impaired participants' ability to localise painful stimuli, therefore suggesting S1 has a role in processing pain location. However, Lockwood et al. (2013) argued that the stimuli used by Grundmann et al. (2011) were not within the painful range and that Porro et al. (2007) used mechanical stimuli that activate tactile as well as nociceptive fibres. This additional tactile sensation, according to Lockwood et al. (2013), may have contributed to pain localisation, and it may have been this tactile location information that the S1 stimulation disrupted.

So far, there have only been two previous studies which have reported a significant effect of TMS over S2 on pain intensity (Lockwood et al., 2013; Valmunen et al., 2009). Valmunen et al. (2009) delivered rTMS over the somatosensory cortices and found that rTMS over S2 increased heat pain thresholds on the face, but S1 did not. However, Valmunen et al. (2009) used thermal contact-heat stimulation, which again involves a combination of both nociceptive and tactile afferent input. Lockwood, Iannetti, & Haggard (2013), discovered that when TMS pulses were administered over S2, the participants' judgement of the pain intensity was reduced relative to the control stimulation (vertex). No significant results were found for pain location however. They therefore concluded that S2 causally contributes to the ability to discriminate the intensity of a painful stimulus. rTMS over S1 had no significant effects on perception of either pain intensity or pain location. Interestingly, Lockwood et al. (2013) found that judgements of intensity were significantly disrupted not only when comparing S2 to vertex stimulation, but also when comparing S2 to S1. Lockwood et al. (2013) therefore argued that this indicates the possibility of distinct roles for S1 and S2 in pain perception. Kanda et al. (2003) also used TMS but found no significant effect over S2 on the reporting of pain location during the administration of a laser stimulus to the hand. Lockwood, Iannetti, & Haggard (2013), however, argued that the task employed by Kanda et

al., (2003) focused on pain detection, as opposed to subjective ratings of pain intensity. There is also evidence that motor cortex (M1) stimulation can also be used for pain relief (Lefaucheur et al., 2001; Lima and Fregni, 2008).

Interestingly, both animal and human studies have demonstrated that S1 activity has a linear relationship with stimulus intensity and subjective pain sensation, therefore suggesting it plays important roles in the perception of pain intensity (Bornhövd et al., 2002; Dong et al., 1989; Frot et al., 2007; Timmermann et al., 2001). These studies have also shown that S2 activity exhibited an S-shaped function (Mochizuki & Kakigi, 2015). For example, a sharp increase in amplitude only when the stimulus intensity is well above the pain threshold. This would therefore suggest that S2 may have a specific role in the recognition of and attention toward painful stimuli rather than intensity processing, which contradicts the findings of Lockwood et al. (2013).

In support of this hypothesis is a study by Timmermann et al. (2001) who used MEG and four different intensities of nociceptive laser stimuli to the right hand to investigate the role of the somatosensory cortices in pain intensity discrimination. They observed that S1 responses closely matched the participants' pain ratings, indicating that the region was able to gradually encode the intensity of painful stimuli. However, S2 responses had a more categorical or binary form, demonstrating a sharp increase in amplitude at intensities above the pain threshold. This may therefore indicate that S2 is activated primarily when the pain intensity is high (above the pain threshold). It may therefore have a function in detecting pain which is of an intensity which requires action (for instance, pulling your hand away from a hot plate), rather than encoding intensity. Timmermann et al. (2001) concluded that the activation pattern of S1 suggests its role in the discriminative perception of pain intensity. In contrast, the activation pattern of S2 observed in their study does not support a significant contribution of S2 to the sensory-discriminative aspects of pain perception.

Overall, after reviewing the findings from both neuroimaging and brain stimulation studies, more solid evidence is in support of the idea that S1 and S2 have different roles in the processing of pain. Despite Lockwood et al. (2013) suggesting that S2 has a crucial function in encoding pain intensity, only one other brain stimulation study has demonstrated a disruption in pain intensity post S2 stimulation. In addition to this, there is much literature up to this point which provides evidence contradicting this idea (Mochizuki & Kakigi, 2015; Timmermann et al., 2001). For instance, the role of S1 is found to be vital in processing pain

intensity (Coghill et al., 1999; Grundmann et al., 2011; Hofbauer et al., 2001; Mochizuki & Kakigi, 2015; Timmermann et al., 2001), whereas S2 has more of a function in processing pain which requires action therefore is only evident when the intensity is relatively high. The existing literature documenting the contributions of S1 and S2 to pain perception however, needs further investigation in order to make any firm conclusions.

1.7.2 The Role of the Somatosensory Cortices in the Itch Process

The process of itch within the brain has been explored for roughly the past 20 years (Hsieh, Hägermark, et al., 1994). Since then, numerous brain imaging studies have been completed using a range of neuroimaging techniques such as, PET, fMRI and magnetoencephalography (MEG). Most of these studies have investigated the neural response to itch-inducing stimuli, such as histamine, cowhage and electrical itch stimuli (Mochizuki & Kakigi, 2015).

In itch research, many regions have been found to be activated by the use of histamine and cowhage-induced itch, including the prefrontal cortex (PFC), supplementary motor area (SMA), premotor cortex (PM), primary motor cortex (MI), the somatosensory cortices, cingulate cortex, precuneus, and insula (Darsow et al., 2000; Drzezga et al., 2001; Herde, Forster, Strupf, & Handwerker, 2007; Hsieh, Hägermark, et al., 1994; Ishiuji et al., 2009; Leknes et al., 2007; Mochizuki et al., 2003, 2007; Papoiu et al., 2011; Walter et al., 2005).

Itch research is similar to that of pain, in the respect that the specific functional roles of the somatosensory cortices are currently not well understood, with some inconsistent findings within the literature. Drzezga et al. (2001), for instance, used PET and histamine skin prick tests to the right forearm. This study is currently the only one which has investigated the brain response to varying intensities of itch stimuli. The results demonstrated that the itch induced a significant activation predominantly in the contralateral S1, but that there was no significant activation of S2. The S1 activity correlated with the stimulus intensity, along with participants' ratings of itch intensity. This, therefore, suggests that S1 has a crucial role in the intensity coding of itch. The role of S2 in the processing of itch perception, however, is still unclear. Drzezga et al. (2001) pointed out however that although the lack of S2 activation was 'striking', the functional significance of S2 is not fully understood for pain either. It may be the case that if the roles of S1 and S2 are similar to pain, maybe the intensity of the histamine prick test is not strong enough to evoke a response in S2. This is supported by the fact that previous itch studies using PET and fMRI did not observe a significant correlation between S2 activity and the intensity of itch stimuli and subjective itch sensations (Drzezga et

al., 2001; Leknes et al., 2007; Mochizuki et al., 2007), apart from one fMRI experiment (Herde et al., 2007).

Another way of exploring the brain regions involved in the sensory processing of itch is by observing which areas are activated when an individual views others experiencing an itch sensation. For example, socially contagious itch can occur in that viewing itch in others induces real itch sensations and scratching in observers (Ikoma, Steinhoff, Ständer, Yosipovitch, & Schmelz, 2006; Niemeier & Gieler, 2004; Papoiu et al., 2011). An fMRI study exploring contagious itch documented significant activation of S1, when participants viewed others scratching the body (Holle et al., 2012). These findings are interesting, as the activation has to be independent of empathy for tactile sensation, caused by the observation of scratching. This is because the effect of empathy observed in the brain for tactile sensation was cancelled out by comparing brain activity between viewing scratching and tapping the body.

Another novel study is a neuroimaging experiment which reported brain activation for both histamine and cowhage induced itch (Papoiu et al., 2012). While brain processing of histamine-induced itch has been investigated in numerous studies (Darsow et al., 2000; Drzezga et al., 2001; Hsieh, Hägermark, et al., 1994; Leknes et al., 2007; Mochizuki et al., 2003, 2009; Pfab et al., 2010; Schneider et al., 2008; Valet et al., 2007; Vierow et al., 2009), only one study so far investigated the brain processing of cowhage-induced itch, in comparison to histamine (Papoiu et al., 2012). The results suggested that both stimuli co-activated a core group of brain structures, including both S1 and S2. Also, the pattern of brain activation observed for histamine itch was in line with the results reported in previous experiments (Darsow et al., 2000; Drzezga et al., 2001; Hsieh, Hägermark, et al., 1994; Leknes et al., 2007; Mochizuki et al., 2003, 2009; Schneider et al., 2008). They concluded, however, that cowhage induced itch appears to induce a stronger and more extensive activation in S1 and S2 than histamine. It would therefore be interesting to directly compare the effects of TMS to the somatosensory cortices when histamine and cowhage are administered.

The only two studies which have successfully used brain stimulation to modulate itch intensity have only been published recently. Both have used transcranial direct current stimulation (tDCS) which is a type of brain stimulation which administers electric currents through delivering a low current to the brain via electrodes. There are different types of

stimulation depending on what behavioural response is desired, for example anodal stimulation which increases the neuronal excitability, cathodal stimulation which decreases it and sham which is used as a control and does not alter cortical excitability (Gandiga et al., 2006).

Knotkova, Portenoy, & Cruciani (2013) reported a single-case study of a patient diagnosed with syringomelia, accompanied by treatment-resistant chronic itch and pain. Syringomelia is when cysts or cavities forms within the spinal cord over a long period of time and often result in pain, paralysis, weakness and stiffness (Williams, 1980). Repeated cathodal stimulation of the somatosensory cortex was found to alleviate itch but not pain for several months, before returning to pre-treatment intensity. A second treatment course involving anodal stimulation of the motor cortex provided similar temporary itch relief. The authors suggested that tDCS can be used as a treatment to successfully reduce itch sensation. This treatment, however, was performed on one patient suffering predominantly from pain, as opposed to itch. This makes it therefore difficult to compare and interpret the findings, particularly with regard to chronic itch patients.

The other brain stimulation study was by Nakagawa et al. (2016) who used 14 healthy participants to explore the use of tDCS to S1, on histamine induced itch. The itch was induced on the left forearm and in the two experimental conditions, the cathode was either placed over ipsilateral S1 and the anode over contralateral S1, or vice versa. Sham stimulation was used as a control condition. The results showed that both experimental conditions temporarily reduced itch intensity. However, because of the fact that both hemispheres were stimulated, it is impossible to conclude the contribution of the ipsi- or contralateral stimulation of S1 to this reduction in itch. Another limitation of tDCS in particular is because of the size of the electrodes used (5 x 5cm), stimulation is not solely limited to the region of interest, but could potentially affect nearby areas such as the motor cortex. Overall, however, these two studies demonstrate the potential of using brain stimulation with regards to itch and hold great implications for future research.

1.7.3 The Role of the IFG in the Itch Process

The function of the IFG is usually associated with language, with BA44 thought to have a crucial role in phonological processing (Skipper, Goldin-Meadow, Nusbaum, & Small., 2007). Despite the fact that relatively little is known about the function of the IFG in the process of itch perception, various neuroimaging studies using histamine on healthy

volunteers have demonstrated its activity, indicating some involvement (Bergeret et al., 2011; Darsow et al., 2000; Drzezga et al., 2001; Herde et al., 2007; Hsieh, Hägermark, et al., 1994; Ishiuji et al., 2009; Kleyn et al., 2012; Leknes et al., 2007; Mochizuki et al., 2007, 2009, 2009; Papoiu et al., 2012). However, due to these studies being of a correlational nature it is still unknown whether this area is crucial within the process.

There is research which indicates that the IFG (specifically Broca's area) is activated during action observation (Fadiga et al., 2006), suggesting that it plays a role in interpreting actions of others. In support of this hypothesis, an fMRI study by Holle et al. (2012) has indicated a link between observation of scratching and the IFG. For example, as previously discussed, watching video clips of someone scratching (relative to control videos of tapping) activated many of the neural regions linked to the physical perception of itch, including the left IFG (BA44). Moreover, activity in the left IFG correlated with subjective ratings of perceived itch and also individual differences in neuroticism. This is interesting as neuroticism itself has therefore been identified as a reliable predictor of individual differences, in subjective feelings of contagious itch. This trait is also known to intensify particular clinical symptoms, such as chronic pain (Conrad et al., 2007).

Other research by Ishiuji et al. (2009) claimed that activation of the PFC may be likely to mediate part of the cognitive dimension of itch processing, associated with the encoding of the attendant stimulus. In addition to this, Mochizuki et al. (2003) used PET and histamine iontophoresis and found that the rCBF in the ACC and IFG positively increased with the concentration of histamine administered, which is in support of previous literature (Darsow et al., 2000; Drzezga et al., 2001).

Overall, although relatively little is known about the specific function of the IFG in both itch and pain, some research does point towards its role in the control of cognition and behaviour and that it may serve an attention-related function which modulates the degree of contagion. It is however, necessary to conduct more studies exploring its particular role in itch, to investigate whether its involvement is crucial.

1.8 Brief Summary and Research Questions

One of the first notable issues raised in the literature review is the fact that there is no consensus on which rating scale is the most reliable when measuring acute itch. The first experiment (Chapter 2) therefore directly compares the re-test reliability of three commonly

used measurement scales. The scales compared will be the pure visual analogue scale (pVAS), where the participant indicates itch intensity on a vertical line ranging from 0 (no itch) to 100 (the most intense itch imaginable). The second scale is a variation of the pVAS, where an additional 'Scratch Threshold' marker is set at 33% of the scale (tVAS, Darsow et al., 1996). The last, is the general Labelled Magnitude Scale (gLMS, LaMotte et al., 2009; Sikand et al., 2009), where the participants judge the magnitude of itch on a vertical line with quasi-logarithmically positioned labels. The scale which generates the least variance in itch intensity ratings between testing sessions will be taken as the most reliable and administered for the following experiments.

A second important question raised by the literature review is whether acute itch is multi-dimensional with regards to its intensity and unpleasantness. It is well documented that chronic itch is of a multidimensional nature, including both sensory and affective elements (International Association for the Study of Pain, IASP; Chen, 2001). However, many laboratory-based experiments investigating acute itch view it implicitly as unidimensional by only measuring the intensity aspect of the sensation. An important question therefore is whether acute and chronic itch differ in their dimensionality. If it is the case that laboratory-based acute itch is unidimensional then the current approach of measuring only the perceived intensity is adequate in capturing the sensation. However, if it is multidimensional, then selectively measuring intensity and disregarding the affective aspects could be problematic, as has been described in the general introduction. Chapters 3 and 4 describe two experiments that speak to this issue, with one chapter exploring this for the case of cowhage (Experiment 2) and the next one for histamine (Experiment 3).

The last question to be addressed is that based on the evidence presented in the literature review, it is concluded that the brain regions specifically involved in the process of itch are yet to be confirmed. Therefore, the primary aim of Experiment 4 (Chapter 5) was to explore which brain areas are causally involved in the process of histamine and cowhage induced itch, when induced in a sample of healthy volunteers.

Chapter 2 Experiment 1: The Re-test Reliability of Three Different Itch Measuring Scales

2.1 Overview

This chapter reports the first experiment which compared the re-test reliability of three commonly used measurement scales for itch. The scale with the least variance in itch intensity ratings between testing sessions was taken as the most reliable and administered for the following experiments.

2.2 Introduction

The reliable measurement of itch intensity is crucial, both in research, as well as clinical contexts. However as aforementioned (see introduction), there is still no absolute consensus of which measurement tools should be employed and validation of these instruments is still ongoing (Phan et al., 2012). Various researchers (Phan et al., 2012; Reich & Szepietowski, 2013) have examined the reliability of different rating scales for assessing chronic itch intensity. However, an evaluation of rating scales for the assessment of acute experimental itch, artificially induced using stimuli such as histamine or cowhage, is currently lacking.

In this experiment, the test-retest reliability of three commonly used rating scales was evaluated. The first scale was the pure visual analogue scale (pVAS), where the participant indicates itch intensity on a vertical line ranging from 0 (no itch) to 100 (the most intense itch imaginable). The second scale was a variation of the pVAS, where an additional 'Scratch Threshold' marker is set at 33% of the scale (tVAS, Darsow et al., 1996). The last, was the general Labelled Magnitude Scale (gLMS; LaMotte et al., 2009; Sikand et al., 2009), where the participants judge the magnitude of itch on a vertical line with quasi-logarithmically positioned labels of "no sensation" at 0, "barely detectable" at 1, "weak" at 6, "moderate" at 17, "strong" at 35, "very strong" at 53 and "strongest imaginable sensation of any kind" at 100. All three scales are identical in range, but differ in the type and number of verbal labels provided.

Various research has documented potential issues when administering scales, such as a misunderstanding of how to use the scale due to it being complex, not self-explanatory, or participants simply being unfamiliar with the tools (see introduction – The effective use of Visual Analogue Scales). The current experiment therefore employed a training session prior to the experimental sessions, similar to that used by LaMotte et al. (2009). This allowed

participants to become familiar with the measurement scale. It also ensured that they experienced the sensation of cowhage, as due to the fact that all participants were healthy with no history of a skin disorder, they may have never experienced an intense itch.

2.3 Methods

2.3.1 Design

The experiment was a mixed design, as participants were randomly assigned to three different Scales and all took part in two experimental Sessions (test-retest). There was a space of approximately one week between the three sessions (7.04 days ± 1.0) to minimise memory effects. In each of the sessions participants were required to rate the intensity of the itch every 15 seconds for 10 minutes.

2.3.2 Participants

After having given written informed consent, 90 participants took part in the study. However, there were only 77 usable datasets as 12 participants did not show a sufficient itch response (i.e., peak itch intensity was rated lower than 15 in the initial familiarisation session) and one was classified as an outlier (itch response above 3 SD of group mean). A total of 77 participants (38 females, 39 males, mean age 24.66 ± 6.5; N=25 in the gLMS group, N=26 in the pVAS and tVAS groups) were therefore included in the statistical analysis. Exclusion criteria included participants with any sensitivity to the left volar arm (e.g., wounds, rashes, swelling or reddening, certain), a history of various illness and disease, for example, skin conditions (e.g., eczema) and mental illness (e.g., depression), and lastly, medication taken 24 hours prior to the experiment which could potentially interact with cowhage, for example some types of pain killers (e.g., aspirin, ibuprofen; please see appendix for full exclusion criteria). The study was approved by the University of Hull Psychology Ethics Committee and performed according to the British Psychology Society, code of human research ethics principles. Participation was remunerated with course credits or a payment of £12 on completion of all three sessions.

2.3.3 Stimuli and Materials

Prior to the experimental sessions, the spicules were counted, ensuring that in each trial 60 - 65 cowhage spicules were applied. The spicules were gently placed into a square area of 16 cm^2 defined by medical tape on the left volar forearm and then rubbed into the skin for 45 seconds. This results in about a third of the spicules (Papoiu et al., 2011) to become lodged into the skin and deliver a cysteine protease that elicits the itch (LaMotte et al., 2009). No

gloves were worn during this process to prevent spicules sticking to them and therefore decreasing the amount intended and in turn, decreasing the validity of the results.

The participants' itch ratings were recorded using three computerised scales designed on Presentation Version 17.0 (www.neurobs.com). Three different scales were used in order to test their reliability (see Figure 2.1). The first scale was a pure VAS (pVAS) ranging from 0 (No itch) to 100 (most intense itch imaginable). The second scale included an additional 'scratch threshold' at one-third of the scale (tVAS; Darsow et al., 1996; Magerl & Handwerker, 1988). In studies where this scale is used, participants were instructed that the scale includes a 'scratch threshold' at one-third (33/100) of the scale. Ratings above this threshold indicate that the participants feel the impulse to scratch. The third scale was a general version of the Labelled Magnitude Scale (gLMS; Bartoshuk et al., 2004; Green et al., 1996; LaMotte et al., 2009). The gLMS requires the participants to judge the magnitude of itch on a computerised vertical line which includes semantic labels of "no sensation" at 0, "barely detectable" at 1, "weak" at 6, "moderate" at 17, "strong" at 35, "very strong" at 53 and "strongest imaginable sensation of any kind" at 100, spaced in a quasi-logarithmic fashion. However, only the semantic labels are visible to the participants. All 3 scales were presented vertically for consistency (no significant difference previously found between horizontal and vertical VAS; Reich et al., 2012). The heart rate was recorded throughout the itch intensity ratings, using a PowerLab 26T (Ad Instruments) via a pulse transducer securely attached to the thumb. This data was not analysed as it was purely added to the experiment so that participants would believe that the true aim of the study was to explore the effects of cowhage on the heartrate.

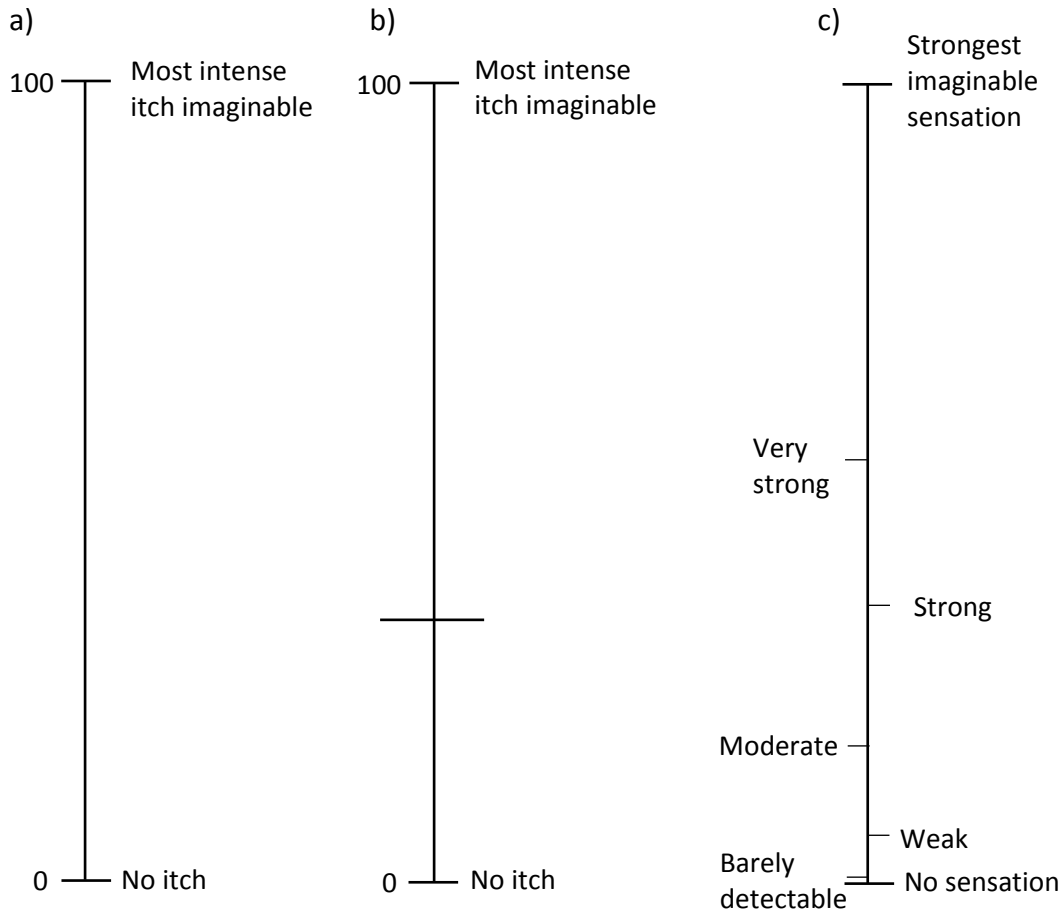


Figure 2.1 Itch intensity measurement scales, a) Pure VAS, b) VAS + scratch threshold and c) gLMS.

2.3.4 Procedure

Participants were informed that the experiment was looking into the effect of itch on the heart rate to minimise demand characteristics. All participants took part in an initial familiarisation session so that they were familiar with the computerised rating scale and the sensation of cowhage-induced itch. It also enabled the exclusion of non-responders (i.e., participants where intensity did not exceed a rating over 15). In the familiarisation session, the participants had to rate a range of itch examples (e.g., an ant bite) both on paper (using the appropriate scale) and then again using the computerised rating scale (see appendix for full questionnaire). On the paper-based scale the experimenter read out each example and the participant then marked on the scale (one scale per example) where they believed reflected the imagined itch intensity. On the computerised version the example was shown on the screen and the participant rated accordingly. This gave participants the opportunity to think about the intensity of personal itch experiences and then practise rating them using the computerised scale, prior to using the same scale to rate cowhage-induced itch.

Participants made their ratings by moving an indicator along the scale using the wheel of the mouse and then clicking using the left button to confirm. Ratings were obtained every 15 seconds and up to 10 minutes after the application of cowhage. The indicator was reset to zero at the beginning of each 15 second interval. The recording of the participants' heart rate began just before the application of cowhage. Participants were not allowed to scratch the itching skin area during the rating period. At the end of each session, the spicules were removed using adhesive tape and a cotton cloth.

2.3.5 Results

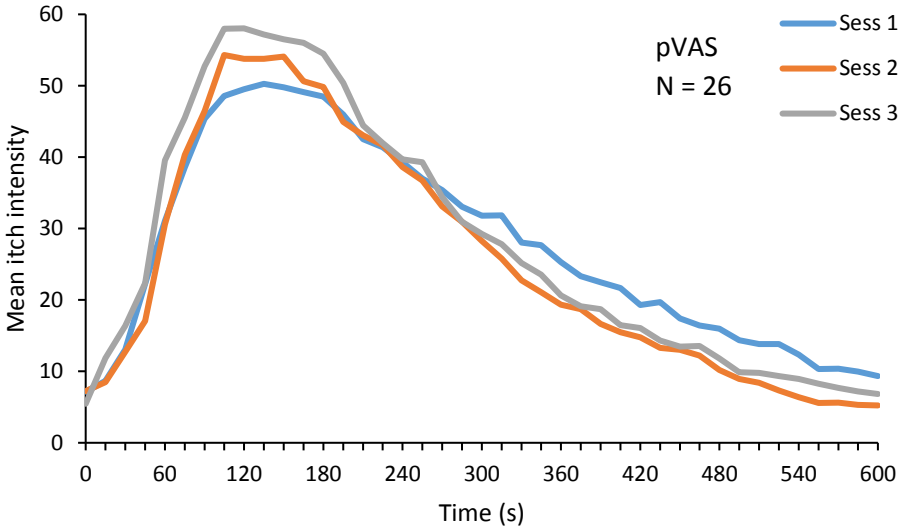
The current experiment compares the re-test reliability of three commonly used rating scales previously described (pVAS, tVAS, gLMS; See Figure 2.1). Acute itch was induced using cowhage spicules over three repeated measurement occasions. Session 1 served as a familiarisation session, whereas the intra-class correlation coefficient (ICC) between sessions 2 and 3 was taken as index of test-retest reliability.

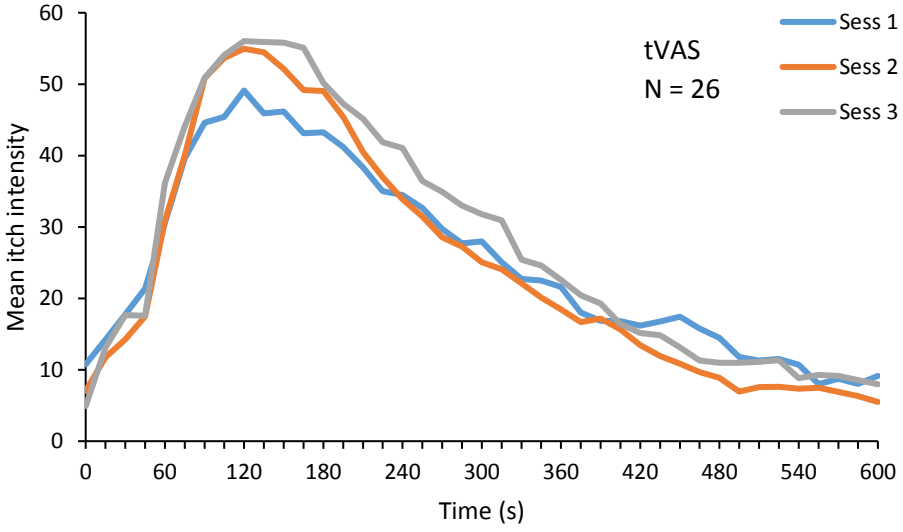
It is evident from the itch ratings that the application of 60 - 65 cowhage spicules is a successful method of inducing itch, with 84% of participants providing a maximum intensity rating of 15 or above. It is also evident that the general pattern of itch evoked follows what is considered to be, the typical itch response induced by cowhage (LaMotte et al., 2009). This is demonstrated by the mean itch over time, across the three scales (see Figure 2.1). Itch

intensity peaks between 1 to 3 minutes after onset and then gradually decreases until the effects of cowhage wear off.

Despite this, there are two notable differences in the reported itch response across the sessions. Firstly, the mean peak intensity ratings slightly increase throughout the sessions (see Figure 2.2) and secondly on average, participants reported the peak itch to occur 186 s (± 94.76) after the onset of skin provocation in session 1, 44 s later than in Session 2 (158 s ± 71.76) and 36 s later than session 3 (150 s ± 58.98; see Figure 2.3). There was therefore a significant delay in the reporting of the itch intensity peak in session 1, in comparison to session 2 and 3 (session 1 vs. session 2, p = .008, session 1 vs. session 3, p =.001, session 2 vs. session 3, p =.335).

This delayed itch intensity peak could potentially be due to the fact that participants have never experienced an itch induced by cowhage and therefore do not have the knowledge that the intensity peaks relatively early (roughly 1 - 3 minutes). The only information they are provided with is that they will rate the itch for 10 minutes, therefore they may be reluctant to rate highly so early on in that time period, as they do not know how long the intensity will increase for. Since the participants in the present study had no prior experience of cowhage-induced itch, we hypothesised that the rating data of the first session (familiarisation) would be qualitatively different from sessions 2 and 3, because participants are initially unsure about the typical time course of cowhage-associated itch.





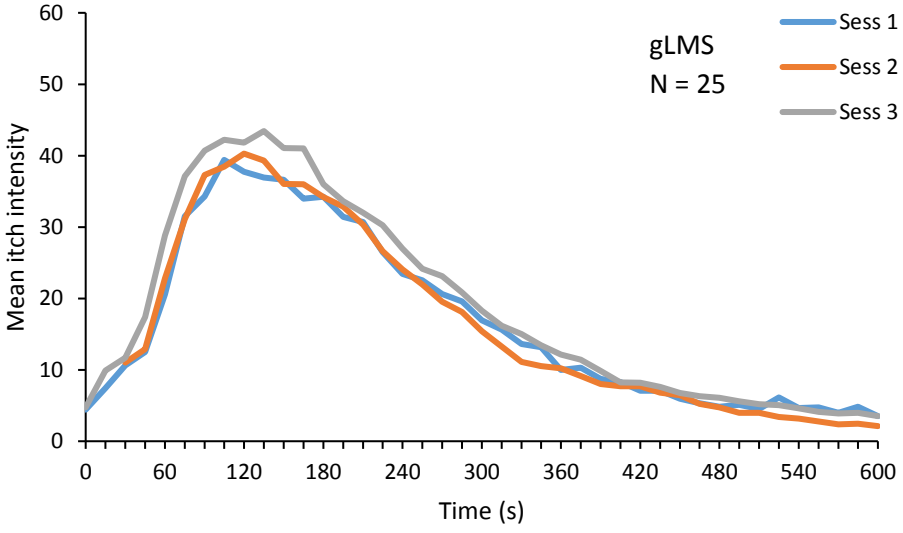


Figure 2.2 Mean itch overtime, across three sessions in pVAS, tVAS and gLMS, respectively.

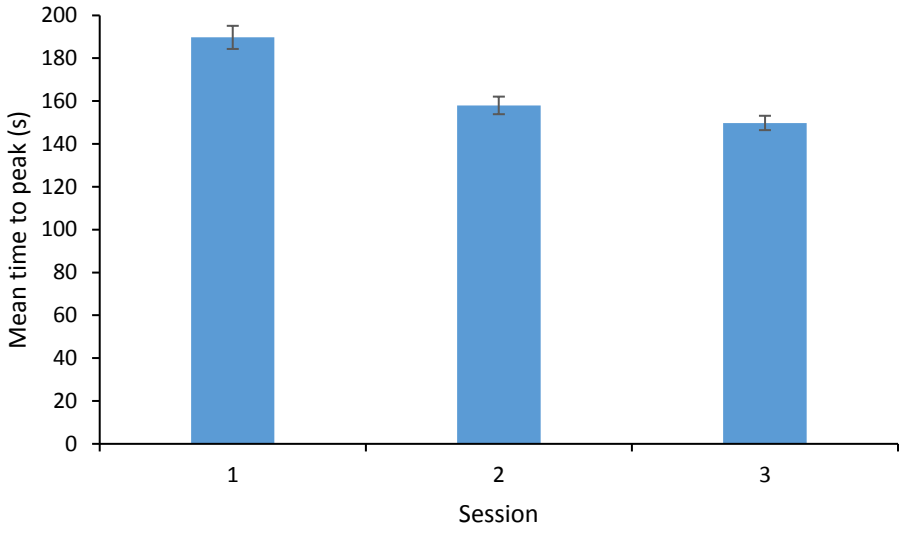


Figure 2.3 Overall mean peak time intervals (± 1 SEM) across three sessions (n=77).

In the following, we analysed the re-test reliability of the mean and peak itch intensity, separately for each rating scale. Reliability was estimated by calculating the intra-class correlation coefficient of the respective scores of Sessions 2 and 3.

The peak and the mean of each time course were used to quantify the overall itch intensity experienced by each participant. The scores did not differ significantly between the sessions (see Table 2.1). The Shapiro-Wilk tests indicated that both the mean and peak scores were normally distributed (all $W > 0.93$, all $p > 0.09$). This reliability analysis was completed in SPSS (Version 23.0, IBM), using a two-way mixed model, using the absolute agreement between measurement sessions (McGraw & Wong, 1996).

Table 2.1 Descriptive statistics of the two itch indices (Mean, Peak) for each session and scale group. Columns 5 and 6 provide the *t* and *p* values of independent samples t-tests comparing sessions 2 and 3.

Scale	Index	*M* (*SD*) Session 2	*M* (*SD*) Session 3	*t*	*p*
pVAS (n=26)	Mean	24.38 (13.34)	27.45 (13.60)	1.11	0.28
	Peak	64.92 (20.99)	66.31 (22.08)	0.32	0.75
tVAS (n=26)	Mean	25.19 (12.05)	27.64 (13.78)	1.14	0.26
	Peak	64.04 (21.20)	67.31 (24.54)	0.99	0.33
gLMS (n=25)	Mean	16.24 (7.38)	18.59 (9.74)	1.83	0.08
	Peak	48.12 (21.64)	49.68 (23.68)	0.65	0.52

As shown in Table 2.2, the gLMS had the highest test-retest reliability. This was the case regardless of which index was used to quantify itch intensity (peak: ICC =.86; mean: ICC =.71). The tVAS had an intermediate reliability (peak: ICC =.73; mean: ICC =.64) and the pVAS was the least reliable scale (peak: ICC =.50; mean: ICC =.45).

Table 2.2 Reliability (as estimated by the Intraclass Correlation Coefficient, ICC) for the 3 scales and 95% Confidence Interval.

Index	Scale	ICC	95% CI
Mean	pVAS	.45	.09 – .71
	tVAS	.64	.35 – .82
	gLMS	.71	.44 – .86
Peak	pVAS	.50	.14 – .74
	tVAS	.73	.48 – .87
	gLMS	.86	.72 – .94

An additional analysis was completed in order to ensure that the higher reliability of the gLMS was not due to the clustering of responses. For instance, participants having a tendency to cluster their responses around the verbal labels of the scale, therefore restricting the spread of the ratings (particularly in comparison to the pVAS and tVAS conditions which only have two labels). This categorical use of the gLMS has been documented previously in the domain of taste perception (Hayes et al., 2013). As demonstrated in Figure 2.2, there is little evidence of categorical rating behaviour in the gLMS group. For example, there is no evidence of peaks around the labelled positions for 'barely detectable', 'weak', and 'moderate', however some evidence is present of response clustering around the labelled positions for 'strong' and 'very strong' positions.

To investigate this further, the rating time-courses of participants in the gLMS condition were individually analysed and it was evident that a limited 2 out of 25 participants were indeed using the scale in a more categorical way, rather than in a continuous fashion (see Figure 2.4). If the reliability of the gLMS were driven by the presence of categorical rating behaviour, then excluding these two subjects would result in a marked reduction of reliability. The reliability indices (ICC) of the gLMS for the full sample, n=25, are .86 and, .71, for peak and mean, respectively. When excluding the two above-mentioned participants exhibiting categorical rating behaviour, these indices are .87, and .72, respectively. Therefore, confirming that the categorical use of the gLMS occurred only in 2 out of 25 participants, and its presence does not impact upon scale reliability.

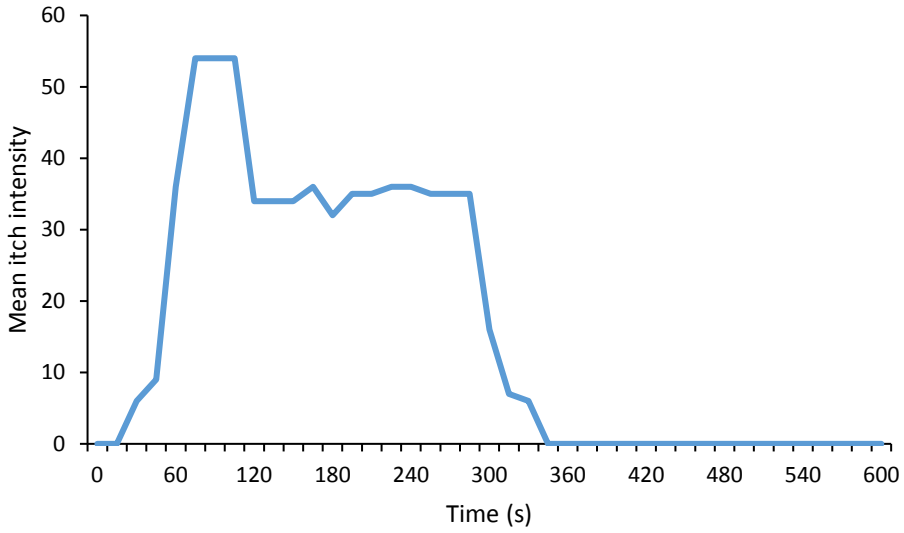


Figure 2.4 Example of a rating time course from a single subject from the gLMS group exhibiting categorical use of the scale (participant 54, session 3).

2.4 Discussion

One of the first items to note is the importance of including a familiarisation session, as the results in the first session were qualitatively different in comparison to Sessions 2 and 3, with a significant delay in reported itch peak. This could potentially be because participants were initially unsure about the typical time course of cowhage-associated itch, due to the fact that participants have never experienced the sensation. They do not have the knowledge that the intensity peaks relatively early (roughly 1 - 3 minutes). The only information they are provided with is that they will rate the itch intensity every 15 seconds up to 10 minutes, therefore they may be reluctant to rate highly so early on in that time period, as they do not know how long the intensity will increase for. This highlights the importance of a familiarisation session to ensure that participants have experienced the itch process induced by cowhage and are confident in their ability to rate it using the computerised scale.

Overall, the gLMS demonstrated the greatest re-test reliability, followed by tVAS and then pVAS. First, it is important to note, that the higher reliability of the gLMS cannot be accounted for by the clustering of responses as the categorical use of the gLMS occurred only in 2 out of 25 participants and their presence within the dataset did not impact upon scale reliability. One explanation for the reliability could be the degree in which a scale is open to interpretation. For example, the pVAS is the most basic, indicating only the minimum and maximum itch intensities and therefore leaving the rest of the scale open to interpretation. The rating of a subjective sensation on a line, however, is a relatively complex process which

can be easily misunderstood, especially when there are no landmarks presented. Research has indicated that the lack of verbal anchors in the pVAS creates ambiguity, as the participant is unsure where exactly they should position their ratings (Gonzalez-Fernandez et al., 2014; Kersten et al., 2012). This unsystematic variation may therefore reduce the reliability of the pVAS. The tVAS includes a scratch threshold marker, providing participants with an additional landmark to guide their reporting of intensity. The gLMS includes 7 verbal indicators and no numerical information, therefore further reducing the interpretation of the scale, through the guidance of various landmarks.

The gLMS is therefore arguably the most self-explanatory scale, with the most guidance, which in turn, generated the highest reliability. This is crucial, not only for acute itch measurement but for clinical trials, where small intensity variations are vital in assessing treatment strategies. The gLMS could therefore, potentially be a suitable alternative to pVAS in these circumstances. It is however, difficult to compare these findings across studies (especially acute vs. chronic) and the different time scales used (for instance: hours vs. weeks). Further studies would be necessary in order to investigate whether these benefits of gLMS also hold true for experimental itch induced in chronic itch patients or for the assessment of chronic itch.

A second explanation for the results is that the verbal labels of the gLMS have an estimated quasi-logarithmic distance between them, determined by a semantic scaling procedure (Green et al., 1996; Green et al., 1993). The scale labels are therefore positioned in a way that mirrors their semantic magnitude, thus generating ratio level data. It is debatable, however, whether the pVAS yields ratio data (Price et al., 1983) or simply ordinal data (for review, see Kersten et al., 2012). Forrest & Andersen (1986) argue that although the VAS ratings are generally converted into either cm or percentage in reality the scale has no true unit of measurement and is therefore only ordinal. It has therefore been argued that the VAS should be analysed as non-continuous using statistical methods for ordinal data (Lund et al., 2005) . In addition to this, there is evidence that rather than providing a linear transformation of the internal representation of stimulus intensity, the pVAS generates only a non-linear representation, with a tendency to generate a compression of ratings at the top end of the scale (Gonzalez-Fernandez et al., 2014). This would therefore suggest that the gLMS may be an appropriate alternative over the pVAS in clinical settings in particular, as it reduces the ceiling effect by decompressing the upper limit of the scale (Gonzalez-Fernandez et al., 2014).

There are therefore at least two possible explanations as to why the gLMS generates the most reliable results. The first, is that due to the number of verbal labels provided there is more guidance during the rating process and therefore less of the scale is open to interpretation. The second, is not the labels themselves but the way they are logarithmically spaced. One way of testing which explanation is the most valid would be to administer two versions of the gLMS. One group of participants would rate itch intensity on the standard gLMS and the other would receive a version of the gLMS which does not have a logarithmic distance between the labels, but simply equal distances. If it is the case that it is the verbal labels alone which increases reliability, there will not be a significant difference between the results. If however, it is the logarithmic spacing, there will be a significant difference, with the standard gLMS generating a significantly higher retest reliability.

In summary, the current findings indicate that the superior reliability makes the gLMS ideally suited to study experimentally induced itch in healthy participants, in comparison to the pVAS and tVAS. However, as scale reliability is not a fixed property, but is also population-dependent (Shrout, 1998) further experiments are necessary in order to explore whether these advantages of the gLMS in assessing experimentally induced itch are also applicable to chronic itch patients or to the clinical assessment of chronic itch intensity. Despite this, the results demonstrated that by employing a sensitive, self-explanatory measurement scale and an effective familiarisation session, the re-test reliability of acute itch perception can be significantly increased.

Chapter 3 Experiment 2: The Subjective Dimensionality of Cowhage-Induced itch

3.1 Overview

This chapter explores the process of cowhage-induced itch with regards to its time course and general itch patterns. It also seeks to find out whether perceived intensity and unpleasantness dissociate in the case of acute cowhage-induced itch. Finally, these findings will inform the transcranial magnetic stimulation experiment (Chapter 5) as it will highlight whether cowhage is a reliable method of inducing itch.

3.2 Introduction

A recent method of inducing itch is the use of cowhage which various researchers have demonstrated is a suitable experimental itch model used to explore a histamine-independent pathway of itch (LaMotte et al., 2009; Papoiu et al., 2011; Rukwied et al., 2013). It is also documented that cowhage induces a more intense itch sensation to that of histamine and that it is the dominating factor in itch perception when both pathways are stimulated simultaneously (Papoiu et al., 2011). In addition to this, it could be argued that cowhage-induced itch has an advantage over histamine, due to its additional nociceptive sensations, similar to that of clinical pruritus (LaMotte et al., 2009). Cowhage was therefore the stimuli chosen for the purpose of this experiment.

It is well documented that chronic itch is of a multidimensional nature, including both sensory and affective elements (International Association for the Study of Pain, IASP; Chen, 2001). Despite this, many laboratory based experiments exploring acute itch view it as unidimensional, only measuring the intensity element of the sensation. An important question therefore, is whether acute and chronic itch differ in their dimensionality. If it is the case that laboratory-based acute itch is unidimensional, then this approach is adequate in capturing the sensation. However, if it is multidimensional, then selectively measuring intensity and disregarding the affective aspects could be problematic, as has been described in detail in the general introduction. If the latter is the case, it would suggest that current experimental paradigms of acute itch are not suitable for investigating the process of chronic itch.

In addition to the itch dimensionality, the current study also explores the participants' heart rate variability (HRV). This is to investigate whether there are any significant differences, induced by the varying doses and reported itch intensity and unpleasantness. Various studies

have measured HRV in order to monitor the autonomic nervous system (e.g., in patients with chronic disease; Dobrek, Friediger, & Thor, 2006; Hassett et al., 2007), which has a crucial role in the stress response. These HRV measures are categorised into a set of parameters that either reflect a primarily sympathetic response, such as stress and anxiety, or a parasympathetic response (e.g., relaxation and calmness; Hayano et al., 1991; Moser et al., 1998).

Various research indicates a dysfunction of the ANS could potentially be a contributing factor to pruritus in patients with AD (Ständer & Steinhoff, 2002). For example, Tran et al. (2010) monitored HRV at 5 min intervals at rest and after each of 3 acute stress tests, which included histamine-induced itch on the forearm, scratching around the itch site and the Trier Social Stress Test. They found that AD patients had a significantly higher heart rate than the controls in all conditions. The High Frequency (HF) component of the power spectrum (reflecting parasympathetic activity) responded rapidly to itch and scratching in healthy controls, but demonstrated a limited adaptability in AD patients. They therefore concluded that AD is a stress-responsive disorder, involving autonomic nervous system dysfunction. AD patients exhibited an overactive sympathetic response to itch and scratching, while the parasympathetic tone was persistently elevated indicating a lack of adaptability in response to stress. As the participants in the current study are healthy volunteers, based on these findings we could hypothesise that the HF component would increase rapidly post-itch induction and may potentially positively correlate with dose increase.

The aims of Experiment 2 are as follows: First, to explore any changes in the time-course and peak of itch intensity/unpleasantness, induced by varying doses of cowhage spicules. Secondly, to examine any dissociation between itch intensity and unpleasantness, which would indicate that they are dissociable dimensions. If the claim is accurate that intensity and unpleasantness are distinct dimensions of acute itch, we should observe instances where they dissociate over time (e.g., the intensity peaks before unpleasantness) or over intensity (e.g., an increase in cowhage spicules may not induce correlated ratings of intensity and unpleasantness). In addition, heart rate variability was measured throughout the experiment in order to detect any significant difference with regards to the dose administered or dimension measured. As aforementioned, it was predicted that the high frequency component will increase rapidly post-itch induction and may potentially positively correlate with dose increase (e.g., the higher the dose, the greater the HF domain).

3.3 Methods

3.3.1 Design

The experiment was a 2 x 3 factorial design, with 2 dimension conditions (intensity and unpleasantness) and 3 dose conditions (low, medium and high). There was a space of one week between the two sessions. Rating order (intensity or unpleasantness first) were counterbalanced between participants (this includes the practice session), along with the site of application (left vs. right volar arm).

3.3.2 Participants

A total of 29 participants (11 male, mean age 20.33 ± 3.67) completed both sessions of the study. Exclusion criteria were identical to Experiment 1. The study was approved by the University of Hull Psychology Ethics Committee and performed according to the British Psychology Society, code of human research ethics principles. Participation was remunerated with 3 hours course credits at a rate of 1.5 hour per session.

3.3.3 Stimuli and Materials

The itch inducing stimuli used were cowhage spicules. Prior to the experimental sessions, the spicules were counted using a (5x) magnifying lens and micro-tweezers, to prevent the accidental application of more spicules than necessary. During the experiment, a visual barrier was used, so that participants could not see their hand during itch induction and rating, in order to control a potential confounding variable (i.e. looking at one's own reddened skin might be itch-inducing in itself; Lloyd, Hall, Hall, & McGlone, 2013). Cowhage spicules were prepared and applied as described in Experiment 1.

The participants' itch ratings were recorded using an adapted version of the Vertical General Labelled Magnitude Scale (gLMS) designed on Presentation. This scale is used by experts in the area (Bartoshuk et al., 2004; Green et al., 1996; LaMotte et al., 2009) and was found to have superior reliability as compared to other rating scales (see Chapter 2). During the intensity session, the participants judged the intensity of the itch on a scale which includes semantic labels of "no sensation" at 0, "barely detectable" at 1, "weak" at 6, "moderate" at 17, "strong" at 35, "very strong" at 53 and "strongest imaginable itch intensity of any kind" at 100, spaced in a quasi-logarithmic fashion, on a vertical line. During the unpleasantness session, participants rated the unpleasantness of the itch. The scale was labelled as follows: "no unpleasantness" at 0, "barely detectable" at 1, "weak" at 6, "moderate" at 17, "strong" at 35, "very strong" at 53 and "strongest imaginable unpleasantness of any kind" at 100. Only the semantic labels however, were visible to the participants. Ratings were made by moving

an indicator along the scale using the wheel of the mouse and then clicking using the left button to confirm the intensity/unpleasantness of the itch. The indicator then reset to zero at the beginning of each 15 second interval, for the participants to provide their next rating. The heart rate was monitored and recorded throughout the experimental sessions using a PowerLab 26T and was collected via a pulse transducer securely attached to the thumb.

3.3.4 Procedure

3.3.4.1 Familiarisation Session

All participants took part in an initial familiarisation session so that they were familiar with the computerised rating scale, the sensation of cowhage-induced itch and the difference between intensity and unpleasantness. In this familiarisation session, participants were informed that there were two aspects of itch which they would have to rate: the intensity, i.e., how strong the itch feels, and the unpleasantness, i.e., how unpleasant, uncomfortable or disturbing the itch is experienced. To ensure participants were fully aware of this distinction, instructions from a pain experiment by Price, McGrath, Rafii, & Buckingham (1983) discriminating between intensity and unpleasantness using visual analogue scales, were adapted. Instructions were as follows.

> "The distinction between these two aspects of itch might be made clearer if you think of listening to a sound, such as a radio. As the volume of the sound increases, I can ask you how loud it sounds or how unpleasant it is to hear it. The intensity of itch is like loudness; the unpleasantness of itch depends not only on intensity but also on other factors which may affect you. There are scales for measuring each of these two aspects of itch. Although some itch sensations may be equally intense and unpleasant, we would like you to judge the two aspects independently."

The participants then had to rate the itch intensity or unpleasantness of a range of itch examples (e.g., an ant bite) both on paper (using the appropriate scale) and then again using the computerised rating scale. This gave participants the chance to think about the intensity/unpleasantness of personal itch experiences and then practise rating them using the computerised scale prior to using the same scale to rate cowhage-induced itch.

Participants then had a practise trial rating rather the intensity or unpleasantness of the cowhage induced itch. They provided their first rating of intensity or unpleasantness as soon as the rubbing of spicules began. Ratings continued up until 10 minutes. The recording of the

participants' heart rate began just before the application of cowhage. The spicules were then removed using adhesive tape and a cotton cloth after 10 minutes.

3.3.4.2 Experimental Sessions

The two experimental sessions included three applications of cowhage each (low 25, medium 65 and high 100 dose) as described above. The interval between the cowhage applications was roughly 10 minutes as this allowed the previous dose to stop itching. The dimension, dose order and site application were counterbalanced. At the end of the second experimental session participants were debriefed and informed of the true nature of the experiment.

The doses were chosen based on Experiment 1 (averaging session 1 and 2, across all 25 participants in the gLMS condition), where 65 spicules generated an estimated a mean itch of 17 (classified as moderate itch according to gLMS quasi-labelling) and a peak itch of 49 (a rating of 53 being the 'strongest itch imaginable'). In addition to this, previous literature documented that cowhage induces additional nociceptive sensations of pricking and burning (Sikand et al., 2009), therefore increasing the spicule dose above 100 may result in an increase of these sensations.

3.3.5 Heart Rate Analysis

The heart rate was measured throughout the itch ratings, in order to detect any significant difference with regards to the dose administered or the dimension rated. The heart rate data was collected from all 29 participants, out of which 28 were usable for the analysis (one was excluded due to noise in the dataset, making the beats per minute uncalculable). The data was analysed using the LabChart 8 Heart Rate Variability (HRV) 2.0 Add-On module, which calculates the variation in the time interval between heartbeats. Ectopic (abnormal) beats were manually removed from the dataset prior to analysis. The HRV module used the Lomb Periodogram nonparametric method for spectral analysis bands.

HRV measures variations around beat-to-beat intervals of the heart rate, and these measurements translate into a set of parameters, reflecting states of sympathetic (stress, anxiety) or parasympathetic (relaxation, calmness) activation within the body (Hayano et al., 1991; Moser et al., 1998). In the frequency domain, power spectral analysis was performed using a Fast Fourier Transform (FFT) algorithm. The software used this algorithm to define the power spectral density (Pagani et al., 1986). Within the spectrum, the high-frequency component (HF) (0.15–0.50 Hz) is an indicator of parasympathetic tone, the low-frequency component (LF) (0.04–0.15 Hz) is a measure of sympathetic/parasympathetic balance, and the very low-frequency component (VLF) (0.003–0.04 Hz) is an indicator of sympathetic

regulation (Electrophysiology, 1996; Furlan et al., 1990; Malliani, Pagani, Lombardi, & Cerutti, 1991; Tran et al., 2010). Due to the fact that there were large variations between subject differences in the total power, the data were normalised. This was achieved by dividing each frequency band by the total power. Time domain parameters were also analysed such as SDNN (standard deviation of normal R-R intervals), RMSSD (root mean square of successive differences) and the total power.

3.4 Results

In order to examine whether cowhage induce itch is dose dependant and can evoke dissociable feelings of intensity and unpleasantness, the itch peak, time to peak and mean were analysed.

3.4.1 Time to Peak

In order to explore any patterns or differences in the time-course induced by the varying doses of cowhage, the time to peak was analysed. As can be seen in Figure 3.1 the mean intensity time to peak decreases in relation to the dose, with the low dose taking the longest time to peak (M = 134.48 s, SD = 80.63 s), followed by the medium dose with a mean decrease of 11.38s (M = 123.10 s, SD = 41.15 s) and then highest dose which decreased by 6.2s (M = 116.90 s, SD = 45.25 s).

The mean time to peak for the unpleasantness dimension however, does not follow this same pattern, as the mean does not decrease as the dose increases. The low dose generated a mean of 113.28 s (SD = 112.72 s), followed by the medium dose (M = 141.21 s, SD = 59.13 s) and then the highest (M = 124.14 s, SD = 37.58 s). The standard deviations interestingly also decrease as the dose increases in the unpleasantness condition, therefore indicating less variance in the time to peak. As demonstrated in Figure 3.1 this is also the case for the standard error, for both dimensions. As the dose increases, the variance decreases, indicated by the standard error bars.

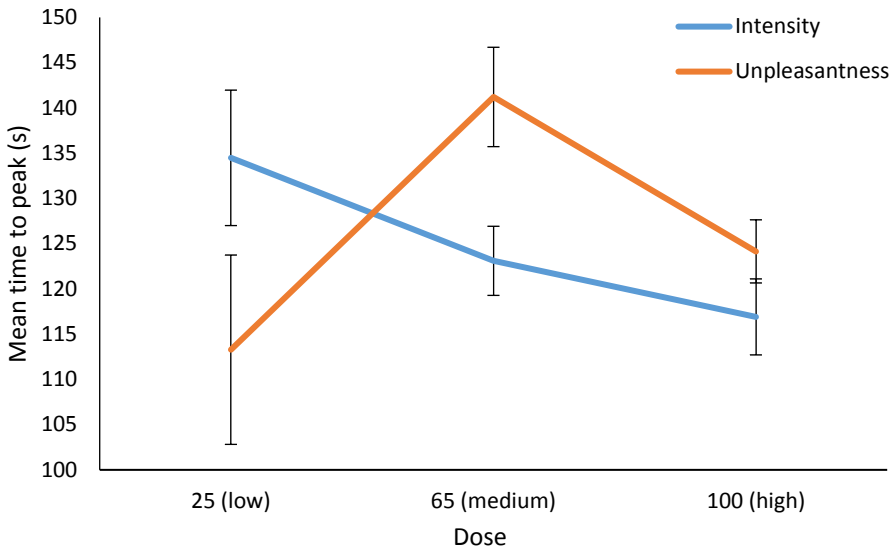


Figure 3.1 Mean peak time intervals (± 1 SEM) across three spicule doses (low: 25, medium: 65, high: 100) and two dimensions (n=29).

The repeated measures ANOVA however, indicated that there was no significant main effect of Dimension $F(1,28) = .018$, $p = .893$ or, after a Greenhouse-Geisser correction, Dose $F(1.48,41.52) = .48$, $p = .567$. There was no significant interaction between the two $F(1.76, 49.4) = 1.65$, $p = .204$.

As there was no significant difference between the dimensions, the variance within the overall mean time to peak (averaging intensity and unpleasantness) was analysed in order to examine any differences across the doses. This was in fact the case, as variance decreased as the dose increased (see Figure 3.1; low: $M = 123.90$ s, $SD = 76.58$ s; medium: $M = 132.16$ s, $SD = 36.33$ s; high: $M = 120.52$ s, $SD = 29.79$ s). The variances were significantly different for the low and medium doses of itch ratings; $t(1,56) = 10.65$, $p = .002$ and the low and high; $t(1,56) = 15.78$, $p = .000$. The variances for the medium and high dose however, were not significantly different; $t(1,56) = .58$, $p = .450$.

3.4.2 Mean Itch

As can be in seen Figure 3.2 the mean intensity increases in relation to the doses, with the low dose generating the lowest mean ($M = 11.27$ s, $SD = 11.71$ s), followed by the medium dose with a mean increase of 3.17 s ($M = 15.91$ s, $SD = 12.74$ s) and then the highest with a slight increase of 1.13 s ($M = 17.04$ s, $SD = 8.73$ s). This general pattern also applies to the mean unpleasantness, with the low dose generating a mean of 7.23 s ($SD = 11.43$ s), followed by the medium dose increasing by 4.91 s ($M = 12.14$ s, $SD = 9.30$ s) and then the highest, with an increase of 4.89 s ($M = 17.03$ s, $SD = 11.66$ s). However, although both mean itch

dimensions increase as the doses gets higher, the mean unpleasantness continues to increase at the same rate (a mean of almost 5 units on the 0 - 100 rating scale), whereas the mean intensity does not increase at the same rate (dropping from an increase of approximately 3 s to 1 s). Therefore the mean intensity appears to level off and stabilise, whereas the unpleasantness continues to increase.

A 2 x 3 repeated measures ANOVA was then computed which indicated a significant main effect of Dose, $F(2,28) = 14.77$, $p = .000$ indicating that the mean itch ratings are significantly dose dependent. However, there was not a significant main effect of Dimension, $F(1,28) = 1.99$, $p = .170$, or an interaction between Dose and Dimensionality, $F(2,28) = 1.37$, $p = .263$.

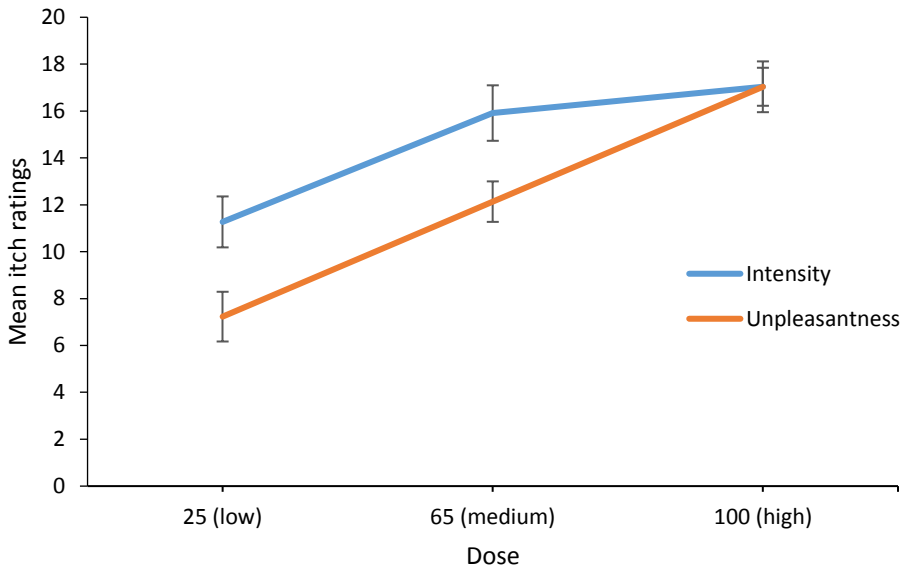


Figure 3.2 Mean itch (± 1 SEM) across three spicule doses (low: 25, medium: 65, high: 100) and two dimensions (n=29).

Following the significant main effect of dose, post-hoc t-tests were performed using Bonferroni correction for multiple testing. There were significant differences between the low and medium dose ($p = .007$) and the low and high dose ($p = .000$). There was however no significant difference between the medium and high dose ($p = .081$).

An additional exploratory analysis was also completed in order to test for linear trends within the data. There was a significant positive linear trend for both the mean itch intensity $F(1,28) = 13.002$, $p = .001$ and mean unpleasantness $F(1,28) = 14.99$, $p = .001$. There was no significant quadratic trend for either intensity or unpleasantness.

3.4.3 Peak Itch

This same pattern with regards to the dose and dimension is also evident in the peak itch (see Figure 3.3), with the low dose generating the lowest itch intensity (M = 33.07, SD = 26.78) followed by the medium dose (M = 44.52, SD = 25.61) and then the highest (M = 50.90, SD = 21.23). Along with the mean unpleasantness, with the low dose generating the lowest mean (M = 21.38, SD = 24.96) followed by the medium (M = 37.59, SD = 22.54) and then the highest (M = 50.66, SD = 29.19).

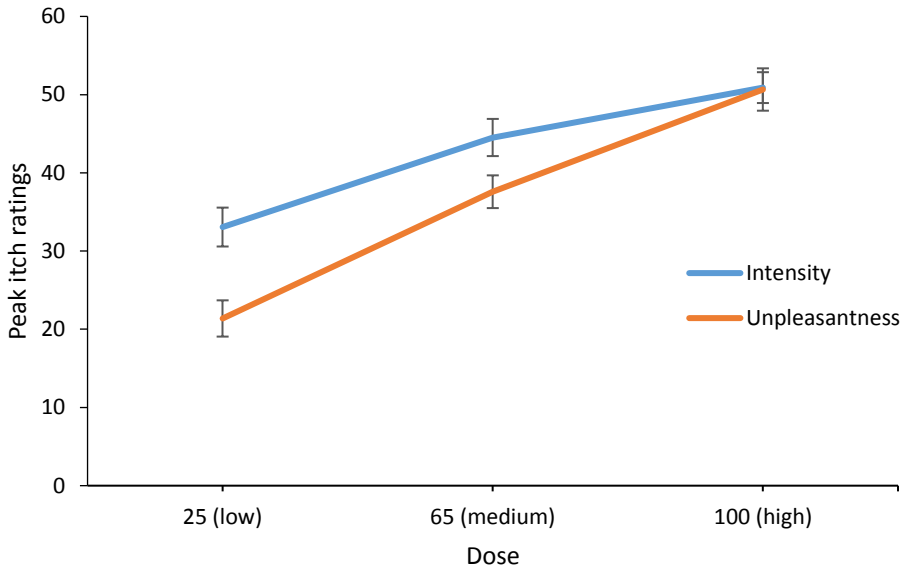


Figure 3.3 Peak itch (± 1 SEM) across three spicule doses (low: 25, medium: 65, high: 100) and two dimensions (n=29).

The 2 x 3 repeated measures ANOVA again indicated a significant main effect of Dose $F(1,28) = 22.02$, $p = .000$, but no significant main effect of Dimension, $F(1,28) = 2.27$, $p = .143$, or interaction, $F(1,28) = 1.85$, $p = .167$. The post hoc test indicated that all doses were significantly different (low and medium at $p = .002$, low and high at $p = .000$, and medium and high at $p = .013$).

There was also a significant positive linear trend for both the peak itch intensity, $F(1,28) = 17.150$, $p = .000$, and unpleasantness, $F(1,28) = 27.624$, $p = .000$. This therefore indicated that as the dose increased, both the itch intensity and unpleasantness significantly increased proportionately for the peak. There was no significant quadratic trend for either of the dimensions.

Overall, both the itch mean and peak demonstrated the same pattern. There was a significant linear increase in ratings as the doses increased, for both the intensity and the unpleasantness

dimension. There was however, only a significant main effect of dose, not for dimension, nor was there any significant interaction.

This dose-response effect (L < M < H) was evident in 38% of the participants when intensity was rated, and in 52% of participants when unpleasantness was rated. When combining the data from both sessions (intensity and unpleasantness), 48% of participants showed a dose-response effect.

3.4.4 Heart Rate Results

The results demonstrated that there were no significant differences between any of the frequency domains (see Table 3.1 for descriptive statistics). Within the VLF bands there was no significant difference between the Dimensions, $F(1,27) = .00$, $p = .990$, or Doses, $F(2,54) = .61$, $p = .549$, nor was there a significant interaction between the two, $F(2,54) = 1.51$, $p = .231$. There was also no significant difference for the LF band (Dimension $F(1,27) = .43$, $p = .517$; Dose $F(2,54) = .23$, $p = .798$; Interaction $F(2,54) = 2.38$, $p = .102$). This was also the case for the HF band (Dimension $F(1,27) = .26$, $p = .616$; Dose $F(2,54) = .58$, $p = .566$; Interaction $F(2,54) = .77$, $p = .469$). There was also no significant differences in time domain parameters such as SDRR (standard deviation of normal R-R intervals) or RMSSD (root mean square of successive differences) for Dose or Dimension, nor was there for the total power $F(1,27) \leq .868$, $p \leq .484$.

Table 3.1 Descriptive statistics of the normalised frequency bands (VLF, LF, HF) for each dimension and dose.

Dimension and dose	M (SD) VLF	M (SD) LF	M (SD) HF
Intensity_Low	.36 (.13)	.39 (.12)	.25 (.14)
Intensity_Med	.34 (.12)	.41 (.11)	.25 (.14)
Intensity_High	.38 (.14)	.39 (.13)	.23 (.16)
Unpleas_Low	.35 (.11)	.40 (.11)	.24 (.15)
Unpleas_Med	.36 (.12)	.37 (.13)	.26 (.17)
Unpleas_High	.36 (.14)	.39 (.12)	.25 (.17)

3.5 Discussion

The current experiment aimed to first explore any patterns which would indicate that cowhage induced itch is dose dependent and second, to examine any differences between intensity and unpleasantness which would suggest that they are dissociable dimensions.

The results demonstrate that there was in fact a significant linear trend for both intensity and unpleasantness related to dosage, for all of the analyses (mean and peak). There was specifically a significant difference (for both dimensions) between the low vs. medium dose, and low vs. high dose for the mean and peak itch. However, there was only a significant difference between the medium vs. high dose for the peak itch, not the mean. This therefore indicates that the doses are ideal for exploring differences in peak itch, however for a significant difference between the medium and high dose, it is likely that the high dose would have to be increased further to see a significant difference for the mean. Alternatively, however, it may be the case that 100 spicules yield 'peak itch' and the intensity rating will not increase for higher doses.

Another possible way to increase itch intensity would be to increase not only the number of spicules but also the surface area in which the spicules are applied (spatial summation). LaMotte et al., for instance, explored this method and found that the peak magnitude of the itch increased as the number of spicules increased from one to seven within a spatial area of 6 cm. The same increase in spicules however did not increase itch magnitude within an area of 1 cm, therefore exhibiting spatial summation.

Although it makes sense that the number of activated afferent nerve fibres would be greater for seven than for one spicule, the firing in a single afferent is not greater when multiple spicules as opposed to just one are inserted according to Johanek et al. (2008). It could be the case, as suggested by LaMotte et al, that afferents activated by cowhage may induce a local, lateral inhibitory effect on activity from neighbouring afferents as they converge on second-order neurons. Therefore stimulation of more distant fibres may avoid this local inhibition and therefore enable spatial summation. This would therefore prevent spatial summation within small areas, such as $<1\ cm^2$, which would explain the results in the LaMotte et al. study. Therefore it would be interesting to explore this method in order to further increase the existing peak and mean itch in the current experiment.

There were no significant differences found between the intensity and unpleasantness dimensions. Despite this, there appears to be a consistent pattern throughout the analyses

(peak and mean; see Figure 3.2 and 3.3) which suggests that although there is no significant difference between the dimensions, they do not follow an identical pattern. For example, as the dose increases the unpleasantness of the itch continues to increase, whilst the intensity increases at a slower rate and appears to be levelling off. It would therefore be interesting to find out if the cowhage dose was decreased (e.g., 10 instead of 25) whether there be a significant difference between intensity and unpleasantness (with the unpleasantness being rated significantly lower than intensity) and if the high dose was increased from 100 to 125, whether this would result in the unpleasantness ratings increasing whilst intensity ratings levelling off (resulting in unpleasantness rated significantly higher than intensity).

One point to note is that this experiment only tests 3 doses and is therefore trying to determine a line of best fit using only 3 data points. A more stable estimate of the dose-response relationship could be made if the number of dose conditions were increased. For example, Darsow et al. (2000) used 9 increasing concentrations of histamine (0.03 - 8%) in order to explore the process of itch intensity on a neural level (see Chapter 5). This would also increase the likelihood of finding a significant difference between the dimensions if one exists, as aforementioned.

There was also no significant difference between any of the HRV measures. This may have been due to the fact that there were such large variations between subject differences in total power. Although the data were normalised in order to reduce this it may be the case that the total power of the HRV measurements was due to the variation in the participants' anxiety. Due to the nature of the experiment some participants may have been quite anxious, particularly when receiving itch inducing stimuli or even at the thought of it. This possibility could have been ruled out or potentially avoided if participants were filtered based on state anxiety questionnaires.

There are also a variety of reasons which could potentially explain why the present study did not significantly dissociate between the dimensions, including the method of cowhage application, the measurement scale implemented and the doses used.

Firstly, it could be the case that the present study did not use the optimal method of applying cowhage spicules. The present study replicated the application mode used by Papoiu et al. (2011), which involved rubbing the spicules into the skin, as opposed to inserting them as LaMotte et al. (2009) did. LaMotte et al. (2009) for instance, inserted the tip of the cowhage spicule into the skin using a spicule applicator, which consisted of 9/10 spicules fixed in a

row along the cut end of a surgical sponge using nail polish. According to LaMotte et al. (2009), this usually resulted in the insertion of the tips of 7 spicules (± 1). For example, the mode of application in the present study could result in many of the spicules simply being moved around on the surface of the skin, therefore potentially decreasing the likelihood of them reliably inducing an itch. Therefore the itch induced may not reflect the number of spicules applied due to the large variance of activated spicules. Cowhage delivery experiments by Papoiu et al. (2011) suggested that the number of cowhage spicules inserted using this rubbing method, represents roughly a third of the total spicules applied.

Another point to make is the possibility of habituation when administering itch stimuli numerous times. For example, the itch intensity/unpleasantness response may reduce as the number of applications increases. However, this possibility was reduced by counterbalancing the order to dose application.

Another alternative explanation could be that cowhage is simply not the best suited itch stimulus to dissociate between intensity and unpleasantness, especially for brain stimulation experiments where the comparison of individual datasets is crucial. For example, although overall there were significant differences between the itch induced by the varying doses, within datasets of individual participants this linear dose-response intensity was only present in 38% of the participants. This could be due to the fact that when using the rubbing method of spicule application, the experimenter cannot guarantee how many spicules actually become lodged into the skin. Even when inserting them via an applicator (LaMotte et al. 2009) it is still not guaranteed that one spicule will induce an itch after the first application. For instance, in the by LaMotte et al. (2009), the probability of a sensation arising from the first insertion of a single spicule was 0.56. However, in the LaMotte et al. (2009) subsequent experiment 38 out of 50 tests generated a sensation on the first insertion; increasing the probability to 0.76. A similar proportion of 0.77 was obtained by Sikand et al. (2008). Therefore even when directly inserting the spicules a 0.56 to 0.77 probability of each spicule can generate a significant proportion of variability. Therefore, the method of application used in the current study will arguably generate significantly greater variation with regards to the spicules activated, particularly when taking into account the claim that activate spicules only represents roughly a third of the total spicules applied (Papoiu et al., 2011). This therefore takes the control of itch induced (which is crucial for a dose-response study) away from the experimenter.

Additional findings from LaMotte et al. (2009) have also demonstrated that there is no significant difference between the insertion of 1 vs. 7 spicules, therefore concluding that the time course and magnitude of itch were similar in response to 1 or 7 spicules. There was however, a significant difference between 7 vs. 28 spicules, with regards to the peak itch. So far no previous study has demonstrated a clear dose-response effect for cowhage. The current study demonstrates this for the first time, which is a significant contribution to the literature on acute itch.

Overall, although cowhage is a valuable method of inducing itch, it may not be reliable enough to draw conclusions on varying doses and finding slight differences between the dimensions of itch. It is therefore clear that work still needs to be put into finding an effective, reliable strategy of dissociating dimensions of itch. A possible alternative stimulus could be the use of histamine, as it may potentially be a more stable and reliable method of inducing itch, as the experimenter has more control of how much histamine is administered. For example, administering a dose with histamine would make it easier to control the dosage. Additionally, we already know that histamine shows a dose dependant increase in itch intensity (Drzezga et al., 2001). However, at this stage in the project it was only possible to get ethical approval for the use of cowhage and not histamine.

Chapter 4 Experiment 3: The Subjective Dimensionality of Histamine-Induced Itch

4.1 Overview

In the previous experiment (Chapter 3) it was concluded that cowhage does not generate intra-individually consistent dose-response effects. Therefore, it is not the ideal stimulus to explore brain processes involved in itch. In this chapter the experiment will be repeated but using the histamine prick test. It is predicted that histamine will have greater intra-individual consistency, due to that fact that the experimenter has much more control over how much of the itch-inducing substance enters the skin, and given the previous reports of a clear dose-response relationship for histamine. The aims will therefore remain as follows: the time course and general itch patterns induced will be analysed, along with a comparison between the two dimensions of itch, to investigate whether they are dissociable dimensions. These finding will inform the TMS experiment (Chapter 5) as it will highlight whether this method of itch induction is consistent across sessions.

4.2 Introduction

In Experiment 2 the dimensionality of itch (intensity vs. unpleasantness) was explored using three varying doses of cowhage. The findings demonstrated that although cowhage induced itch had a significant linear mean dose-response intensity across 30 participants, at the individual level, a dose-response effect was only evident in 38% of the participants. There was also no significant difference between the dimensions across or within participants. It was therefore decided that cowhage may not be the best suited itch stimuli to dissociate between intensity and unpleasantness for the future TMS experiment (chapter 5), where the comparison of individual datasets is crucial.

A possible alternative stimulus could be the use of histamine, which is the prototypical stimuli used in acute experimental itch studies and is known to be reliable and stable (Drzezga et al., 2001; Mochizuki et al., 2003). In addition to this, the experimenter has more control of how much histamine is administered than when using cowhage. This is supported by findings proving that histamine induced-itch is significantly dose dependent (Drzezga et al., 2001; Mochizuki et al., 2003). So far, however, there have only been four research groups, which have studied histamine-induced acute itch as a multidimensional sensation (Drzezga et al., 2001; Kleyn et al., 2012; Mochizuki et al., 2003; Walter et al., 2005).

The first aim was therefore to explore any changes in the time-course and peak of itch intensity/unpleasantness, induced by three varying doses of histamine concentrations. The second aim was to specifically examine whether histamine can successfully evoke dissociable feelings of intensity and unpleasantness. If the claim is accurate that intensity and unpleasantness are distinct dimensions of acute itch, we should observe instances where they dissociate overtime (e.g., the intensity peaks before unpleasantness) or over intensity (e.g., an increase in the dose may not induce correlated ratings of intensity and unpleasantness). HRV was also measured throughout the experiment in order to detect any significant difference with regards to the dose administered or dimension measured.

4.3 Methods

4.3.1 Design

The experiment used a 2 x 3 factorial within subjects design, with 2 dimension conditions (intensity and unpleasantness) and 3 dose conditions (low, medium and high). There was a space of one week between the two sessions. Rating order (intensity or unpleasantness first) were counterbalanced between participants, along with the site of application (left or right volar arm).

4.3.2 Participants

A total of 30 participants (8 male, mean age 20.7 ± 3.7) completed both sessions of the study. The exclusion criteria included participants with any sensitivity to the left or right volar arm (e.g., wounds, rashes, swelling or reddening, certain), a history various illness and disease, for example, skin conditions (e.g., eczema), disease of the heart or blood vessels (e.g., cardiovascular disease), and lastly, medication taken 48 hours prior to the experiment (e.g., antihistamines; please see appendix for full exclusion criteria). The study was approved by the University of Hull Psychology Ethics Committee and performed according to the British Psychology Society, code of human research ethics principles. Participation was remunerated with 3 hours course credits; 1.5 hour per session or £12; £6 per session.

4.3.3 Stimuli and Materials

The histamine prick test consists of the application of a single drop of histamine dihydrochloride (Drzezga et al., 2001; Theunis, Black, Degouy, Schmitt, & Misery, 2008). The concentration of histamine was dependent on the condition: low (0.01%), medium (0.1%) and high (1%). The skin was then pricked through this drop with the tip of a sterile lancet (ALK, Sweden). In all dose conditions the histamine prick test was performed on the left/right volar forearm. During the experiment, a visual barrier was used, so that participants

could not see their hand during itch induction and rating, in order to control a potential confounding variable (i.e. looking at one's own reddened skin might be itch-inducing in itself; Lloyd et al., 2013). All other experimental details (rating procedure, acquisition of Heart Rate data, Familiarisation Session) were identical to Experiment 2.

4.4 Results

In order to examine whether histamine induce itch is dose dependent and can evoke dissociable feelings of intensity and unpleasantness, the itch peak and mean were analysed.

4.4.1 Time to peak

In order to explore any patterns or differences in the time-course induced by the varying doses of histamine, the time to peak was analysed. As can be in seen Figure 4.1 the mean intensity time to peak increases in relation to the doses, with the low dose taking the least time to peak (*M* = 101.50 s, *SD* = 119.59 s), followed by the medium dose with a mean increase of 5.5 s (*M* = 107 s, *SD* = 72.07 s) and then highest dose which increased by 27 s (*M* = 134 s, *SD* = 104.59 s).

The mean time to peak for the unpleasantness dimension, also follows this same pattern. The low dose generated a mean of 88 s (*SD* = 95.74 s), followed by the medium dose (*M* = 95 s, *SD* = 64.53 s) and then the highest (*M* = 141s, *SD* = 95.73 s).

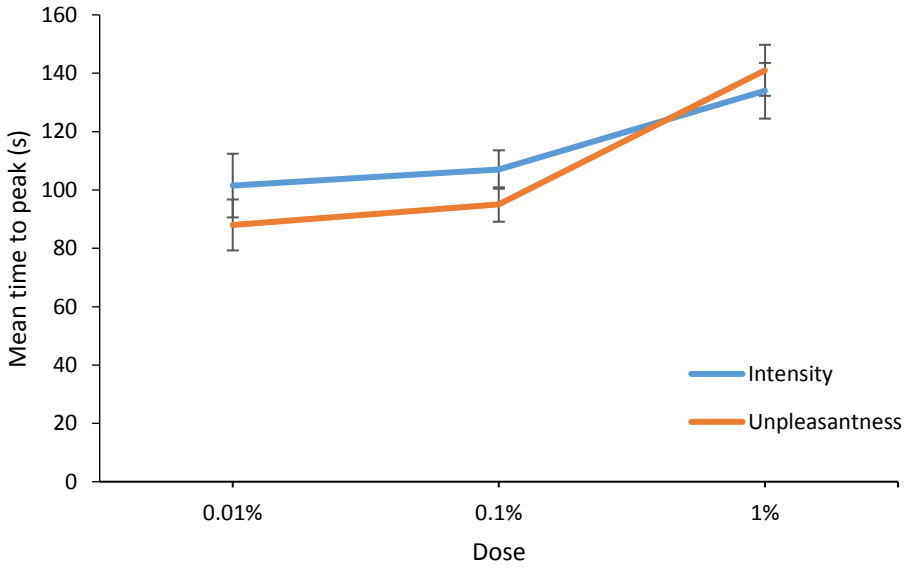


Figure 4.1 Mean peak time intervals (± 1 SEM) across three doses and two dimensions (n=30).

The repeated measures ANOVA however, indicated that there was a significant main effect of Dose $F(2,58) = .6.40$, $p = .003$. There was however, no significant main effect of

Dimension $F(1,29) = .157$, $p = .695$ or a significant interaction between the two $F(2,58) = .300$, $p = .742$.

Following the significant main effect of dose, post-hoc tests were performed using Bonferroni correction for multiple testing. There were significant differences between the low and high dose ($p = .013$), and the medium and high dose ($p = .017$). There was however no significant difference between the low and medium dose ($p = 1.00$).

4.4.2 Mean itch

As can be in seen Figure 4.2 the mean intensity increases in relation to the doses, with the low dose generating the lowest mean ($M = 2.59$, $SD = 4.18$), followed by the medium dose with a mean slight increase of 3.65 s ($M = 6.24$, $SD = 10.06$) and then the highest with a larger increase of 6.81 ($M = 13.05$, $SD = 13.00$). This general pattern also applies to the mean unpleasantness, with the low dose generating a mean of 3.59 ($SD = 7.09$), followed by the medium dose slightly increasing by 1.84 ($M = 5.43$, $SD = 7.60$) and then the highest, with an increase of 6.38 ($M = 11.81$, $SD = 13.45$).

A 2 x 3 repeated measures ANOVA was then computed which, after a Greenhouse-Geisser correction, indicated a significant main effect of Dose, $F(1.58, 45.74) = 19.77$, $p = .000$ indicating that the mean itch ratings are significantly dose dependent. However, there was not a significant main effect of Dimension, $F(1,29) = .18$, $p = .67$, or an interaction between Dose and Dimensionality, $F(1.88, 54.48) = .72$, $p = .484$.

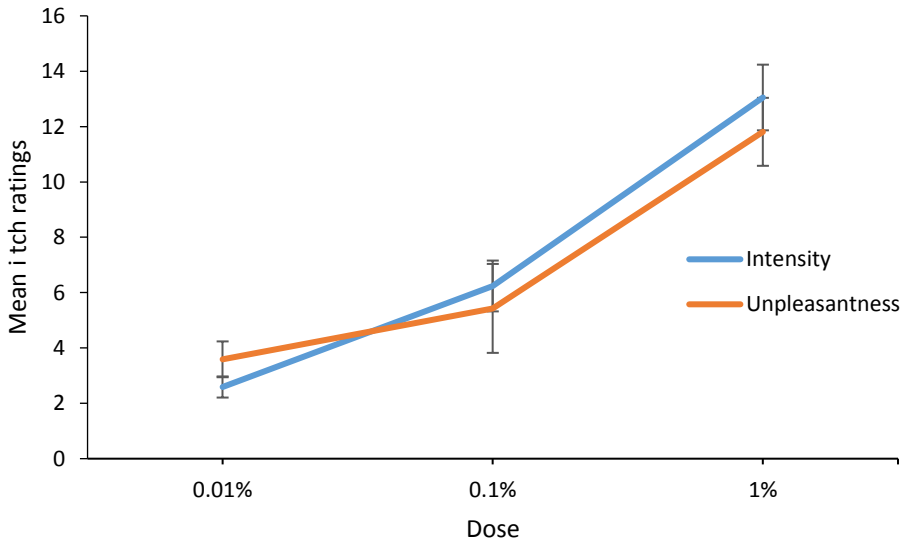


Figure 4.2 Mean itch (± 1 SEM) across dose and dimension (n=30).

Following the significant main effect of dose, post hoc tests were performed using Bonferroni correction for multiple testing. There were significant differences between the low and high dose (p = .000), and the medium and high dose (p = .001). There was however no significant difference between the low and medium dose (p = .057).

Additional exploratory analysis was also completed in order to test for linear trends within the data. There was a significant positive linear trend for both the mean itch intensity $F(1,29) = 26.61$, p = .000 and mean unpleasantness $F(1,29) = 15.69$, p = .000. There was no significant quadratic trend for either intensity or unpleasantness.

4.4.3 Peak itch

This same pattern with regards to the dose and dimension is also evident in the peak itch (see Figure 4.3), with the low dose generating the lowest itch intensity (M = 8.50, SD = 11.17) followed by the medium dose (M = 16.93, SD = 18.76) and then the highest (M = 27.73, SD = 23.04). Along with the mean unpleasantness, with the low dose generating the lowest mean (M = 9.47, SD = 15.89) followed by the medium (M = 15.90, SD = 21.34) and then the highest (M = 25.90, SD = 24.04).

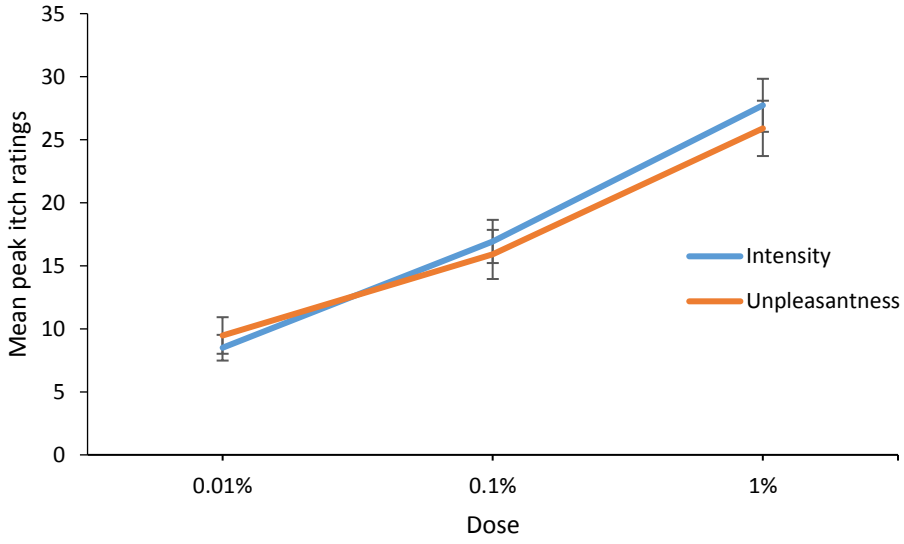


Figure 4.3 Mean peak itch (± 1 SEM) across dose and dimension (n=30).

The 2 x 3 repeated measures ANOVA again indicated a significant main effect of Dose $F(2,58) = 23.19$, p = .000, but no significant main effect of Dimension, $F(1,29) = .23$, p = .636, or interaction, $F(2,58) = .39$, p = .678. The post hoc test indicated that all doses were significantly different (low vs. medium at p .017, low vs. high at p .000, and medium vs. high at p .000).

There was also a significant linear trend for both the peak itch intensity, $F(1,29) = 30.19$, $p = .000$, and unpleasantness, $F(1,29) = 27.23$, $p = .000$. This therefore indicated that as the dose increased, both the itch intensity and unpleasantness significantly increased proportionately for the peak. There was no significant quadratic trend for either of the dimensions.

Overall, both the itch mean and peak demonstrated the same pattern. There was a significant linear increase in ratings as the doses increased, for both the intensity and the unpleasantness dimension. There was however, only a significant main effect of dose, but no main effect of dimension, nor was there any significant interaction.

This dose-response effect (L < M < H) was evident in 63% of the participants, with regards to the mean itch intensity and 50% for the mean itch unpleasantness. The peak itch intensity demonstrated 57% of participants showed this pattern of itch response, in comparison to 37% for the peak itch unpleasantness ratings.

4.4.4 Heart Rate Results

The heart rate data was collected from all 30 participants, out of which 26 were usable for the analysis (4 participants were excluded due to noise in one or more of the six datasets, which made it impossible to calculate the beats per minute).

Table 4.1 Descriptive statistics of the normalised frequency bands (VLF, LF, HF) for each dimension and dose.

Dimension and dose	M (SD) VLF	M (SD) LF	M (SD) HF
Intensity_Low	.40 (.14)	.38 (.13)	.22 (.12)
Intensity_Med	.39 (.09)	.40 (.09)	.22 (.10)
Intensity_High	.35 (.94)	.41 (.11)	.23 (.11)
Unpleas_Low	.41 (.13)	.39 (.11)	.20 (.08)
Unpleas_Med	.43 (.12)	.37 (.13)	.19 (.11)
Unpleas_High	.38 (.10)	.41 (.10)	.20 (.08)

The repeated measures ANOVA demonstrated that there was no significant main effect of Dimension in the VLF band: $F(1,25) = 2.766$, $p = .109$, but there was for Dose, $F(2,50) = 3.755$, $p = .03$. There was however, no significant interaction between the two $F(2,50) = .494$, $p = .613$. As has been mentioned previously, the VLF band is an indicator of sympathetic

regulation (Electrophysiology, 1996; Furlan et al., 1990; Malliani et al., 1991; Tran et al., 2010).

The post-hoc tests demonstrated that the medium dose was associated with significantly greater power in the VLF band (M = .41, SD = .076) than the high dose (M = .37, SD = .076), however no significant difference was observed between the VLF low vs. high (p = .226) or low vs. medium doses (p = 1.0; see Figure 4.4).

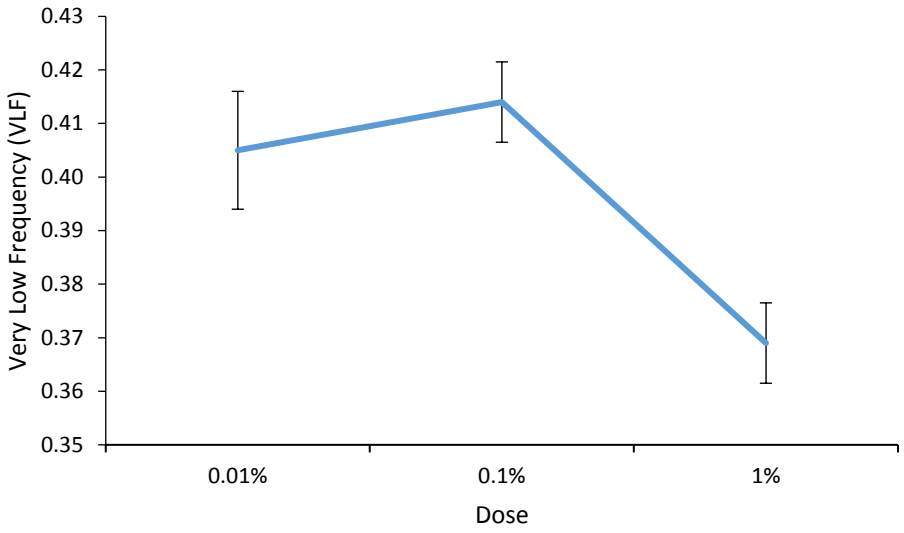


Figure 4.4 Very low frequency band (VLF) across 3 doses (± 1 SEM).

There was no significant difference for the LF domain (Dimension $F(1,25) = .011$, $p = .917$; Dose $F(2,50) = 2.712$, $p = .076$; Interaction $F(2,50) = .920$, $p = .405$). This was also the case for the HF domain (Dimension $F(1,25) = 2.443$, $p = .131$; Dose: $F(2,50) = .601$, $p = .552$; Interaction: $F(2,50) = .052$, $p = .949$). There were also no significant differences in time domain parameters such as SDNN or RMSSD for Dose or Dimension, nor was there for the total power.

4.5 Discussion

The current experiment aimed to first explore any patterns which would indicate that histamine induced itch is dose dependent and second, to examine any differences between intensity and unpleasantness which would suggest that they are dissociable dimensions.

The results demonstrate that there was a significant linear trend for both intensity and unpleasantness found in all of the analyses, therefore supporting the existing literature that histamine concentrations result in a linear increase in itch sensation (Drzezga et al., 2001; Mochizuki et al., 2003). For the peak itch, all of the doses were significantly different from

one another, in that the high dose was significantly greater than the medium dose, and the medium was significantly greater than the low dose. However, for the mean itch although the low vs. medium dose and low vs. high were significantly different, medium vs. high just missed significance ($p = 0.057$). This therefore indicates that the doses are ideal for exploring differences in peak itch, however for a significant difference to be observable for the mean, the doses may need to be more varied.

There were no significant differences found between the intensity and unpleasantness dimensions, or any Dose by Dimension interactions. Despite this, there appears to be a consistent pattern throughout the analyses (peak and mean; see Figure 4.1 and 4.2) suggesting that although there is no significant difference between the dimensions, they are not the same. For example, unpleasantness is rated slightly (but not significantly) higher than intensity for the low dose, but then intensity is rated increasingly greater for the medium and high dose, respectively. This could suggest that when there is a mild histamine induced itch, the unpleasantness and uncomfortableness is perceived as more noticeable than the itch intensity, reflected in its higher rating.

It would be interesting to include additional histamine concentrations, to see if they would evoke a significant interaction. For example, if a weaker histamine concentration was added (e.g., 0.001%) would there be a significant difference between intensity and unpleasantness (with the unpleasantness being rated significantly higher than intensity, in comparison to 0.01% or 0.1%)? Also, if a high concentration was included (e.g., 2% +), would this generate a significant interaction? For instance, if the intensity continued to increase whilst the unpleasantness continued to level off or slightly increase at a slower rate. However, research by Drzezga et al. (2001) would suggest that increasing the dose beyond 1% may not necessarily increase the itch intensity ratings. For example, Drzezga et al. (2001) administered 9 doses from 0.03 to 8%, however the doses exceeding 1% (2, 4 and 8%) actually resulted in a reduced peak for both itch intensity and unpleasantness. The 1% concentration interestingly created the greatest peak itch out of the 9 doses. This may therefore suggest that all of the itch receptors are fully saturated at 1%, therefore increasing the concentration will not increase the output (itch intensity) beyond this point. This however is merely speculation and further investigation would be necessary to draw any firm conclusions. One limitation in this respect is the fact that ready-made histamine solutions are only available in 1%, 0.1% and 0.01% concentrations. Therefore, in order to use a different

concentration, the solution would have to be created on site by the experimenter, which was not approved by the University of Hull Psychology Ethics Committee.

Another potential issue with increasing the histamine concentration, is the fact that the duration of itch can typically last up to 10 minutes. Therefore, by increasing the number of doses, the testing time would considerably increase. Also, the physiological symptoms of the histamine prick test can remain longer than the immediate itch (Lewis & Zotterman, 1927). Therefore, if a higher concentration was added, the likelihood of inducing a flare/wheal lasting post-testing would be increased, which could influence the ratings of unpleasantness for any following dose conditions administered on the same arm.

An alternative method to varying the concentration, could be changing the method of histamine application. In Histamine Iontophoresis, for example, one could manipulate the dose via altering the current (Darsow et al., 1996). Mochizuki et al. (2003), for instance, used a concentration of 0.01% histamine with iontophoresis (Millicoulomb; mC: 120, 1 mA X 120 s) in the intense itching condition, in comparison to the current study which administered the concentration as the lowest dose. The mean and peak itch, however, were not reported in the Mochizuki et al. (2003) study, therefore it is impossible to draw firm conclusions. Despite this, it may be an alternative induction of itch worth exploring, specifically if a further study was conducted in order to investigate any dimension differences.

Numerous researchers have argued that the sensory and affective dimensions of pain perception are associated with different neuronal pathways where phasic and tonic nociceptive information are independently processed (Rainville et al., 1992). On a peripheral level, the sensation of a well-localised, sharp pricking or stinging type of pain (e.g. stinging or pricking) correlates with the activation of Aδ nociceptor units, as opposed to the processing of a more delayed, dull, or burning pain which is related to the activation of C-polymodal nociceptors (Ochoa and Torebjork, 1989). For example, as previously discussed, an experiment by Rainville et al. (1992) demonstrated that the sensory-discriminative and affective elements of pain evoked by the phasic pain stimuli (contact heat and electric shock) were significantly different, with participants reporting the perceived unpleasantness lower than the intensity. There was however, no significant difference between the rated dimensions evoked by the tonic pain stimuli (muscle ischemia or cold-water immersion). This is interesting as there does not seem to be this same distinction between the types of itch evoked from varying stimuli (e.g. tonic vs phasic), as itch tends to be longer in duration (lasting

around 8-10 minutes in the lab). This lack of distinction between these types of information which are possibly mediated via different pathways may therefore potentially explain the lack of a significant difference in the current experiments (2 and 3).

Arguably one of the one most important findings in the current experiment was that there were not only clear differences between the itch induced by the varying doses across participants, but this pattern was also relatively consistent with individual participants. This linear dose-response was therefore more stable than in Experiment 2 (with 63% in comparison to 38% of participants showing this response for the mean intensity). One reason for this could be that the use of the histamine prick test ensured that there was more control over how much histamine was administered, which was not possible when administering cowhage spicules for reasons previously mentioned in Chapter 3. Another possibility could potentially be that due to the additional cowhage-induced sensations of pricking and burning (Sikand, Shimada, Green & LaMotte, 2009), it may be more difficult for individuals to independently rate the itch intensity and ignore these nociceptive sensations. Regardless of the reason however, histamine, as the literature suggests, appears to be a valuable method of inducing itch, that generates intra-individually consistent dose-response effects (Drzezga et al., 2001; Mochizuki et al., 2003), more so than cowhage.

An additional finding supporting the literature is the fact that cowhage took less time to reach the mean maximum rating than histamine, which is to be expected due to the known rapid itch intensity onset and peak of cowhage-induced itch (LaMotte et al., 2009; Papoiu et al., 2011). What however, is particularly interesting is the fact that as the histamine dose increases, so does the time taken for the itch intensity to peak, which is the opposite of which was observed with cowhage (increased dose resulting in less time taken to peak). This would be another interesting avenue to explore, however very few studies investigating histamine or cowhage induced itch specifically report information on the latency to peak, therefore making it difficult to interpret the current results in light of previous research.

Another potential explanation for the lack of a significant interaction firstly, could be the type of scale used. For example, it could be argued that the gLMS is effectively more restricted in its range because of how its verbal labels are arranged, so participants may be likely to just use the lower end of the scale to make ratings (see Chapter 3 discussion for further details). Secondly, it could be argued that the lack of an interaction between Dose and Session could be that the dimensions are simply not dissociable in acute itch, as administering varying

doses of both cowhage and histamine failed to induce a significant interaction. For example, intensity and unpleasantness ratings may have a strong correlation and therefore there would not be any additional information from the affective dimension, which was not already provided by the intensity ratings.

A final note to make is the clear differences and challenges faced when attempting to dissociate between the dimensions in acute sensations, in comparison to chronic sensations. It is widely accepted that both chronic pain (Chapman et al., 2001; Chen, 2001) and itch (Zachariae et al., 2008) are multidimensional, involving both sensory and affective components. The current question however, is whether the affective dimension also exists independently from intensity in acute itch.

One possibility is that much of the research in chronic conditions which dissociates these dimensions may be influenced by the 'secondary' affect (e.g., the consequences of the pain or itch). For example, Price et al. (1984) individuals suffering pain associated with a serious threat to health, have been showed to rate their pain affect significantly greater than those whose pain was not as threatening, despite both providing the same intensity ratings (Price et al., 1984). Similarly, Price et al. (1987) found that when women in labour focused on the impending birth of the child, giving birth was associated with much less pain unpleasantness, than when focusing on pain or avoiding pain. Findings such as these, indicate that the selective influence of the cognitive factors (e.g., anticipation or expectation) on pain affect or unpleasantness, is greatly dependent upon the impact of cognitive focus. This 'secondary' affect linked with chronic or clinical conditions is generally not associated with acute sensations, especially in experimental settings.

Despite this, some studies have been able to selectively manipulate the 'primary' pain unpleasantness (without changing intensity), by altering the cognitive focus in acute pain via suggestibility. (Rainville et al., 1997) for example successfully used hypnotic suggestions, to modulate (both increase and decrease) pain unpleasantness selectively, without manipulating the perceived pain intensity. In addition to this, a later study by Rainville et al. (1999), demonstrated that the manipulation of pain unpleasantness ratings was largely independent of variations in perceived pain intensity. They also found a significant correlation between stimulus-evoked heart rate increase and ratings of pain unpleasantness selectively, without increasing the intensity. This therefore demonstrates that ratings generated by the dimensions of primary pain cannot only be successfully dissociated, but that there could potentially be a

direct functional interaction between pain affect and autonomic activation. The overall results therefore show that hypnotic suggestions can be successfully used as a form of cognitive intervention, to selectively modulate the affective dimensions of pain.

This demonstrates that hypnotic suggestions can be used as a tool in cognitive intervention, to selectively modulate the affective dimensions of pain. This technique could potentially assist in the dissociation between dimensions and changes in physiological responses to acute itch. In turn, if this was successful, there may be a possibility of implementing this in a TMS experiment. For example, TMS to brain areas associated with the affective dimensions (e.g., the dlPFC) may interfere with the cognitive ability to perceive the itch as unpleasant (while unaltering the intensity ratings), in comparison to the control area which has no link to itch. This could therefore provide an interesting approach to discover which parts of the brain would be likely to be involved in the affective dimension of pain.

Overall, it was concluded that histamine is an appropriate itch-inducing stimuli for the purpose of the following TMS experiment and under the time constraints not to continue exploring different methods of attempting to evoke significantly different dimensions of itch. This experiment, along with the cowhage experiment reported in Chapter 3, have informed the following TMS experiment, in that it has been decided to only measure the intensity dimension of itch. This is because Experiments 3 and 4 suggest that itch unpleasantness ratings provide little (if any) information over and above that already provided by intensity ratings. It has also led to the conclusion to use the 1% concentration of histamine in the following TMS experiment, due to the low itch ratings induced in the current experiment along with the previously mentioned issues with increasing the dose beyond 1%.

In summary, the current experiment provided further evidence to suggest that histamine induced itch via the prick test is dose dependent, however further investigation would be necessary to explore the theory that acute itch is multidimensional in nature. The findings therefore are not sufficient evidence to conclude that itch is unidimensional. As aforementioned, further exploration would possibly require the administration of additional doses, an alternative itch-induction technique or the manipulation of cognitive focus.

Chapter 5 Experiment 4: Exploring the Process of Itch in the Brain, using Transcranial Magnetic Stimulation

5.1 Overview

This chapter presents a TMS study conducted on healthy participants. The primary purpose of this experiment is to use TMS to investigate which brain areas have a necessary role in the process of histamine and cowhage induced itch. This hypothesis was tested by stimulating the somatosensory cortices (S1 and S2) and the inferior frontal gyrus (IFG; BA44) and comparing the obtained itch ratings with the control condition.

5.3.4 Selecting the ROIs for the Current TMS Study

In order to gain an estimation of brain areas involved in itch perception, a coordinate-based meta-analysis of all published neuroimaging studies using Ginger Ale 2.3 (www.brain.org) was performed. Only experiments which induced acute itch in healthy volunteers, using either histamine, cowhage or electrical modes of itch induction, were included in the analysis. The contrasts compared an itch condition with a baseline condition of no itch, for instance, cowhage vs. saline. Contrasts that combined itch with an additional modulatory stimulus, such as, itch with scratching vs. itch-only, were not included. Overall, a total of 17 contrasts taken from 14 separate publications met these inclusion criteria and were therefore included (Bergeret et al., 2011; Darsow et al., 2000; Drzezga et al., 2001; Herde et al., 2007; Hsieh, Hägermark, et al., 1994; Ishiuji et al., 2009; Kleyn et al., 2012; Leknes et al., 2007; Mochizuki et al., 2003, 2007, 2009; Papoiu et al., 2012).

The Activation Likelihood Estimation (ALE) assesses the overlap between foci based on modelling them as probability distributions centred at the respective coordinates. The algorithm, as implemented in Ginger Ale 2.3, allows random-effects inference therefore enabling generalisation of the results to the entire population of studies analysed. Ginger Ale then provided an ALE ($p < 0.01$, corrected), using the input of the coordinates of 17 contrasts. This revealed the activation of a network of regions, including the somatosensory cortices, the left IFG (BA44), the anterior insular and the inferior and mid cingulated cortices. Due to the fact that the itch inducing stimuli was to be administered to the right hand in the current study, an additional meta-analysis was performed, using only studies that applied itch to the right arm (Darsow et al., 2000; Drzezga et al., 2001; Hsieh, Hägermark, et al., 1994; Ishiuji et al., 2009; Papoiu et al., 2012; Valet et al., 2007), which demonstrated activation of both S2 and the IFG.

As the meta-analysis showed activation of the IFG and S2, and the literature from both experimental pain and itch studies, provided relatively strong evidence supporting the role of S1 in the processing of stimuli intensity, those three (contralateral S1, contralateral S2 and ipsilateral IFG) were selected as target regions for TMS. In line with most previous neuroimaging studies on itch, the right volar forearm was used for itch induction, which means that left S1, left S2 and right IFG were the TMS target areas. In addition to this, although the vertex is usually considered gold standard as a control stimulation region, it is relatively close to brain regions which are documented to have a role in itch manipulation (e.g., S1), therefore it was decided to not use vertex stimulation, as it may interfere with its activity. An area was chosen in the midline of the superior parietal lobe, based on the lack of literature linking it to any itch processing.

The aim of the current study is to explore any differences in the perceived itch intensity after brain stimulation to the somatosensory cortices (S1 and S2) and the inferior frontal gyrus (BA44), in comparison to the control site, using the histamine prick test. It was predicted that perceived itch intensity after brain stimulation to the somatosensory cortices and the IFG will significantly reduce, in comparison to the control condition. It was also predicted that the skin responses (e.g., induced wheal and flare) and the psychophysical data from the HR would correlate with the itch intensity findings (Darsow, Ring, Scharein, & Bromm, 1996). For instance, TMS stimulation of the experimental ROIs could significantly reduce the wheal and flare reaction and decrease the HR, in comparison to the control.

5.4 Methods

5.4.1 Design

The experiment consisted of four one hour TMS sessions, approximately one week apart. The TMS sessions consisted of three experimental: left S1 (MNI coordinates: -32, -35, 64), left S2 (-47, -21, 13), right IFG (51, 10, 7) and one control session (SPL -3, -62, 71; see Figure 5.1). Additionally, an initial familiarisation session was conducted, as in previous studies. Order of the TMS sessions was counterbalanced across participants, using a balanced Latin Square design (one ROI per session). The order of application of the stimuli was histamine then cowhage. This sequence was based on the fact that the prior study on cowhage (Experiment 2) demonstrated a lack of clear dose-response relationship within participants' individual datasets (38 %), in comparison to the histamine in Experiment 3 (63 %). We therefore prioritised the histamine response and ensured the TMS effects lasted for at least the full duration of the histamine induced itch.

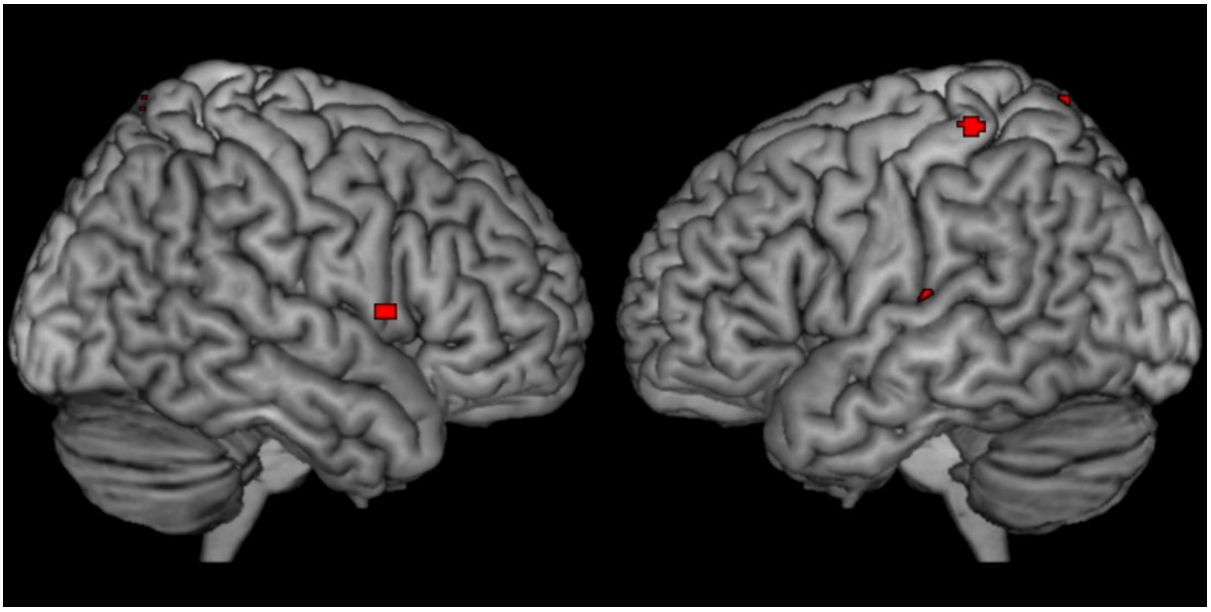


Figure 5.1 Sagittal view of MNI coordinates used for the current TMS experiment. Left image: right IFG (MNI coordinates: 51, 10, 7). Right image: left S1 (-32, -35, 64), left S2 (-47, -21, 13) and (SPL -3, -62, 71).

5.4.2 Participants

A total of 20 participants took part in the study, however one was not available to complete the last two sessions. Therefore, the final sample size was 19 participants who completed all sessions of the study (6 male, mean age 25.5 ± 5.7). All were right handed as determined by the Edinburgh Handedness Questionnaire (Mean Laterality Coefficient = 82.13, SD = 20.62 , (Oldfield, 1971), had normal or corrected-to-normal vision and had no history of neurological or psychiatric disorders. The exclusion criteria included participants with any sensitivity to the left or right volar arm (e.g., wounds, rashes, swelling or reddening, certain), a history various illness and disease, for example, skin conditions (e.g., eczema), disease of the heart or blood vessels (e.g., cardiovascular disease), and lastly, medication taken 48 hours prior to the experiment (e.g., antihistamines; see appendix for full exclusion criteria). The study was approved by the University of Hull Psychology Ethics Committee and performed according to the British Psychology Society, code of human research ethics principles. All participants took part in a trial TMS session prior to consenting to take part in the study. Participation was remunerated with £40 on the completion of the study. If participants did not complete the study, they received partial payment of £8 per hour.

5.4.3 Stimuli and materials

The histamine prick test consists of the application of a single drop of 1% histamine dihydrochloride to the forearm (Drzezga et al., 2001; Florian Pfab et al., 2006; Theunis et al., 2008). The skin was then pricked through this drop with the tip of a sterile lancet (ALK, Sweden). The histamine prick test was performed on the right volar forearm. A visual barrier

was used so that participants could not see their hand during itch induction and rating, in order to control a potential confounding variable (i.e. looking at one's own reddened or swelling skin might be itch-inducing in itself; Lloyd et al., 2013). All other experimental details (rating procedure, acquisition of Heart Rate data, Familiarisation Session) were identical to Experiment 2 and 3.

5.4.4 MRI Data Acquisition and Analysis

TMS navigation required high resolution (1 x 1 x 0.6 mm) T1 weighted 3D structural MRI data, with sagittal slices covering the whole head and brain, of each individual participant. The scans were performed at Hull Royal Infirmary using a GE medical systems scanner with a field strength of 3 Tesla.

MRI data were analysed using BrainVoyager 20.2 (QX 3.2; BrainInnovation, Maastricht, the Netherlands). A 3D anatomical projection was created. The high-resolution volumes were used for surface reconstruction of both hemispheres for each participant. The surface reconstruction was performed, in order to recover the exact spatial structure of the cortical sheet to improve the visualisation of the defined ROIs in relation to the anatomy of each participant. MNI coordinates of the three target areas and the control area were defined as regions of interest (ROIs) using Marsbar (marsbar.sourceforge.net) and SPM12. These ROIs were then backprojected from MNI space into each participant's native brain space, using SPM12's inverse transformation function. Subject-specific ROIs were then imported into BrainVoyager and superimposed on the surface reconstruction of the two hemispheres, and defined as targets during neuronavigation. This ensured precise stimulation of each target region in each participant.

5.4.5 TMS Procedure

Participants took part in a TMS taster session prior to the experiment, where 20 s (half of the stimulation time used during the proper experiment) of stimulation was performed on S2 and IFG, which are the areas that usually cause the most discomfort. This enabled all participants to provide full informed consent. The rest of the familiarisation session was the same as in the previous studies (Experiment 1, 2 and 3).

At the start of each TMS session, participants' heads were coregistered to their structural brain imaging data, using the BrainVoyager TMS Neuronavigator (Brain Innovation BV). This enabled precise, continuous online coil navigation, to target the specific anatomical region of interest, on the cortical surface pre-defined based on the individual MRI scan. A

Magstim Rapid² stimulator was used to generate repetitive magnetic pulses. The pulses were delivered with a standard 70 mm figure-8 coil. A cTBS train of 804 pulses (268 bursts, each burst consisting of three pulses at 30 Hz, repeated at intervals of 100 ms), which lasted for 40 s was administered. Participants were provided with an earplug and chewing gum, to reduce the discomfort during stimulation. These cTBS parameters were based on Nyffeler et al. (2008) and Nyffeler, Cazzoli, Hess, & Müri (2009).

Each session involved the histamine prick test as described above, directly after brain stimulation. The diameter of the wheal and flare induced by the test was also measured and recorded at the end of the 10 minute rating period. After the histamine itch induction, cowhage was then administered. At the end of the last session participants were debriefed and informed of the true nature of the experiment.

5.4.6 Heart Rate Analysis

The heart rate was recorded and analysed as described in Experiment 2.

5.5 Results

In order to examine whether stimulation to S1, S2, the IFG could significantly modulate histamine and cowhage induce itch intensity, in comparison to the control (SPL), the peak, time to peak and mean were analysed. As the three a-priori effects of interest were S1, S2 and the IFG vs. control site stimulation, planned contrasts were used in order to test for significant differences in the itch intensity generated.

A virtual lesion induced by TMS is a temporary and relatively mild disruption of the activation of the ROI and does not completely diminish it. Therefore, TMS experiments need to be able to reveal slight differences, in order to see if it has a therapeutic effect. It is therefore important to reduce and limit large potential variations within the data, to the best of our ability. For example, there can be large variations of itch across participants, as no one experiences it the same. Therefore, certain assumptions have to be made. For instance, we assume here that everyone uses the scale in the same way (e.g., all individuals perceive a 'strong itch' as the same) and that all participants have the same itch threshold. In pain studies, the pain intensity can be adapted to each individual's subjective threshold, therefore creating a pain baseline. This, however, is not possible for itch research, due to the induction process taking up to 10 minutes. It is only possible, to our knowledge, to normalise the results post testing within each participants' dataset. This was completed by dividing the results (e.g., mean or peak) of each individual by that particular participants' overall peak score

(across all experimental sessions), thereby normalising each participant's dataset, in relation to their maximum itch intensity reported.

5.5.1 Histamine Results

5.5.1.1 Time Course of Histamine Induced Itch

In order to explore how stimulation to the target areas modulated the overall itch induction pattern over the time-course of the itch (10 minutes), a line graph was plotted to directly compare each dataset overtime. As can be seen in Figure 5.2, not only does the control have a higher peak, but it also slightly increases in the last 2 minutes of itch induction in comparison to the other brain regions. Overall, S1 appears to have a lower mean itch intensity than the other ROIs, particularly within the first 5 minutes.

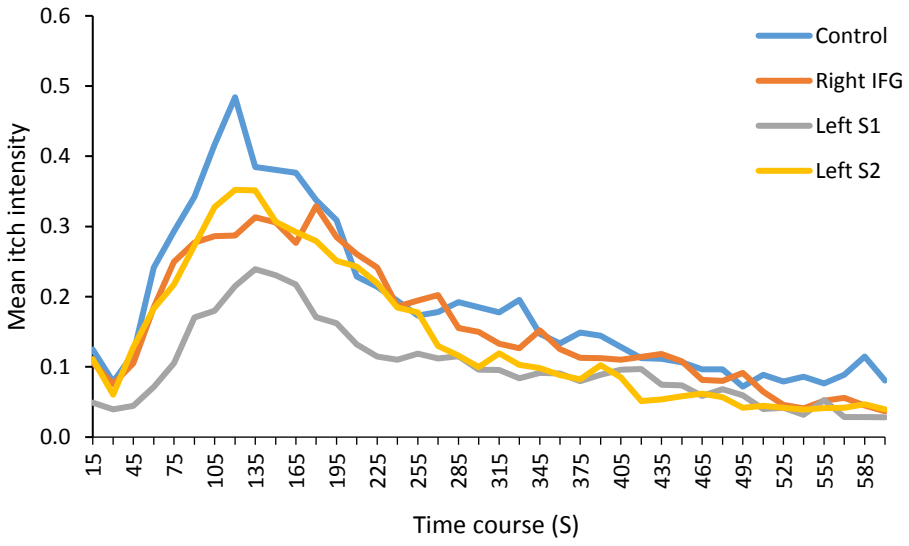


Figure 5.2 Time course of mean itch intensity for histamine across all 4 stimulation conditions.

5.5.1.2 Mean Itch Intensity

As can be in seen Figure 5.3 the brain region which generated the highest itch mean intensity was the control (M = .19, SD = .15), followed by the IFG (M = .15, SD = .17) and then S2 (M = .14, SD = .15). Overall, stimulation to S1 resulted in the lowest itch intensity (M = .10, SD = .12). The planned contrasts demonstrated that there were no significant differences between the mean itch for the control and IFG $t(18) = 1.102$, $p = .285$ or the control and S2 $t(18) = .631$, $p = .536$. However S1 had a significantly smaller mean itch intensity than the control, $t(18) = 2.695$, $p = .015$.

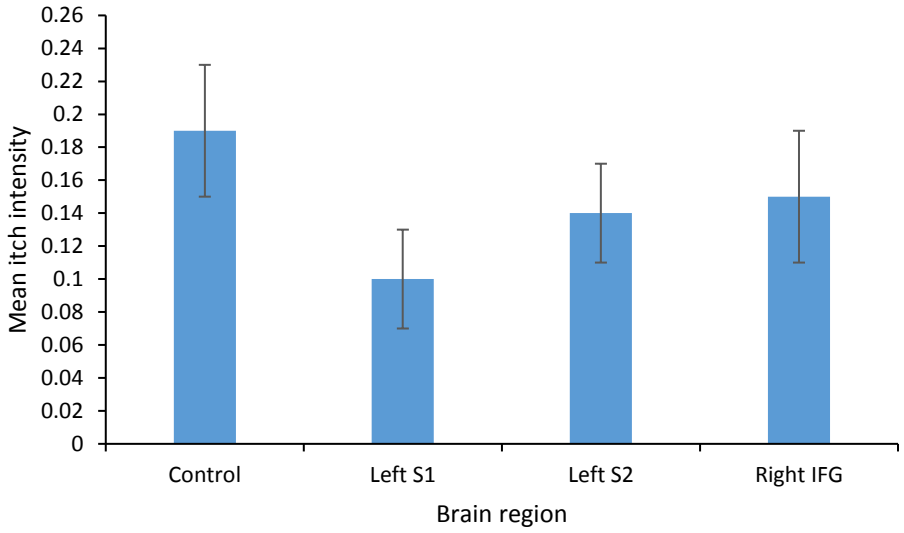


Figure 5.3 Mean itch intensity (± 1 SEM) for histamine across all 4 stimulation conditions.

5.5.1.3 Peak Itch Intensity

As can be in seen Figure 5.4 the brain region which generated the highest itch peak intensity was the control (M = .68, SD = .37), followed by S2 (M = .51, SD = .38), and then IFG (M = .45, SD = .42). Overall, stimulation to S1 resulted in the lowest itch peak itch intensity (M = .37, SD = .35). There were no significant differences between the peak for the control and IFG $t(18) = 1.620$, $p = .123$ or the control and S2 $t(18) = 1.215$, $p = .240$. The control however generated a significantly greater peak that S1 $t(18) = 2.468$, $p = .024$.

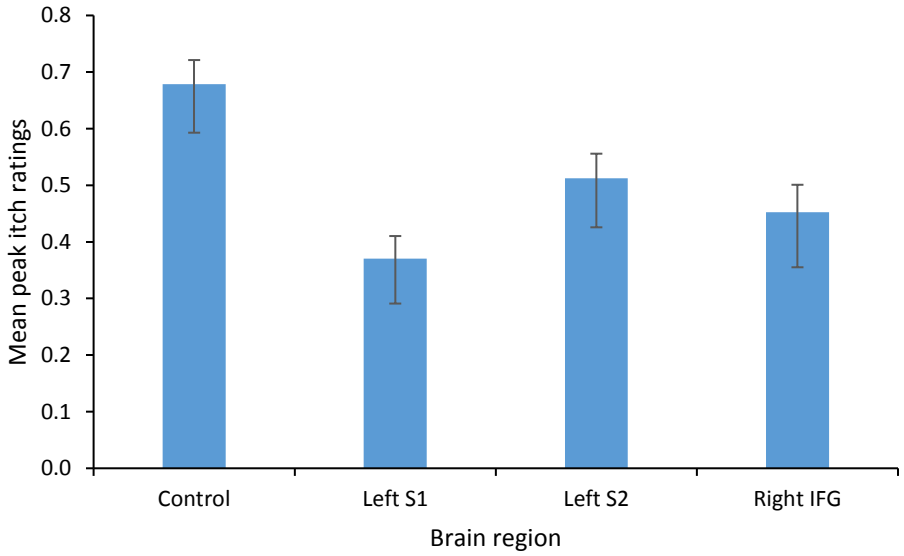


Figure 5.4 Mean peak itch intensity (± 1 SEM) for histamine across all 4 stimulation conditions.

5.5.1.4 Histamine Induce Wheal

The largest wheal diameter (measured in millimetres) was observed in the control condition (M = 3.42, SD = 1.8), followed by S2 (M = 2.63, SD = 1.57) and then the IFG conditions (M = 2.16, SD = 1.8; Fig. 5.5). Overall, stimulation to S1 resulted in the smallest wheal size (M = 2.05, SD = 1.68). Planned contrasts demonstrated that there were no significant differences between the wheal diameter for the control and S2 $t(18) = 1.385$, $p = .183$. There was however a significant difference between the control and S1 $t(18) = 3.311$, $p = .004$, as well as the control and the IFG $t(18) = 2.413$, $p = .027$.

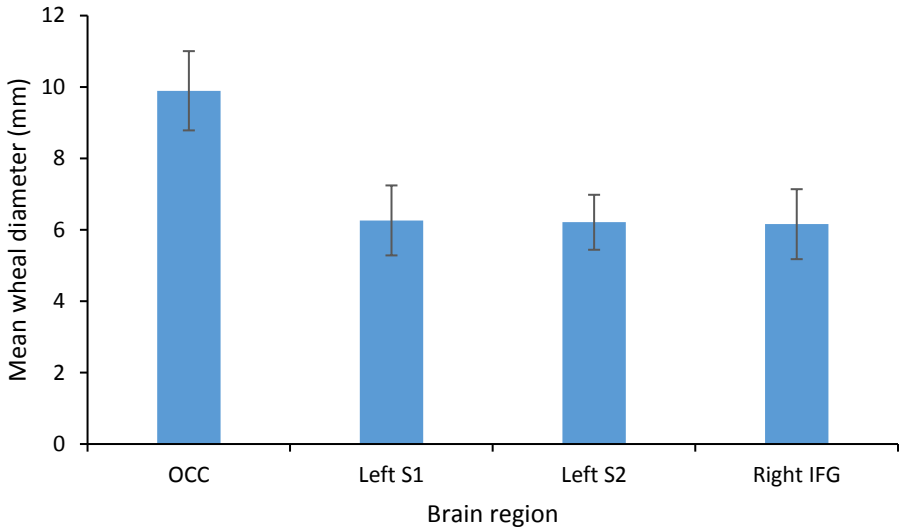


Figure 5.5 Mean wheal induced (± 1 SEM) post histamine prick test, across all 4 stimulation conditions.

5.5.1.5 Histamine Induced Flare

The largest flare diameter was observed in the control condition (M = 9.89, SD = 9.67), followed by S1 (M = 6.26, SD = 8.55), then S2 (M = 6.21, SD = 6.72) and lastly, the IFG (M = 6.16, SD = 8.56; Fig. 5.6). Planned contrasts demonstrated that there were no significant differences between the flare diameter for the control and IFG $t(18) = 1.301$, $p = .210$, the control and S2 $t(18) = 1.419$, $p = .173$, or between the control and S1 $t(18) = 1.166$, $p = .259$.

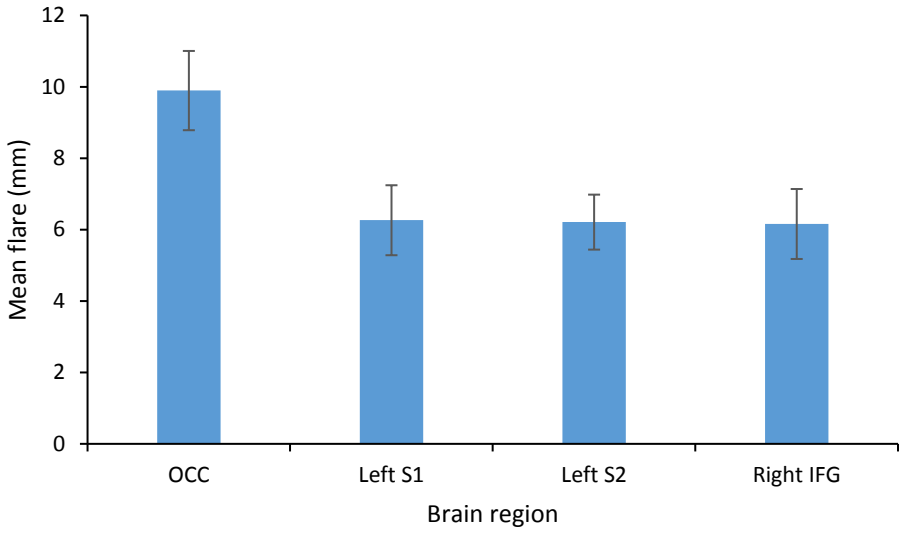


Figure 5.6 Mean flare induced (± 1 SEM) post histamine prick test, across all 4 stimulation conditions.

5.5.1.6 Heart Rate Results

The results demonstrated that there were no significant differences between any of the frequency domains. Within the VLF bands there was no significant difference between, the control and IFG $t(18) = .653$, $p = .522$, the control and S1 $t(18) = .952$, $p = .354$ or the control and S2 $t(18) = 2.065$, $p = .054$. There was also no significant difference for the LF band (control vs IFG $t(18) = -.501$, $p = .622$; control vs S1 $t(18) = .057$, $p = .955$; control vs S2 $t(18) = -1.161$, $p = .261$). This was also the case for the HF band (control vs IFG $t(18) = -.432$, $p = .671$; control vs S1 $t(18) = -1.979$, $p = .063$; control vs S2 $t(18) = -1.320$, $p = .204$). There was also no significant differences for the total power, average rate or in time domain parameters such as SDNN (standard deviation of normal R-R intervals) or RMSSD (root mean square of successive differences) for any of the brain regions.

Table 5.1: Descriptive statistics of the normalised frequency bands (VLF, LF, HF) for each brain region stimulated.

Brain region	M (SD) VLF	M (SD) LF	M (SD) HF
Control	.41 (.17)	.38 (.13)	.21 (.15)
Right IFG	.38 (.13)	.40 (.13)	.22 (.12)
Left S1	.37 (.12)	.38 (.11)	.25 (.13)
Left S2	.33 (.10)	.42 (.11)	.24 (.13)

5.5.2 Cowhage Results

5.5.2.1 Time Course of Cowhage Induced Itch

In order to explore how stimulation to the brain regions modulated the overall itch induction pattern over the time-course of the itch (10 minutes) a line graph was plotted to directly

compare each condition over time. As can be seen in Figure 5.7, all conditions appear to stay relatively the same for the first minute. The peaks appear to be slightly different, with itch ratings after the control and IFG stimulation being slightly higher than after stimulation of the somatosensory cortices. IFG and S2 seem to peak slightly prior to the control and S1.

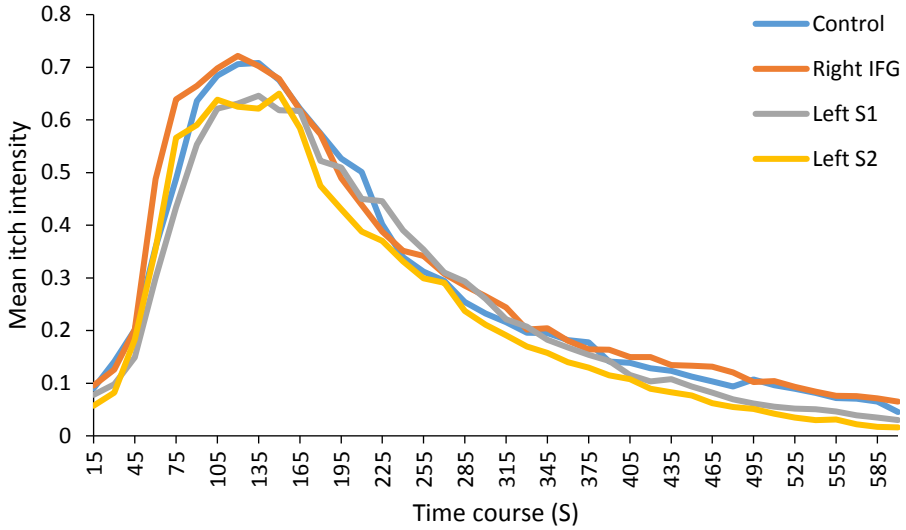


Figure 5.7 Time course of mean itch intensity for cowhage across all 4 conditions.

5.5.2.2 Mean Itch Intensity

As can be in seen Figure 5.8 the brain region which generated the highest itch mean intensity was the IFG (M = .29, SD = .15), followed by the control (M = .27, SD = .12) and then S1 (M = .25, SD = .15). Overall, stimulation to S2 resulted in the lowest itch intensity (M = .23, SD = .09). There were no significant differences between the mean itch intensity for the control and IFG $t(18) = -.284$, $p = .784$, the control and S1 $t(18) = .774$, $p = .449$ or the control and S2 $t(18) = 1.807$, $p = .088$.

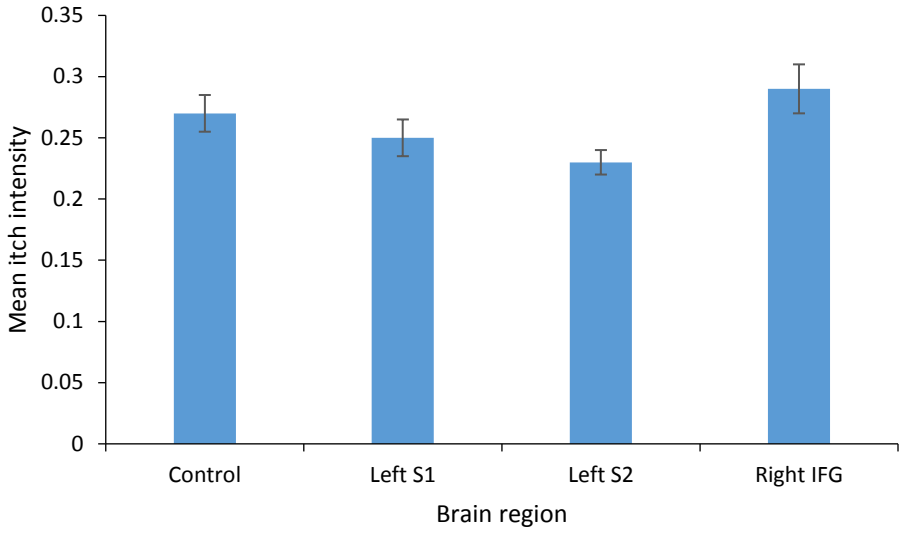


Figure 5.8 Mean itch intensity (± 1 SEM) for cowhage across all 4 stimulation conditions.

5.5.2.3 Peak Itch Intensity

As can be in seen Figure 5.9 the brain region which generated the highest itch peak intensity was the IFG (*M* = .85, *SD* = .20), followed by the control (*M* = .80, *SD* = .21), and then S2 (*M* = .79, *SD* = .19). Overall, stimulation to S1 resulted in the lowest itch peak itch intensity (*M* = .77, *SD* = .28). There were no significant differences between the time to peak for the control and IFG $t(18) = -.747$, $p = .465$ or the control and S1 $t(18) = .516$, $p = .612$, or the control and S2 $t(18) = .297$, $p = .770$.

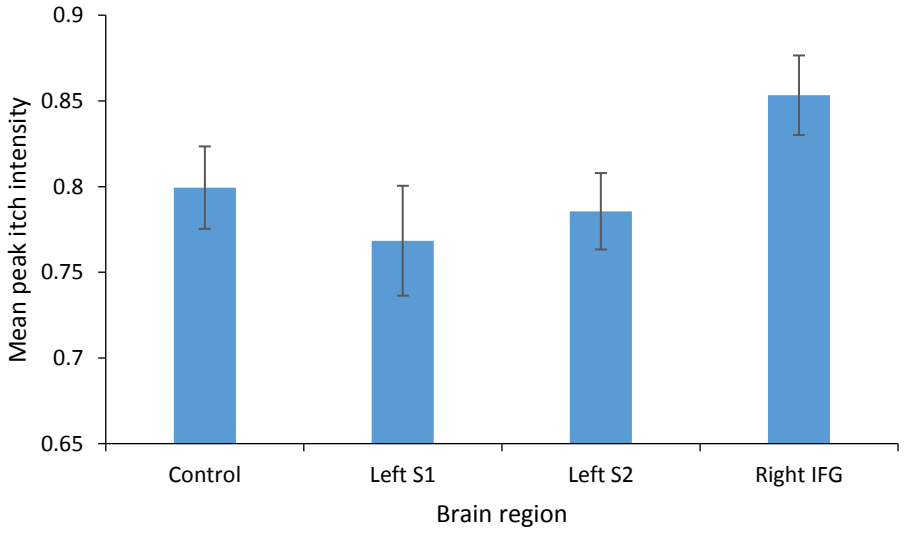


Figure 5.9 Mean peak itch intensity (± 1 SEM) for cowhage across all 4 stimulation conditions.

5.6 Discussion

The current experiment aimed to explore any differences in the perceived itch intensity after TMS to the somatosensory cortices (S1 and S2) and the right IFG, in comparison to stimulation of a control site (SPL), using both the histamine prick test and cowhage. It was predicted that the itch intensity post stimulation of the experimental brain areas would be significantly reduced, in comparison to the control site.

The results demonstrate that TMS to S1 significantly reduced the itch intensity, when administered via the histamine prick test. The size of the wheal, a typical skin reaction after the histamine prick test, was also a significantly reduced after S1 and IFG stimulation. There was, however, no significant difference in the intensity ratings or the skin response (wheal and flare) with regards to S2, nor was there a significant reduction in the flare post IFG stimulation, in comparison to the control. There was also no difference in itch intensity or skin response, for any of the brain regions stimulated when cowhage was administered. Lastly, there was no significant difference between any of the heart rate analyses performed.

First, there are a few potential reasons as to why no effects of brain stimulation on itch response were found when administering cowhage. One possible explanation is the lack of consistency in cowhage induced itch. For example, in Experiment 2 only 38% of participants demonstrated a dose dependent response to cowhage, in comparison to 63 %, when using histamine (Experiment 3). Also, there is a possibility that the TMS effects may have worn off or reduced when cowhage was administered, due to not counterbalancing the stimuli as exploring histamine induced itch was the priority. Therefore the minimum time between TMS and cowhage itch induction was approximately 12 minutes, as it took 10 minutes to rate the histamine induced itch and then a couple of minutes to apply the tape to the skin, as a barrier for the cowhage. Note, however, that the after effects of cTBS can last for approximately 20 minutes following 20 seconds of stimulation (Huang et al., 2005) and the present study administered 40 seconds of cTBS.

Various neuroimaging experiments documented significant activation of S1 when administering histamine in healthy participants (Darsow et al., 2000; Drzezga et al., 2001; Herde, Forster, Strupf, & Handwerker, 2007; Ishiuji et al., 2009; Papoiu, Coghill, Kraft, Wang, & Yosipovitch, 2012; Valet et al., 2008) These correlational methods however cannot infer causality. Experiments administering brain stimulation can go beyond correlational approaches and directly aid the development of research on itch towards the ability to make causal inferences as to which areas are necessary for the central nervous generation of itch.

Previous studies have demonstrated that tDCS and TMS interventions have successfully modulated experimentally induced pain sensations in healthy subjects (Antal et al., 2008; Boggio, Zaghi, Lopes, & Fregni, 2008; Csifcsak et al., 2009; Hansen et al., 2011; Porro et al., 2007; Reidler et al., 2012; Terney et al., 2008). So far, only two studies have successfully manipulated itch using brain stimulation, which highlights the novelty and importance of the current findings.

The first was by Knotkova et al. (2013) who administered a tDCS treatment course on a patient suffering from syringomelia which involved primary symptoms of untreatable chronic pain, accompanied by itch. The tDCS was administered over 13 months and resulted in a reduction of itch for several months but not pain. They concluded that the tDCS successfully reduced itch sensation, however the experiment was performed on only one patient primarily suffering from chronic pain, as opposed to itch. This, therefore, makes it difficult to compare and interpret the findings, particularly with regards to chronic itch patients.

The second study was conducted by Nakagawa et al. (2016) who also used tDCS, in a sample of 14 healthy participants. A histamine-induced itch was evoked on the left forearm and in the two experimental conditions, the cathode was either placed over ipsilateral S1 and the anode over contralateral S1, or vice versa. They found that tDCS to the contralateral S1 area temporarily suppressed the itch response. These results correlated with the current experiment as they found a significant difference between the reported itch peak, which is promising. However, although there are notable similarities between the findings, it is crucial that more brain stimulation experiments are performed, in order to get a clear idea of whether S1 has a crucial role in itch intensity processing. Also, as previously mentioned, due to the fact that the stimulation was bi-hemispheric, the contribution of ipsi- vs. contralateral S1 cannot be concluded without further investigation.

Despite the fact that numerous neuroimaging studies have demonstrated a significant activation of S2, the present experiment did not significantly reduce itch intensity to this area. This may potentially point towards the functional roles of the somatosensory cortices differing with regards to processing itch. For example, although S1 is associated with the sensory and discriminative aspect of itch and pain, it has been suggested that S2 may have a higher order of functions, such as processing the cognition of and attention to itch (Mochizuki & Kakigi, 2015). For instance, various neuroimaging studies have not reported a significant correlation between S2 activity and the stimulus intensity of itch (Drzezga et al.,

2001; Leknes et al., 2007; Mochizuki et al., 2007), except for one fMRI study by (Herde et al., 2007).

The results of the current study in addition to findings by Drzezga et al. (2001), who documented a correlation between histamine dose and S1, but not S2 activity, would support the hypothesis of Mochizuki & Kakigi (2015) that S1 and S2 may have differing functions in the field of itch too. This however would need further investigation in order to make any firm conclusions.

The current study also found no significant manipulation of itch, post IFG stimulation. Despite the fact that the IFG is significantly activated in numerous acute itch experiments (including in the meta-analysis), its functional role is, at this point, not well understood. The current results would indicate that it does not play a crucial role in the processing of itch intensity. However, this is not to say that it does not play a role in other aspects, such as stimulus localisation (Ishiuji et al., 2009) or motor planning of the scratch response (Darsow et al., 2000; Hsieh et al., 1994). It could also have a function in the emotional processing of contagious itch (Holle et al., 2012), therefore may reduce itch caused by observing someone else scratching. This would therefore be interesting to explore further.

One notable limitation of the experiment with regards to the flare data is the way in which it was measured. Flare diameter was measured visually using a ruler. However, the shape of the flare can be very difficult to measure in some instances, due to its irregular shape. It is therefore highly possible that the current method of measurement was not accurate enough and a potential reduction of the flare post S1 stimulation could have been missed. A more sensitive method of measurement, such as laser doppler flowmetry (Heyer et al., 1989), could have therefore been employed to increase accuracy.

In summary, the results indicate that the histamine prick test of 1% and cTBS are ideal for exploring the role of S1 in itch perception, but not for S2 or the IFG. It is still controversial whether S2 encodes stimulus intensity, due to the lack of evidence in both itch and pain research. More literature appears to point in the direction of S2 playing a role in processing the recognition of the sensations, as opposed to the intensity. It may, therefore not be surprising that the current study did not find any significant difference in itch intensity after S2 stimulation. However, this would need more investigation to draw any conclusions and it may simply be the case that the current method used in the experiment was not ideal for exploring the role of S2.

More exploration will therefore be necessary, in order to evaluate the roles of these brains regions in itch perception. Modulating neural activity through brain activation provides more direct and causal evidence to understand the role of each region. Further experiments administering TMS and tDCS which modulate cortical excitability will therefore be extremely useful and play important roles in advancing the understanding of the cerebral mechanism of itch.

Chapter 6 Summary and General Discussion

6.1 Overview

This chapter summarises the main findings from the experiments presented in this thesis. The implications of these findings will also be discussed and future directions are proposed.

6.2 Research Aims

In Chapter 2, Experiment 1 directly compared the re-test reliability of three commonly used measurement scales. The scales compared were the pure visual analogue scale (pVAS), where the participant indicates itch intensity on a vertical line ranging from 0 (no itch) to 100 (the most intense itch imaginable). The second scale was a variation of the pVAS, where an additional 'Scratch Threshold' marker is set at 33% of the scale (tVAS, Darsow et al., 1996). The last was the general Labelled Magnitude Scale (gLMS, LaMotte et al., 2009; Sikand et al., 2009), where the participants judge the magnitude of itch on a vertical line with quasi-logarithmically positioned labels.

The dimensionality of both cowhage and histamine induced itch were investigated in Experiment 2 and 3 (Chapters 3 and 4), respectively. The aim was to explore any changes in the time-course, mean and peak of itch intensity and unpleasantness, which would indicate that the dimensions are independent of one another.

Based on the evidence presented in the literature review in Chapter 1, it was concluded that the brain regions specifically involved in the process of itch are yet to be confirmed. Evidence was presented in favour of using cTBS to explore the role of the somatosensory cortices and the IFG in the itch intensity process. Therefore, the primary aim of this thesis was to test which of these brain areas have a necessary function in the process of histamine and cowhage induced itch (Chapter 5).

6.3 Summary of Research Findings

6.3.1 Chapter 2

In Chapter 2, the experiment directly compared the re-test reliability of three commonly used measurement scales. The scales compared were the pure visual analogue scale (pVAS), where the participant indicates itch intensity on a vertical line ranging from 0 (no itch) to 100 (the most intense itch imaginable). The second scale was a variation of the pVAS, where an additional 'Scratch Threshold' marker is set at 33% of the scale (tVAS, Darsow et al., 1996). The last, was the general Labelled Magnitude Scale (gLMS, LaMotte et al., 2009; Sikand et

al., 2009), where the participants judge the magnitude of itch on a vertical line with quasi-logarithmically positioned labels. The gLMS generated the least variance in itch intensity ratings between testing sessions and was therefore taken as the most reliable and administered for the following experiments.

6.3.2 Chapter 3

Chapter 3 explored cowhage induced itch with regards to its time-course and dimensionality. The aims of this chapter were (1) to explore any changes in the time-course and peak of itch intensity/unpleasantness, induced by varying doses of cowhage spicules and (2) to examine any dissociation between itch intensity and unpleasantness, which would indicate that they are dissociable dimensions. The results demonstrated that there was a significant linear trend for both intensity and unpleasantness, however there was no significant difference between the dimensions. It was concluded that although cowhage is a valuable method of inducing itch, it does not generate intra-individually consistent dose-response effects. Therefore, it was considered to not be the ideal stimulus to explore brain processes involved in itch.

6.3.3 Chapter 4

In chapter 4, Experiment 2 was repeated but using the histamine prick test procedure. The aims therefore remained as follows; (1) to explore any changes in the time-course and peak of itch intensity/unpleasantness, induced by three varying doses of histamine concentrations, and (2) to examine whether histamine can successfully evoke dissociable feelings of intensity and unpleasantness. The results demonstrated that there was a significant linear trend for both intensity and unpleasantness, however there was no significant difference between the dimensions. It was therefore concluded that histamine is a reliable form of itch induction, which generates intra-individually consistent dose-response effects. Based on these results, it was decided that histamine is an appropriate stimuli to use in the following TMS experiment (Chapter 5) and that only the intensity should be measured, as the unpleasantness dimension did not appear to add any additional information.

6.3.4 Chapter 5

Chapter 5 presented a TMS study, investigating which brain areas have a necessary function in the process of histamine and cowhage induced itch. The aim was to explore any differences in the perceived itch intensity, after brain stimulation to the somatosensory cortices (S1 and S2) and the IFG, in comparison to the control (SPL). The results demonstrated that TMS to S1 significantly reduced the itch intensity, when administered via the histamine prick test. There was also a significant reduction of the wheal induced for both S1 and the IFG. There was however, no significant reduction in the intensity ratings in the S2

and IFG condition nor was there a significant reduction in the flare for any of the brain areas. There was also no significant difference in itch intensity or skin response, for any of the brain regions stimulated when cowhage was administered. In summary, the results indicate that S1 has a crucial role in the processing of itch intensity, and that the histamine prick test and cTBS are ideal for exploring this. More investigation is necessary, however, to explore the role of S2 and the IFG in itch perception.

6.4 What Can be Done to Improve Reliability of Acute Itch Measurements

6.4.1 Findings of the Present Study

The aim of Experiment 1 was to find the most reliable method of measuring acute laboratory-based itch. It compared the re-test reliability of three commonly used measurement scales. The scales were the pure visual analogue scale (pVAS), the 'Scratch Threshold' visual analogue scale (tVAS; includes scratch threshold marker at 33%; Darsow et al., 1996) and the general Labelled Magnitude Scale (gLMS, LaMotte et al., 2009; Sikand et al., 2009), which included quasi-logarithmically positioned labels. The gLMS generated the least variance in itch intensity ratings between testing sessions, and was therefore taken as the most reliable.

6.4.2 Discussion and Potential Impact

The first notable finding was the importance of including a familiarisation session, prior to an experiment on acute itch measurement. This includes participants practising using the rating scale prior to the experiment and experience the sensation of the itch stimuli. The importance of this was evident in the results, as the first session generated ratings which were qualitatively different in comparison to Sessions 2 and 3, with a significant delay in the itch peak. This therefore indicates that participants may be initially unsure about the typical time course of cowhage induced itch, as the only information they are provided with is that they will rate the intensity for 10 minutes. This may therefore result in the reluctance to provide high ratings early on in that time period, as they do not know that the intensity peaks roughly in the first three minutes of induction. This highlights the importance of a familiarisation session to ensure that participants have experienced the itch process induced by cowhage and are confident in their ability to rate it using the computerised scale.

In summary, the gLMS demonstrated the greatest re-test reliability, followed by the tVAS and then pVAS. There are at least two potential explanations for the superior reliability of the gLMS. The first is how much of the scale is open to interpretation. For example, the greater the number of verbal labels, the more guidance the participant has. The pVAS provides the most basic information, indicating only the minimum and maximum itch intensities. The rest

of the scale is therefore left for the participant to interpret. Studies have demonstrated that the lack of verbal labels in the pVAS creates ambiguity, resulting in the participant being unsure of where exactly on the scale they should place their ratings (Gonzalez-Fernandez et al., 2014; Kersten et al., 2012). In the tVAS, there is an additional marker in the form of a scratch threshold indicator, therefore providing the participant with additional guidance. The gLMS is arguably the most self-explanatory scale, as it includes 7 verbal indicators and no numerical information, therefore further reducing the interpretation of the scale. The gLMS therefore, provides the greatest guidance, which in turn, generated the highest reliability.

The second explanation is that it is may not be the number of labels themselves but the way they are logarithmically spaced in the gLMS that increases scale reliability. The verbal labels of the gLMS have an estimated quasi-logarithmic distance between them, determined by a semantic scaling procedure developed by Green et al. (1993). The scale labels are therefore situated in a way that reflects their semantic magnitude, therefore producing ratio level data. In contrast, it is debatable whether in fact the pVAS yields ratio data (Price et al., 1983) or just ordinal level data (Kersten et al., 2012). Despite the fact that the ratings generated by the VAS are usually converted into cm or percentages, according to Forrest & Andersen (1986) the scale is simply ordinal, as it has no true unit of measurement. From this perspective, ratings data from the VAS should be analysed as non-continuous using statistical methods for ordinal data as opposed to ratio (Lund et al., 2005). Also, research suggests that rather than providing a linear transformation of the internal representation of stimulus intensity, the pVAS generates only a non-linear representation and has a tendency to generate a compression of ratings at the top end of the scale (Gonzalez-Fernandez et al., 2014). Overall, the verbal labelling and positioning of the gLMS has generated the most reliable results, in comparison to the pVAS and tVAS.

6.4.3 Outlook

As previously mentioned findings suggest that the pVAS has a tendency to generate a compression of ratings at the top end of the scale (Gonzalez-Fernandez et al., 2014). This indicates that the gLMS could potentially be a suitable alternative in clinical settings in particular. This is because it would reduce the ceiling effect through decompressing the upper limit of the scale (Gonzalez-Fernandez et al., 2014). This holds great importance, not just for generating reliable measurements for acute itch, but also clinical trials where slight intensity changes are crucial in assessing treatment success. However, it is very difficult to directly compare these findings across studies. For example, some experiments induce acute itch and

others use chronic itch patients. In addition to this, various time scales are used, such as hours in comparison to weeks.

The current findings demonstrate that the gLMS has greater reliability, making it suitable for experiments which artificially induce itch in healthy participants, in comparison to pVAS and tVAS. However, due to the fact that scale reliability is not a fixed property, but is also population-dependent (Shrout, 1998) additional studies are vital to investigate whether these advantages of the gLMS are also applicable to experiments inducing itch in chronic itch patients or to the clinical assessment of chronic itch intensity. Despite this, the results demonstrated that by employing a sensitive, self-explanatory measurement scale and an effective familiarisation session, the retest reliability of acute itch perception can be significantly increased.

6.5 Acute itch: One Dimension or Multiple Dimensions?

6.5.1 Findings of Present Studies

The dimensionality of both cowhage and histamine induced itch were investigated in Experiment 2 and 3, respectively. The aim was to explore any changes in the time-course, mean and peak of itch intensity and unpleasantness, which would indicate that the dimensions are independent of one another. The findings however, demonstrated that there was no significant difference between the dimensions, for either of the stimuli. It was therefore concluded that only the intensity dimension should be measured in the brain stimulation experiment, as the unpleasantness dimension did not appear to add any additional information.

6.5.2 Discussion and Potential Impact

As there were no significant differences found between the intensity and unpleasantness dimensions, the question of whether acute itch is multidimensional or unidimensional presents itself. It could be the case that the dimensions are simply not dissociable in acute itch. For example, intensity and unpleasantness ratings for both histamine and cowhage induced itch may highly correlate and therefore there would not be any additional information from the affective dimension, which was not already provided by the intensity ratings.

In order to further test this theory more doses of both stimuli could be administered. However, there are some potential issues with this for both cowhage and histamine. For example, as Experiment 2 demonstrated, cowhage was not reliable on an individual level, therefore adding more doses would not solve this problem. Also, with regards to histamine,

research suggests that increasing the dose beyond 1% may not necessarily increase the itch intensity ratings. Drzezga et al. (2001) for instance, administered doses up to 8%, however the doses exceeding 1% (2, 4 and 8%) actually resulted in a reduced peak for both itch intensity and unpleasantness. Another issue with adding doses, is the fact that ready-made histamine solutions are only available in 1%, 0.1% and 0.01% concentrations. Therefore, in order to use a different concentration, the solution would have to be mixed by the experimenter, which raises additional ethical challenges.

Another important point when exploring dimensionality is that despite the fact that chronic itch is known to be multidimensional, it has clear differences from acute itch. For example, chronic conditions have great associations with 'secondary' affectiveness (e.g., consequences of the itch or pain). A good example of this, is a study by Price et al. (1984) who demonstrated that patients suffering with pain associated with a serious threat to health, reported significantly greater pain affectiveness than those whose pain was not as threatening, despite both reporting the same intensity. This highlights the important influence cognitive factors such as expectations have on pain unpleasantness. This 'secondary' affect which is linked with chronic and clinical conditions is generally not associated with acute sensations, particularly in experimental settings, such as the current studies in this thesis.

However, it is important to highlight that some studies have been successfully manipulated the 'primary' pain unpleasantness independent of the intensity, by altering the cognitive focus in acute pain through hypnotic techniques. For instance, hypnotic suggestions have successfully modulated (increased and decreased) pain unpleasantness selectively, without altering the perceived pain intensity (Rainville, Duncan, Price, Carrier, & Bushnell., 1997). This therefore demonstrates that hypnotic suggestions can in reality be successfully used as a form of cognitive intervention, to selectively modulate the affective dimensions of pain. It would therefore be interesting to explore whether this technique could be used in the domain of itch.

6.5.3 Outlook

As previously mentioned, it has been demonstrated that hypnotic suggestions can be used to selectively modulate the affective dimensions of pain. This technique could potentially assist in the dissociation between the dimensions and changes in physiological responses to acute itch. In turn, if this was successful, there may be a possibility of implementing this in a TMS experiment. For example, TMS of brain areas associated with the affective dimensions (e.g., the dlPFC) may interfere with the cognitive ability to perceive the itch as unpleasant (without

altering the intensity ratings). This could therefore provide an interesting approach to discover which parts of the brain would be likely to be involved in the affective dimension of itch.

The dlPFC in particular has been linked with the affective side of pain and is generally associated with the cognitive, attentional and emotional processing of painful stimuli (Bornhövd et al., 2002; Coghill et al., 1999). It also plays a crucial role in anxiety, depression (Avery et al., 2007), and unpleasantness, in relation to pain (Freund, Stuber, Wunderlich, & Schmitz, 2007). For example, it is activated during painful states and in turn potentially modulates structures involved in the emotional perception of pain, including the ACC, insula, and amygdala (Lorenz, Minoshima, & Casey, 2003). Therefore, brain stimulation of the dlPFC may interfere with the emotional processing of pain, by actively exerting control on pain perception, through modulating these cortico-subcortical and cortico-cortical pathways (Lorenz et al., 2003).

The dlPFC is also involved in pain observation and empathy, as neuroimaging studies exploring the pain response of distress, caused by witnessing others experiencing pain, have demonstrated activation in the dlPFC, ACC and the anterior insula (Fregni, Freedman, & Pascual-Leone, 2007; Lefaucheur, Drouot, Keravel, & Nguyen, 2001). Rêgo et al. (2015) further explored the emotional reactions elicited by pain observation but administering tDCS to the dlPFC on healthy volunteers. Compared to sham stimulation, both left-cathodal/right-anodal and left-anodal/right-cathodal tDCS significantly decreased hostility, sadness and self-pain perception. These decreased sensations after tDCS to the left and right dlPFC therefore further suggest its role in personal distress modulation, associated with pain and generally the unpleasantness aspect surrounding it. These results are in line with previous tDCS experiments, which showed that stimulation over the left dlPFC decreased distress in volunteers seeing images of other people in pain (Boggio, Zaghi, & Fregni, 2009). For instance, Boggio et al. (2009) found that left dlPFC anodal stimulation, decreased self–discomfort and unpleasantness judgments when viewing pictures of people with injuries.

In another interesting brain stimulation experiment, Borckardt et al. (2007) explored the idea of the dlPFC and its links to control. They specifically investigated the effects of TMS, over the left dlPFC (compared with sham TMS as the control) on the analgesic effects of perceived pain controllability. They found that the dlPFC suppressed the analgesic benefits of perceived control on the emotional dimension of pain, but interestingly, not the

sensory/discriminatory dimension. These findings therefore suggest that TMS over the left dlPFC may interrupt the perceived control of the emotional dimension of experimental pain experience. In addition to this, Krummenacher, Candia, Folkers, Schedlowski, & Schönbächler (2010) administered 1 Hz rTMS to both the left and right dlPFC, prior to inducing expectation-based placebo analgesia. They discovered that treatment expectation increased pain threshold and tolerance, but that low-frequency rTMS blocked this analgesic effect.

The neural basis of the affective dimension of itch is similar to that of pain, with evidence suggesting many of the same areas are involved in its control and modulation. Mochizuki et al. (2003) for example, used PET and histamine iontophoresis. They found that the rCBF in the ACC, the dlPFC, the posterior parietal cortex and the premotor cortex positively increased with the concentration of histamine administered, which is in support of previous literature (Darsow et al., 2000; Drzezga et al., 2001).

The PFC has also been linked to have a role in scratching. Yosipovitch, Ishiuji and Patel (2008), for example, reported that repetitive scratching increased brain activity in the dlPFC, while activity in the ACC and PCC was reduced. It is therefore possible that scratching is perceived as inhibiting itch and therefore as rewardable and pleasurable, which is reflected through the activation of these brain areas. In addition to this, literature has also indicated a link between observation of scratching and the dlPFC. For example, as previously discussed, watching video clips of someone scratching (relative to control videos of tapping) activated, as indicated by functional neuroimaging, many of the neural regions linked to the physical perception of itch, including prefrontal (BA44). Moreover, activity in the left dlPFC correlated with subjective ratings of perceived itch. Similar to pain research, this suggests that the dlPFC has a crucial role in processing itch information. This is supported by Ishiuji et al. (2009), who claimed that activation of the PFC may be likely to mediate part of the cognitive dimension of itch processing, associated with the encoding of the attendant stimulus.

As previously highlighted, a method of investigating whether a particular brain region has a specialised role in itch perception (e.g., sensory/affectiveness), is through selectively modulating a dimension and observing the difference in brain activity. Psychological factors, like expectancy, are known to significantly influence ratings, as well as brain activity in areas

associated with the affective dimensions of both itch (Napadow et al., 2015) and pain (Kong et al., 2008; Tracey, 2010).

The role of verbal suggestion in nocebo effects on itch, for example, has been explored in a study by Napadow et al. (2013), using the histamine prick test on AD patients. The results demonstrated that patients reported more itch from the saline solution, when they expected a real allergen, than when they were informed that it was saline. Interestingly, the fMRI data showed that similar brain regions were activated when saline was applied whilst patients expected a real allergen, as with the previously applied real allergen. For example, there was greater activation in the striatum and the dlPFC, which are regions previously linked to placebo- or nocebo-induced brain processes, related to pain and its regulation (Enck, Benedetti, & Schedlowski, 2008).

Scholz & Hermanns, (1994) found that AD patients reported higher itch ratings, when histamine administration was accompanied by negative verbal suggestions, with regards to the skin response and itch perception. Napadow et al. (2015) further explored this theory of expectancy using fMRI, which demonstrated that nocebo-induced itch generated greater itch sensation, in comparison to the control, along with an increased activity of the dlPFC. This research is crucial in understanding the role of psychological factors involved in the manipulation of itch perception and indicating which brain mechanisms are selectively involved.

Desbordes et al. (2015) also explored the role of the dlPFC in AD patients. They interestingly discovered that increased connectivity between the superior parietal lobule (SPL) and the dlPFC was associated with a decrease in perceived itch. Patients with greater increase in SPL–dlPFC connectivity reported a lower increase in itch sensation. This result indicated that greater interaction between the SPL and dlPFC could potentially limit an itch sensation via enhanced top-down regulation. This suggests that the observed increased connectivity could serve as a protective mechanism, limiting perceived itch severity in AD patients. The dlPFC and SPL sub-regions could have an involvement in the control network, supporting executive processing of cognitive control (Vincent, Kahn, Snyder, Raichle, & Buckner, 2008), particularly in chronic itch disorders. In other words, patients who are able to regulate increased information exchange within this network were better able to limit the severity of the perceived itch. It would therefore be interesting to see whether a disruption of the dlPFC

(e.g., via brain stimulation) would interfere with itch perception. For instance, if dlPFC activity in AD is disrupted, would this result in an increase in itch perception in AD patients?

A study by Ishiuji et al. (2009), however, did not find any significant activation of the ACC and dlPFC, with histamine-induced itch in healthy controls. This therefore contradicts previous studies, using PET and BOLD fMRI in healthy subjects (Darsow et al., 2000; Drzezga et al., 2001; Herde et al., 2007; Hseish et al., 1994; Leknes et al., 2007; Mochizuki et al., 2007; Valet et al., 2007; Walter et al., 2005). Ishiuji et al. (2009) suggested that possible explanations for this could potentially be the differences in the severity of itch between studies. In the group of AD patients, Ishiuji et al. (2009) observed a significant correlation between the percentage change of brain activation in dlPFC and disease severity (r^2 = 0.30,$P < 0.03$). This was the first study which demonstrated an association between severity of a chronic itch state and itch-induced brain activity (dlPFC, ACC and insula). These findings indicate that these three brain regions may potentially be promising targets for future drugs or brain stimulation which reduce activity in these areas, therefore decreasing the augmented perception of itch in AD and other forms of chronic itch and pain. More studies are needed, however, to further explore itch and the dlPFC, especially any differences in its role in healthy participants in comparison to chronic itch patients.

Moreover, with regards to itch research, various studies have explored the role of verbal suggestion in nocebo effects. Napadow et al. (2013), for instance, used the histamine prick test on AD patients and found that they reported greater itch from the saline solution when they expected a real allergen, as compared to when they were told it was saline. The fMRI data indicated that similar brain areas were activated when saline was applied whilst patients expected a real allergen, as with the previously applied real allergen. It would therefore be interesting to test this further through repeating the TMS study in both healthy volunteers and AD patients, whilst using suggestibility techniques to enhance the affective dimension, in order to see if TMS to the dlPFC then reduces the itch intensity. This would not only enable us to test whether the enhancement of the affective dimension of itch can modulate the involvement of this brain region, but also provides the opportunity to directly compare its role in AD patients and healthy participants.

Although numerous research provides evidence suggesting the vital role of the dlPFC, more brain stimulation studies need to be completed, in order to gain a clear coherent picture of its role in the pain and itch perception process. The evidence however, suggests that it would be

a good brain area to target, in order to modulate the affective dimension of both pain and possibly itch.

6.6 Acute itch: Which Brains Areas are Crucial?

6.6.1 Findings of Present Study

The primary purpose of this experiment was to administer TMS in order to investigate which brain areas have a necessary function in the process of histamine and cowhage induced itch. This was tested by stimulating the somatosensory cortices (S1 and S2) and the IFG, and comparing the perceived itch ratings with the control condition (SPL). The results demonstrated that TMS to S1 significantly reduced the itch intensity, when administered via the histamine prick test. There was also a significant reduction of the wheal induced for S1 and the IFG condition. There was, however, no significant difference in the intensity ratings or the wheal response with regards to S2 and the IFG. There was also no significant reduction in the flare for any of the experimental brain areas post histamine induced itch. In addition to this, there was also no significant difference in itch intensity or skin response, for any of the brain regions stimulated when cowhage was administered. In summary, the results indicate that S1 has a crucial role itch the processing of itch intensity, and that the histamine prick test and cTBS are ideal for exploring this. More investigation is necessary however, to explore the role of S2 and the IFG in itch perception.

6.6.2 Outlook

The first notable question is whether S1 and S2 have differing roles in the processing of itch. As it has been suggested that S2 is primarily activated when stimulus intensity is high, a possible way to investigate this query would be to replicate the current TMS experiment but using an itch substance which induces a higher intensity of itch. It has been found that cowhage evokes a more intense itch response than histamine both in the current thesis and work by Papoiu et al. (2012) who reported that cowhage induced itch appears to induce a stronger and more extensive activation in S1 and S2 than histamine. However, due to the fact that the current method of ‘rubbing’ in the spicules into the skin did not prove to be consistent, a more accurate alternative would be necessary. For example, inserting the spicules rather than simply placing them onto the skin (LaMotte et al., 2009; for further details see Experiment 2). This would therefore be an interesting way of further exploring the role of S2 in the specific process of itch and compare it to that of histamine.

The second important question which this chapter addresses is which specific role the IFG serves in itch processing, as it is currently largely undefined. Research indicates that it is

activated during action observation (Fadiga et al., 2006) which suggests its function in interpreting actions of others. In addition to this, research by Holle et al. (2012) indicated a link between observation of scratching and activation of the IFG, in an fMRI study. It is however, necessary to conduct more studies in order to explore its role in contagious itch. One potential way of testing this, could be to incorporate the study by Holle et al. (2012) into a brain stimulation experiment and explore whether stimulation of the IFG significantly reduces reported itch intensity, when observing someone scratching.

Overall, more exploration is necessary to evaluate the roles of these brains regions in itch perception. Modulating neural activity through brain activation provides promising causal evidence to understand the role of each region. Further experiments administering TMS and tDCS to manipulate brain activity will be extremely useful and play an important role in advancing the understanding of the cerebral mechanism of itch.

6.7 General Summary and Conclusions

The first notable issue presented in the literature review is the fact that there is no consensus on which rating scale is the most reliable when measuring acute itch. The first experiment (Chapter 2) therefore directly compared the re-test reliability of three commonly used measurement scales (pVAS, tVAS and gLMS). The gLMS generated the least variance in itch intensity ratings between testing sessions and was therefore taken as the most reliable and administered for the following experiments.

The second important question raised by the literature review is whether acute itch is multi-dimensional, with regards to its intensity and unpleasantness. The dimensionality of both cowhage and histamine induced itch were therefore investigated in Experiment 2 and 3, respectively. The aim was to explore any changes in the time-course, mean and peak of itch intensity and unpleasantness, which would indicate that the dimensions are independent of one another. The findings however, demonstrated that there was no significant difference between the dimensions, for either of the stimuli. It was therefore concluded that only the intensity dimension should be measured in the brain stimulation experiment, as the unpleasantness dimension did not appear to add any additional information.

The third question was based on the evidence presented in the literature review that indicated that the brain regions specifically involved in the process of itch are yet to be confirmed. The primary purpose of this experiment was to therefore administer cTBS in order to investigate which brain areas have a necessary function in the process of histamine and cowhage induced

itch. This was tested by stimulating the somatosensory cortices (S1 and S2) and the IFG, and comparing the perceived itch ratings with the control condition (SPL). The results demonstrated that TMS to S1 significantly reduced the itch intensity, when administered via the histamine prick test. There was also a significant reduction of the wheal induced for S1 and the IFG condition. There was however, no significant difference in the intensity ratings or the wheal response with regards to S2 and the IFG. There was also no significant reduction in the flare for any of the experimental brain areas post histamine induced itch. In addition to this, there was no significant difference in itch intensity or skin response, for any of the brain regions stimulated when cowhage was administered. In summary, the results indicate that S1 has a crucial role in the processing of itch intensity, and that the histamine prick test and cTBS are ideal for exploring this. More investigation is necessary however, to explore the role of S2 and the IFG in itch perception.

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Appendix A

Full Exclusion Criteria for Taking Part in Experiments Involving the Application of Cowhage

- Any allergy against the cowhage plant (also known ‘velvet bean’)
- A skin condition (e.g., eczema, psoriasis)
- Suffer from diabetes or currently take medication to control blood sugar level
- Currently taking any drugs with a blood-thinning effect (e.g., aspirin, ibuprofen, naproxen, heparin, clopidogrel)
- Suffer from Parkinson’s Disease
- Have taken any of the following drugs the last 7 days (Ritalin, amphetamine (e.g., speed), methamphetamine (e.g., crystal meth), cocaine)
- A history or currently suffer from schizophrenia, depression or any other mental illness
- Have a disease of the heart or blood vessels (cardiovascular disease)
- Currently receive drug treatment against high blood pressure
- Currently be taking Mono Amine Oxidase Inhibitors (a drug often prescribed for depression)
- Have a liver disease
- Have skin cancer (melanoma)
- Have a stomach ulcer or intestinal ulcers
- Had a surgery within the last two weeks, or have one planned in the two weeks following the experimental sessions
- History of any mental illness (e.g., psychosis)
- Currently pregnant or breastfeeding
- Received any medication/topical treatment on the forearm (e.g., regular application of corticosteroids on the forearm)
- Any of the following in the test area: Wounds, rashes, swelling or reddening, scars or tattoos

Appendix B

Full Exclusion Criteria for Taking Part in Experiments Involving the Application of Histamine

Taken any of the medication/drugs in the past 48 hours:

- Antihistamines (e.g., as a treatment for hayfever)
- Beta blockers (e.g., for treatment of heart condition)

Do you currently suffer from or have a history of any of the following:

- Fainting during medical procedures (e.g., flu shots or immunization shots)
- An allergy
- An acute or chronic skin condition (e.g., eczema, psoriasis)
- Any disease of the heart or blood vessels (cardiovascular disease)
- Low blood pressure
- Fever
- Asthma
- Histamine intolerance
- Any of the following in the test area: Wounds, rashes, swelling or reddening, scars or tattoos
- Any medication/topical treatment on the forearm (e.g., regular application of corticosteroids on the forearm)

Are you hypersensitive to any of the following substances:

- Histamine (spinach, sauerkraut, certain types of sausage and cheese are rich in histamine)
- Phenol (many types of berries and fruit are rich in phenol)
- Glycerol (milk, clotted cream, puddings and yogurt are rich in glycerol)
- Sodium Hydroxide (a.k.a. lye or caustic soda, used for preparation of pretzels, chinese noodles, also used in production of soft drinks)

Additional criteria

- Currently pregnant or breastfeeding

Appendix C

Full Exclusion Criteria for Taking Part in Experiments Involving TMS

- History of any neurological or psychiatric conditions
- Suffered from epilepsy, febrile convulsions in infancy or had recurrent fainting
- An immediate or distant family suffering from epilepsy?
- Ever undergone a neurosurgical procedure (including eye surgery)?
- Currently pregnant
- Currently have tooth or ear ache

<u>Do you currently have any of the following fitted to your body?</u>

- Heart Pacemaker
- Cochlear implant
- Medication pump
- Surgical clips

Appendix D

Full List of Itch Examples Used in the Familiarisation Session

- An insect bite (e.g., from an ant or a mosquito)
- Prickly heat (an itchy rash of small, raised red spots caused by heat)
- Insect crawling up your arm (e.g., ant or fly)
- Itch from a scar
- Chickenpox
- Itchy scalp (e.g., from dry scalp or hair dye)
- Athletes foot
- Sunburn
- Wearing a woolly jumper
- A label in an item of clothing
- Watching a video of someone else scratch their arm (how itchy does it make you feel?)
- Listening to a scratching noises (How itchy does it make you feel?)

Appendix E

Original Results for Experiment 4 (TMS) Prior to Being Normalised

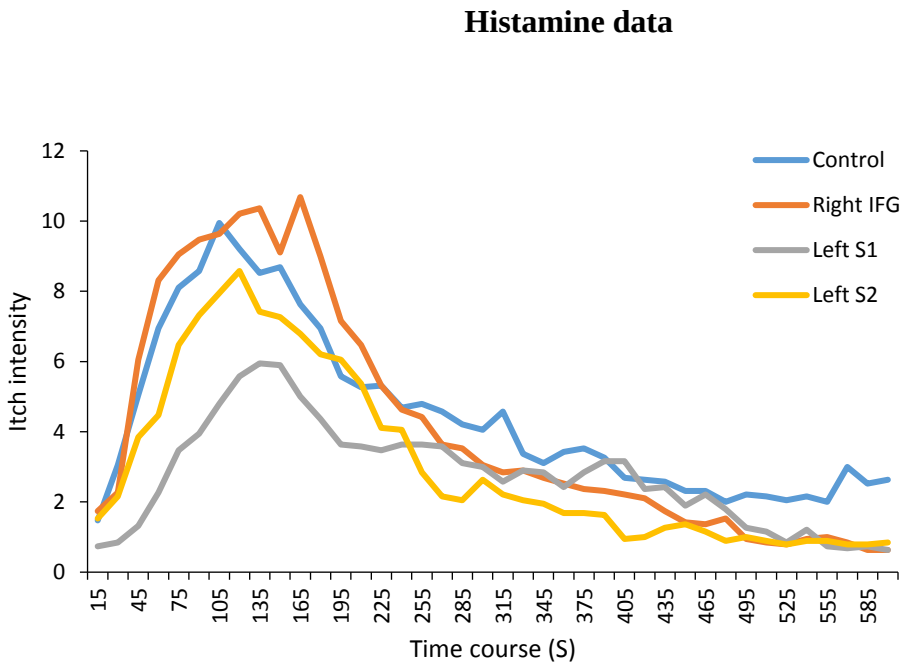

Figure E.1 Time course of mean itch intensity for histamine across all 4 stimulation conditions.

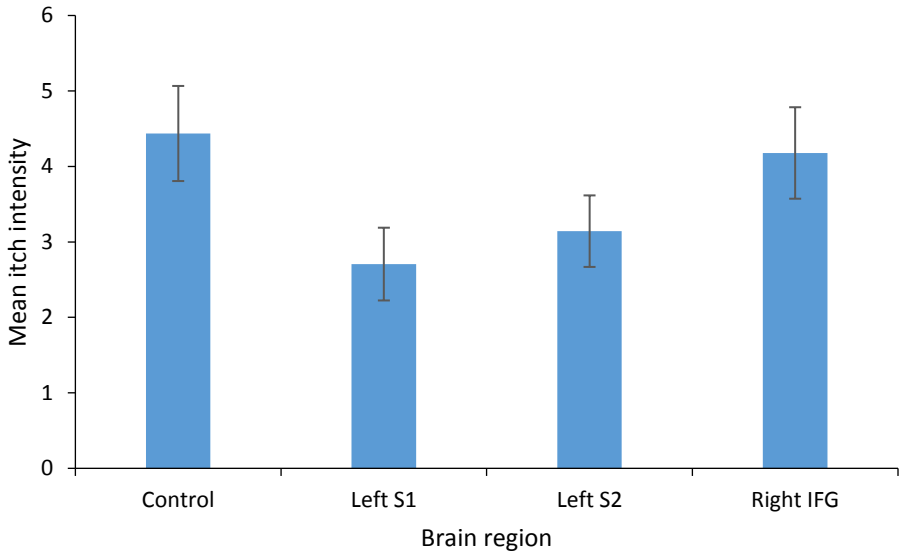

Figure E.2 Mean itch intensity (± 1 SEM) for histamine across all 4 stimulation conditions.

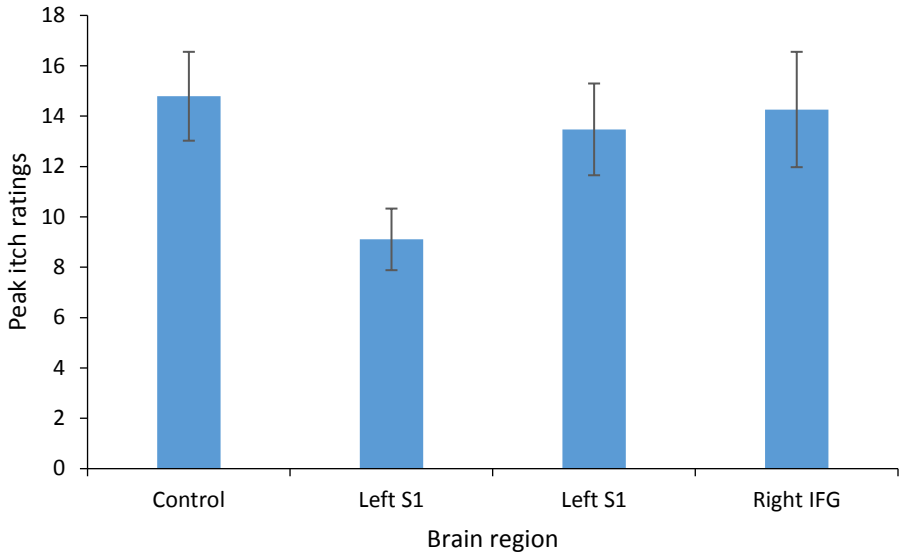

Figure E.3 Mean peak itch intensity (± 1 SEM) for histamine across all 4 stimulation conditions.

Cowhage data

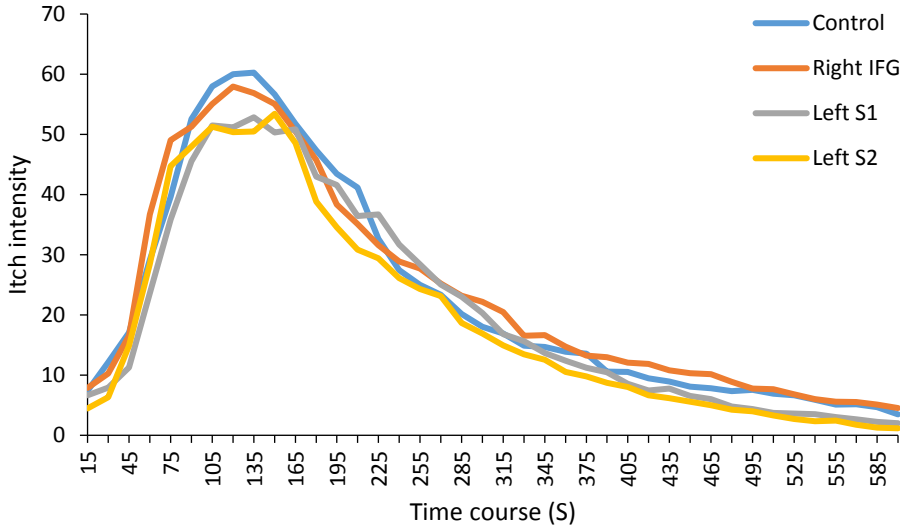

Figure E.4 Time course of mean itch intensity for cowhage across all 4 conditions.

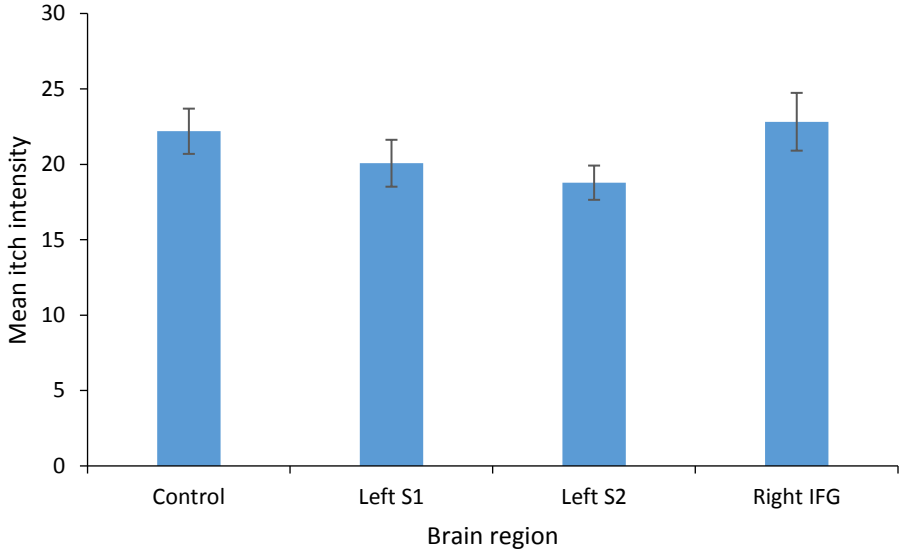

Figure E.5 Mean itch intensity (± 1 SEM) for cowhage across all 4 stimulation conditions.

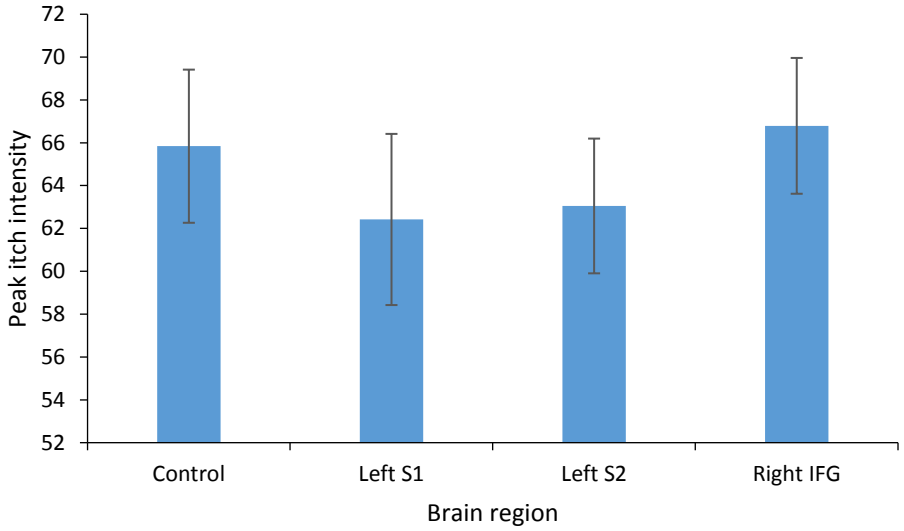

Figure E.6 Mean peak itch intensity (± 1 SEM) for cowhage across all 4 stimulation conditions.