Stress-Activated Lateral Hypothalamic Neurons Suppress Itch

A Thesis

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by

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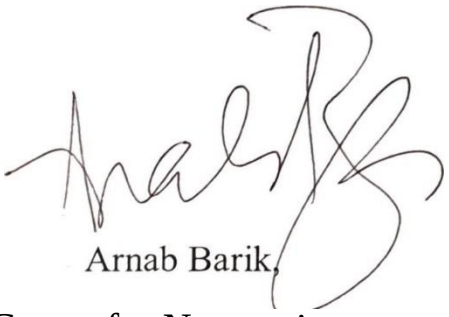
Certificate

This is to certify that this dissertation entitled Stress-Activated Lateral Hypothalamic Neurons Suppress Itch towards the partial fulfilment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune represents study/work carried out by Manojeet Pattanayak at Indian Institute of Science, Bangalore under the supervision of Arnab Barik, Assistant Professor, Centre for Neuroscience, during the academic year 2024-2025.



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This thesis is dedicated to my parents & my friends who always kept me motivated.

Declaration

I hereby declare that the matter embodied in the report entitled Stress-Activated Lateral Hypothalamic Neurons Suppress Itch are the results of the work carried out by me at the Centre for Neuroscience, Indian Institute of Science, Bangalore, under the supervision of Arnab Barik and the same has not been submitted elsewhere for any other degree.

Manojeet Pattanayak

Date: 26/3/2025

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Abstract

The bidirectional relationship between stress and itch represents a critical but incompletely understood aspect of pruritic skin disorders that affects millions of patients worldwide.

This thesis identifies a specific population of lateral hypothalamus neurons activated by restraint stress (LHStress neurons) that play a crucial role in stress-modulation of itch. Using the TRAP2 system in mice, we demonstrate that acute restraint stress significantly suppresses scratching behavior in both acute chloroquine-induced ($P = 0.0092$, $n = 8$) and chronic imiquimod-induced ($P = 0.0053$, $n = 7$) itch models. Chemogenetic activation of LHStress neurons reduced scratching in both models ($P < 0.005$), while silencing these neurons increased scratching behavior ($P < 0.02$) and abolished stress-induced itch suppression. Through viral tracing and targeted manipulations, we identified the LH^{Stress}→lateral/ventrolateral periaqueductal gray pathway as both necessary and sufficient for stress-induced itch suppression. Notably, LH^{Stress} neurons exhibited significant plasticity during chronic itch, developing novel scratch-related activity patterns ($P = 0.0113$, $n = 10$) and increased intrinsic excitability not observed in acute conditions.

These findings reveal a specific neural circuit mechanism underlying stress-itch interactions and provide a framework for understanding why stress often exacerbates pruritic disorders in clinical settings. The identification of experience-dependent plasticity in these circuits offers potential targets for therapeutic intervention aimed at breaking the vicious cycle between stress and itch that characterizes many chronic pruritic conditions.

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Contributions

Contributor name	Contributor role
Arnab Barik	Conceptualization Ideas
Jagat Narayan Prajapati	Methodology
Devangshu Nandi	Software
Arnab Barik	Validation
Jagat Narayan Prajapati	Formal analysis
Manojeet Pattanayak	Investigation
Arnab Barik	Resources
Jagat Narayan Prajapati	Data Curation
Manojeet Pattanayak	Writing - original draft preparation
Manojeet Pattanayak	Writing - review and editing
Jagat Narayan Prajapati	Visualization
Arnab Barik	Supervision
Arnab Barik	Project administration
Arnab Barik	Funding acquisition

Chapter 1 Introduction

Itch represents one of the most debilitating and prevalent symptoms that patients who have skin with inflammatory conditions experience.(Yosipovitch and Bernhard, 2013) According to recent studies, stress and itching have a complicated two-way relationship in which both can have a major impact on one another.(Arck *et al.*, 2006) The vicious cycle of itching and scratching, which is fed by underlying inflammation, helps in maintenance of this link, albeit the exact molecular processes are still unclear.(Mack and Kim, 2018) Emerging evidence further reveals specific neural circuits mediating anxiety-like behaviours in chronic itch, providing new insights into this complex relationship.(Sanders *et al.*, 2019) This thesis investigates the neurobiological mechanisms underlying stress modulation of itch perception, with particular focus on hypothalamic regulation and associated neural circuits.

Neurobiological Basis of Itch

Sensation and Transmission Pathways

Despite sharing some neuroanatomical pathways, itch is described as an unpleasant sensation that makes one want to scratch, which represents a different sensory modality as compared to pain.(Ikoma *et al.*, 2006) Itch signals originate primarily from the skin through activation of specialized receptors and are carried to the CNS via different afferent nerve fibres.(Han and Dong, 2014) These signals travel to the dorsal horn of spinal cord via the dorsal root ganglia before ascending to higher brain centres for processing and perception.(Bautista *et al.*, 2014)

Pruritus can be categorised into four types based on origin: pruritoceptive (arising from skin inflammation), neuropathic (from nervous system damage), neurogenic (from systemic disorders affecting the central nervous system), and psychogenic (from psychological factors including stress).(Twycross *et al.*, 2003) This classification system helps guide both research approaches and therapeutic interventions.

Peripheral and Central Mechanisms

At the peripheral level, various pruritogens activate itch-specific receptors on sensory nerve endings.(Schmelz, 2010) Primary pruritogens include histamine (acting through H1 and H4 receptors), proteases (activating protease-activated receptors), and numerous neuropeptides, cytokines, and chemokines.(LaMotte *et al.*, 2014) Once activated, these receptors initiate intracellular signalling cascades leading to action potential generation and transmission through specialized primary afferent fibres.(Shim and Oh, 2008)

Centrally, itch processing involves complex neural circuits in the brain as well as spinal cord. In the spinal cord, GRP or gastrin-releasing peptide, and its receptor, also called GRPR, play crucial roles in transmission of itch, with GRPR-expressing neurons constituting a labelled line for itch signals.(Sun *et al.*, 2009) Important brain regions involved in itch processing

include the thalamus, somatosensory cortex, anterior cingulate cortex, and limbic structures, which collectively contribute to both sensory and affective dimensions of itch perception.(Papoiu *et al.*, 2012)

Stress Response Systems and Neural Circuitry

Definition and Physiological Impact

Stress can be defined as a state of threatened homeostasis eliciting physiological and behavioural adaptations aimed at restoring balance.(McEwen, 2007) The duration and intensity of stress exposure critically determine its effects: acute stress generally elicits adaptive responses, while chronic stress often leads to maladaptive outcomes(Chrousos, 2009) with significant health implications. Stressors may be physical (injury, infection), psychological (fear, anxiety), or social (discrimination, isolation), each activating overlapping yet distinct neural pathways.(Ulrich-Lai and Herman, 2009)

Interpersonal stressors, such as discrimination, can trigger cascading biological processes beginning with perceived social exclusion and registering as social pain.(Eisenberger, 2012) Such stressors increase sympathetic nervous system activation and upregulate the hypothalamic-pituitary-adrenal (HPA) axis, leading to increased physiological wear and tear and elevated risks of cardiometabolic conditions.

Neuroendocrine Activation

The body responds through two primary systems for stress: the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic-adrenal-medullary (SAM) axis.(Ulrich-Lai and Herman, 2009) The HPA axis coordinates a slower but more sustained stress response through glucocorticoid secretion. Concurrently, the SAM axis mediates immediate "fight-or-flight" responses through sympathetic activation and catecholamine release.(Goldstein, 2010)

Neurons in the paraventricular nucleus of the hypothalamus release CRH, also called corticotropin-releasing hormone, which initiates the HPA axis.(Herman *et al.*, 2016) CRH helps in release of adrenocorticotropic hormone (ACTH) by stimulating the anterior pituitary, which triggers the adrenal cortex to secrete glucocorticoid.(Vale *et al.*, 1981) These glucocorticoids (corticosterone in rodents, cortisol in humans) exert widespread effects throughout the body, including metabolic regulation, immune modulation, and neural functional alterations.

Neural Circuits in Stress Processing

The brain's response to stress involves interconnected structures including the prefrontal cortex, hippocampus, amygdala, and hypothalamus.(Dedovic *et al.*, 2009) The amygdala serves as the central threat detector, while the hippocampus contributes to contextual

processing and HPA axis regulation.(Herman *et al.*, 2005) The prefrontal cortex exerts top-down control over emotional responses and stress reactivity.(Arnsten, 2009)

Recent research has highlighted the lateral hypothalamus's importance in stress processing. Using TRAP2 transgenic mice, researchers have identified lateral hypothalamus neurons activated by restraint stress (LH^{Stress} neurons) that interconnect with brain regions involved in stress perception and modulation.(Bonnavion *et al.*, 2016) These neurons represent a critical node in orchestrating appropriate physiological and behavioural responses to stressors.

The Bidirectional Relationship Between Stress and Itch

Acute Stress and Itch Suppression

Acute stress has been demonstrated to suppress itch perception through activation of stress-responsive neural circuits.(Valet *et al.*, 2008) This phenomenon, similar to stress-induced analgesia, is chiefly regulated by descending inhibitory pathways originating the brain to the spinal cord, involving endogenous opioids, noradrenaline, and serotonin, which collectively inhibit itch transmission at the spinal level.(Akiyama *et al.*, 2011)

Research using rodent models demonstrates that acute restraint stress can reduce scratching behaviour in response to pruritogens, suggesting an adaptive function in temporarily suppressing itch sensation. This suppression may represent an evolutionary adaptation allowing organisms to prioritize attention and resources toward addressing immediate threats rather than attending to less urgent sensations like itch**.**

Chronic Stress and Itch Exacerbation

In stark contrast to acute stress effects, chronic stress often exacerbates itch symptoms.(Hall *et al.*, 2012) Patients with chronic pruritic conditions such as atopic dermatitis and psoriasis frequently report stress as a trigger for symptom flares.(Chrostowska-Plak *et al.*, 2013) Studies confirm that individuals suffering from chronic itch have significantly higher stress levels compared to those without itch, highlighting the reciprocal relationship between these conditions.

Chronic stress may enhance itch perception through several mechanisms, including neuroinflammation, altered immune function, and disruption of skin barrier integrity.(Peters *et al.*, 2011) Prolonged HPA axis activation leads to dysregulation of immune responses, potentially upregulating pro-inflammatory cytokines that sensitize peripheral nerve endings to pruritogens and lower the threshold for itch perception.(Cohen *et al.*, 2012)

Chronic Itch and Anxiety Comorbidity

Chronic itch frequently coexists with anxiety symptoms, forming a self-perpetuating cycle of itch-anxiety comorbidities that are challenging to treat(Schneider *et al.*, 2006).Recent studies have identified specific neural circuits underlying this connection, highlighting the role of the lateral septum (LS) in mediating chronic itch-induced anxiety which mainly consists of GABAergic neurons (Sanders *et al.*, 2019). This finding helps explain why individuals with chronic pruritus often experience psychological distress, which further worsens their condition.

Research using mouse models has demonstrated that chronic itch is associated with increased activity and synaptic plasticity from the thalamic nucleus reuniens (Re) onto LS GABAergic neurons in form of excitatory projections (Mu *et al.*, 2017). Selectively inhibiting the Re → LS circuit via chemogenetic approaches significantly reduces anxiety induced by chronic itch without affecting restraint stress-induced anxiety, indicating that distinct neural pathways regulate different stress responses.

Additionally, LS GABAergic neurons provide monosynaptic inhibition to GABAergic neurons in the lateral hypothalamus (LH), contributing to the regulation of chronic itch-induced anxiety. This Re → LS → LH pathway represents a promising therapeutic target for disrupting the itch-anxiety cycle that characterizes many chronic pruritic conditions.

Shared Mediators and Pathways

Stress and itch share several neurobiological mediators and pathways, providing a mechanistic basis for their interaction.(Arck *et al.*, 2006) These include:

1. Neuropeptides: Substance P and calcitonin gene-related peptide (CGRP) are involved in both stress responses and itch transmission. Stress can enhance the release of these neuropeptides, which promote itch sensation through direct and indirect mechanisms. (Peters *et al.*, 2006)
2. Mast cells: These immune cells respond to both stress mediators and pruritogens. Stress-induced release of neuropeptides such as CRH and substance P can trigger mast cell degranulation, leading to histamine release and other itch-inducing mediators.(Theoharides *et al.*, 2012)
3. Neurotrophin signalling: Upregulation of BDNF, also called brain-derived neurotrophic factor, and NGF, nerve growth factor, occurs in response to both stress and pruritic stimuli, promoting neuronal sensitization and enhanced itch perception.(Tominaga *et al.*, 2007)
4. Cytokine networks: Pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α are elevated in both chronic stress and chronic itch conditions, potentially serving as biological links between these conditions.

The Vicious Itch-Scratch Cycle

The relationship between stress and itch is often perpetuated by the itch-scratch cycle, wherein scratching provides temporary relief but ultimately worsens inflammation and skin

barrier disruption, leading to increased itch sensitivity. Stress amplifies this cycle by lowering the threshold for itch perception, increasing scratching behaviour, and exacerbating skin inflammation.

The psychological components of this cycle are equally significant, as the distress associated with unrelieved itch generates additional stress, creating a self-perpetuating loop. Breaking this cycle represents a key challenge in managing chronic pruritic conditions, requiring integrated approaches addressing both physiological and psychological dimensions of itch.

Neurobiological Mechanisms Linking Stress and Itch

Hypothalamic Regulation

The hypothalamus serves as a critical interface between nervous and endocrine systems, coordinating responses to both stress and itch.(Saper and Lowell, 2014) Within this structure, distinct neuronal populations respond to different stressors and modulate various physiological outputs, including itch perception. Recent research using TRAP2 transgenic mice has identified lateral hypothalamus neurons (LH^{Stress} neurons) that play a key role in stress-induced itch modulation.(Guenthner *et al.*, 2013)

The lateral hypothalamus contains diverse neuronal populations regulating functions including arousal, feeding, reward, and stress responses. Chemogenetic activation of LH^{Stress} neurons decreases scratch responses under both acute and chronic itch conditions in mice, while their inhibition produces opposite effects. Interestingly, these neurons do not respond to acute itch-induced scratching, suggesting they don't directly encode scratching behaviour.

Role of the Periaqueductal Gray in Itch Modulation

The periaqueductal gray (PAG) represents a midbrain structure crucial for pain and itch modulation.(Chen *et al.*, 2016) It receives inputs from various brain regions, including the hypothalamus, amygdala, and prefrontal cortex, and sends projections to the rostral ventromedial medulla, which in turn modulates spinal cord neurons involved in itch transmission.(Gao *et al.*, 2019)

Viral anterograde and retrograde tracing reveals that LH^{Stress} neurons project densely to the lateral and ventrolateral PAG (l/vlPAG), regions known to be involved in itch modulation. Activation of LH^{Stress} neuron terminals in the l/vlPAG suppresses itch in mice, indicating the LH-PAG circuit is essential for stress-induced modulation of itch sensation.

Neurochemical Mediation

Several neurochemical mediators facilitate communication between stress and itch pathways.(Slominski *et al.*, 2013) CRH, the principal mediator of the HPA axis, modulates itch perception both centrally and peripherally.(Tsatsanis *et al.*, 2007) In the skin, CRH can

activate mast cells, inducing degranulation and release of pruritogens such as histamine and tryptase.(Cao *et al.*, 2005)

Endogenous opioids demonstrate complex effects on itch perception: μ-opioid receptor agonists can induce itch when administered centrally, while κ-opioid receptor agonists exert anti-pruritic effects. Stress-induced release of endogenous opioids may therefore contribute to the complex relationship between stress and itch through differential receptor activation patterns.

Neurotransmitters such as serotonin and norepinephrine, involved in descending inhibitory pathways, are also modulated by stress and influence itch transmission. The balance between these systems determines whether stress will suppress or enhance itch perception in specific contexts.

Immune System and Microbiota Involvement

The immune system serves as a critical mediator between stress and itch through its effects on inflammation and neural sensitization.(Hall *et al.*, 2012) Stress modulates immune function via both the HPA axis and sympathetic nervous system, affecting cytokine production, chemokine release, and other inflammatory mediator patterns.(Glaser and Kiecolt-Glaser, 2005)

In itch contexts, stress-induced immune alterations can enhance mast cell activation, increase pro-inflammatory cytokine production, and alter T-cell responses. These changes sensitize peripheral nerve endings to pruritogens and promote neurogenic inflammation, contributing to enhanced itch perception.

The skin microbiota has emerged as another potential mediator between stress and itch.(Grice and Segre, 2011) Stress can alter skin microbiome composition and function, which influences skin barrier integrity, immune responses, and neural sensitivity. The skin microbiota produces various neurochemicals, including acetylcholine, histamine, and catecholamines, which modulate itch perception through direct and indirect mechanisms.

Clinical Implications and Research Methodologies

Stress-Induced Itch in Dermatological Disorders

The stress-itch relationship has significant implications for managing various skin disorders.(Dalgard *et al.*, 2007) Conditions such as atopic dermatitis, psoriasis, and chronic urticaria frequently show exacerbation during periods of increased stress, with patients consistently reporting stress as a primary trigger for disease flares.(Chrostowska-Plak *et al.*, 2013) Understanding the neurobiological mechanisms underlying this relationship can inform development of targeted interventions.

The concept of the "skin-brain axis" acknowledges the bidirectional communication between the central nervous system and skin, providing a framework for understanding how

psychological factors influence cutaneous processes and vice versa.(Arck *et al.*, 2006) This framework supports integrated approaches to treating stress-exacerbated skin conditions, combining dermatological and psychological interventions.

Current and Emerging Therapeutic Approaches

Current therapeutic strategies for stress-induced itch include both pharmacological and non-pharmacological approaches.(Ständer *et al.*, 2007) Pharmacological interventions target specific mediators involved in the stress-itch interface, including antihistamines, anti-inflammatory agents, and neuromodulators. Antidepressants, particularly selective serotonin reuptake inhibitors (SSRIs) and tricyclic antidepressants, show efficacy in certain pruritic conditions, potentially by modulating both mood and neurochemical pathways involved in itch transmission.(Yosipovitch and Bernhard, 2013)

Non-pharmacological approaches include education about the itch-scratch cycle, habit reversal training to reduce scratching behaviour, and stress management techniques.(Evers *et al.*, 2005) A multidisciplinary approach involving dermatologists, psychologists, and other healthcare providers is often most effective in addressing the complex interplay between stress and itch.

Advanced Research Techniques

Animal models have been instrumental in advancing our understanding of neurobiological mechanisms underlying the stress-itch relationship. Various stress paradigms, including restraint stress, social defeat stress, and chronic unpredictable mild stress, have been combined with models of acute and chronic itch to investigate their interaction.

Genetic approaches provide powerful tools for investigating molecular mechanisms underlying the stress-itch relationship. The development of the TRAP2 (Targeted Recombination in Active Populations 2) system represents a significant advance, allowing permanent labelling and subsequent manipulation of neurons activated during specific time windows, enabling researchers to identify and study neurons activated by specific stimuli such as stress.

Fiber photometry allows real-time recording of neural activity in freely behaving animals, complemented by viral tracing methods that reveal anatomical connections between neural populations. Optogenetic and chemogenetic techniques further enhance our ability to manipulate specific neural circuits with temporal precision, establishing causal relationships between neural activity patterns and behavioural outcomes.

Future Research Directions

Future research should focus on further elucidating the specific neural circuits and molecular pathways mediating stress-induced modulation of itch. Particular attention should be paid to plastic changes in neural circuits during chronic stress and chronic itch conditions, which

may explain the transition to chronic stress-induced itch exacerbation from acute stress-induced itch suppression.

New insights into the role of the Re → LS → LH pathway in modulating anxiety resulting from chronic itching offer promising prospects for focused therapies. Future studies should investigate whether modulating this pathway can alleviate both itch and anxiety symptoms in clinical populations, potentially breaking the vicious cycle that characterizes many chronic pruritic conditions.

The potential for targeting specific components of the stress-itch interface for therapeutic intervention represents an exciting frontier. Pharmacological agents that selectively modulate specific receptors or signalling pathways involved in stress-induced itch may offer more precise control with fewer side effects than current broad-spectrum approaches.

Finally, translational research bridging the gap between preclinical models and clinical applications will be essential for developing effective interventions for stress-exacerbated pruritic conditions. This will require collaborative efforts between neuroscientists, immunologists, dermatologists, and psychologists to address the multifaceted nature of the stress-itch relationship.

Research Objectives and Hypothesis

Based on previous findings suggesting lateral hypothalamic involvement in stress modulation of itch, this thesis investigates the neural circuit mechanisms underlying this relationship. Specifically, we hypothesize that lateral hypothalamus neurons activated by restraint stress (LH^{Stress} neurons) play a critical role in modulating itch perception through connections with the periaqueductal gray and other brain regions involved in stress processing and sensory modulation.

Using TRAP2 transgenic mice to capture neurons activated by restraint stress, we aim to characterize the anatomical connections, physiological properties, and behavioural significance of these LH^{Stress} neurons in the context of both acute and chronic itch conditions. Through a combination of behavioural assays, in vivo calcium imaging, and circuit-specific manipulations, we seek to elucidate the mechanisms by which stress influences itch perception, with potential implications for treating stress-exacerbated pruritic disorders.

Chapter 2 **Materials and Methods**

Experimental Animals

Animal Housing and Care

All experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC) of the Indian Institute of Science (IISc), Bengaluru, and conducted in accordance with institutional guidelines for animal experimentation. Adult male TRAP2 (Targeted Recombination in Active Populations 2) mice, expressing the tamoxifen-inducible Cre recombinase under the control of the activity-dependent Fos promoter (Fos2A-iCreERT2) were used for all experiments. Animals were housed in groups of 3-5 per cage in climate-controlled rooms (23 ± 1°C, 55 ± 5% humidity) under a 12-hour light/dark cycle (lights on at 7:00 AM). Standard laboratory chow and water were provided ad libitum. All behavioural tests were conducted during the light phase of the cycle.

Genotyping of Fos2A-iCreERT2 Mice

Genomic DNA was extracted from ear clippings (2-3 mm) obtained from 21-day-old mice. Tissue samples were digested for an hour at 95°C in lysis buffer containing 50 mM NaOH and 5 mM EDTA followed by Tris HCL and dd H2O. Following digestion, samples were centrifuged at 13,000 rpm for 1 minute, and kept in 4ºC. DNA precipitated in the meantime.

PCR amplification was performed using the following primers: 5'-AAGACCTCTAGGCTCCGAGACT-3' (forward) and 5'-GGCAGTTCTTGCGACCTTCT-3' (reverse). The PCR cycling conditions consisted of an initial denaturation at 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 58°C for 30 seconds, and extension at 72°C for 45 seconds, with a final extension at 72°C for 5 minutes. The amplified products were separated on a 2% agarose gel containing ethidium bromide and visualized under UV illumination. The presence of a 500 bp band confirmed the Fos2A-iCreERT2 genotype.

Genetic Labelling of Activated Neurons

TRAPing Procedure for Restraint Stress Activated Neurons

For capturing neurons activated by restraint stress (LH^{Stress} neurons), we employed the TRAP2 system, which allows for permanent genetic access to neurons active during a specific time window. Eight-week-old male Fos2A-iCreERT2 mice were individually placed in ventilated 50 ml conical tubes (with a 0.5 cm diameter hole at the bottom for tail protrusion and several 0.25 cm holes near the top for ventilation) for 60 minutes to induce restraint stress. Control mice were left undisturbed in their home cages.

Immediately preceding the restraint session, both experimental and control mice received a single intraperitoneal injection of 4-hydroxytamoxifen (4-OHT, 50 mg/kg dissolved in corn oil containing 10% ethanol) to induce Cre-mediated recombination specifically in neurons activated during the stress experience. Following 4-OHT administration, mice were returned to their home cages and allowed to recover for at least 5 days before any subsequent experimental procedures to ensure complete clearance of 4-OHT and adequate expression of Cre-dependent viral constructs or reporter genes.

Itch Models

Acute Itch Induction

For acute itch experiments, mice were habituated to the testing environment (clear acrylic testing chambers, 10 × 10 × 10 cm) for 15 minutes prior to pruritogen administration. Acute itch was induced by subcutaneous injection of chloroquine (200 μg in 50 μl PBS) into the nape of the neck using a 30-gauge needle. Following injection, mice were immediately placed back into the testing chamber, and scratching behaviour was video-recorded for 30 minutes. Scratching bouts were defined as lifting the hind paw to the injection site and rapidly moving it back and forth. A bout ended when the animal either lowered its paw to the floor or began a grooming sequence. The total number of scratching bouts was counted by an experimenter blinded to the treatment conditions.

Chronic Itch Model Through Imiquimod-Induced Psoriasis-Like Inflammation

For the chronic itch model, we employed a well-established protocol using topical application of imiquimod to induce psoriasis-like skin inflammation. The fur on the nape of the neck (approximately 2 × 3 cm area) was shaved two days prior to treatment initiation. A daily dose of 62.5 mg of commercially available 5% imiquimod cream (equivalent to 3.125 mg of the active compound) was applied to the shaved area for 7 consecutive days.

Skin inflammation was assessed daily by measuring erythema, scaling, and thickening. Spontaneous scratching behaviour was observed on days 0, 3, and 7 of treatment. On day 8,9,10 mice were used for either behavioural testing or tissue collection for histological and molecular analyses.

Behavioural Assessment

Open Field Test

The open field test was conducted to assess anxiety-like behaviour and general locomotor activity. Mice were placed individually in the centre of a square open field arena (50 × 50 × 40 cm) with white floors and walls, and allowed to explore freely for 10 minutes. All sessions were conducted under consistent lighting conditions (150 lux). Animal movement was

recorded using a ceiling-mounted camera and analysed with DeepLabCut tracking software (Tübingen, Germany).

Parameters quantified included: total distance travelled (cm), average velocity (cm/s), time spent in the centre zone (defined as the central 25 × 25 cm area), number of entries into the centre zone, and percentage of time spent in the centre relative to the periphery. Between trials, the apparatus was thoroughly cleaned with 0.1% acetone to eliminate olfactory cues.

Light-Dark Box Test

The light-dark box test was utilized to further evaluate anxiety-like behaviour. The apparatus consisted of a square box (20 × 20 × 45 cm) divided into two equal compartments: a brightly illuminated chamber (400 lux) with white walls and floor, and a dark chamber (10 lux) with black walls and floor. The compartments were connected by a small opening (7 × 7 cm) allowing mice to move freely between chambers.

Mice were placed in the centre of the light compartment facing away from the dark compartment and allowed to explore both chambers for 10 minutes. The following parameters were recorded: latency to first entry into the dark compartment, number of transitions between compartments, and time spent in each compartment. The apparatus was cleaned with 0.1% acetone between trials.

Conditioned Place Preference Test

A biased conditioned place preference (CPP) paradigm was employed to assess the rewarding or aversive properties of chemogenetic stress. The CPP apparatus consisted of a rectangular chamber (20 × 20 × 20 cm) divided into two distinct compartments of equal size by a removable partition. One compartment had striped walls with a striped floor, while the other had black walls with a cross textured floor.

The protocol consisted of three phases: (1) Pre-conditioning phase (day 1): mice were allowed to freely explore both compartments for 15 minutes to determine baseline compartment preference; (2) Conditioning phase (days 2-4): mice received injections (DCZ, 50 µL in 950 µl saline) and were immediately confined to their non-preferred compartment for 30 minutes in the morning session, while receiving vehicle injections (50 µl PBS) and confined to their preferred compartment for 30 minutes in the afternoon session; (3) Post-conditioning phase (day 5): mice were allowed to freely explore both compartments for 15 minutes with the partition removed.

CPP score was calculated as the difference in time spent in the drug-paired compartment during the post-conditioning phase versus the pre-conditioning phase. All sessions were video-recorded and analysed using DeepLabCut software.

Surgical Procedures and Viral Injections

Stereotaxic Surgery

For targeted manipulation of LH^{Stress} neurons, mice underwent stereotaxic surgery for viral injections 5-7 days after 4-OHT administration. Animals were anesthetized with isoflurane (4% for induction, 1.5-2% for maintenance) delivered in oxygen (1 L/min) and positioned in a stereotaxic frame (RWD Instruments, China). Body temperature was maintained at 37°C using a heating pad. After confirming adequate anaesthesia by absence of toe-pinch reflex, the scalp was shaved, sterilized with saline and H2O2, and a midline incision was made to expose the skull.

Bregma and lambda were aligned in the same horizontal plane, and small craniotomies were performed at the appropriate coordinates for the lateral hypothalamus (LH): AP -1.5 mm, ML ±1.0 mm, DV -5.2 mm from bregma. For viral injections targeting the LH, a Hamilton syringe with a 33-gauge needle was lowered to the target coordinates, and the virus was infused at a rate of 100 nl/min using a microinjection pump. Following injection, the needle was left in place for an additional 10 minutes to allow for virus diffusion before being slowly withdrawn.

For bilateral injections into the lateral/ventrolateral periaqueductal gray (l/vlPAG) for terminal manipulation experiments, the following coordinates were used: AP -4.5 mm, ML ±0.6 mm, DV -2.8 mm from bregma.

Viral Constructs

Purpose	Viral Construct	Titer (vg/ml)	Injection Volume (nl per site)
Chemogenetic Activation	AAV-DJ-EF1α-DIO-hM3Dq-mCherry	5×10^{12}	300
Chemogenetic Inhibition	AAV-DJ-EF1α-DIO-hM4Di-mCherry	5×10^{12}	300
Neuronal Silencing	AAV-DJ-EF1α-DIO-Kir2-eGFP	3×10^{12}	300
Optogenetic Inhibition	AAV-DJ-EF1α-DIO-eNpHR3.0-eYFP	3×10^{12}	300
Control Experiments	AAV-DJ-EF1α-DIO-mCherry / AAV-DJ-EF1α-DIO-eGFP	3×10^{12}	300
Calcium Imaging	AAV-DJ-EF1α-DIO-GCaMP8s	2×10^{12}	300

Table 1. List of Viral Constructs used

Post-operative Care

Following surgery, the incision was closed with surgical staples. Animals were placed on a heating pad until full recovery from anaesthesia and then returned to their home cages. Mice were monitored daily for signs of distress, weight loss, or infection and allowed to recover for at least 3 weeks before behavioural testing to ensure adequate viral expression.

Chemogenetic and Optogenetic Manipulations

Chemogenetic Activation and Inhibition

For chemogenetic manipulation experiments, mice expressing hM3Dq (excitatory DREADD) or hM4Di (inhibitory DREADD) in LH^{Stress} neurons received intraperitoneal injections of the DREADD agonist deschloroclozapine (100 µl of DCZ 50 µl in 950 µl saline) or vehicle (saline) 15 minutes prior to behavioural testing. For control experiments, mice expressing fluorescent reporter proteins (mCherry or eGFP) underwent identical procedures.

For site-specific chemogenetic activation of LH^{Stress} terminals in the l/vlPAG, mice received bilateral infusions of DCZ (100 µl of DCZ 50 µl in 950 µl saline) or vehicle directly into the l/vlPAG through previously implanted guide cannulae 15 minutes before testing.

Optogenetic Inhibition

For optogenetic inhibition of LH^{Stress} terminals in the l/vlPAG, mice expressing eNpHR3.0 in LH^{Stress} neurons were implanted with bilateral optical fibres (200 µm diameter, 0.39 NA) targeted to the l/vlPAG (AP −4.5 mm, ML ±0.6 mm, DV −2.3 mm from bregma). Following recovery, optical inhibition was achieved using a 593 nm yellow laser delivering continuous light (8-10 mW output power at fibre tip) attached to lightweight fibre optic cables. Light stimulation was delivered for 5-minute epochs during behavioural testing, alternating with 5-minute epochs without stimulation.

Statistical Analysis

All data were analysed using GraphPad Prism 9 software (GraphPad Software, USA). Results are presented as mean ± SEM. Statistical significance was determined using appropriate tests based on experimental design and data distribution. For two-group comparisons, two-tailed Student's t-tests were employed. For multiple group comparisons, one-way or two-way analysis of variance (ANOVA) was used. For repeated measures data, repeated measures ANOVA was applied. Statistical significance was set at $p < 0.05$. Sample sizes were determined based on previous studies with similar experimental approaches to ensure adequate statistical power.

Chapter 3 **Results and Discussion**

Results

Characterizing the Role of Lateral Hypothalamus in Stress Modulation of Itch

Acute Restraint Stress Suppresses Itch in Mice

To investigate the relationship between stress and itch, we first examined whether acute stress affects itch perception in mice. Adult mice were subjected to acute restraint stress by confining them in ventilated restraint tubes for 1 hour, followed by assessment of scratching behaviour in both acute and chronic itch models.

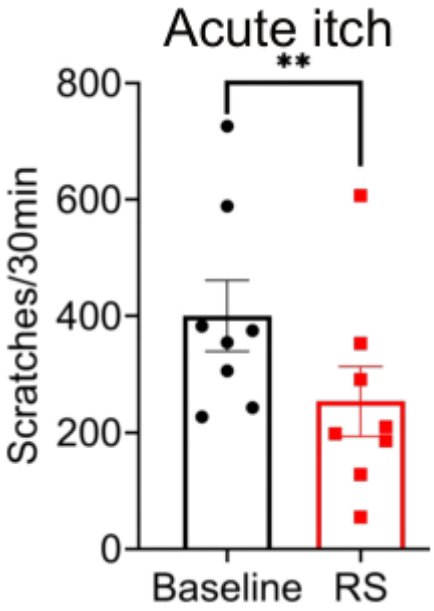

Figure 4 Effect of acute stress on chloroquine-induced scratching compared to baseline

In the acute itch model, subcutaneous injection of chloroquine (CQ) into the nape of the neck produced robust scratching behaviour in unstressed mice. However, mice that underwent acute restraint stress prior to CQ injection exhibited significantly fewer scratching bouts compared to unrestrained controls ($P = 0.0092$, $n = 8$; Figure 1). This suppression of scratching behaviour suggests that acute stress reduces the perception or expression of itch sensation.

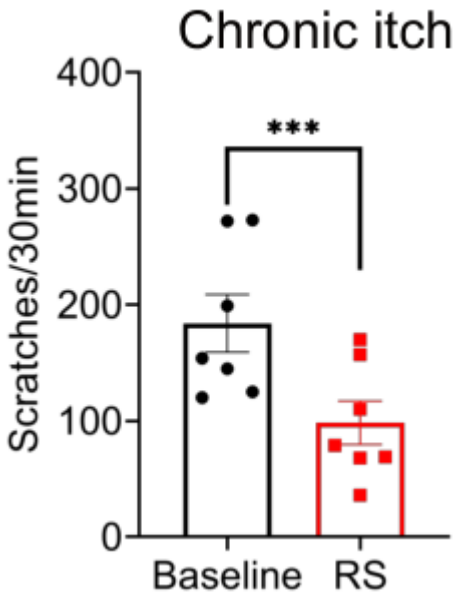

Figure 5 Effect of acute on imiquimod induced spontaneous scratching compared to baseline spontaneous scratching.

Similarly, we tested the effect of acute restraint stress on spontaneous scratching in a chronic itch model. Mice were treated with daily topical application of imiquimod (IMQ) cream to the nape of the neck for 7 days to induce psoriasis-like inflammation and chronic itch. When subjected to acute restraint stress on day 7, these mice also showed significant reduction in spontaneous scratching behaviour compared to their pre-stress baseline ($P = 0.0053$, $n = 7$; Figure 2). These results demonstrate that acute stress suppresses itch perception in both acute and chronic itch conditions.

Identification and Characterization of Lateral Hypothalamus Neurons Activated by Restraint Stress

To identify and manipulate neural populations involved in stress-induced itch modulation, we employed the TRAP2 (Targeted Recombination in Active Populations 2) system to capture neurons activated by restraint stress. Fos2A-iCreERT2 transgenic mice underwent 1-hour restraint stress followed by intraperitoneal injection of 4-hydroxytamoxifen (4-OHT) to induce Cre recombination specifically in activated neurons.

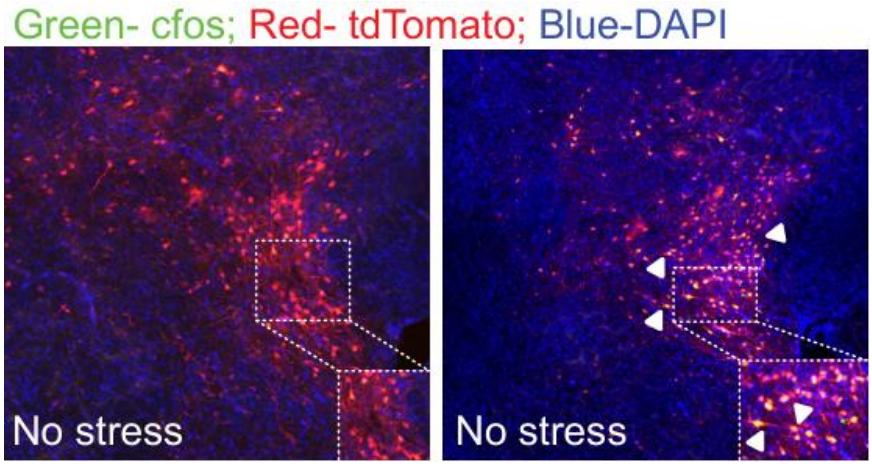

Figure 6 Colocalization of Fos and tdT-positive neurons was demonstrated by confocal images of coronal brain sections from infected mice following restraint stress or no stress and stained with Fos (green), tdT-positive neurones (red), and DAPI (blue).

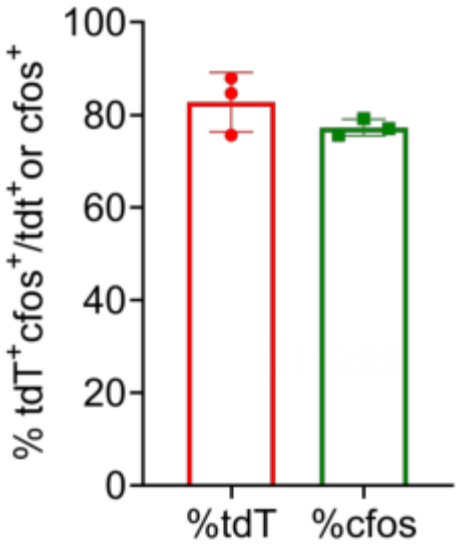

Figure 7 Colocalization analysis of the overlap between the Fos and tdT expressing neurons in LH

Immunohistochemical analysis of brain tissue revealed significant activation of neurons in the lateral hypothalamus (LH) during restraint stress, as indicated by Fos expression. When mice were subjected to a second restraint session 1 week later, we observed substantial overlap between neurons expressing the genetic tag (tdTomato) and those expressing Fos protein (Figure 3), confirming successful capture of a stress-responsive neuronal ensemble in the LH, which we termed LH^{Stress} neurons.

LH^{Stress} Neurons Exhibit Anxiogenic Properties

To examine the behavioural functions of LH^{Stress} neurons, we injected a Cre-dependent adeno-associated virus (AAV) expressing the excitatory designer receptor exclusively activated by designer drugs (DREADD) hM3Dq-mCherry into the LH of Fos2A-iCreERT2 mice. Following restraint stress and 4-OHT administration, this approach allowed selective chemogenetic activation of LH^{Stress} neurons.

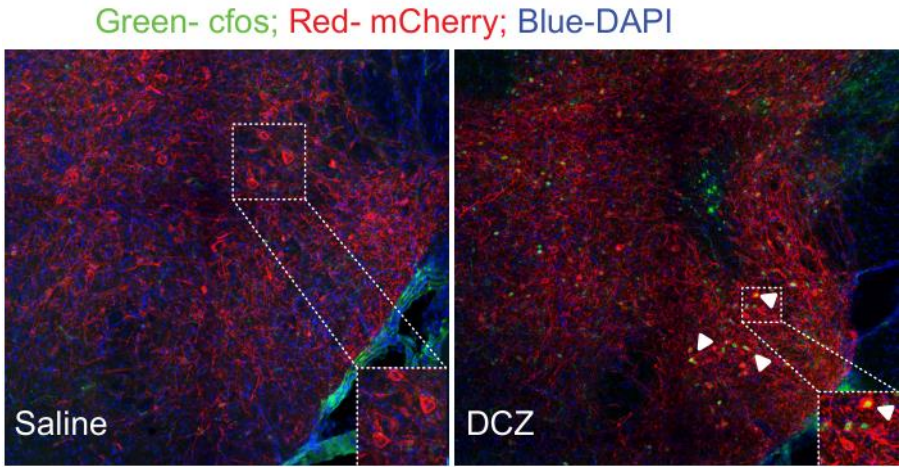

Figure 8 Following saline/DCZ injection, confocal pictures of coronal brain slices from infected mice labelled with DAPI (blue) showed extensive induction of Fos (green) and mCherry-positive neurones (red).

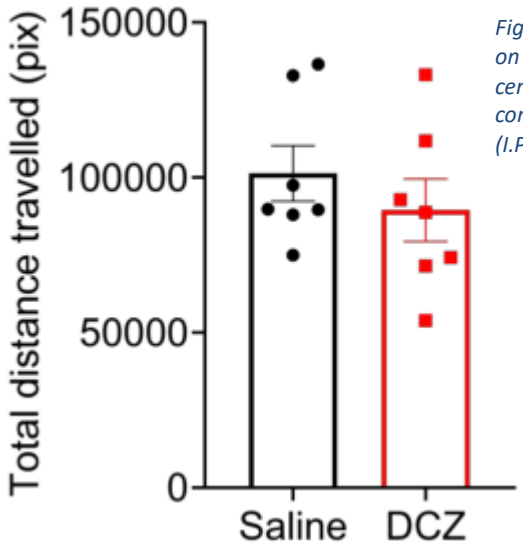

Figure 9 Effect of DCZ on time spent in central square as compared to saline (I.P.)

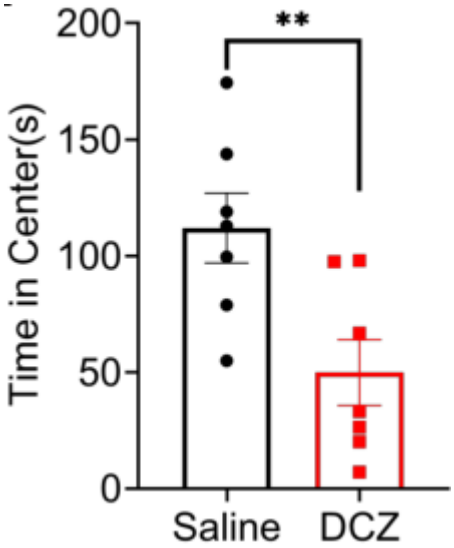

Figure 10 Effect of DCZ on time spent in centre in OFT as compared to Saline (I.P.)

Activation of LH^{Stress} neurons by intraperitoneal injection of deschloroclozapine (DCZ, 0.1 mg/kg) induced significant Fos expression throughout the LH (Figure 5), confirming effective recruitment of the target neuronal population. Behavioural assessment revealed that DCZ administration significantly reduced time spent in the centre of an open field arena compared to saline controls (P = 0.0011, n = 7; Figure 7), without affecting total distance travelled (P = 0.3318, n = 7; Figure 6). Similarly, in the light-dark box test, DCZ-treated mice spent significantly less time in the light chamber compared to saline-treated controls (P = 0.0001, n = 7; Figure 8).

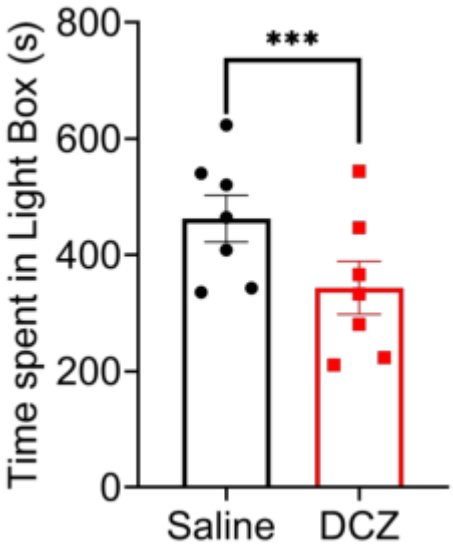

Figure 11 Effect of DCZ on time spent in light chamber as compared to saline (I.P.)

These results indicate that selective activation of LH^{Stress} neurons induces anxiety-like behaviour in mice, suggesting these neurons encode negative emotional states associated with stress exposure.

LH^{Stress} Neurons Bidirectionally Modulate Itch Perception

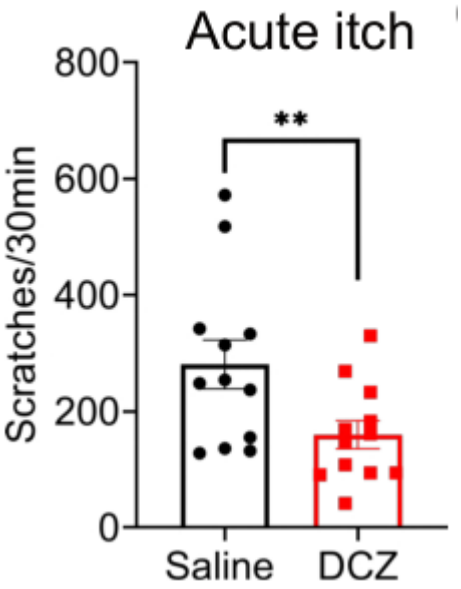

Figure 13 Effect of DCZ on CQ induced scratching as compared to saline (I.P.)

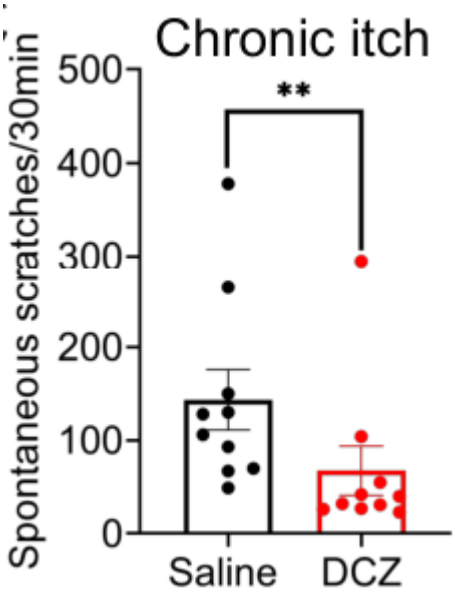

Figure 12 Effect of DCZ on IMQ induced spontaneous scratching as compared to saline (I.P.)

Having established that LH^{Stress} neurons mediate anxiety-like behaviour, we next investigated their role in itch perception. Chemogenetic activation of LH^{Stress} neurons by DCZ administration significantly reduced scratching responses to acute CQ-induced itch compared to saline controls (P = 0.0035, n = 12; Figure 10). Similarly, activation of these neurons

attenuated spontaneous scratching in the IMQ-induced chronic itch model (P = 0.0042, n = 10; Figure 9).

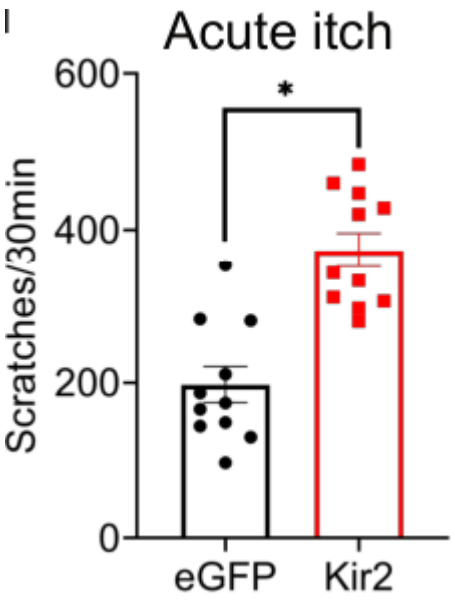

Figure 14 Effect of silencing LHstress neurons using Kir2.1 channels on CQ induced scratching as compared with eGFP

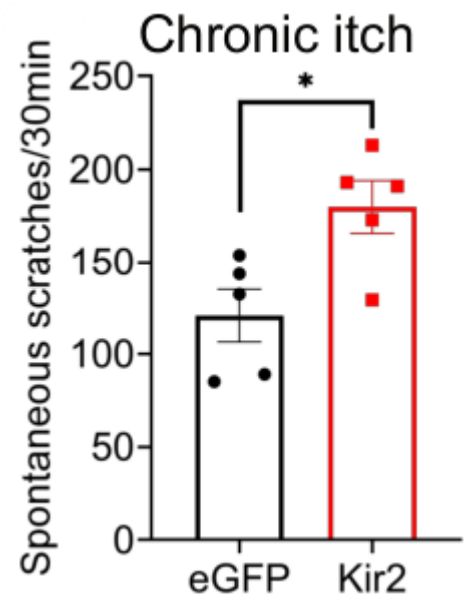

Figure 15 Effect of silencing LHstress neurons using Kir2.1 channels on IMQ induced spontaneous scratching as compared with eGFP.

To examine the necessity of LH^{Stress} neurons in itch processing, we silenced these neurons by expressing the inward-rectifying potassium channel Kir2.1. Mice expressing Kir2.1 in LH^{Stress} neurons exhibited significantly increased scratching behaviour in response to CQ injection compared to control mice expressing eGFP (P = 0.0001, n = 11; Figure 12). Likewise, silencing LH^{Stress} neurons enhanced spontaneous scratching in the IMQ-induced chronic itch model (P = 0.0184, n = 5; Figure 11).

These bidirectional manipulations demonstrate that LH^{Stress} neurons exert an inhibitory influence on itch perception, with activation suppressing and inhibition enhancing scratching behaviour in both acute and chronic itch conditions.

LH^{Stress} Neurons Are Required for Stress-Induced Suppression of Itch

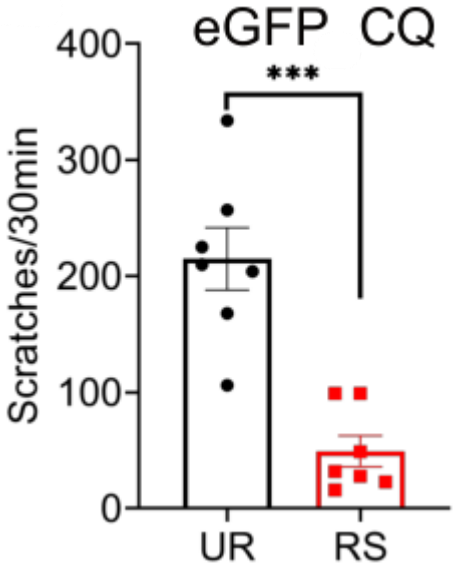

Figure 17 Effect of Restrain Stress (RS) on CQ induced itch as compared to unrestrained (UR).

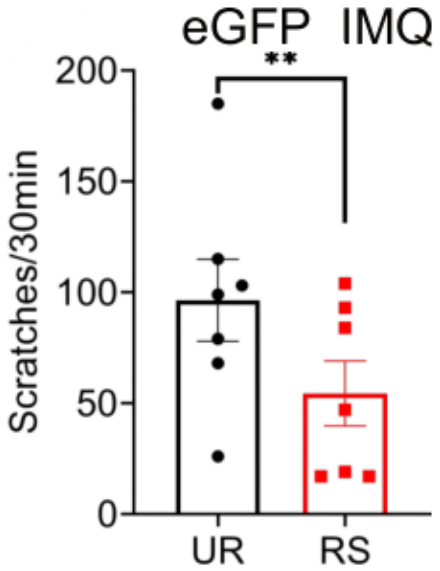

Figure 16 Effect of Restrain Stress (RS) on IMQ induced chronic itch as compared to unrestrained (UR).

To determine whether LH^{Stress} neurons are necessary for stress-induced suppression of itch, we silenced these neurons using Kir2.1 and assessed the effect of acute restraint stress on itch behaviour. In control mice expressing eGFP, acute restraint stress significantly reduced CQ-induced scratching (P = 0.0001, n = 7; Figure 13) and IMQ-induced spontaneous scratching (P = 0.0053, n = 7; Figure 14), consistent with our initial observations.

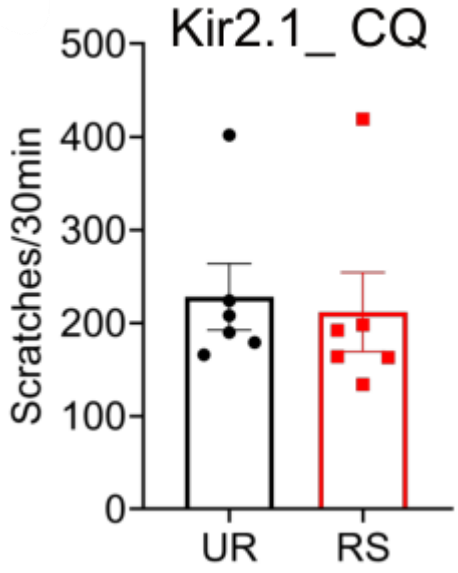

Figure 18 Effect of silencing LHstress neurons on restrain stress induced suppression of CQ induced acute itch as compared to unrestrained

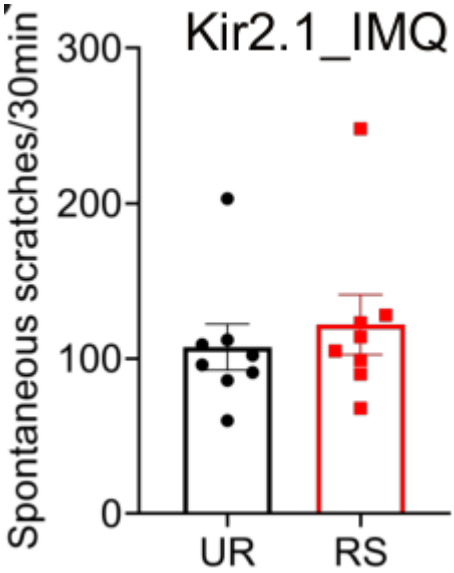

Figure 19 Effect of silencing LHstress neurons on restrain stress induced suppression of IMQ induced chronic itch as compared to unrestrained

Strikingly, silencing LH^{Stress} neurons with Kir2.1 abolished the suppressive effect of restraint stress on scratching behaviour in both acute and chronic itch models (Figure 15 & 16). These results demonstrate that LH^{Stress} neurons are necessary for stress-induced suppression of itch, functioning as a key neural substrate for stress-itch interactions.

LH^{Stress} to vlPAG Projections Mediate Itch Suppression

Based on the dense projections from LH^{Stress} neurons to the l/vlPAG, we investigated whether this pathway mediates the anti-pruritic effects of these neurons. We injected Cre-dependent hM3Dq-mCherry into the LH of Fos2A-iCreERT2 mice and implanted guide cannulae targeting the l/vlPAG for localized drug delivery.

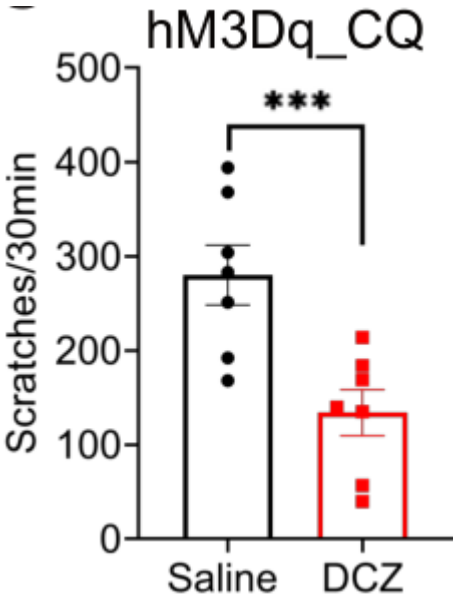

Figure 20 Impact of DCZ infusion on acute scratching caused by CQ in contrast to saline infusion

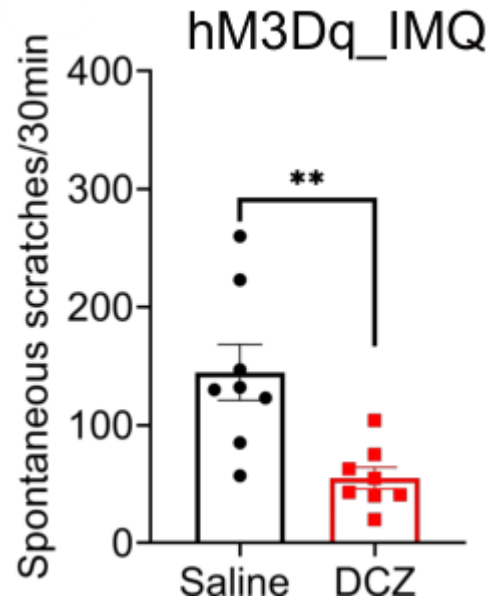

Figure 21 Impact of DCZ infusion on chronic scratching caused by IMQ in contrast to saline infusion

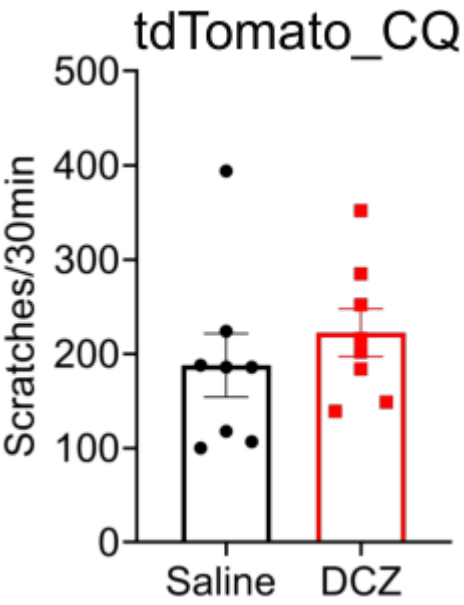

Figure 23 DCZ or saline infusion had no effect on CQ induced scratching in tdTomato infected mice (Control)

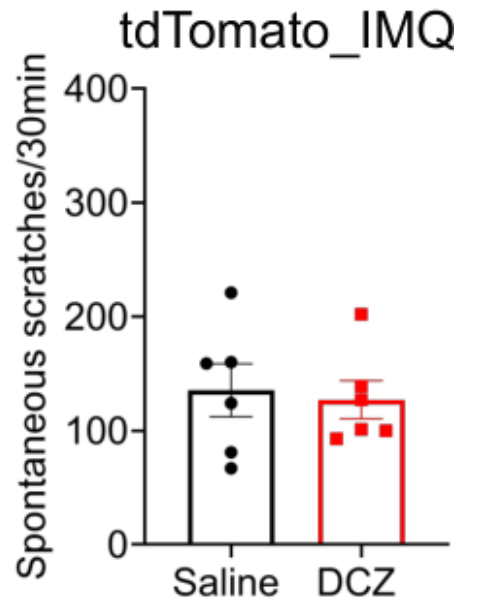

Figure 22 DCZ or saline infusion had no effect on IMQ induced scratching in tdTomato infected mice

Remarkably, selective activation of LH^{Stress} neuron terminals in the l/vlPAG through local DCZ infusion significantly reduced scratching responses in both CQ-induced acute itch (P = 0.0001, n = 7; Figure 17) and IMQ-induced chronic itch models (P = 0.0012, n = 8; Figure 18). Control mice expressing tdTomato showed no changes in scratching behaviour following DCZ administration (Figure 19 & 20), confirming the specificity of this effect.

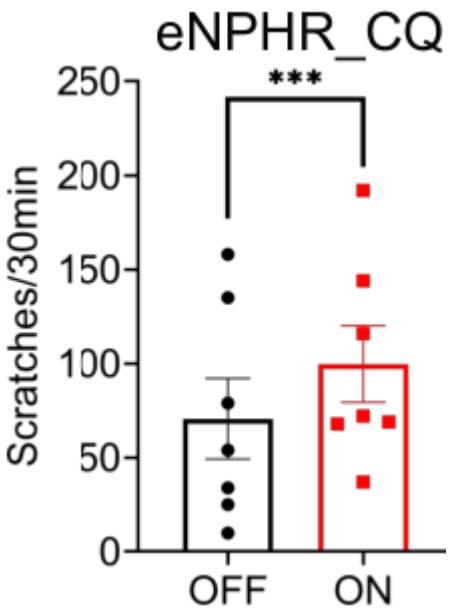

Figure 25 Optogenetic inhibition (laser ON) exacerbates CQ induced scratching as compared to laser OFF

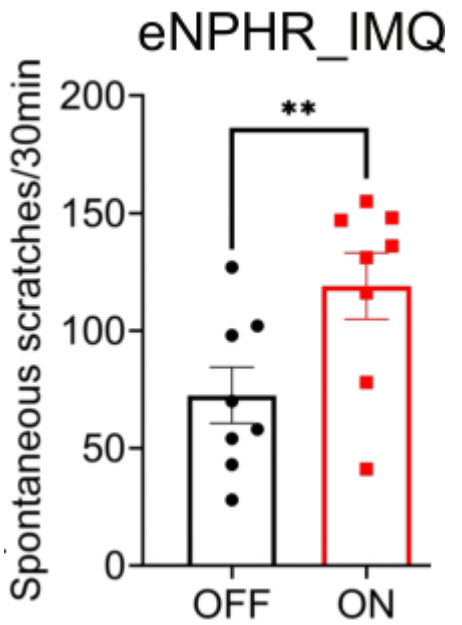

Figure 24 Optogenetic inhibition (laser ON) exacerbates IMQ induced scratching as compared to laser OFF

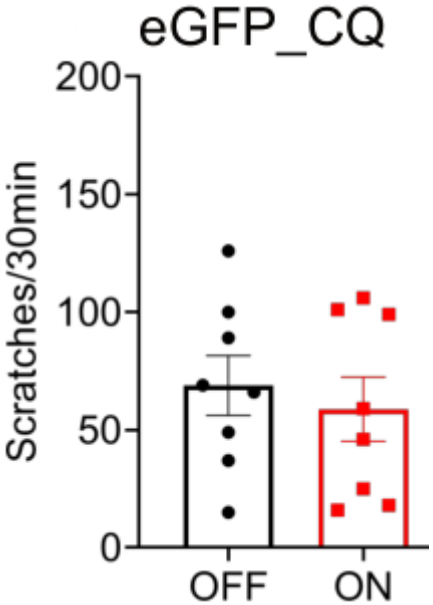

Figure 27 No Difference was observed in CQ induced acute scratching in tdTomato infected mice when compared between Laser ON and OFF

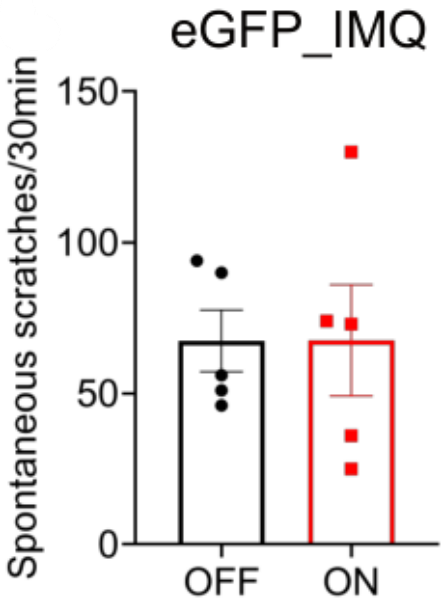

Figure 26 No Difference was observed in IMQ induced chronic scratching in tdTomato infected mice when compared between Laser ON and OFF

To further validate the necessity of this pathway, we expressed the inhibitory opsin eNpHR3.0 in LH^{Stress} neurons and implanted optical fibres targeting the l/vlPAG. Optogenetic inhibition of LH^{Stress} terminals in the l/vlPAG significantly increased scratching behaviour in both CQ-induced (P = 0.0009, n = 7; Figure 22) and IMQ-induced models (P = 0.0024, n = 8; Figure 21), while light delivery had no effect in control mice expressing eGFP (Figure 23 & 24).

These findings demonstrate that the LH^{Stress} →l/vlPAG pathway is both necessary and sufficient for the suppression of itch by LH^{Stress} neurons, identifying a key circuit mechanism for stress-induced itch modulation.

LH^{Stress} Neurons Exhibit Plastic Changes in Response to Chronic Itch

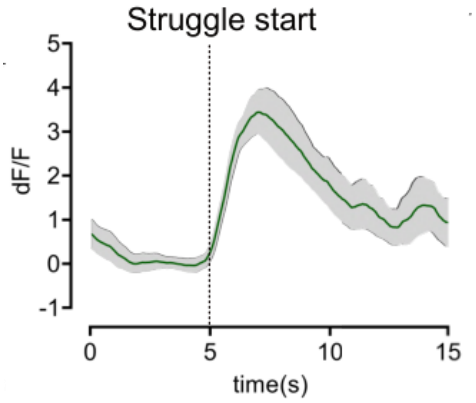

Figure 28 Increase in neural activity observed by higher calcium fluorescence in LHstress neurons when mice start to struggle under restrain stress

To investigate the dynamic activity patterns of LH^{Stress} neurons during itch processing, we performed fibre photometry recordings using the calcium indicator GCaMP8s. As expected, LH^{Stress} neurons showed robust calcium signals during restraint stress (Figure 28), confirming their stress responsivity.

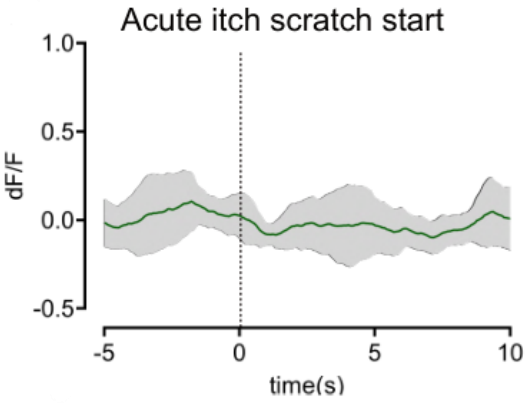

Figure 30 Neural activity of LHstress neurons during acute itch

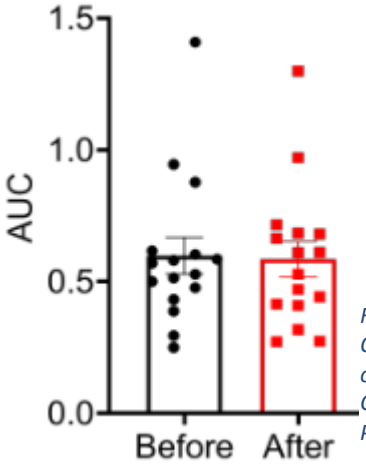

Figure 29 Quantification of Area under Curve for Photometry

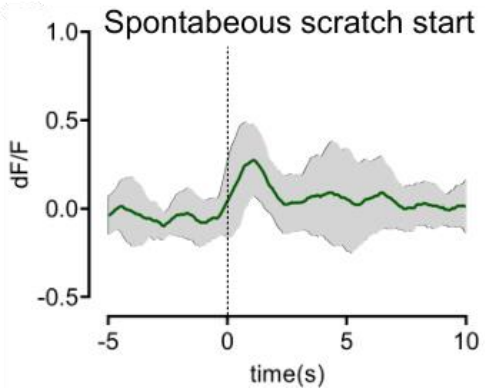

Figure 32 Neural activity of LHstress neurons during IMQ chronic itch

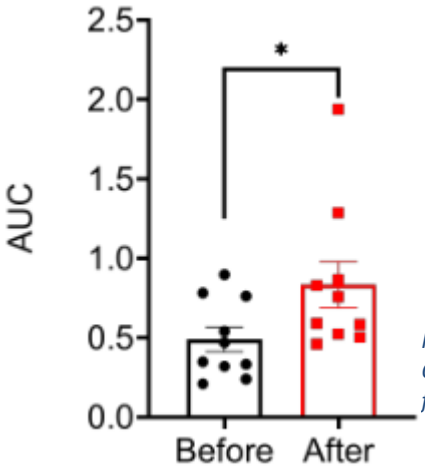

Figure 31 Quantification for fig.32

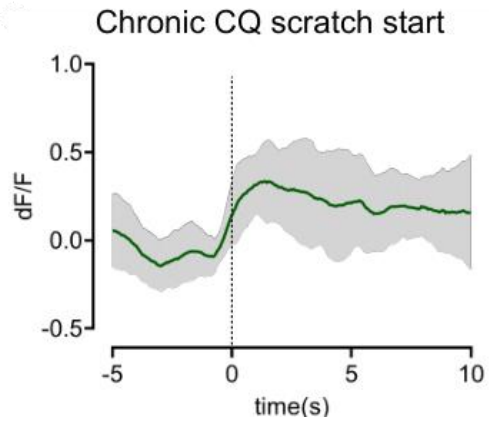

Figure 34 Neural activity of LHstress neurons during CQ evoked chronic itch

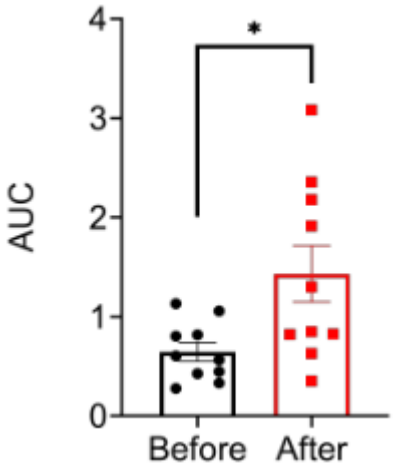

Figure 33 Quantification for fig.34

Interestingly, these neurons did not show significant changes in activity during acute CQ-induced scratching (Figure 29 & 31), suggesting they do not directly encode scratching behaviour in naive conditions. However, in mice with IMQ-induced chronic itch, LH^{Stress} neurons exhibited increased calcium activity during both spontaneous scratching episodes (Figure 30 & 32) and CQ-evoked scratching ($P = 0.0113$, $n = 10$; Figure 33 & 34).

This emergence of scratch-related activity in LH^{Stress} neurons specifically in the context of chronic itch suggests experience-dependent plasticity in these neurons, potentially reflecting adaptation to persistent pruritic stimuli

Discussion

Neural Circuits Underlying Stress Modulation of Itch

Our study identifies a specific population of lateral hypothalamus neurons activated by restraint stress (LH^{Stress} neurons) that play a crucial role in mediating stress-induced suppression of itch. Using the TRAP2 system, we selectively captured and manipulated these

neurons, revealing their causal role in the bidirectional modulation of both acute and chronic itch. These findings provide important insights into the neural mechanisms underlying the complex relationship between stress and somatosensory processing.

The lateral hypothalamus has long been recognized as a critical integrator of diverse physiological and behavioural functions, including feeding, arousal, reward, and stress responses. Our findings extend the functional repertoire of this brain region to include modulation of pruriceptive signalling. The identification of a discrete, genetically accessible ensemble of stress-responsive neurons within this structure offers a valuable entry point for dissecting the neural circuitry underlying stress-itch interactions.

Our anatomical tracing experiments revealed that LH^{Stress} neurons are embedded within an extended brain network involving regions associated with both stress processing and sensory modulation. The diverse inputs to these neurons suggest they integrate information from multiple sources to coordinate appropriate physiological and behavioural responses to stressors. The output projections of LH^{Stress} neurons to regions like the PAG, dorsal raphe, and locus coeruleus position them to influence descending modulatory pathways that regulate sensory processing at multiple levels of the neuraxis.

The LH^{Stress}→PAG Circuit: A Critical Pathway for Itch Suppression

A major finding of our study is the identification of the LH^{Stress}→l/vlPAG pathway as a key circuit mechanism for stress-induced itch suppression. The PAG has been extensively studied for its role in pain modulation, but its involvement in itch processing has received comparatively less attention. Our results demonstrate that this pathway is both necessary and sufficient for the anti-pruritic effects of LH^{Stress} neurons, as evidenced by the bidirectional modulation of scratching behaviour through chemogenetic and optogenetic manipulations of LH^{Stress} terminals in the l/vlPAG.

The l/vlPAG is known to participate in descending control of spinal cord circuits via projections to the rostral ventromedial medulla, which in turn modulates dorsal horn neurons involved in itch transmission. Our findings suggest that stress activates LH^{Stress} neurons, which then engage this descending inhibitory pathway to suppress itch perception at the spinal level. This mechanism is conceptually similar to stress-induced analgesia, wherein stress activates endogenous pain inhibitory systems to suppress pain perception.

Interestingly, our results also demonstrate that LH^{Stress} neurons exhibit anxiogenic properties, as their activation induced anxiety-like behaviour in the open field and light-dark box tests. This dual role in promoting anxiety while suppressing itch suggests these neurons may coordinate multiple aspects of the stress response, potentially facilitating adaptive behavioural states during threatening situations by simultaneously enhancing vigilance and suppressing distracting sensory stimuli like itch.

Plastic Changes in LH^{Stress} Neurons During Chronic Itch

Perhaps one of the most intriguing findings of our study is the emergence of scratch-related activity in LH^{Stress} neurons specifically in the context of chronic, but not acute, itch. While these neurons did not respond to scratching behaviours in naive mice, they showed significant calcium responses during both spontaneous and evoked scratching in mice with IMQ-induced chronic itch. This functional adaptation was accompanied by increased intrinsic excitability, as revealed by ex vivo electrophysiological recordings.

These plastic changes suggest that persistent pruritic stimulation induces functional remodelling of stress-responsive neural circuits. Such plasticity may represent a compensatory mechanism to counteract the enhanced itch signalling associated with chronic pruritic conditions. Alternatively, it could reflect pathological rewiring that contributes to the comorbidity between chronic itch and stress-related disorders commonly observed in clinical populations.

The bidirectional relationship between stress and chronic itch is well-documented in clinical settings, with patients reporting stress as a trigger for itch flares and chronic itch contributing to psychological distress. Our findings provide a potential neural substrate for this vicious cycle, wherein chronic itch induces plasticity in stress-responsive hypothalamic circuits, potentially altering their responsivity to subsequent stressors and modulating their influence on itch perception.

Implications for Understanding Stress-Itch Interactions in Clinical Conditions

Our results have significant implications for understanding the neurobiological basis of stress-itch interactions in clinical conditions. Pruritic skin disorders such as atopic dermatitis and psoriasis are frequently exacerbated by stress, yet the mechanisms underlying this relationship have remained poorly understood. By identifying specific neural circuits and demonstrating their causal role in stress-induced itch modulation, our study provides a framework for developing targeted interventions for stress-exacerbated pruritic disorders.

The finding that LH^{Stress} neurons exert anti-pruritic effects may seem counterintuitive given the clinical observation that stress often worsens itch symptoms. However, this apparent discrepancy can be reconciled by considering the distinction between acute and chronic stress. While our study primarily examined acute restraint stress, which activated LH^{Stress} neurons and suppressed itch, chronic stress may have different or even opposite effects on these circuits. Chronic stress could potentially lead to dysregulation or desensitization of this pathway, compromising its anti-pruritic function and contributing to stress-induced itch exacerbation in clinical settings.

Additionally, our observation of plastic changes in LH^{Stress} neurons during chronic itch suggests that the relationship between stress and itch circuits is not static but evolves with disease progression. This dynamic interaction may explain why stress management interventions show variable efficacy in different patient populations and at different stages of disease.

Limitations and Future Directions

While our study provides important insights into the neural mechanisms of stress-itch interactions, several limitations and open questions remain. First, we focused primarily on restraint stress, which represents just one form of stressor. Different types of stressors (physical, psychological, social) may engage distinct neural circuits and have differential effects on itch perception. Future studies should explore how diverse stressors modulate itch and whether they converge on common neural pathways.

Second, our investigation centred on the lateral hypothalamus, but stress is known to activate multiple brain regions. Additional stress-responsive areas, such as the amygdala, bed nucleus of the stria terminalis, and prefrontal cortex, likely contribute to stress-induced itch modulation through parallel or interacting pathways. A comprehensive mapping of stress-itch circuits across the brain would provide a more complete understanding of this complex relationship.

Third, while we demonstrated plastic changes in LH^{Stress} neurons during chronic itch, the molecular mechanisms underlying this plasticity remain unexplored. Identifying the signalling pathways and transcriptional programs that drive these adaptive changes could reveal potential therapeutic targets for breaking the stress-itch cycle in chronic conditions.

Conclusion

In conclusion, our study reveals a previously unrecognized role for lateral hypothalamus neurons in mediating stress-induced suppression of itch through projections to the periaqueductal gray. These findings provide a neural circuit mechanism for the bidirectional relationship between stress and itch, with important implications for understanding and treating stress-exacerbated pruritic disorders. The plastic changes observed in these circuits during chronic itch further highlight the dynamic nature of stress-itch interactions and suggest potential avenues for therapeutic intervention. By elucidating the specific neural substrates underlying stress modulation of itch, this work contributes to a growing appreciation of the complex interactions between emotional states and somatosensory processing in both health and disease.

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