The Thermal Grill Elicits Central Sensitization

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Short Title: TGI induces secondary hyperalgesia

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Abstract

The thermal grill, in which innocuous warm and cool stimuli are interlaced, can produce a paradoxical burning pain sensation—the thermal grill illusion (TGI). While the mechanisms underlying TGI remain unclear, prominent theories point to spinal dorsal horn integration of innocuous thermal inputs to elicit pain. It remains unknown whether the TGI activates peripheral nociceptors, or solely thermosensitive afferents and is integrated within the spinal cord. Different types of sensitization have established mechanisms and can inform TGI mechanisms: if the TGI elicits (1) primary hyperalgesia, peripheral nociceptors are activated; (2) secondary hyperalgesia in the absence of primary hyperalgesia, spinal integration is required; and (3) brush allodynia, wide-dynamic range neurons are involved in mediating the TGI. Here, we determine whether the TGI elicits primary hyperalgesia, secondary hyperalgesia or brush allodynia. Fifty-two participants underwent individually calibrated phasic thermal grill stimulation. We found that the TGI elicited primary hyperalgesia, but only in participants with component temperatures in the noxious range (<19°C and >41°C). The TGI also elicited secondary hyperalgesia, even in participants with strictly innocuous thermal inputs. No participants developed brush allodynia. We observed sex differences in primary hyperalgesia: only males exhibited thermal grill-induced primary hyperalgesia. These findings suggest that the TGI is integrated in the spinal dorsal horn, likely mediated by heat-pinch-cold (HPC) neurons, and, to some degree, by primary nociceptive afferents in males. This study shows that the TGI may have sex-dependent mechanisms and determines that HPC cells are involved in the illusory sensation of pain from innocuous thermal inputs.

Keywords

Pain, Altered States, Sensory Physiology, Somatosensory system, Primary afferents, paradoxical heat, illusion, thermal

Introduction

The thermal grill illusion (TGI) is a paradoxical sensory phenomenon causing a perception of pain and burning heat in response to alternating warm and cool innocuous stimuli [4,27,61,67,68]. A key feature of this illusion is the perception of synthetic heat, meaning the overall temperature perception is hotter than either stimulus (warm or cool) in isolation [27]. This experience of heat pain with the TGI has been frequently compared to the burning sensation commonly associated with cold pain [7]. The thermal grill elicits the illusion of heat in as few as 20-25% of individuals [58].

The mechanisms driving the illusory perception of pain in TGI remain a source of debate. There exist two prevailing theories on the mechanism behind the illusion: the addition theory and disinhibition theory [27,61]. Both theories posit that the illusion is driven by C-fiber activity, and central integration, but through different mechanisms. The addition theory posits the TGI is induced by the linear summation of convergent warm and cool input to second-order wide dynamic range (WDR) neurons in the dorsal horn of the spinal cord [35,36]. In contrast, the disinhibition theory posits that TGI is caused by the disinhibition of second order heat-pinch-cold (HPC) neurons. Normally, HPC neurons are inhibited by second-order fibres that encode cool stimuli (COOL). However, when a warm stimulus is introduced, the warm primary afferent inhibits the COOL fibres, thus disinhibiting HPC neurons. HPC fibres primarily receive input from C-polymodal nociceptors (CPNs) [33], which also encode cool stimuli below the thermoneutral zone (~24˚C) [10,11,19,21-23]. Therefore, the TGI is primarily thought to be caused by spinal or supraspinal interactions [12,21,23,28,29,31,35,36,50,53], but it remains unknown whether innocuous warm and cool stimulations activate nociceptive primary afferents [61].

To determine whether TGI activates primary nociceptors, or whether the integration is wholly in the CNS can be determined through the investigation of sensitized states. Specifically, primary hyperalgesia would indicate primary nociceptor activation; whereas secondary hyperalgesia, in the absence of primary hyperalgesia, would indicate central integration [41,70]. Furthermore, the second-order neuron encoding the TGI can be delineated through investigation of brush allodynia (also known as stroking hyperalgesia) which is mediated by WDR cells [16,56].

Therefore, the aims of this study are to determine whether the TG elicits primary, secondary and/or stroking hyperalgesia. Based on extant theories and evidence, we hypothesize that there will be secondary hyperalgesia, but not primary hyperalgesia. We are agnostic of whether stroking hyperalgesia can be elicited by the thermal grill, which would determine whether WDRs are involved. This study is the first to use altered states to disentangle the mechanisms of the TGI.

Methods

Participants

Sixty-seven participants between the ages of 18 and 45 completed the study approved by the University of Toronto Research Ethics Board (Protocol#: 45598). Exclusion criteria for the study were (1) current or history of chronic pain; (2) major neurological or psychiatric diseases; (3) vascular diseases; (4) skin diseases (i.e. psoriasis or open sores); (5) self-reported opioid use; (6) daily use of medication known to affect pain sensitivity (e.g., NSAIDS, anxiolytic, antispastic, acetaminophen); (7) a score ≥20 on the Beck's Depression Inventory II (BDI-II >20) [9]; (8) indication of suicidal ideation (>1 on item 9 of the BDI-II); (9) a score of <28 on Mini Mental State Exam (MMSE) [30]; and (10) pain intensity ratings <5/100 for the calibrated thermal grill stimulation (described in detail in the *Thermal Grill Calibration* section). Of the 67 participants, 15 were excluded due to being a TGI "non-responder" (TGI pain intensity <5/100; n=14) or scored >25 on the BDI-II (n=1). The final sample included in the study comprised 52 healthy participants (26 females, 26 males; mean age ± SD: 23.5 ± 4.8 years). This sample is larger than that required based on the effect size of a pilot study (n= 5, Cohen's d=1.444). Based on this effect size, we would need a minimum sample of 7 participants, or 14 participants to allow for adequately powered sex disaggregated analyses. We purposefully oversampled to ensure reliable results [39].

Experimental Design

Participants underwent a single behavioural session lasting one hour. After consenting to procedures, participants were first screened for depression with the BDI-II [9] and cognitive impairment with the MMSE [30], as per the exclusions above. Next, they completed questionnaires for measures known to be associated with pain sensitivity.

Beck's Depression Inventory-II (BDI-II) is a questionnaire that measures participants' depression levels. The questionnaire consists of 21 items, scored on a 4-point Likert scale ranging from 0-3. Scores can range from 0-63 [9].

The *Mini Mental State Examination (MMSE)* is a questionnaire that assesses participants' alertness and cognitive impairments. The exam consists of 28 questions asking participants to follow the experimenter's directives. Scores can range from 0-28 [30].

State-Trait Anxiety Inventory (STAI) is a questionnaire that measures participants' state and trait anxiety. The first 20 questions ask participants questions to determine their anxiety level at the current moment (state anxiety). The following 20 questions are used to determine the participants general level of anxiety (trait anxiety). State and trait anxiety levels are computed separately. Questionnaire scores range from 20-80, for each subscale [64].

The *Pain Catastrophizing Scale (PCS)* measures participants' emotions and thoughts when experiencing pain. Catastrophizing score is calculated on a 4-point Likert scale across 13 items. The questionnaire has three validated subscales measuring magnification, rumination, and helplessness when experiencing pain. Scores can range from 0-52 [65].

The *Fear of Pain Questionnaire-III (FPQ-III)* measures participants' fears towards painful experiences. The questionnaire consists of 30 items, scored on a 5-point Likert scale ranging from 1 (not at all) to 5 (extreme). Scores can range from 30-150 [51].

The *Somatosensory Amplification Scale (SSAS)* measures participants' tendency to detect and focus on physiological states and uncomfortable bodily sensations (i.e. heart rate). The questionnaire consists of 10 items, scored on a 5-point Likert scale ranging from 1 (strongly disagree) to 5 (strongly agree). Scores can range from 10-50 [8].

The *Toronto Academic Health Science Network (TAHSN)* demographics questionnaire asks participants to self-report personal demographics information: age, sex, gender, race, sexual orientation, and spiritual or religious affiliation, disability status, gender, sex, sexual orientation, and family income.

Participants first had their cold and heat pain thresholds (CPT and HPT, respectively) measured on the left volar forearm. Next, we identified component temperatures for the thermal grill. Next, we determined whether participants were TGI responders. TGI responders then qualitatively described the sensation using the descriptors list in the McGill Pain Questionnaire (MPQ) [52]. Participants then underwent baseline sensitization measures at the stimulation site. Next, participants underwent phasic thermal grill (phTG) stimulation, followed by post-stimulus sensitization measures.

Thermal Stimulation Paradigm

For all thermal stimulations, we used the QST.Lab TCS II thermal stimulating device (Strasbourg, France) with a T11 thermode. This thermode has five separate "grills" that can be independently controlled and set to different temperatures. We only used 4 of the 5 grills to have a stimulation area (3.7 cm x 2.5 cm = 9.25cm^2), and to ensure equal number of grills for cool and warm. The fifth grill as set to the fixed baseline temperature (32°C). For TG stimulations, grill 1 is set to baseline, grills 2 and 4 to the calibrated warm component temperature (WCT), and grills 3 and 5 to the calibrated cool component temperature (CCT). The thermode is placed on top of the volar forearm and held in place by the experimenter for all stimulations, applying no additional downward pressure beyond the gravitational force on the thermode.

Pain Threshold Calibration

Participants' CPT and HPT are determined on their left volar forearm. Stimulation baseline was set to 32°C. CPT was determined with the staircase method, progressively decreasing the temperature until the participants report pain, before incrementally increasing until the mildest temperature that consistently elicits a painful percept is found. Specifically, participants first received a 5s (25°C/s ramp rate) stimulus at 25°C, if the participant reports no pain, the stimulus is dropped 5°C repeatedly until pain is reported. To avoid any sensitization, the thermode was moved to one of three locations along the participants arm between each stimulation, starting the most proximal and moving distally before returning to proximal. Once the participant reports pain to the cold stimulus, the temperature is increased by 1°C until the CPT is determined—i.e., defined here as the mildest (closest to baseline) cold temperature that consistently elicits a binary painful percept across three consecutive stimulation trials. The coldest temperature participants were given was 0°C. To avoid any sensitization participants were then given a 2-minute break before HPT determination using a similar method. The initial 5s (25°C/s ramp rate) stimulus is set to 35°C and increased in 5°C increments until pain is reported, then reduced by 1°C until the HPT is determined. Again, the thermode was moved between each stimulation. Here, HPT is defined as (closest to baseline) hot temperature that consistently elicits pain across three consecutive stimulation trials. The hottest temperature participants were given was 45°C.

Thermal Grill Calibration

Studies have found that 20-60% of individuals find the TGI painful [12,58,59]; therefore, to explore the individual differences in the pain percept associated with the illusion, participants received individually calibrated thermal grill component temperatures. The thermal grill calibration was used to find WCT and CCT that were consistently reported as non-painful by participants. For the CCT, stimulation began 1°C above the participant's CPT, if the participants reported pain, the stimulus was increased 1°C repeatedly until a stimulation temperature that is not painful over three trials was identified. Again, to avoid sensitization, the thermode was moved between stimulations. Then following a 2-minute break, the same paradigm was repeated to determine WCT, with the first stimulus 1°C below participants HPT, and repeatedly reduced by 1°C. The minimum CCT used was 5°C and the maximum WCT used was 42°C. After determining participants warm and CCT, participants were given another 2-minute break before receiving three thermal grill stimulations, to ensure they report the thermal grill as painful. Participants were prompted to provide pain intensity and unpleasantness ratings after each stimulation. Pain intensity ratings were on a verbal numerical rating scale (vNRS) with anchors 0="no pain at all" and 100="the worst imaginable pain". Stimulus unpleasantness ratings were also on a vNRS but with anchors 0="not unpleasant at all" and 100="the most unpleasant sensation imaginable". There is no consensus on criteria to determine TGI responders and non-responders. We have a employed a modified version of TGI calibration, as described by Mitchell et al. [53], to capture the individual variability in pain percepts and responsiveness to the TGI. If participants provided a mean pain intensity rating less than 5/100 across the three trials, the study

session was terminated, and the participant deemed a TGI non-responder. Finally, after determining TGI responsiveness, participants are given one final thermal grill stimulation and prompted to select descriptor words from the MPQ to describe the TGI sensation. Within the MPQ, descriptors are grouped together under word sets. If a participant selected multiple descriptors within the word set, the most intense word was scored.

Phasic Thermal Grill (phTG) Stimulation Paradigm

To elicit sensitization, participants received a phTG stimulation on their right volar forearm. The phTG stimulation consisted of 8 calibrated 40s thermal grill stimuli, with a 20s inter-stimulus interval. Throughout the paradigm participants are prompted to rate their pain intensity and stimulus unpleasantness at every 10s (four times during each phasic stimulation, a total of 32 ratings for the phTG paradigm).

Innocuous Brush Stimulus (Brush Allodynia)

To determine whether phTG stimulation can induce brush allodynia, participants received calibrated brush stimulation (SENSELab Brush 05, Somedic SENSELab AB, Sösdala, Sweden) before (pre-phTG) and after (post-phTG) phTG stimulation, within the stimulation site. Participants receive three brush strokes post-phTG and are prompted to give a binary response about whether they felt pain to the prompt: “Is it painful, yes or no?” If participants reported pain to the brush stimulus then they had brush allodynia.

Primary Mechanical Hyperalgesia

To determine primary mechanical hyperalgesia, participants receive a mechanical punctate pinprick with a 512mN probe (PinPrick stimulator, MRC Systems GmbH, Heidelberg, Germany) pre- and post-phTG stimulation, within the stimulation site. Pre-phTG, participants receive three punctate pinprick stimuli over the stimulation site of grills 3, 4, and 5. Participants are prompted to rate their pain intensity using the vNRS after each stimulation. Post-phTG, participants undergo the same procedure. The magnitude of primary mechanical hyperalgesia is calculated as the difference between means of post-phTG pain intensity ratings and pre-phTG pain intensity ratings.

Secondary Hyperalgesia

Pre-phTG, a secondary hyperalgesia map was drawn onto the participant’s right volar forearm (Figure 1). Post-phTG, a 256mN PinPrick probe is used to stimulate each dot along the eight radial arms starting with the most proximal line and moving counterclockwise. Stimulations begin at the furthest dot and move inwards at a rate of ~1Hz. Participants are prompted to notify the researcher when they experience an increase in intensity of the pinprick stimulation, compared to the previous ones of along the same radial arm. The area of secondary hyperalgesia

was calculated using ImageJ [60], by taking the area of sensitivity around the stimulation site and subtracting the area of the thermode.

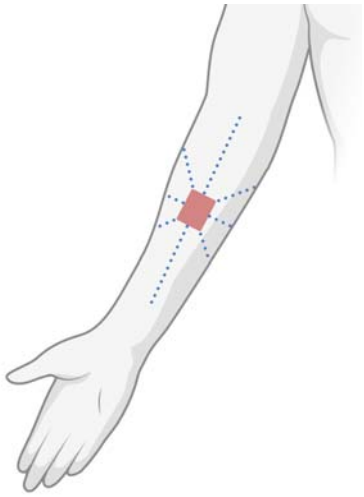

Figure 1: Secondary hyperalgesia map. The map consists of 8 arms radiating outwards from the stimulation site, with each arm containing 14 dots at 0.5cm increments (total distance in each direction = 7cm).

Statistical Analyses

All frequentist statistical analyses were performed using SPSS Statistics v29.0.2.0 (IBM SPSS Statistics, Armonk, NY, USA) and all data visualization was performed using GraphPad's Prism v10 (GraphPad Software, Boston, Massachusetts USA). Bayesian statistical analyses were performed using JASP v.0.19.2 (JASP Team, 2024) to allow for the direct quantification of evidence for or against the null hypothesis [72]. All Bayesian analyses were run with 10,000 permutations, where appropriate. The Shapiro-Wilk test was run to test whether outcome measures were normally distributed. We investigate whether there are sex differences in sample characteristics and whether phTG induces sensitization, as measured by our three primary outcome measures: brush allodynia, primary mechanical hyperalgesia, and secondary hyperalgesia.

Sample characteristics

Analyses were performed for the whole group, as well as in sex-disaggregated groups. Independent samples t-tests, or Mann-Whitney U tests if the variable was not normally distributed, were run to ensure male and female participants did not differ significantly in age, CPT, HPT, WCT, CCT, and questionnaire measures. Significance was set at $p<0.05$.

Brush Allodynia

No statistical test was performed to determine brush allodynia, as no participant reported pain to the brush stroke in the stimulation site.

Primary Mechanical Hyperalgesia

To test whether the phTG induced primary mechanical hyperalgesia, averaged pre- and post-phTG pain intensity ratings were compared. As both pre- and post-phTG pain intensity ratings to the 512mN mechanical pinprick were not normally distributed ($p<0.001$), a related-samples Wilcoxon signed rank test was run, with significance set at $p<0.05$. A Bayesian Wilcoxon signed rank test was also run to determine the strength of evidence for the null and alternative hypotheses. To determine whether there were any differences in primary mechanical hyperalgesia within each sex, the same analyses was performed with sex disaggregated samples. We further investigated if there were sex differences in primary hyperalgesia. As there is no non-parametric equivalent to a repeated measures ANOVA, we computed the difference in ratings between post-phTG and pre-phTG and performed a Mann-Whitney U test to compare primary hyperalgesia between groups. Again, to determine the strength of evidence for the null and alternative hypotheses, a Bayesian Mann-Whitney U test was performed.

Secondary Hyperalgesia

To test whether PHP induced secondary hyperalgesia, a one-sample t-test was run comparing the area of secondary hyperalgesia in each individual with a test value set to 0. A Bayesian one-sample t-test was again run to determine strength of evidence for the hypotheses. To determine whether there were any differences in secondary hyperalgesia within each sex, the same analyses was performed with sex disaggregated samples. We also performed independent samples t-test was run to compare male and female participants' area of secondary hyperalgesia, with significance set at $p<0.05$. Again, a Bayesian independent samples t-test was performed to determine the strength of evidence in favour of the hypotheses.

Contribution of WCT and CCT to primary outcome measures

To determine whether component temperatures affected primary outcome measures, Bayesian ANCOVAs were run with participants' WCT and CCT as covariates. For primary hyperalgesia, a repeated measures Bayesian ANCOVA was performed.

Analysis of sensitization in a subsample with WCT and CCT in the innocuous range

As some stimuli were putatively in the noxious range, even if perceived as non-painful by participants, we wanted to determine whether participants who received strictly innocuous stimuli (warm <41˚C and cool >19˚C) from the thermal grill developed primary mechanical and/or secondary hyperalgesia. This resulted in a subsample of n=7 (2 males, 5 females). All measures were normally distributed ($p>0.05$).

For primary hyperalgesia, we performed a paired t-test in this subgroup, with significance set at $p<0.05$. Bayesian paired t-tests were also run to determine the strength of the evidence in support of the null and alternative hypotheses. For secondary hyperalgesia, we performed a one-sample t-test in this subgroup, with significance set at $p<0.05$. Bayesian one sample t-tests was also run to determine the strength of evidence for the null and alternative hypotheses.

Associations between TG-induced sensitization and psychosocial measures

Finally, exploratory Bayesian correlation analyses were performed to determine whether there was evidence to support the hypothesis that psychosocial measures (BDI-II, STAI State, STAI Trait, PCS, FPQ, and SSAS) were related to TGI pain intensity ratings (average rating during phTG), magnitude of primary hyperalgesia, and secondary hyperalgesia. Further, as all measures

except for secondary hyperalgesia were not normally distributed ($p<0.05$), Kendall's tau correlations were performed.

Results

Sample Demographics and Questionnaire Data

Fifty-two sex-matched participants (26 females, 26 males; age ±SD: 23.5 ±4.8 years) were included in the final analysis. Table 1 provides a summary of demographic information of the sample, including self-reported gender, sexual orientation, race, and religious or spiritual affiliation. Data from participants psychological and behavioural questionnaire data is summarized in Table 2.

Table 1: Demographic information of the study sample. Participants completed a self-report demographics questionnaire comprising age, sex, gender, sexual orientation, race, and religious/spiritual affiliation.

Age (mean ± SD)	
Female	23.2 ±4.44
Male	23.9 ± 5.24:
Sex	
Female	26
Male	26
Gender	
Man	26
Woman	26
Other	0
Sexual Orientation	
Asexual	1
Bisexual	1
Gay	2
Heterosexual	46
Prefer not to specify	2
Race	
Aboriginal	1
Asian – East	14
Asian – South	9
Asian – South East	4
Black	3
Latin American	1
Middle Eastern	4
White/European	15
Prefer not to specify	1
Religion or Spiritual Affiliation	
I do not have a religious/spiritual affiliation	26
Buddhism	2
Christianity	4
Christian Orthodox	2
Protestant	1
Roman Catholic	4
Hinduism	4
Islam	7
Spiritual	2

Table 2: Sample psychosocial questionnaire measures. Group and sex-specific summaries of psychosocial questionnaire data, including means and ranges.

Questionnaire (Maximum score)	Male Mean (± SD)	Female Mean (± SD)	All Participants Mean (± SD)	Range
BDI-II (63)	5.96 (±4.52)	5.04 (±5.81)	5.42 (±5.17)	0 - 19
MMSE (30)	30 (±0)	30 (±0)	30 (±0)	30
STAI – State (80)	18.00 (±5.56)	16.54 (±6.59)	17.27 (±6.08)	19 - 54
STAI – Trait (80)	17.04 (±5.30)	16.12 (±5.62)	16.58 (±5.43)	20 - 60
PCS (52)	10.04 (±7.56)	9.83 (±10.97)	10.08 (±9.29)	0 - 34
FPQ-III (150)	73.52 (±17.59)	81.35 (±22.38)	77.69 (±20.27)	34 - 136
SSAS (50)	23.00 (±5.10)	24.08 (±7.03)	23.54 (±6.11)	13 - 40

Calibrated Thermal Grill Component Temperatures

Participants underwent CPT and HPT calibration using a method of levels to determine their thermal pain ranges. Participants had a median [IQR] HPT of 43.5°C [42.5-44.0] (Figure 2A), and CPT of 2.0°C [0.0-11.0] (Figure 2B).

Participants received an individually calibrated TG stimulus. The median CCT was 8.0°C [5.0-16.3]; males had a median CCT of 10.5°C [5.0-15.0], and females had a median of 6°C [5.0-17.5], with no significant difference between groups (p=0.780). Participants had a median WCT of 41.0°C [40.0-42.0]; males had a median WCTs of 41°C [40.1-42.0], and females had a median of 41.0°C [40.0-42.0], with no significant difference between groups (p=0.662). With individual calibration of TGI component temperatures, we observed a responder rate of 78%. Participants' CCT and WCT are summarized in Figure 2C.

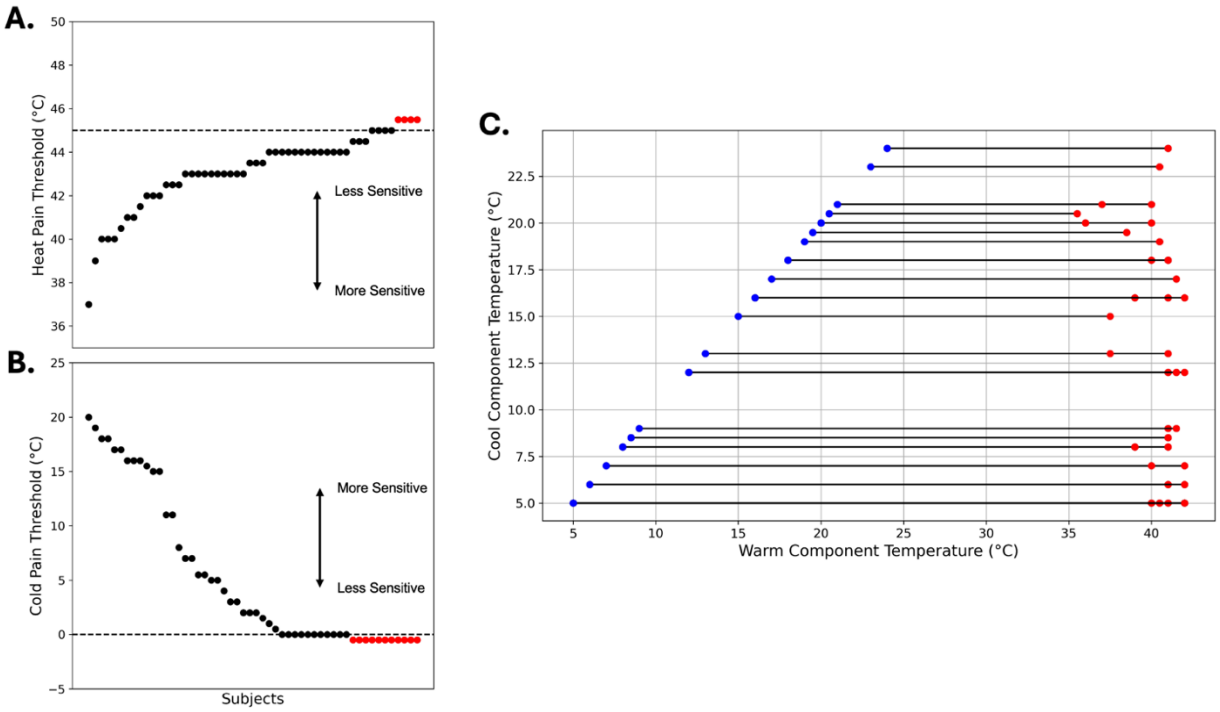

Figure 2: Participants' noxious thermal stimulus thresholds and TGI stimulation temperatures. (A) Heat pain threshold and (B) cold pain thresholds for each participant. Red dots indicate participants whose HPT or CPT were outside the range of stimulation temperatures, indicated by the dotted lines (maximum 45°C and minimum 0°C). (C) TGI component temperatures and differences: blue dots represent the cool component temperature, red dots represent the warm component temperature, and the lines connecting them represent the magnitudes of difference of component temperatures. Dotted lines indicate noxious thresholds for both the heat and cold directions.

TGI Qualitative Sensations

Participants completed the MPQ following a thermal grill stimulation, to explore the qualitative descriptions commonly used to categorize sensation evoked by the TGI. The frequencies of descriptors used are provided in Figure 3. Thermal sensory descriptors were most commonly reported, with "hot" (51.9%), "cold" (44.2%) and "cool" (34.6%), followed by other sensory descriptors such as "tingling" (25%) and "spreading" (21.2%). Evaluative terms such as "annoying" (30.7%) and "troublesome" (9.6%) were also frequently reported. Affective descriptors, including "fearful" (3.8%) or "dreadful" (1.9%), were among the least frequently selected. Participants also selected descriptors within the miscellaneous categories. The frequency of all MPQ descriptors selected is provided in Table S1.

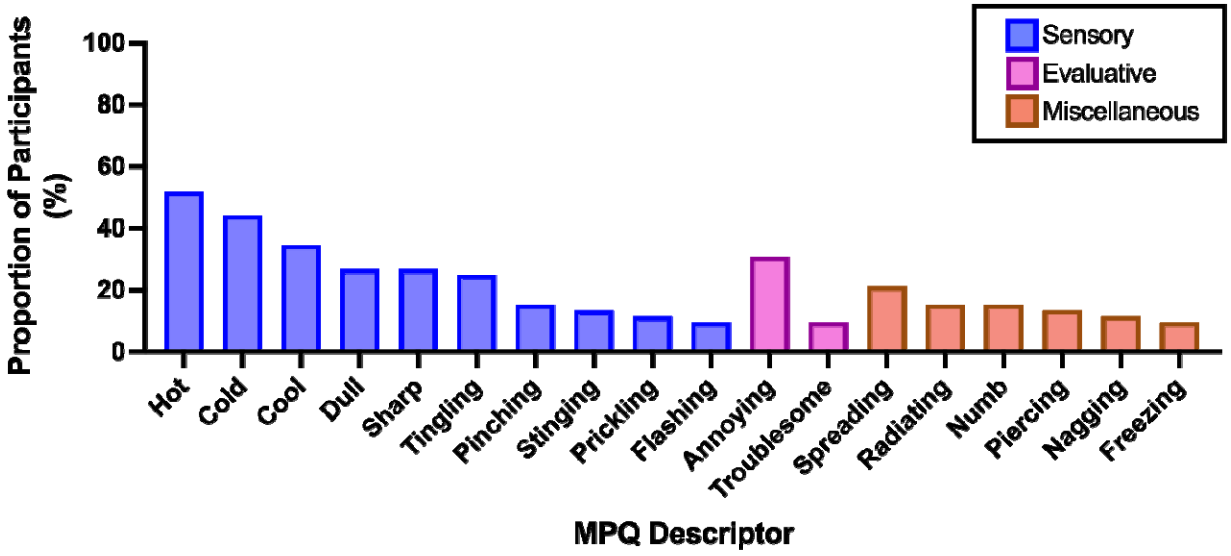

Figure 3: Frequency of descriptors from the McGill Pain Questionnaire used to describe the Thermal Grill Illusion in Responders. Percent frequencies of the most used descriptors (>5 participants or ~10% of sample) from the McGill Pain Questionnaire (MPQ) used to describe the thermal grill illusion. Descriptors are categorized as sensory (blue), evaluative (green), miscellaneous (purple), or affective (of which none met the cutoff) dimensions.

Brush Allodynia

No participants indicated any pain sensations to an innocuous brush stimulus within the stimulus site post-phTG stimulation, therefore no participant developed brush allodynia.

Primary Hyperalgesia

Both pre-phTG and post-phTG measures were not normally distributed (both $p<0.001$), thus non-parametric tests were used. Pre-phTG average pain intensity ratings ranged from 0.3 to 56.7, with a median rating of 9.15 [2.23–18.30]. Post-phTG average pain intensity ratings ranged from 0 to 80, with a median rating of 14.20 [5.00–30.43]. Post-phTG pain intensity ratings were significantly higher than pre-phTG ($z=-3.729$, $p<0.001$, one-tailed, Pearson correlation effect size $r=-0.517$; Figure 4).

To determine evidence for the null or alternative hypotheses, a Bayesian Wilcoxon signed rank test was performed. The analysis provided extreme evidence in favour of the alternative hypothesis ($BF_{+0}= 865.185$; $R_{hat}=1.004$), indicating that post-phTG pain intensity was higher than pre-phTG pain intensity ratings.

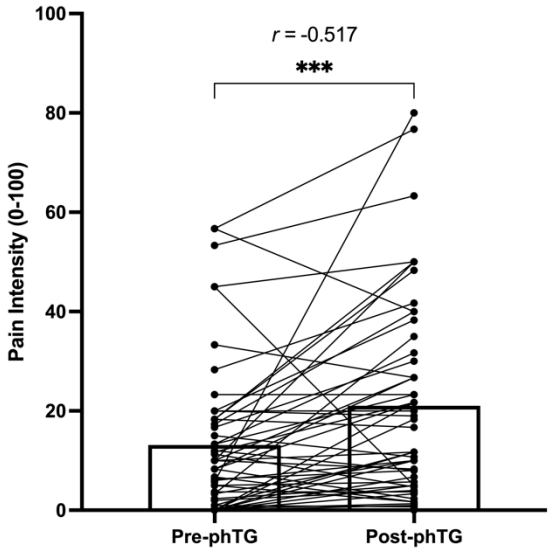

Figure 4: Phasic thermal grill elicits primary hyperalgesia. Pain intensity to a 512mN Pinprick before phasic thermal grill (pre-phTG) and after phTG (post-phTG) within the area of stimulation. Bars indicate median values, and each pair of dots connected by a line represents a participant. Abbreviations: *d* = Cohen's *d* effect size, *r* = Pearson's correlation effect size, vNRS = verbal numerical rating scale, ***=p<0.001.

Secondary Hyperalgesia

Fifty-one out of 52 participants developed secondary hyperalgesia, where the area of sensitization outside of the stimulation site is greater than 0cm^2. The area of secondary hyperalgesia was normally distributed (p=0.081), thus parametric analyses were run. Participants' area of secondary hyperalgesia ranged from 0 to 93.7 cm^2 (mean ±SD=47.17 ±25.38 cm^2). The area of secondary hyperalgesia evoked by phTG deviated significantly from null hypothesis of 0 cm^2 (t=13.404, p<0.001, one-tailed; Cohen's *d*=1.859; Figure 5B). A Bayesian one-sample t-test revealed that there is extreme evidence in favour of the alternative hypothesis (BF_{+0}= 4.474x10^{15}; error %=4.941x10^{-19}), indicating the thermal grill does elicit secondary hyperalgesia.

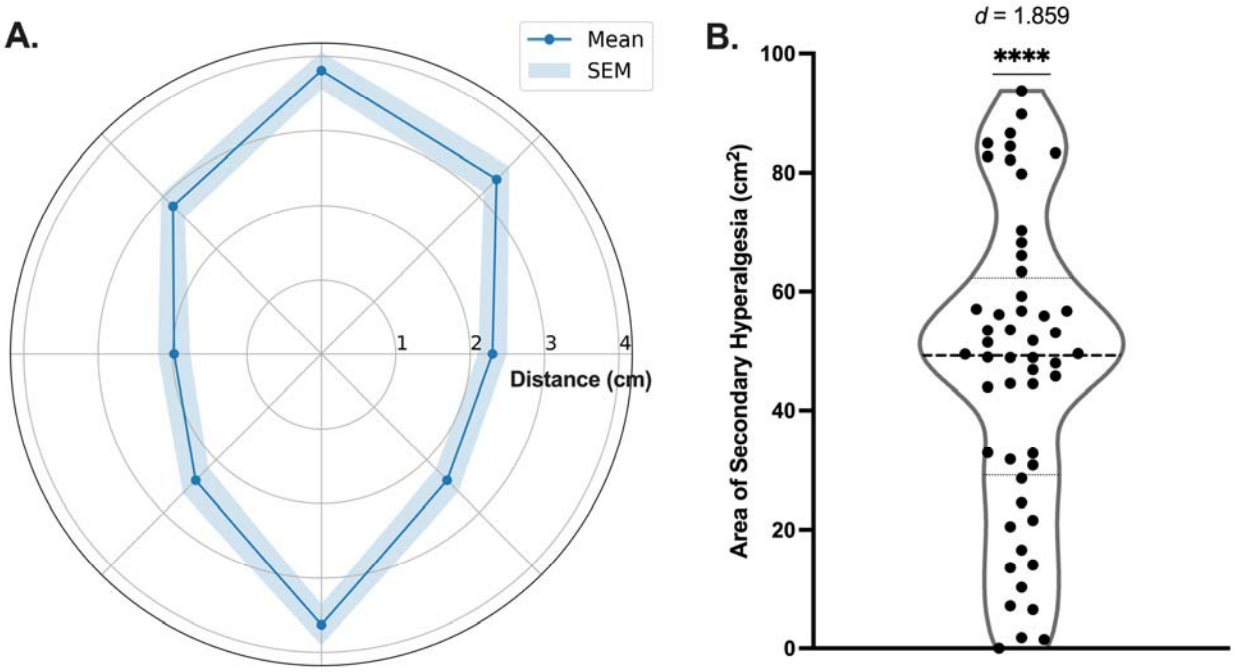

Figure 5: The thermal grill elicits secondary hyperalgesia. (A) The mean (blue line, shaded area: SEM) area of secondary hyperalgesia elicited by the thermal grill illusion. (B) A one-tailed t-test indicates that there is a statistically significant area of secondary hyperalgesia (cm^2) elicited by the thermal grill in all participants, with group median in thick dashed line and quartiles in a thinner dotted line. Abbreviations: *d* = Cohen's *d*, ****=p<0.0001.

Contribution of Component Temperatures to TGI-Induced Sensitization

Despite all participants reporting both the WCT and CCT as not painful, it is still plausible that component temperatures fall within noxious ranges. Therefore, we sought to determine whether component temperatures were associated with primary and secondary hyperalgesia. A Bayesian repeated measures ANOVA with WCT and CCT as covariates was performed to determine whether the difference in mechanical pinprick pain ratings pre- versus post-phTG was influenced by component temperatures. The factor of time (pre-phTG vs post-phTG) had the highest posterior probability (P(M|data)=34.5%), compared to the model that included WCT (P(M|data)=18.8%), CCT (P(M|data)=29.3%), or both (P(M|data)=14.2%). Bayes factors (BFs) further confirmed weaker evidence for models including WCT (BF_{10}=0.544), CCT (BF_{10}=0.848), or both (BF_{10}=0.411), compared to the model with only the factor of time (BF_{10}=1) indicating component temperatures does not explain the significant difference between post-phTG and pre-phTG, indicating that component temperatures are not associated with primary hyperalgesia (Table S2).

A Bayesian ANCOVA for area of secondary hyperalgesia showed the null model (without WCT and CCT) had the highest posterior probability (P(M|data)=55.5%), compared to the model including WCT (P(M|data)=18.2%), CCT (P(M|data)=18.6%), or both (P(M|data)=7.6%). BFs

further confirmed weaker evidence for models including WCT (BF_{10}=0.329), CCT (BF_{10}=0.336), or both (BF_{10}=0.138), compared to the null model (BF_{10}=1) that WCT or CCT does not explain the observed effect of secondary hyperalgesia following phTG (Table S2). To further explore the role of potential noxious component temperature inputs on primary outcome measures, analyses for primary and secondary hyperalgesia were rerun for all participants whose WCT and CCT were within the innocuous range (CCT>19° and WCT<41°C; N=7). All primary outcome measures were normally distributed (p > 0.05), thus parametric tests were used.

For participants with innocuous component temperature inputs, there was with no significant increase in mechanical pain ratings post-phTG compared to pre-phTG (t = -1.684, p = 0.072, one-tailed, *d*=-0.637l; Figure 6A). A Bayesian paired t-test, showed anecdotal evidence in favour of the null hypothesis, indicating the TG did not elicit primary mechanical hyperalgesia (BF_{-0}= 1.761; error %=8.697x10^{-6}). There was a significant greater area of secondary hyperalgesia than 0cm^2 (t=4.185, p=0.003, one-tailed, *d*=1.582; Figure 6B). A Bayesian one-sample t-test revealed that there is strong evidence in favour of the TG eliciting secondary hyperalgesia (BF_{+0}= 20.422; error %=8.372x10^{-7}).

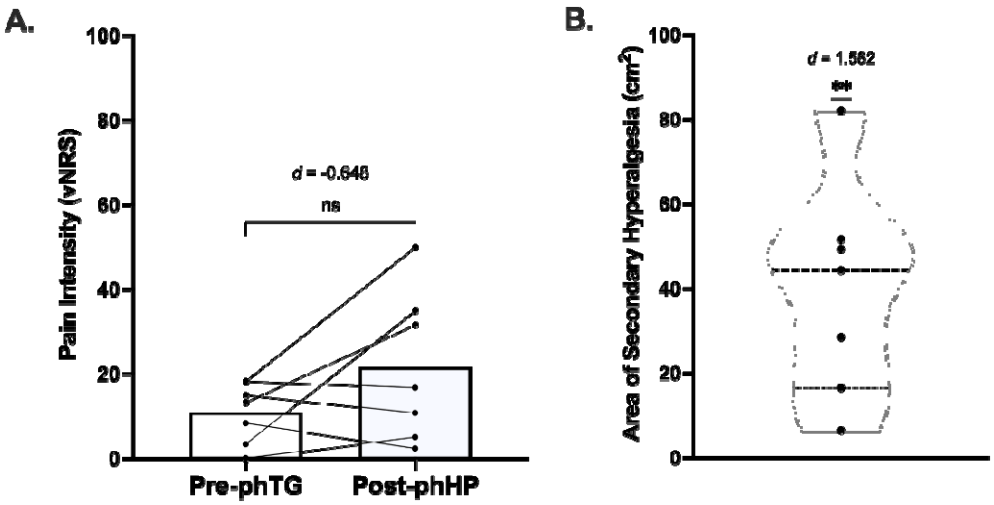

Figure 6: Innocuous component temperatures elicit secondary, but not primary hyperalgesia. (A) Primary hyperalgesia measures comparing mechanical punctate pain intensity pre- and post-phTG within the area of stimulation in participants with innocuous component temperatures (WCT <41°C and CCT >19°C), with median values highlighted with the bars. (B) Violin plot of area of secondary hyperalgesia (cm^2) in participants with innocuous component temperatures, with group median in thick dashed line and quartiles in a thinner dotted line. Abbreviations: *d* = Cohen's *d*, **=p<0.01.

Sex Disaggregated and Sex Differences Analyses

Females showed no significant differences between pre- and post-phTG pain ratings (z=-1.83, p=0.034, one-tailed, *r*=-0.346; Figure 5A), and thus did not exhibit primary hyperalgesia.

Bayesian analyses showed anecdotal evidence for this observation (BF_{-0}= 1.62; R_{hat}=1.001). In contrast, males showed a significant increase in pain ratings post-phTG (z=-3.33, p<0.001, one-tailed, *r*=-0.653; Figure 5A), exhibiting primary hyperalgesia. Bayesian analyses further indicate extreme evidence in favour of the alternative hypothesis (BF_{-0}=427.57; R_{hat}=1.003). However, there was no significant difference in magnitude of primary hyperalgesia between sexes (U=411.0, z=1.34, p=0.18; two-tailed, r=0.185), with Bayesian analyses showing anecdotal-to-moderate evidence in favour of the null hypothesis of no difference between sexes (BF_{10}=0.33; R_{hat}=1).

Both female (t=8.36, p<0.001, one-tailed, *d*=1.639; Figure 7B) and male (t=11.11, p<0.001, one-tailed, *d*=2.179; Figure 7B) participants showed a significant deviation in area of secondary hyperalgesia from the null hypothesis. Bayesian analyses showed extreme evidence in favour of the alternative hypothesis that both males (BF_{+0}=5.05x10^{8}; error%=6.07x10^{-12}) and females (BF_{+0}=2.47x10^{6}; error%=4.26x10^{-9}) developed secondary hyperalgesia. When comparing areas of secondary hyperalgesia between sexes, there was a trend towards males having larger areas, but this observation was not significant (t=-1.94, p=0.058, two-tailed, d=-0.538). There exists anecdotal evidence for this observation (BF_{10}=0.88; error%=0.008).

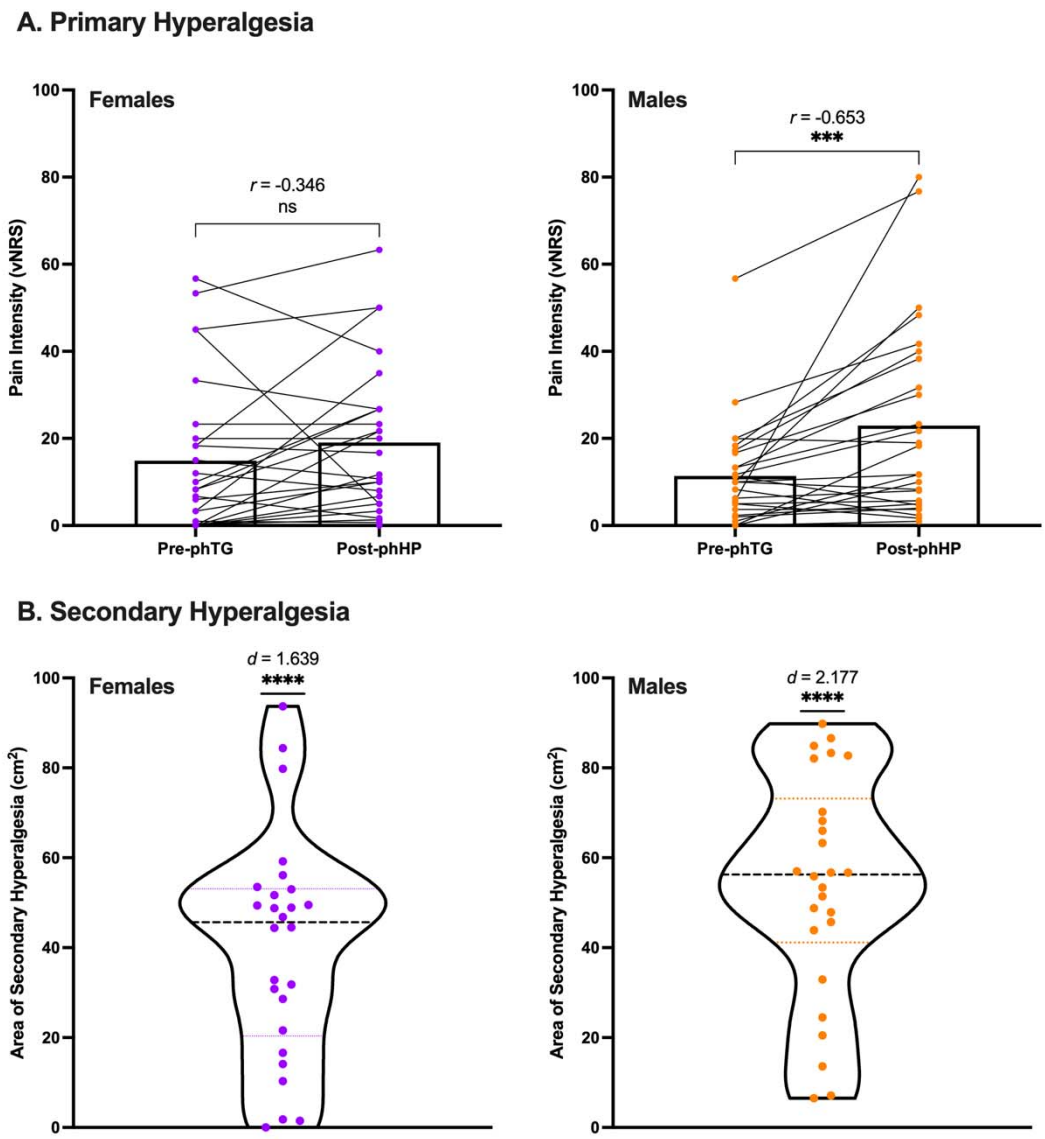

Figure 7: Sex disaggregated analyses of TGI-induced primary and secondary hyperalgesia. (A) Female (purple) and male (orange) primary hyperalgesia measures pre- and post-phTG. Bars represent median values. (B) Females (purple) and males (orange) area of secondary hyperalgesia (cm^2), with group median in thick dashed line and quartiles in a thinner dotted line (B). Abbreviations: *d* = Cohen's *d*, *r* = Pearson's correlation effect size, ***=p<0.001, vNRS= verbal numerical pain score, ****=p<0.0001.

Psychosocial Influences on TGI Sensitization

To explore whether thermal grill sensitization is associated to psychosocial measures, Bayesian correlations were performed between outcome measures—primary and secondary hyperalgesia, and TGI pain intensity during phTG—and questionnaire measures (BDI-II, STAI Trait, STAI State, PCS, FPQ-III, SSAS). There existed moderate evidence in favour of the null hypothesis—

i.e., that psychosocial measures were not correlated with outcome measures ($3 < BF_{01} < 6$) (Figure S1).

Discussion

The TGI, where interlaced innocuous warm and cool stimuli elicit a painful sensation, can reveal the neural underpinnings of thermonociceptive integration and perception. The peripheral and spinal mechanisms of the TGI remain unknown. One approach to answering these outstanding questions is to determine whether the TGI elicits altered states, such as brush allodynia, primary hyperalgesia and secondary hyperalgesia, each of which have clearly established mechanisms [56,70]. This study aimed to determine whether the TGI elicits peripheral and/or central sensitization. Specifically, if the TGI elicits primary hyperalgesia this indicates that the innocuous thermal inputs of the illusion activate peripheral nociceptors [41,70]; if the TGI elicits brush allodynia, then we can infer that WDR cells are involved [56]; and, lastly, if the TGI elicits secondary hyperalgesia, but not primary hyperalgesia or brush allodynia, then this implies the integration occurs entirely at the level of the dorsal horn, likely in HPC cells [20,21]. We report that the TGI elicits primary and secondary hyperalgesia but not brush allodynia. Furthermore, when inputs were firmly in the innocuous range (WCT <41°C and CCT >19°C), only secondary hyperalgesia was observed. These data suggest that the TGI does not require integration by WDR cells but does require integration in the dorsal horn likely by HPC cells. Primary hyperalgesia was significantly greater in males than females, suggesting sex-dependent mechanisms for the TGI: males may integrate innocuous thermal inputs in the periphery, whereas females integrate these signals in the dorsal horn of the spinal cord. Finally, it remains unknown whether top-down factors influence the TGI percept, and we show that psychosocial factors known to affect pain sensitivity do not affect TGI sensitization measures.

There are two prominent theories about how the TGI elicits pain: the addition theory [12,35,36] and the disinhibition theory [21,23]. Both theories posit the TGI is mediated by primary C-fibre afferents, and that integration occurs in the central nervous system[61]. However, the question of whether these C-fibres are nociceptive or not remains unclear. Furthermore, one theory posits that the second-order neurons mediating the TGI are WDR neurons [12,35,36], whereas the other posits that HPC neurons are responsible [18,21-23].

The thermal grill, classically consisting of 40°C and 20°C, is largely thought to be innocuous [61]. The perception of pain when thermal component inputs are combined, and the absence of pain when isolated, is hallmark to the illusory phenomenon. We show the experience produces paradoxical burning and cold percepts, consistent with previous findings [6,12,38]. Further, the percepts evoked are typically sensory or evaluative, but not affective, in dimension. The paradoxical perception of pain with a fixed combination of warm and cool thermal inputs is not present in all individuals—only between 20-60% of people find the illusion painful (termed “TGI responders”) [12,58,59]. Consistent with several recent studies, we show that individually calibrated component temperatures can greatly improve TGI responsiveness [1,2,26,28,44,53]. Humans’ heat pain thresholds are ~42°C and thought to be mediated by a the vanilloid transient receptor potential type 1 (TRPV1), a class of temperature-sensitive receptor channels that mediate thermal encoding [40,54]. Cold pain remains less understood, and thresholds are thought to be ~15°C and mediated by TRPA1 [43,45,57]. While cold pain is proposed to be mediated at least in some part by Aδ fibres [24,25,62,63], ischemic block studies have proposed a prominent

role of CPNs [32,73,75]. Our observation of the abolition of primary hyperalgesia in participants with CCT >19°C, could indicate that the group-level observations were driven by cold component temperatures that bled into the noxious range. It is worth noting, however, that covariate analysis showed that component temperatures did not have a significant impact on primary hyperalgesia, and thus the innocuous subsample could be underpowered. CPNs are thought to encode noxious stimuli outside the thermoneutral zone (<24°C)[10,13,18] and microneurography studies have observed firing <19°C [14] and >39°C [69]. Furthermore, evidence suggests that CPNs exhibit sensitization following mild tissue injury [69] and mild deviations outside the thermoneutral zone [10]. Therefore, there remains a possibility that the TGI does still involve some form of peripheral integration, as observed through the group-level observation of primary hyperalgesia. A potential candidate proposed to mediate the TGI peripherally are type-2 bimodal C-fibres (C2) [49], which respond to warming (>38°C) and cooling (<24°C) into the noxious ranges [13]. However, C2 fibres are mechanically insensitive and thus do not account for the primary mechanical hyperalgesia observed [13]. Therefore, the most likely candidate mediating are C-mechano-heat-cold fibres which begin firing <19°C and thus would account for the lack of primary hyperalgesia observed with CCT >19°C [14].

The observation of secondary hyperalgesia, especially in the innocuous temperature subsample, indicates that there is some form of integration of the TGI within the dorsal horn. While secondary hyperalgesia is not a direct measure of central sensitization, it is a widely used behavioural correlate [47], and thus used alongside the TGI could be used to explore altered nociceptive processing [2,61,66]. There currently exist three classes of second-order fibres that are thought to mediate pain: nociceptive specific (NS), WDR, and HPC neurons [18]. NS cells respond to noxious mechanical and heat stimuli. Similarly, HPC neurons are insensitive to low-threshold mechanical stimulation, but show graded responses to noxious heat, mechanical, and cold stimuli [15,18-20,22,77]. Conversely, WDR neurons respond to innocuous and noxious mechanical, heat, and cold [3,16,17,34]. Of these second-order fibres, HPC and WDR neurons have observed to sensitize in response to phasic noxious stimulation or tissue injury [17,20,37,55]. A key feature of secondary hyperalgesia is an increase in pain response to mechanical stimuli, but not to thermal stimuli. As such secondary hyperalgesia is thought to be mediated by a mechanosensitive second-order fibre [41,46,70]. Therefore, the second-order fibres proposed to mediate the TGI in the addition and disinhibition theories could exhibit secondary hyperalgesia. The primary distinction between these two fibre classes is their responses to innocuous mechanical stimulation—especially in sensitized states where they mediate brush allodynia [74]. Given our observation that no participant reported pain to the innocuous brush stimulation following phTG, it is clear the TGI does not require WDRs, which are the primary mediators of tactile allodynia [3,56]. Therefore, the TGI is most likely integrated within the dorsal horn by HPC neurons. Tied with the observations of primary hyperalgesia with CCT <19°C, likely activating CPNs [10], and role of HPC neurons in secondary hyperalgesia, our findings are supportive and consistent with the disinhibition theory rather than the addition theory.

There does appear to be a difference in TGI altered states between sexes. Specifically, the TGI did not elicit primary hyperalgesia, regardless of component temperature inputs, in females. However, the degree of peripheral sensitization observed was not statistically different across the sexes. Whether there exist sex differences in the perception or mechanism of the TGI, remain

poorly understood. A study by Averbeck et al. [5] found that females showed more pronounced responses to the TGI than males, hinting at more complex sex differences in thermal perception. Specifically, studies have reported that females have higher sensitivity to innocuous and noxious thermal stimuli [42,48]. However, it remains unknown whether there are any sex differences in peripheral sensitization. We also observed anecdotal evidence in favour of males having larger areas of secondary hyperalgesia compared to females, however, this difference could be due to differences in forearm size when calculating the area of sensitization. However, we did not collect forearm size and thus could not adjust for this potential confound. Therefore, future studies are needed to parse out any sex differences in TGI sensitization, and whether mechanistic differences exist.

While we have shown that the TGI can elicit secondary hyperalgesia, and thus central sensitization, further research is required to tease apart the influence of noxious component temperature inputs on TGI-induced altered states. Further, while we have shown that the TG can induce primary hyperalgesia, specifically in males, there exists the possibility that thermal grill component temperatures bled outside the thermoneutral zone (and potentially into the noxious range), despite being reported as non-painful by participants. Therefore, further investigation is needed to determine whether the classic thermal grill activates peripheral nociceptive afferents, and thus a noxious input.

Despite these limitations, the current study is the first to show that the TGI can induce sensitization. We used these altered states to parse the peripheral and spinal mechanisms of the TGI, showing that the TGI induces central sensitization, and is likely mediated by the HPC, and not WDRs. Given that altered thermonociceptive processing is common in chronic pain conditions[71,76] and that the TGI has been used to study these differences[2,66], our findings suggest that the TGI could be a valuable tool for investigating pathological pain states and their underlying mechanisms.

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