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Thermal taster status: Temperature modulation of cortical response to sweetness perception

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ABSTRACT

Temperature is known to impact taste perception, but its reported effect on sweet taste perception in humans is inconsistent. Here, we assess whether thermal taste phenotype alters the temperature modulation of the brains' response to sweet samples and sweet taste perception. Participants ($n = 24$ balanced for thermal tasters (TT) and thermal non-tasters (TnT), 25 ± 7 years (mean ± SD), 10 males) underwent a thermal taste phenotyping session to study responses to cooling and warming of the tongue using a thermode. In a separate session, functional Magnetic Resonance Images (fMRI) were collected during sweet samples (87 mM sucrose) delivery at two temperatures ('cold' (5 ± 2 °C) and 'ambient' (20 ± 2 °C)) and the perceived sweetness intensity rated.In the phenotyping session, TTs had heightened perceptual temperature sensitivity to cooling and warming of the tongue using a thermode compared to TnTs. Although there was no significant effect during the fMRI session, the fMRI response to the 'cold sweet' sample across all participants was significantly increased in anterior insula/frontal operculum and mid-insula compared to the 'ambient sweet' sample, likely to reflect the perceptual difference to temperature rather than taste perception. TTs showed significantly increased fMRI activation patterns compared with TnTs and an interaction effect between thermal taster status and sample temperature, with TTs showing selectively greater cortical responses to 'cold sweet' samples compared to TnTs in somatosensory regions (SI and SII).The increase in cortical activation in somatosensory cortices to the 'cold sweet' stimulus correlated with perceptual ratings of temperature sensitivity to the thermode. The results highlight the importance of investigating the effects of thermal taster phenotype across a range of temperatures representing the reality of consumer consumption to beverages.

1. Introduction

Temperature is known to impact taste perception [1,2,3], level of acceptance [4] and emotional response [5, 6] to many foods and beverages. For example, lemonade and beer are perceived as more palatable when served cold [6, 7], whilst coffee, which can be consumed warm or cold, has a U-shape hedonic function with its minimum hedonic value at ambient temperature. Although psychophysical studies in humans show that changing the temperature of a taste solution can modulate gustatory perception [1-3], reported effects of temperature on sweet taste perception have been inconsistent. For example, some studies show a clear linear relationship between increasing temperature and increasing sweetness perception [1, 8], whilst others show no effects [9, 10]. The human T1R2-T1R3 sweet taste receptor (STR) plays an important role in recognizing sweet-tasting sugars, resulting in the release of intracellular heterotrimeric G protein that in turn leads to the sweet taste perception. Studies on the influence of temperature on sweet taste response have largely focused on the peripheral nervous system and psychophysical

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effects, few studies have assessed the effect of temperature on the cortical response to sweet taste in the brain. Electrophysiology studies by Wilson and Lemon [11] showed that warming sucrose solutions to 30 °C increased gustatory neuron responses in the nucleus of the solitary tract (NTS) and the medulla, whilst cooling to 16 °C or 18 °C inhibited gustatory neuron responses. These studies provide evidence of temperature-taste interactions of sweet taste at the central level, but the impact of temperature on sweet taste perception in the human brain and how this varies with individual taster status has not been explored.

Sensitivity to taste and oral sensations including temperature varies greatly between individuals, and has been explained by phenotypic and genetic differences [12,13,14]. Thermal taster status is of interest as there is an association between temperature and taste perception in these individuals. Thermal taster status is a taste phenotype first reported in 2000 [15] whereby a thermally-induced taste sensation (thermal taste) may be elicited when the tongue is thermally stimulated in the absence of gustatory stimulus. Those perceiving a taste are termed "thermal tasters" (TTs). To phenotype participants, the tongue is cooled or warmed rapidly using a thermode placed on the tongue [15]. Thermal taste sensations perceived include prototypical tastes of sweet, sour, salt, umami, and bitter, or other oral sensations (such as mint, metallic, spicy) [12, 16]. The reported taste sensations and temperature range at which they are experienced also vary across individuals [17]. In contrast, thermal non-tasters (TnTs) perceive only a change in temperature and no taste response. The prevalence of TTs in the population is reported to be between 20%[12] to 50%[15] highlighting its wide relevance, but the mechanism behind thermal taste is yet unclear. This phenomenon could arise peripherally from temperature-sensitive gustatory nerve fibres on the tongue [18], or centrally as TTs may have more temperature sensitive neurons where taste and temperature converge in the brain producing a higher gain within the afferent system, and thus a thermally-induced taste [15, 19]. Perceptual studies have shown that TTs display elevated taste perception with heightened responsiveness to pure taste samples (sweet, bitter, sour and salty) at supra-threshold levels[12, 19, 20], temperature stimuli [12, 16], metallic and astringent sensations [12, 21], and aroma samples[19]. However, other studies failed to find a significant impact of thermal taster phenotype on intensity responsiveness to some taste and trigeminal stimuli [12, 16, 22]. Only a single human fMRI study has explored the impact of thermal taster phenotype on the brain response to taste and somatosensory stimulation [22]. In that study, the cortical response of TTs and TnTs to cold (6 °C) gustatory (sweet) samples at varying levels of trigeminal stimulation elicited by carbonation (CO_2) was studied. The TT group perceived gustatory and trigeminal samples as significantly more intense than TnTs and were significantly more discriminating of the CO_2 level. fMRI data revealed that the TT group showed elevated cortical activation to the un-carbonated sweet sample compared to the TnT group in taste, oral somatosensory and reward processing areas of the brain.

Here, our primary aim was to determine how brain responses in the two thermal taster phenotypes – thermal tasters (TTs) and thermal non-tasters (TnTs) – are modulated by taste/temperature interactions using sweet samples of cold (5 °C) and ambient (20 °C) temperature. The temperature of the samples was chosen to reflect beverages commonly consumed in soft drinks, whilst providing a large temperature difference.

2. Methods

2.1. Participants

The study was approved by the University of Nottingham Medical School Research Ethics Committee. All participants gave written informed consent before enrolling in the study. Recruitment questionnaires screened any volunteers with contraindications to MRI safety or those who had a known taste dysfunction. Twenty-four healthy participants (12 TTs and 12 TnTs, 10 males), age 25 ± 7 years (mean ± SD) were recruited from a pool of participants ($n = 130$) previously screened for 6-n-propylthiouracil (PROP) and thermal taster status. Although, current evidence suggests that the phenomena of PROP and thermal taster status are likely independent [12, 16], we choose to exclude PROP non-tasters to reduce individual variability in oral perception and heterogeneity in brain responses to taste samples from this phenotype as demonstrated in Eldeghaidy et al., [23]. Participants were invited to take part in two sessions, one for validation of phenotyping and one for functional MRI, on separate days.

2.2. PROP and thermal taster status classification

All 24 participants were invited to a phenotyping session to reconfirm their previously determined taste phenotype. PROP taster status was defined based on the bitterness intensity ratings of 0.32 mM PROP (Sigma Aldrich, UK) prepared in deionised water from a reverse osmosis unit, presented and classified according to a method described by Lim, et al.[24]. Perceived intensity was rated on the general Labelled Magnitude Scale (gLMS) [25], a continuous category-ratio scale (no sensation, barely detectable, weak, moderate, strong, very strong, and strongest imaginable sensation of any kind) recommended for valid taste comparisons across individuals. Participants were instructed to mark along a continuous vertical line to register their perceived intensity of sensations. Training on use of the gLMS was given prior to data collection, as described in Yang et al.[16].

Thermal Taster Status was assessed using a Medoc Pathway with intra-oral ATS (advanced thermal stimulator) thermode (Medoc, Israel) applied to the tip of the tongue, as described previously in Hort et al. [22]. Participants placed the intra-oral thermode (6 mm diameter round surface) on the anterior tongue tip, the area which has shown to be most responsive to thermal taste [15] and where fungiform papillae are most densely innervated [26]. Participants were instructed to hold the thermode firmly on the tongueduring all temperature trials.Two warming and two cooling trials were delivered following the procedure of Bajec and Pickering [12]. All temperature changes occurred at a rate of 1 °C/s, with the surface temperature of the tongue being at approximately 35 °C during the baseline [27]. The warming trials commenced at 35 °C, cooled to 15 °C and re-warmed to 40 °C where it was held for 1 s (work by Cruz and Green [15] showed that precooling the tongue (15–20 °C) is important to trigger the thermal taste sensation during the warming stimulation). The cooling trials started at 35 °C, cooled to 5 °C where it was held for 10 s before rising to baseline (35 °C). Participants were instructed to 'attend' to the temperature increasing from 15 to 40 °C during the warming trial, and from 35 to 5 °C of the cooling trial. At the end of each trial, the participant rated the intensity of the warming and cooling temperature when it reached its maximum on a gLMS [16], respectively. If a taste/s was perceived, a second gLMS was presented so each of the perceived taste qualities (sweet, salty, bitter, sour, umami, 'metallic' and 'other') and the intensity of the taste(s) could be rated. The study by Skinner et al. 2018 [17] demonstrates the variations in the taste quality and temperature range over which thermal taste is perceived for such warming and cooling trials among thermal tasters. Warming trials always preceded cooling trials to avoid possible adaptation from the intense, sustained cold stimulation [19], and participants were told to wait until tongue temperature and sensation had returned to normal before proceeding onto the next trial, with a minimum of two minutes rest between trials. Thermal tasters were classified

as those participants who perceived a taste, above weak on the gLMS scale (> 6 on the gLMS, equivalent to >0.78 on the log scale) during both replicates of either the warming or cooling trial. TnTs were classified as those participants who only perceived temperature and no associated taste during warming/cooling of the tongue.

2.3. Tastant samples and stimulus delivery

Two sweet taste samples were prepared on the morning of the fMRI scan session, 'cold sweet' and 'ambient sweet'. The taste samples consisted of 87 mM sucrose (Silver Spoon, UK), prepared with Evian water (Evian, Danone, France) which were delivered to participants at cold (5 ± 2 °C) and ambient (20 ± 2 °C) temperatures. Cold samples were stored in a freezer for approximately 1 hour to reach 5 °C, and then stored in the refrigerator to maintain the temperature.

The tastants were delivered to the participants whilst inside the scanner using nozzles placed in the middle of the subjects' mouth which were connected to an automated spray delivery system [28]. The delivery system was placed outside the scanner room, controlled by Presentation software (Neurobehavioral Systems, http:// www.neurobs.com) which was triggered by the scanner TTL pulse. To maintain the temperature of the cold sample during scanning, the reservoir of the cold sample was surrounded with an ice pack, and ice cubes made from 87 mM sucrose solution were added to the bottle frequently to maintain the solution temperature. The temperature of each solution was monitored via afiber optic thermometer throughout the scan session. The cold sample was flushed through the tube immediately before each subject to eliminate any solution that may have warmed inside the tube.

2.4. fMRI collection

2.4.1. fMRI paradigm

Participants were instructed to have a light breakfast on the day of the scan session and restrict eating or drinking any strong flavoured food for 2 h prior to the scan. One cycle of the fMRI paradigm is shown schematically in Fig. 1. In each cycle, 3 mL of each sample was delivered over a 3 s period in a pseudo-random order. Following sample delivery, participants were instructed by a visual cue to swallow (Presentation Software, Neurobehavioral System, San Francisco, US). Surface electromyography (EMG) was acquired concurrently with the fMRI data acquisition [23] to determine the exact time of swallow of the sample, thus allowing the duration each sample remained in the mouth to be calculated and subsequently used in the fMRI data analysis. At 10 s following sample delivery, 3 mL of still mineral water (Evian, Danone, France**)** 'water wash' at ambient temperature was delivered (1 mL/s) to clear the oral cavity of any lingering sample. After the wash, participants were instructed to rate the perceived sweetness intensity of each sample on a 4-point category scale, using a button press of 1, 2, 3 or 4 corresponding to weak, moderate, strong and very strong, subjects were instructed to equate these levels to those used on the gLMS scale on which they had been previously trained. Subjects were given clear instructions to consider the level of taste perception to the sucrose stimulus following the sample delivery and retained this for the 17 s period before the button response. This time period was included to ensure a clean haemodynamic response could be collected in response to the taste stimulus, without any confounding effects on the haemodynamic response function due to the button press on the 4-point category scale.

A delay of 8.8 s was allowed before repeating the entire cycle, resulting in the total cycle duration of 28.8 s. For each subject, 18 cycles of delivery of the 'cold sweet' and 'ambient sweet' sample were delivered in a randomised design.

2.4.2. fMRI data acquisition

MRI data was acquired on a 3 T Philips Achieva scanner with a 16-ch receive coil. fMRI data was collected using a double-echo gradient-echo, echo-planar-imaging (GE-EPI) acquisition: TE = 25/40 ms, TR = 2500 ms, flip angle (FA) 85°, 3 mm isotropic spatial resolution, 192 × 192 mm^2 field of view (FOV), SENSE factor 2 in the right-left (RL) direction, and 36 slices aligned parallel with AC-PC plane. Following the fMRI data collection, a multi-echo T_2*-weighted dataset was collected (TE: 11, 30, 49, 68, and 87 ms; TR: 10 s) for combining the dual-echo GE-EPI fMRI data. In addition, a T_1-weighted MPRAGE image (1 mm isotropic resolution; TE/TR = 8.3/3.8 ms, FA = 8°, SENSE factor = 2, 160 slices, 256 × 256 matrix) was collected to aid registration of the fMRI data to MNI space.Each participant's scan took approximately 30 min to complete.

2.5. Data analysis

2.5.1. Analysis of the perceptual data

All statistical analysis of the perceptual data was performed using SPSS version 21 (SPSS IBM, USA). A Shapiro-Wilk test was used to test the normality of the data. Parametric data is expressed as mean (± SD) and non-parametric as median (interquartile range, IQR). For any statistical test performed a $p<0.05$ was considered statistically significant.

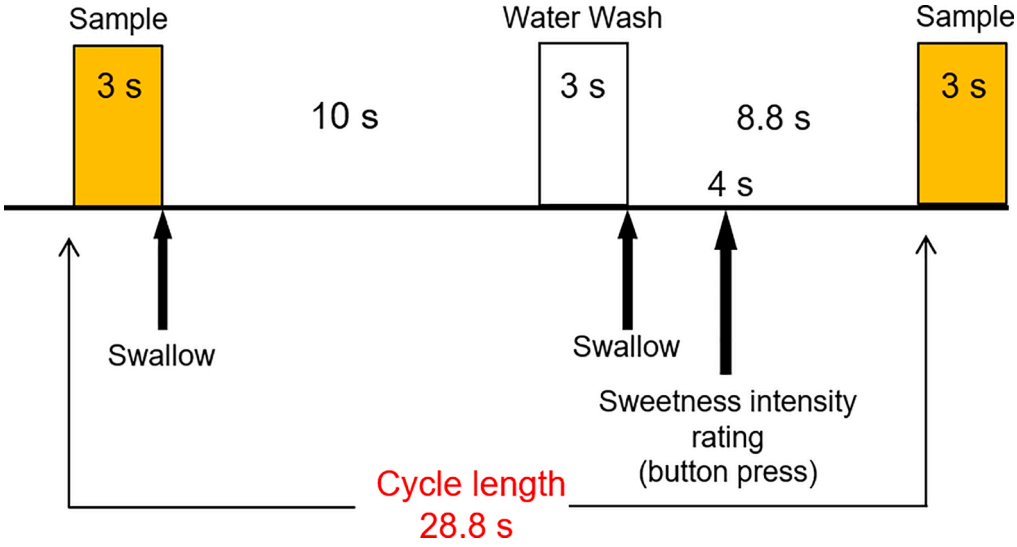

Fig. 1. One cycle of the fMRI paradigm. 3 mL of each sample was delivered over a 3 s period. Subjects were instructed by a small visual cue to swallow. At 10 s following sample delivery, a water wash was delivered. Subjects were then asked to rate the perceived sweetness of the sample received by a button press. A delay of 8.8 s was allowed before repeating the cycle.

To re-confirm thermal taster status, the quality and intensity of any perceived thermally-induced taste reported during the phenotyping session was assessed, and the percentage of thermally-induced taste quality sensations for the warming and cooling temperature trials was collated. gLMS intensity ratings for thermally-induced taste and temperature were $\log_{10}$ transformed and averaged across the two replicates (0 ratings were adjusted to 0.4 prior to transformation). The mean perceived temperature intensity during the cooling and warming trials was calculated for the TT and TnT group. A two-way ANOVA was used to assess the effect of group (TT, TnT) and thermode temperature (warming, cooling) on the temperature intensity rating and any interaction.

Perceived sweetness intensity ratings for the 'cold sweet' and 'ambient sweet' samples recorded during the fMRI session were collated. Since 18 replicates were collected during the fMRI paradigm, this allowed the assessment of the effect of the number of repeats on sweetness perception rating. For each participant, the sweetness intensity rating for the 'cold sweet' and 'ambient sweet' sample was assessed for the first trial only (as typically used in perception studies) and also the mean of the 18 replicates. Data for the first trial and mean of 18 replicates were then grouped for TTs and TnTs to assess the effect of thermal taste phenotype on sweetness perception rating. A two-way ANOVA compared the effect of group (TT, TnT) and sample ('cold sweet', 'ambient sweet') on the sweetness intensity rating and any interaction for both the first trial and mean of 18 replicates.

To assess trial-by-trial modulation, sweetness intensity ratings (mean (±SD) across participants) were plotted against trial number and a linear regression (r) computed for each individual. To assess whether thermal taster status impacted on sweetness adaptation, the median linear regression coefficient across trials was evaluated for the 'cold sweet' and 'ambient sweet' samples for each group (TT, TnT) (a Shapiro-Wilk normality test showed the data to be non-normal, [29] and a nonparametric 2-tailed Wilcoxon test assessed significant differences between group (TT and TnT) and sample temperature (cold and ambient).

2.5.2. Analysis of fMRI data

fMRI data was processed using in-house software and SPM (Statistical Parametric Mapping, Wellcome Department of Imaging Neuroscience; www.fil.ion.ucl.ac.uk/spm). First, the double-echo fMRI data were combined voxel-wise in a weighted summation based on the T_2* maps [30]. T_2* maps were formed from a linear, weighted least squares fit of the multi-echo data set. The fMRI data was then corrected for slice timing and motion. Individual motion parameter plots were visually inspected to ensure no subject moved > 1 voxel during the fMRI scan. Data were normalised to the MNI template, and spatially smoothed with an 8 mm Full width at half maximum (FWHM).

A first level general linear model (GLM) analysis was performed for each subject to generate contrast maps to the 'cold sweet' and 'ambient sweet' samples, using the time each sample remained in the mouth calculated from the EMG trace as the sample duration. This sample duration was convolved with a canonical hemodynamic response function (HRF) of the fMRI signal, and the data temporally filtered with a 60 s high pass filter cut-off. The 'water wash', button press response and motion parameters were included as covariates of no interest.

A second level random effects (RFX) group analysis was then performed. First, group statistical maps to the 'cold sweet' and 'ambient sweet' samples were generated across all participants, regardless of thermal taste phenotype. A two-sample *t*-test between 'cold sweet' and 'ambient sweet' samples was then performed to determine whether temperature modulates the cortical response to sweet samples independent of thermal taste phenotype (threshold $p < 0.001$, cluster size (k) > 20). Subsequently, to determine whether temperature modulates the response to sweet samples differently across thermal tasters groups, a paired *t*-test between the 'cold sweet' and 'ambient sweet' samples was performed for each of the TT and TnT groups, and a two-sample *t*-test performed between the TTs and TnT groups to 'cold sweet' and 'ambient sweet' samples (threshold p ⟨ 0.005 uncorrected, k ⟩ 20).

Region of interest (ROI) analysis was performed based on *a priori* brain regions related to taste and temperature to interrogate the beta (β) value of the taste and temperature responses, representing the magnitude of the stimulus fMRI response to the 'cold sweet' and 'ambient sweet' samples. These ROIs comprised the insula [subdivided into 8 mm spheres centered on the anterior-mid insula (40, 10, −2), mid insula (40, 0, 0) and posterior (44, −32, 12)] (Eldeghaidy et al. 2011), oral somatosensory cortex [primary somatosensory cortex, SI an 8 mm sphere centered at (60, −6, 20), and secondary somatosensory cortex, SII (BA 43)] using WFU PickAtlas (SPM) [31]. The mean of the top 5 % of the β-value was calculated for each ROI, as performed in prior studies [32, 33]. This analysis approach ensured the assessment of the activity in

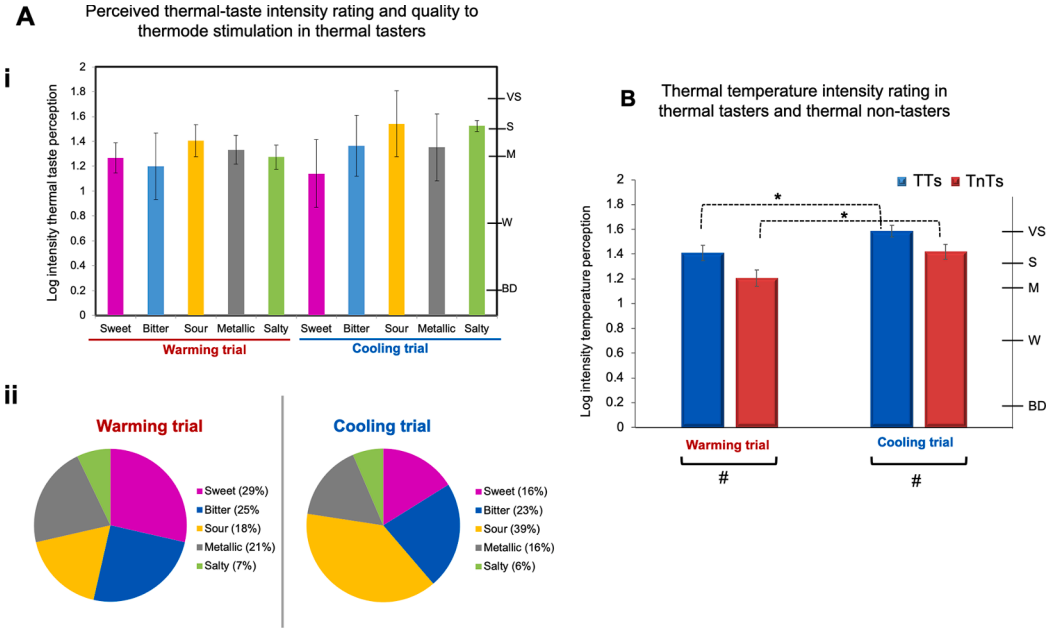

Fig. 2. Perceptual responses collected during the phenotyping sessionacross 11 TTs and 11 TnTs. A) (i) Taste intensity and (ii) distribution of taste quality experienced by thermal tasters during thermal stimulation (warming and cooling) of the tongue. Error bars show standard error. Secondary scale indicates labels on the gLMS: BD = barely detectable, W = weak, M = moderate, S = strong, VS = very strong. B) Temperature intensity rating of thermal taster (TTs) and thermal non-taster (TnTs) groups during thermal stimulation of the tongue. Error bars show standard error. Secondary scale indicates labels on the gLMS A 2-way ANOVA showed significantly higher temperature intensity ratings in the TT than TnT group (F= 9.83; p = 0.003), and significantly higher responses for the cooling trial (F = 10.94; p = 0.002). * indicates significant difference between temperature trials for TT and TnT and # indicates significant difference between TT and TnT groups across temperature trials.

each functional area with a high signal-to-noise ratio, while accounting for any between-subject functional variability (for example, arising due to differences in cortical folding patterns). A 2-way Multivariate ANOVA (MANOVA) test ($\alpha = 0.05$) was performed to assess the effect of group (TT, TnT) and sample ('cold sweet', 'ambient sweet') on the ROI cortical responses and any interaction effects.

To assess whether sweetness intensity reported to the 'cold sweet' and 'ambient sweet' samples during the fMRI scan session was associated with cortical activation to the 'cold sweet' and 'ambient sweet' samples, a Pearson correlation analysis was performed between each subject's 'sweetness intensity' rating and the ROI β-values to 'cold sweet' and 'ambient sweet' samples. To assess whether subjective perceptual temperature sensitivity was associated with the cortical response to the 'cold sweet' and 'ambient sweet' samples, a Pearson correlation analysis was performed between each subject's 'temperature intensity' rating reported to the warming and cooling temperature trials during their phenotyping session and the ROI β-values to 'cold sweet' and 'ambient sweet' samples.

3. Results

3.1. Perceptual responses

During phenotyping, thermal tasters reported the intensity of thermally-induced tastes between weak and strong on the gLMS, with an average intensity rating across all tastes of just below moderate (Fig. 2A (i)). Sweet taste quality was the most frequently reported (29%) thermal taste during the warming trial, and sour taste (39%) during the cooling trial (Fig. 2A(ii)). During thermal stimulation of the tongue, TTs perceived the temperature intensity of warming and cooling applied to the tongue as significantly more intense than TnTs ($F = 9.83$, $p = 0.003$), whilst the temperature intensity for cooling of the tongue was significantly greater than for warming ($F = 10.94$, $p = 0.002$), Fig. 2B. However, there were no interaction effects between thermal taster phenotype (TT/TnT) and trial type (warming/cooling).

Fig. 3 shows the mean sweetness intensity rating for the 'cold sweet' and 'ambient sweet' samples delivered during the fMRI session for the first trial (Fig. 3A) and mean across all 18 trials (Fig. 3B) for each thermal taster group. No significant effect of either thermal taster phenotype (first trial: $F = 0.123$, $p = 0.727$; mean of 18 trials: $F = 1.50$, $p = 0.23$) or sample temperature (first trial: 1.74, $p = 0.194$; mean of 18 trials: $F = 0.8$, $p = 0.38$) was found on sweetness intensity perception, or any interaction effects (first trial: 0.123, $p = 0.194$; mean of 18 trials: $F = 0.34$, $p = 0.56$). Interestingly, sweetness intensity perception shows a different response for the first trial compared to the mean across the 18 trials. The first trial shows a trend of increased sweetness perception for the 'ambient sweet' sample compared to the 'cold sample' for TTs, whilst for the mean of the 18 trials the 'cold sweet' sample shows a trend of increased sweetness intensity perception in TTs.

The perception of sweetness intensity across the 18 replicates showed a significant adaptation/reduction of the sweetness intensity rating to the 'ambient sweet' sample ($r = -0.41$, $p = 0.014$), but no significant adaptation/reduction to the 'cold sweet' sample, Fig. 4A. There was no significant difference in adaptation regression coefficients between thermal taster groups for the 'cold sweet' ($p = 0.657$) or 'ambient sweet' ($p = 0.959$) samples, Fig. 4B.

3.2. fMRI responses

3.2.1. Modulation of the brain's response with sweet sample temperature

The group activation maps for all participants (both TT and TnT groups) in response to the 'cold sweet' samples are shown in Fig. 5A. Strong responses are seen in somatosensory cortices (SI and SII), anterior and mid insula areas, anterior cingulate gyrus (ACC), amygdala, and thalamus. Fig. 5B shows that temperature modulated the cortical response across all participants to sweet taste, with increased activations in bilateral anterior insula [(44, 16, −10, $Z = 3.76$); (50, 14, −4, $Z = 3.33$)] and left mid insula [(−36, −6, 2), $Z = 3.47$] to the 'cold sweet' sample compared to the 'ambient sweet' sample ('cold sweet' >'ambient sweet'). No brain regions showed increased activation to the 'ambient sweet' sample compared to the 'cold sample'.

3.2.2. Thermal tasters have heightened brain responses to the sweet samples compared with thermal non-tasters

Fig. 6 shows the brain responses comparing 'cold sweet' and 'ambient sweet' samples for the thermal tasters and thermal non-tasters groups. Temperature modulated cortical responses to 'sweet' stimuli differently between thermal tasters groups. In TTs (Fig. 6A), the 'cold sweet' sample resulted in a significant increase in activation in bilateral anterior insula [(42, 16, −10), $Z = 3.03$; (−44, 18, −4), $Z = 2.94$)], left frontal operculum [(−50, 12, −4), $Z = 2.90$], and precentral gyrus [(−36, −2, 48) $Z = 2.96$] compared to the 'ambient sweet' sample. In TnTs, the 'cold sweet' sample resulted in a significant increase in activation in the right posterior insula [(42, −8, 10)$Z = 3.10$], bilateral mid frontal gyrus [(30, 50, −10), $Z = 2.89$; (−12, 60, 0)$Z = 3.19$], right superior temporal gyrus [(36, 6, −18), $Z = 3.11$], and left mid temporal gyrus [(−50, −16, −16), $Z = 3.74$] compared to the 'ambient sweet' sample.

The brain's response to both the 'cold sweet' and 'ambient sweet' samples was compared between TTs and TnTs. Fig. 7 shows the

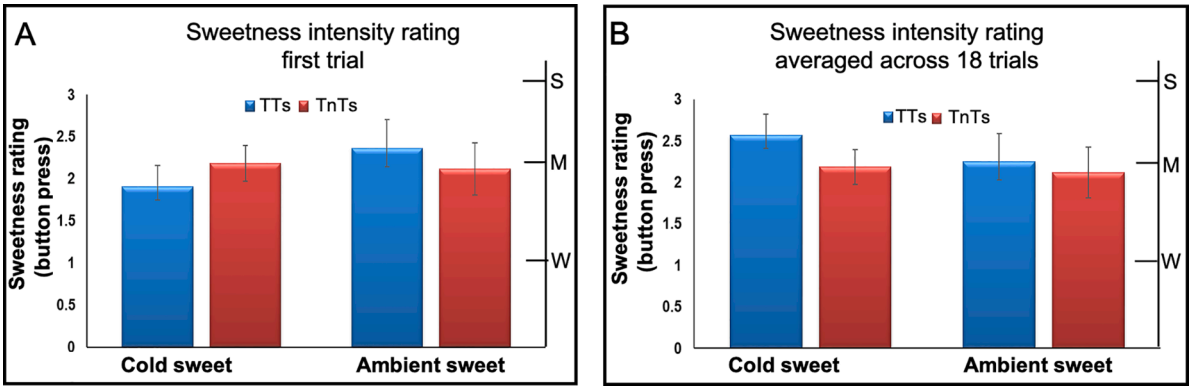

Fig. 3. Perceptual responses of sweetness intensity perceived during the fMRI scan session for the 'cold sweet' and 'ambient sweet' samples across all participants in the TT and TnT group for the first trial A) and averaged across 18 trials B). Error bars show standard error. A 2-way ANOVA model showed no significant difference in sweetness intensity ratings with thermal taster group (first trial: $F = 0.123$, $p = 0.727$; averaged 18 trials: $F = 1.50$, $p = 0.23$) or sample temperature (first trial: 1.74, $p = 0.194$; averaged 18 trials: $F = 0.8$, $p = 0.38$) on sweetness intensity perception, or any interaction effects between thermal taster groups and sample temperature (first trial: 0.123, $p = 0.194$; averaged 18 trials: $F = 0.34$, $p = 0.56$). Secondary scale indicates modified gLMS: W = weak (1), M = moderate (2), S = strong (3).

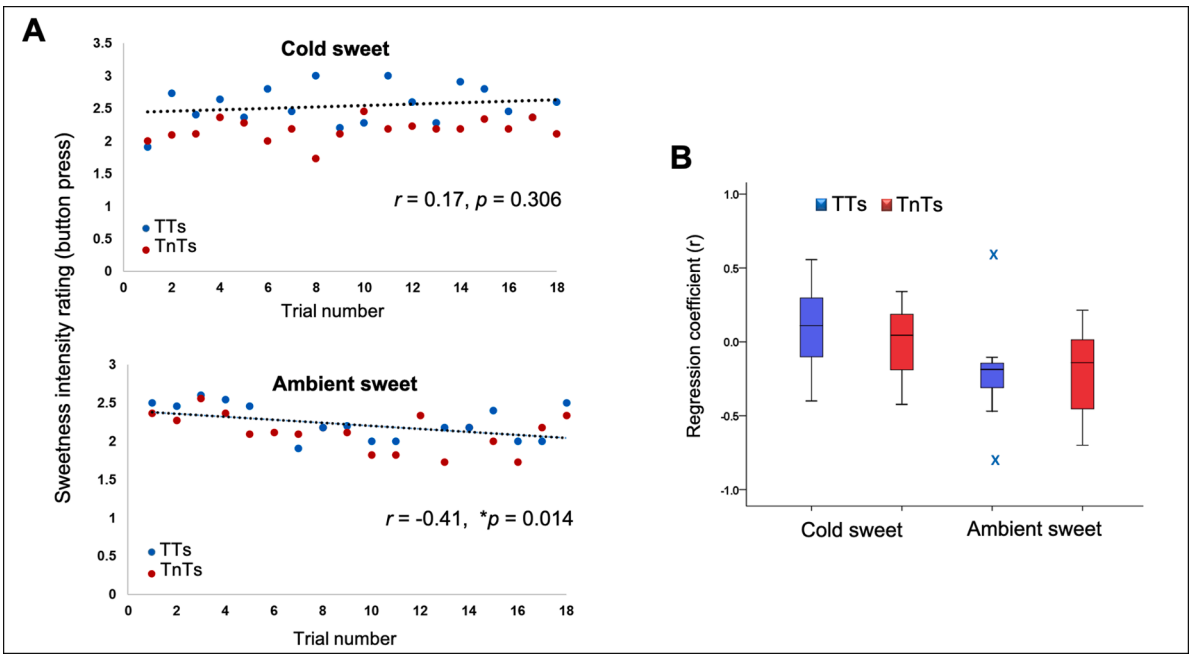

Fig. 4. Assessment of adaption of perception of sweetness intensity of fMRI trials. (A) Mean sweetness intensity ratings for 'cold sweet' and 'ambient sweet' samples across fMRI trials for thermal tasters (TTs) and thermal non-tasters (TnTs). A significant reduction of sweetness intensity over repeated trials was found for the 'ambient sweet' sample, which is not seen for the 'cold sweet' sample. (B) Comparison of regression coefficients for 'cold sweet' and 'ambient sweet' samples in TT and TnT groups. Median, first, and third quartiles together with minor and major outliers (x) are shown. No significant difference in adaptation with respect to sample temperature ($p = 0.657$) or group ($p = 0.959$) was found.

differential activation maps between TTs and TnTs to the 'cold sweet' and 'ambient sweet' samples. TTs showed increased cortical responses to the sweet samples regardless of the sample temperature. For the 'cold sweet' sample, a greater fMRI response was found in TTs compared to TnTs in somatosensory areas including bilateral somatosensory cortices: SI [(54, −10, 30) $Z = 6.4$; (−50, −10, 34) $Z = 4.9$] and SII [(60, −6, 14) $Z = 3.14$; (−64, −16, 12) $Z = 3.39$)], in addition to right superior temporal gyrus [(66, −20, 8) $Z = 4.14$]. For the 'ambient sweet' sample an increase in fMRI response was found in bilateral SI [(52, −12, 32) $Z = 7.1$; (−52, −10, 38) $Z = 6.3$], and right superior temporal gyrus [(62, −16, 6) $Z = 3.03$] in TTs compared with TnTs. There were no brain areas which displayed greater brain responses in TnT compared to TT.

AMANOVA test revealed a main significant effect of sample temperature ($F = 18.15$, $p<0.001$) and TT groups ($F = 6.29$, $p = 0.001$) on ROI cortical activation (Fig. 8) according to the Wilks' Lambda test. Overall, the cortical response to the 'cold sweet' sample was significantly stronger than the 'ambient sweet' sample, and responses in TT's were significantly higher than for TnTs. When assessing individual

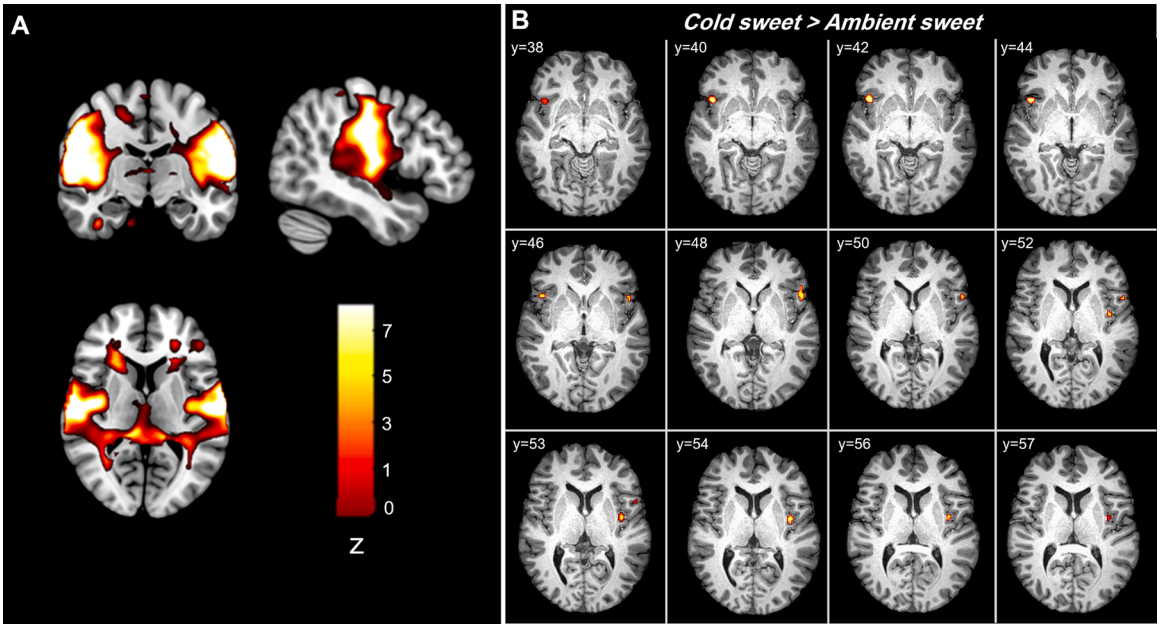

Fig. 5. A) Random effects group (RFX) maps showing the cortical response to the 'cold sweet' samples across all participants ($n = 22$). B) RFX differential maps to temperature of the sweet samples (cold > ambient) across all participants ($n = 22$). Maps displayed at $p < 0.001$ uncorrected, $k > 20$.

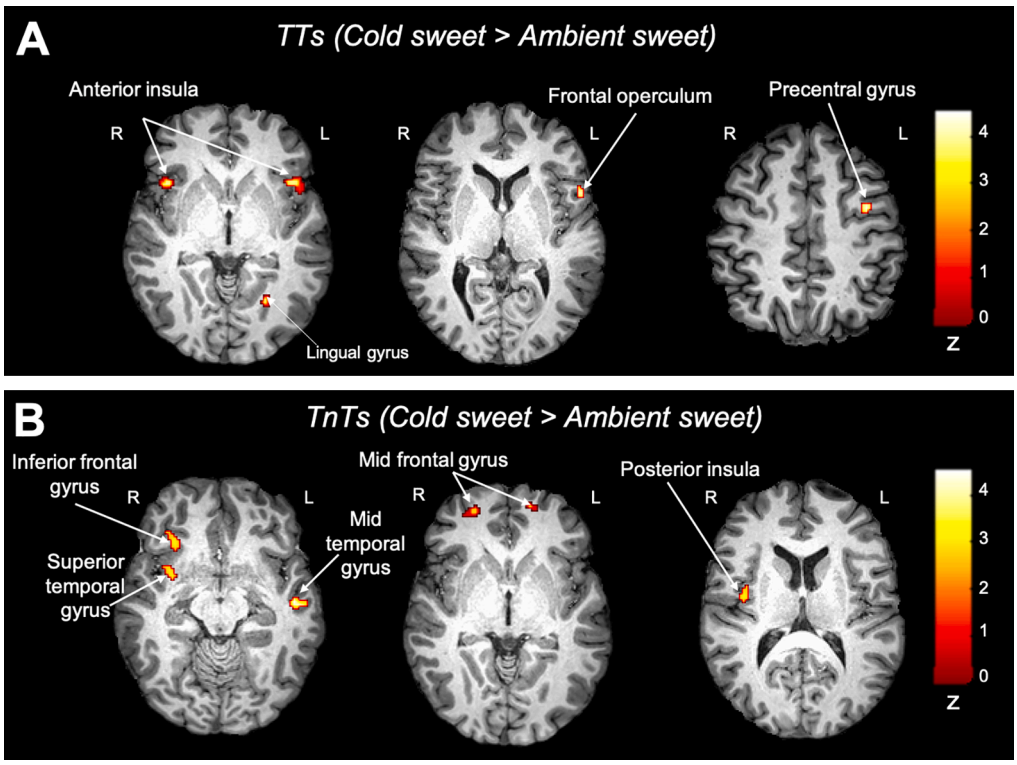

Fig. 6. Random effects group (RFX) differential maps for cold sweet >ambient sweet. A) thermal tasters (TTs) ($n = 11$) and (B) thermal non-tasters (TnTs) ($n = 11$). Maps displayed at $p < 0.005$ uncorrected, $k > 20$.

dependent variables, the effect of sample temperature and TT group was significant for all ROIs (anterior-mid insula (sample temperature: $F = 40.4$, $p < 0.001$; TT group: $F = 8.9$, $p < 0.005$), SI (sample temperature: $F = 8.4$, $p = 0.006$; TT group: $F = 4.8$, $p = 0.035$) and SII (sample temperature: $F = 19.4$, $p < 0.001$; TT group: $F = 11.3$, $p = 0.002$)) except for posterior insula (sample temperature: $F = 3.21$, $p = 0.081$; TT group: $F = 0.50$, $p = 0.482$). On studying interaction effects, a significant interaction between sample temperature and thermal taster status ($F = 3.77$, $p = 0.011$) was found across all ROIs, with a higher response to the 'cold sweet' sample in TTs. When assessing individual dependent variables, the interaction effect between sample temperature and TT group was only significant for SII ROI ($F = 9.29$, $p = 0.004$).

3.2.3. Correlation of subjective sweetness and temperature sensitivity with fMRI responses

No significant correlation was found between the individuals' (TTs and TnTs) sweetness intensity rating to the 'cold sweet' and 'ambient sweet' sample collected during the fMRI session and any of the cortical responses assessed in the ROI analysis. Fig. 9 assesses whether subjective sensitivity to temperature - as measured in the phenotyping session from the temperature intensity ratings in response to the cooling and warming thermode trials - was associated with the brains' response to the 'cold sweet' and 'ambient sweet' samples in SI and SII ROIs. A significant correlation was found between the SII β-values to the 'cold sweet' sample and sensitivity to the cooling ($r = 0.685$, $p < 0.001$) and warming

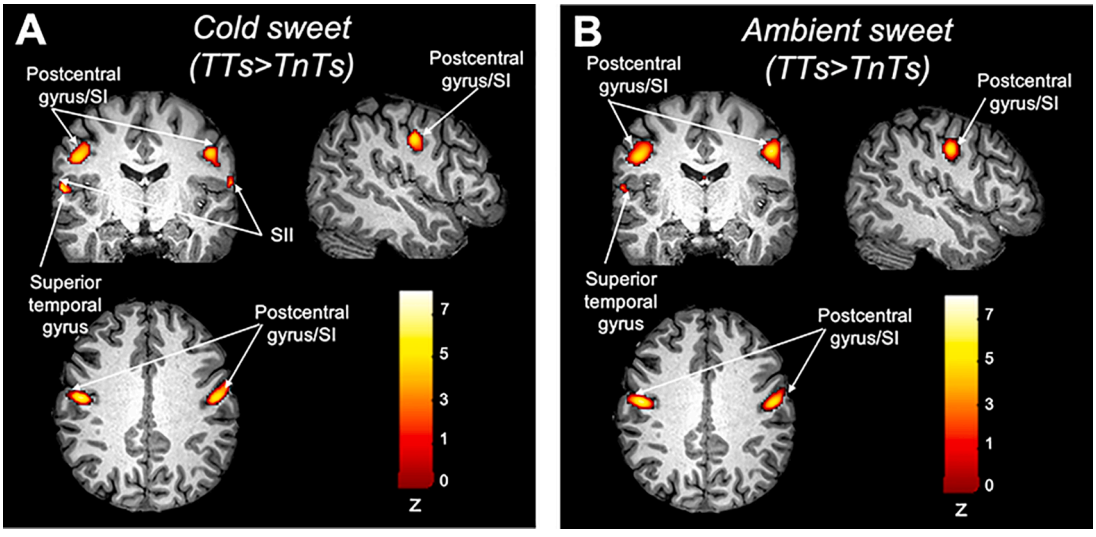

Fig. 7. Random effect group (RFX) differential maps for TTs >TnTs in response to (A) cold sweet sample and (B) ambient sweet sample, displayed at $p < 0.005$ uncorrected, $k > 20$.

($r = 0.634$, $p = 0.003$) trial, Fig. 9, but no significant correlation was found in SI. For the 'ambient sweet' sample, temperature intensity rating during the cooling trials was significantly correlated with SII β-values (cooling trial: $r = 0.44$, $p = 0.039$).

4. Discussion

4.1. Effect of thermal taster phenotype on perception of temperature and sweetness intensity

In agreement with previous perceptual studies [12, 16], thermal tasters perceived the temperature intensity of the thermode during the warming and cooling phenotyping trials to be significantly more intense than thermal non-tasters (Fig. 2). In addition, temperature intensity of the cooling trials was perceived significantly higher than the warming trials, across both groups.

During the fMRI session, temperature had no significant effect on the mean sweetness intensity perception rating measured (Fig. 3), and sweetness perception rating was not significantly different between thermal taster phenotypes. One possible reason for the lack of significance in our perceptual results is that we modified the continuous gLMS used in sensory studies to a four-point button press scale (weak, moderate, strong and very strong) to allow assessment of sweetness intensity perception during the fMRI scan session. This four-point integer scale will have reduced sensitivity to detect the small differences in sweetness intensity perception, and the use of a continuous gLMS should be considered for future fMRI studies. However, this finding of no significant effect of temperature on sweetness intensity is in agreement with Bajec et al. [34] and Schiffman et al. [35]. Conversely, other studies have shown that changing the temperature of a sweet solution does modulate sweetness perception significantly according to a linear relationship [1, 2, 3]. Discrepancies in findings between studies including the current study may arise due to differences in the sugar concentration, temperature of samples used across studies, number of repeats of sample delivery, sample size or rating scale.

In this study, sweetness intensity ratings of the 'cold sweet' and 'ambient sweet' samples collected during the fMRI scan session were evaluated over the first (single) trial, as typically used in sensory experiments, and over $n = 18$ replicates used in fMRI studies. Although sweetness intensity perception showed no significant difference between the 'cold sweet' and 'ambient sweet' samples, interestingly altered sweetness intensity perception responses were observed for the first trial compared with mean of the 18 trials. The trend of increased sweetness intensity perception for the first trial of the 'ambient sweet' sample in TTs in this study is in agreement with Schiffman et al. [35] and Bartoshuk et al. [36]who reported an increaseing trend of sweetness intensity rating for ambient (20 °C) and warm (50 °C) sweet samples compared with cold (6 °C and4 °C) sweet samples of the same sucrose concentration as used in the current study. Whereas, the trend of increased sweetness intensity perception for the 'cold sweet sample' across the 18 trials is in agreement with Bajec et al. (33), who measured sweetness intensity over 5 trials at cold (5 °C) and warm (35 °C) temperature. It is also important to note that the concentration of the sweet sample in the study by Bajec et al. was higher (250 mM) than that used in the current study (87 mM). The sweetness perception of the 18 trials reported in this study is closer to the real-life beverage consumption experience compared to a single trial perception, and the different trend between a single trial compared with 18 trials in thermal tasters highlights the importance of evaluating samples with repeated exposure in sensory perception studies. It would be of interest to explore the effect of repeated exposure ona wider range of sucrose concentrations to determine impact on sweet taste perception and whether modulations between thermal taster phenotypes are concentration dependent and more pronounced for greater sweetness concentrations.

The collection of a large number of sweet sample repeats allowed us to explore the effect of repeated delivery of 'cold sweet' and 'ambient sweet' samples on sweetness intensity perceptionon a trial-by-trial basis. The 'ambient sweet' samples showed a small, but significant attenuation in sweetness intensity rating across repeat trials (Fig. 4A(ii)) whereas no significant suppression in sweetness intensity rating was found for the 'cold sweet' sample (Fig. 4A(i)). Previous studies have assessed the effect of adaptation on sweetness perception and showed increased adaptation at cold compared with ambient and warm temperatures [37, 38, 39]. However, the majority of these studies assessed adaptation through dipping the tongue tip into sugar solutions for few seconds [37, 38] or through the sip and spit method [37, 39]. No study to-date has assessed the effect of sweetness adaptation on a trial-by-trial basis using a spray delivery system at two different temperatures. The effect of temperature on taste adaptation shown in this study may reveal new insights into sweetness taste perceptionand the effects of selective adaptation on

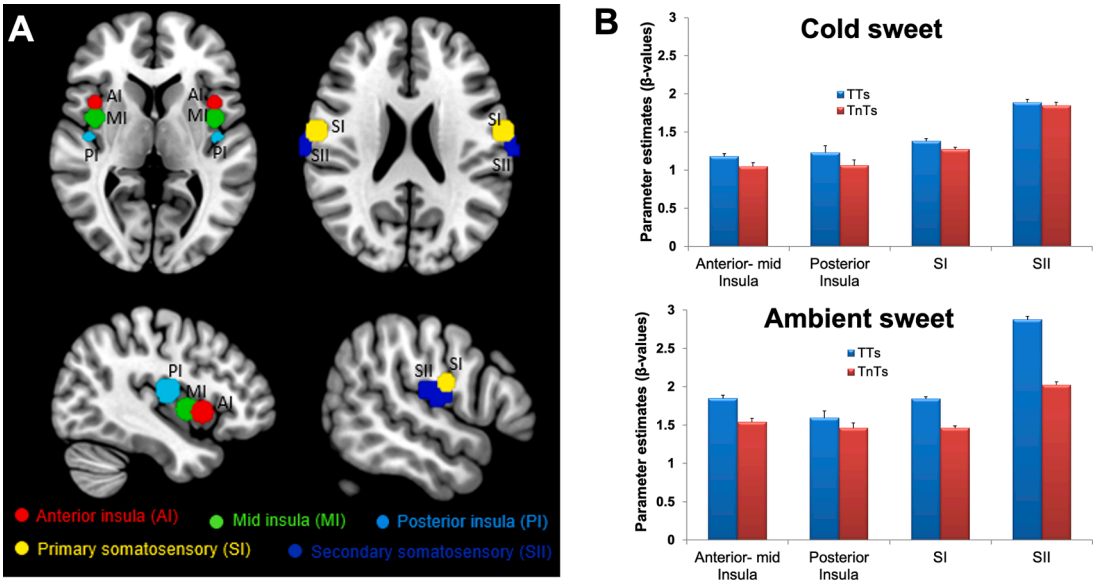

Fig. 8. A) Region of interest (ROI) interrogated in the fMRI analysis. B) ROI analysis for cold sweet and ambient sweet samples across thermal tasters (TTs) and thermal non-tasters (TnTs). A 2-way MANOVA test with TT groups and sample temperature showed a significant effect of sample temperature ($F = 18.15$, $p < 0.001$) and TT groups ($F = 6.29$, $p = 0.001$), and a significant interaction between sample temperature and TT group ($F = 3.77$, $p = 0.011$).

coding sweetness. Adaptation to a single taste stimulus may enhance sensitivity near adapted levels to improve detection of abrupt changes in ambient stimulus concentration. The weakening of ambient stimuli over time may serve as a dynamic coding function [40] by rapidly shifting the gustatory emphasis from the adapted stimulus to another distinct stimulus. Compounds that do not cross-adapt and activate independent taste receptors would then become more salient and identifiable.

4.2. Modulation of the brain's response by the temperature of sweet samples

Our fMRI data showed robust brain responses for 'cold sweet' and 'ambient sweet' samples across all subjects in both taste, reward and oral somatosensory areas (Fig. 5). The response to the 'cold sweet' sample was heightened compared to the 'ambient sweet' sample in anterior and mid- insula areas. The anterior insula is the primary taste cortex, with representation of taste, oral texture, and temperature [41, 42], whereas the mid-insula is an oral somatosensory area shown to respond to textural attributes of stimuli [23], and well as temperature [43]. Functional neuroimaging studies have previously documented function convergence between taste and somatosensory modalities [43, 44, 45] in the insula and oral somatosensory areas. To our knowledge, only one human neuroimaging study by Guest et al. has assessed the influence of changing temperature (5, 20 and 50 °C) on cortical representations [43]. In this, the authors reported an increased activation in the anterior insula taste cortex (identified by glucose taste stimuli), and mid insula, as well as the somatosensory cortex in response to cold, ambient and warm water samples. This study reported that the pleasantness of the stimulus liquids varied, with post-hoc tests showing that the 50 °C water solution was found less pleasant than the 5 °C and 20 °C solution, for which rated pleasantness did not differ. In the current study, we limit the sweet samples to 'cold' and 'ambient' temperatures, which represent the temperatures that most commonly beverages are served and consumed. Further, the study of Guest et al. was limited to water samples alone and did not explore whether the changes in temperature modulated the perception or activity in samples of a given taste attribute. Their finding of an increased fMRI response for 'cold water (5 °C)' > 'hot water (50 °C)' in anterior taste insula is in-line with the increased response in anterior insula in the current study for 'cold sweet' > 'ambient sweet', however the study of Guest et al. did not find a significant difference in the anterior insula for 'cold water (5 °C)' > 'ambient water (20 °C)'. The lack of significant difference in the anterior insula for 'cold water (5 °C)' > 'ambient water (20 °C)' in the Guest et al. study may be due to the fact their samples were water and so did not have enhanced sweetness at cold temperature, and the difference in the anterior insula for 'cold water (5 °C)' > 'hot water (50 °C)' may reflect temperature effects. In macaques, some neurons in the anterior taste insula are known to be tuned to oral temperature, some to taste, and some to both oral temperature and taste [46]. Here, the increased fMRI response to 'cold sweet' as compared to the 'ambient sweet' sample in the insula area likely reflect the effect of temperature as well as sweetness perception (taste), however, we cannot distinguish/uncouple these effects.

The perceptual data collected during the fMRI session showed a trend of increased sweetness intensity perception for the 'cold sweet' sample compared with the 'ambient sweet' sample in TTs. In addition, a significant attenuation in participants' rating of sweetness intensity perception was found for the 'ambient sweet' sample (Fig. 3c) which was not found for the 'cold sweet' sample, this may have caused a decrease in the fMRI response to the 'ambient sweet' sample compared with the 'cold sweet' sample.

4.3. A heightened cortical response in thermal tasters for cold sweet samples

Previous perceptual studies have shown that TTs have heightened perception to pure taste and oral temperature stimuli compared with

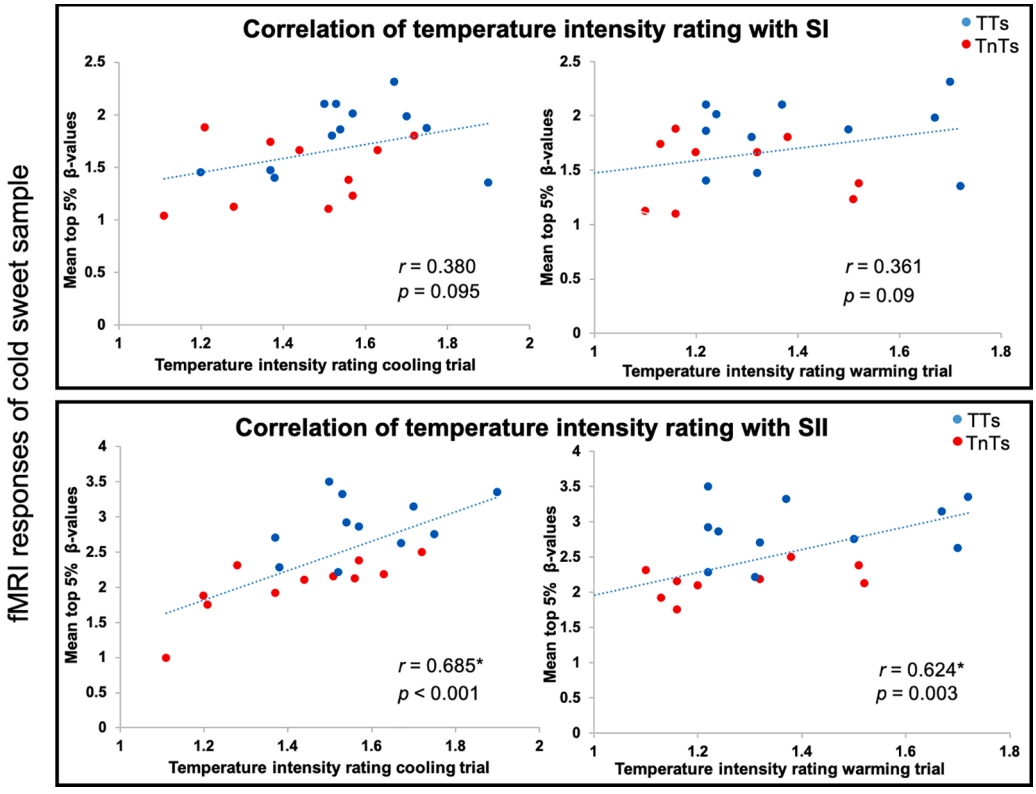

Fig. 9. Correlation of temperature intensity rating collected in response to the thermode warming and cooling trials during the phenotyping session, with ROI β-values to the 'cold sweet' sample in SI and SII. Blue data points show thermal tasters (TTs) and red data points show thermal non-tasters (TnTs). A significant correlation is seen in SII and a trend in SI, no other areas showed a significant correlation.

TnTs [12, 16, 19, 20]. Here, temperature modulated cortical responses to 'sweet' stimuli differently between thermal taster groups. In TTs, the 'cold sweet' sample induced increased cortical activation compared to the 'ambient sweet' sample in anterior insula, frontal operculum (taste area) and precentral gyrus (oral somatosensory areas), whilst the TnTs showed an increased response in posterior insula (oral somatosensory area), temporal gyrus (taste and somato-gustatory area) and frontal areas. The difference in activation patterns observed between TTs and TTs suggests that samples containing both taste and trigeminal stimulation/input are processed differently by the groups, supporting the previous findings of Hort et al. [22] for sweet carbonated samples.

Comparing the response between thermal taster phenotype, TTs showed a heightened cortical response compared to TnTs to the 'cold sweet' (primary and secondary somatosensory cortices) and 'ambient sweet' (primary somatosensory cortex) sample (Fig. 7), and an interaction effect in oral somatosensory areas (Fig. 8). These results are in general agreement with previous fMRI findings for 'cold carbonated sweet' samples [22]. The source of such individual differences in oral perception between thermal taster phenotype is more likely to be a central origin and unrelated to differences in the density of fungiform papillae. Evidence from recent studies[12, 47] has reported no association between thermal taster status and fungiform papillae density. The results of increased fMRI activation in taste and oral somatosensory areas in response to temperature and taste in this study support the hypothesis postulated by Green and colleagues (19, 20) ofthe central gain mechanism (greater excitability in gustatory and somatosensory brain regions in response to oral sensations) in thermal tasters compared with thermal non-tasters. It will be of interest in future studies to investigate whether the altered perception and fMRI responses seen between TTs and TnTs is related to differences in the brain's morphology between these groups.

4.4. Correlation between 'temperature intensity' thermal taster rating and fMRI responses

We show a significant correlation between the subjective temperature intensity rating perceived from the 'cooling trial' in the phenotyping session and the cortical responses in SI and SII to the 'cold sweet' sample (Fig. 9), but this was not seen for the 'ambient sweet' sample. Perceptual data collected during the thermal taste phenotyping session, and previous perceptual studies have shown that TTs perceive the temperature intensity of both warming and cooling trials as significantly more intense [16, 48], as well as being more sensitive to temperature changes than TnTs [16]. These findings suggest that the differences in activation in somatosensory areas between TTs and TnTs in response to temperature stimuli reported in the current study likely reflects heightened oral sensitivity to temperature reported in TTs and is a direct result of the increased cortical activation in oral somatosensory areas.

5. Limitations of the study

In this study, we did not collect control (tasteless/artificial saliva) samples at both 'cold' and 'ambient' temperatures to uncouple whether the response in the insula was due to temperature or taste perception. To address this specific question, control samples at both 'cold' and 'ambient' temperatures would be required to allow the comparison of the difference between the 'control trials at ambient and cold temperature' to the difference in 'sweet trials at ambient and cold temperature'. This would require a considerable increase in scan duration for each sample condition for sufficient trials, and double the number of sample conditions, which would have to be collected in a randomised order. Doing this would potentially limit reliable data being generated to address our primary goal of differences with taste phenotype, due to the potential of increased head motion effects with a long scan duration/ use of a large number of samples and thus fluid consumption/potential cross-over of adaptation effects and the need torandomize the order of delivery of sample conditions. This was outside the main scope of the study, which was to ascertain differences in process between thermal tasters and thermal non-tasters, but will be considered in future studies. Having now shown a difference between thermal tasters and thermal non-tasters, a follow-on study to address this question in thermal tasters alone could be performed.

Another limitation of this study was that perceptual ratings of temperature intensity to the fMRI samples were not collected. However previous studies have shown the perceived temperature intensity of a cold sample is significantly higher in TTs than for ambient samples [16]. Therefore, the increase in responses to cold sweet sample compared with ambient sample (cold > ambient) in the insula, frontal operculum and somatosensory cortices are more likely to be due to the perceptual differences in temperature than taste. It is also important to note that, although sweetness intensity perception ratings collected during the fMRI session (Fig. 3) showed no significant difference between the 'cold sweet' and 'ambient sweet' samples, thermal tasters showed an increased trend of sweetness intensity perception to the 'cold sweet' samples, which could also contribute the anterior insula activation.

6. Conclusion

This study has used brain imaging to demonstrate for the first time the effect of temperature ('cold' 5 °C versus 'ambient' 20 °C) on the perceptual and cortical response to sweetness in humans, and the effect of thermal taste phenotype. The temperature of the sweet samples modulated the brain's response in both taste and somatosensory areas, with a greater response for colder samples. There was an interaction effect between thermal taster status and temperature, with TTs showing selectively greater cortical responses to 'cold sweet' samples compared to TnTs. In summary, TTs demonstrate a temperature dependent central response to sweet perception compared to TnTs. These results contribute in understanding food perception and the impact of thermal taster status on perceiving everyday food and beverages products that require serving at cold temperatures, such as beer and cold soft drinks.

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Declaration of Competing Interest

The authors declare no competing financial interests.

References

[1] L. Bartoshuk, K. Rennert, J. Rodin, et al., Effects of temperature on the perceived sweetness of sucrose, Physiol. Behav. 28 (1982) 905–910.
[2] K. Lipscomb, J. Rieck, P. Dawson, Effect of Temperature on the Intensity of Basic Tastes: sweet, Salty and Sour, J. Food Res. 5 (2016) 1.
[3] C. Ross, K. Weller, Effect of serving temperaure on the sensory attributes of red and white wines, J. Sens. Stud. 23 (2008) 398–416.
[4] A.V. Cardello, The role of the human senses in food acceptance, London: Blackie Academic & Professional. (1996).
[5] R. Pramudya, H. Seo, Influences of product temperature on emotional responses to, and sensory attributes of, coffee and green tea beverages, Front. Psychol. 8 (2018) 2264.
[6] Q. Yang, R. Dorado, C. Chaya, et al., The impact of PROP and thermal taster status on the emotional response to beer, Food Qual. Prefer. 68 (2018) 420–430.
[7] A. Cardello, O. Maller, Acceptability of water, selected beverages and foods as a function of serving temperature, J. Food Sci. 47 (1982) 1549–1552.
[8] A. Calviño, Perception of sweetness: the effects of concentration and temperature, Physiol. Behav. 36 (1986) 1021–1028.
[9] M. Bajec, G. Pickering, N. DeCourville, Influence of stimulus temperature on orosensory perception and variation with taste phenotype, Chem. Percept. 5 (2012) 243–265.
[10] S. Schiffman, E. Sattely-Miller, B. Graham, et al., Effect of temperature, pH, and ions on sweet taste, Physiol. Behav. 68 (2000) 469–481.

[11] D. Wilson, C. Lemon, Modulation of central gustatory coding by temperature, J. Neurophysiol. 110 (2013) 1117–1129.
[12] M. Bajec, G. Pickering, Thermal taste, PROP responsiveness, and perception of oral sensations, Physiol. Behav. 95 (2008) 581–590.
[13] J. Hayes, S. Keast, Two decades of supertasting: where do we stand? Physiol. Behav. 104 (2011) 1072–1074.
[14] L. Bartoshuk, V. Duffy, I. Miller, PTC/PROP tasting: anatomy, psychophysics, and sex effects, Physiol. Behav. 56 (1994).
[15] A. Cruz, B. Green, Thermal stimulation of taste, Nature 403 (2000) 889–892.
[16] Q. Yang, T. Hollowood, J. Hort, Phenotypic variation in oronasal perception and the relative effects of PROP and thermal taster status, Food Qual. Prefer. 38 (2014) 83–91.
[17] M. Skinner, S. Eldeghaidy, R. Ford, et al., Variation in thermally induced taste response across thermal tasters, Physiol. Behav. 188 (2018) 67–78.
[18] K. Talavera, K. Yasumatsu, T. Voets, et al., Heat activation of TRPM5 underlies thermal sensitivity of sweet taste, Nature 438 (2005) 1022–1025.
[19] B. Green, P. George, Thermal taste' predicts higher responsiveness to chemical taste and flavor, Chem. Senses 29 (2004) 617–628.
[20] B. Green, M. Alvarez-Reeves, P. George, et al., Chemesthesis and taste: evidence of independent processing of sensation intensity, Physiol. Behav. 86 (2005) 526–537.
[21] G.J. Pickering, A. Moyes, M.R. Bajec, et al., Thermal taster status associates with oral sensations elicited by wine, Aust. J. Grape Wine Res. 16 (2010) 361–367.
[22] J. Hort, R. Ford, S. Eldeghaidy, et al., Thermal taster status: evidence of cross-modal integration, Hum. Brain Mapp. 37 (2016) 2263–2275.
[23] S. Eldeghaidy, L. Marciani, F. McGlone, et al., The cortical response to the oral perception of fat emulsions and the effect of taster status, J. Neurophysiol. 105 (2011) 2572–2581.
[24] J. Lim, L. Urban, B.G. Green, Measures of individual differences in taste and creaminess perception, Chem. Senses 33 (2008) 493–501.
[25] L. Bartoshuk, V. Duffy, B. Green, et al., Valid across-group comparisons with labeled scales: the gLMS versus magnitude matching, Physiol. Behav. 8 (2004) 109–114.
[26] M. Shahbake, I. Hutchinson, D. Laing, et al., Rapid quantitative assessment of fungiforrn papillae density in the human tongue, Brain Res. 1052 (2005) 196–201.
[27] T. Spierings, M. Peters, A. Plasschaert, Surface temperature of oral tissues. A review, J. Biol. Buccale 12 (1984) 91–99.
[28] L. Marciani, J.C. Pfeiffer, J. Hort, et al., Improved methods for fMRI studies of combined taste and aroma stimuli, J. Neurosci. Methods 158 (2006) 186–194.
[29] S. Shapiro, M. Wilk, An analysis of variance test for normality (complete samples), Biometrika 52 (1965) 591–611.
[30] S. Posse, S. Wiese, D. Gembris, et al., Enhancement of BOLD-contrast sensitivity by single-shot multi- echo functional MR imaging, Magn. Reson. Med. 42 (1999) 87–97.
[31] J.A. Maldjian, P.J. Laurienti, R.A. Kraft, et al., An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets, Neuroimage 19 (2003) 1233–1239.
[32] G. Fernandez, S. Weis, B. Stoffel-Wagner, et al., Menstrual cycle-dependent neural plasticity in the adult human brain is hormone, task, and region specific, J. Neurosci. 23 (2003) 3790–3795.
[33] G. Mitsis, G. Iannetti, T. Smart, et al., Regions of interest analysis in pharmacological fMRI: how do the definition criteria influence the inferred result, Neuroimage 40 (2008) 121–132.
[34] M. Bajec, G. Pickering, N. DeCourville, Influence of stimulus temperature on orosensory perception and variation with taste phenotype, Chem. Percept. 5 (2012) 243–265.
[35] S. Schiffman, E. Sattely-Miller, B. Graham, et al., Effect of temperature, pH, and ions on sweet taste, Physiol. Behav. 68 (2000) 469–481.
[36] L. Bartoshuk, K. Rennert, J. Rodin, et al., Effects of temperature on the perceived sweetness of sucrose, Physiol. Behav. 28 (1982) 905–910.
[37] B. Green, D. Nachtigal, Orosensory factors in taste perception: effects of active tasting and solution temperature, Physiol. Behav. 107 (2012) 488–495.
[38] B. Green, D. Nachtigal, Temperature Affects Human Sweet Taste via At Least Two Mechanisms, Chem. Senses 40 (2015) 391–399.
[39] H. Schifferstein, J. Frijters, Sweetness Does Not Habituate During a Sip-and-Spit Experiment, Physiol. Behav. 51 (1992) 331–336.
[40] M. Frank, H. Goyert, B. Formaker, et al., Effects of selective adaptation on coding sugar and salt tastes in mixtures, Chem. Senses 37 (2012) 701–709.
[41] R. Shura, R. Hurley, K. Taber, Insular cortex: structural and functional neuroanatomy, J. Neuropsychiatry Clin. Neurosci. 26 (2014) 276–282.
[42] E. Rolls, Functions of the anterior insula in taste, autonomic, and related functions, Brain Cogn. 110 (2015) 4–19.
[43] S. Guest, F. Grabenhorst, G. Essick, et al., Human cortical representation of oral temperature, Physiol. Behav. 92 (2007) 975–984.
[44] B. Cerf-Ducastel, P. Van de Moortele, P. MacLeod, et al., Interaction of gustatory and lingual somatosensory perceptions at the cortical level in the human: a functional magnetic resonance imaging study, Chem. Senses 26 (2001) 371–383.
[45] K. Rudenga, B. Green, D. Nachtigal, et al., Evidence for an intgrated oral sensory module in the human ventral insula, Chem. Senses 35 (2010) 693–703.
[46] J. Verhagen, M. Kadohisa, E. Rolls, The primate insular/opercular taste cortex: neuronal representations of the viscosity, fat texture, grittiness, temperature and taste of foods, J. Neurophysiol. 92 (2004) 1685–1699.
[47] S. Eldeghaidy, D. Thomas, M. Skinner, et al., An automated method to detect and quantify fungiform papillae in the human tongue: validation and relationship to phenotypical differences in taste perception, Physiol. Behav. 184 (2018) 226–234.
[48] M.R. Bajec, G. Pickering, Thermal taste, PROP responsiveness, and perception of oral sensations, Physiol. Behav. 95 (2008) 581–590.