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Thermal taster status: evidence of cross-modal integration.

Abbreviated title: Cortical response in thermal taster status

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Abstract

Thermal taster status refers to the finding that, in some individuals, thermal stimulation of the tongue elicits a phantom taste. Little is known regarding the mechanism for this, it is hypothesised to be a result of cross-wiring between gustatory and trigeminal nerves whose receptors co-innervate papillae on the tongue.

To address this, we use functional magnetic resonance imaging to perform the first study of whether the cortical response to gustatory-trigeminal samples is altered with thermal taster status. We study the response to cold (6°C) gustatory (sweet) samples at varying levels of trigeminal stimulation elicited by CO_2 (no CO_2, low CO_2, high CO_2) in thermal taster (TT) and thermal non-taster (TnT) groups, and evaluate associated behavioural measures.

Behaviourally, the TT group perceived gustatory and trigeminal stimuli significantly more intense than TnTs, and were significantly more discriminating of CO_2 level. fMRI data revealed elevated cortical activation to the no CO_2 sample for the TT group compared to TnT group in taste, oral somatosensory and reward areas. In TnTs, a significant positive modulation in cortical response with increasing level of CO_2 was found across taste, somatosensory and reward areas. In contrast, in TTs, a reduced positive modulation with increasing level of CO_2 was found in somatosensory areas (SI, SII), whilst a significant negative modulation was found in taste (anterior insula) and reward (ACC) areas. This difference in cortical response to trigeminal stimuli supports cross-modal integration in TTs, with gustatory and trigeminal nerves highly stimulated by cold gustatory samples due to their intertwined nature.

Introduction

The perception of taste is known to vary widely across individuals. There are many factors that contribute to an individual's taste perception and subsequent food preferences, including the density of taste papillae on the tongue and genetic differences in taste receptors (Bajec and Pickering, 2010; Hayes and Keast, 2011). Functional magnetic resonance imaging (fMRI) studies report that the primary taste cortex is located within the anterior insula/frontal operculum (Small et al., 1997; Small et al., 1999; Veldhuizen et al., 2011) with secondary projections to the orbitofrontal cortex (OFC) (Francis et al., 1999), amygdala (O'Doherty et al., 2001), anterior cingulate cortex (ACC) (Small et al., 2003), ventral striatum (O'Doherty et al., 2003), and dorsolateral prefrontal cortex (Kringelbach et al., 2004). However, few studies have investigated the impact of taste phenotype on the primary gustatory cortex and oral somatosensory areas. Eldeghaidy *et al.* (Eldeghaidy et al., 2011) showed a significant increase in the cortical BOLD response to oral fat in 6-n-propylthiouracil (PROP) tasters in key taste, texture, and reward processing areas (super-taster > taster > non-taster).

A new taste phenotype known as "thermal taster status" has been described (Cruz and Green, 2000). Thermal stimulation of small areas of the tongue has been shown to elicit a "phantom" taste in some individuals, ~ 30-50% of the population (Bajec and Pickering, 2008; Cruz and Green, 2000; Green and George, 2004; Yang et al., 2014). Since its discovery, behavioural differences have been reported between subjects who perceive a phantom taste, termed 'thermal tasters' (TTs), and those who do not, termed 'thermal non-tasters' (TnTs), (Bajec and Pickering, 2008; Cruz and Green, 2000; Green and George, 2004; Green et al., 2005; Pickering et al., 2010a; Pickering et al., 2010b). Thermal tasters have been shown to be more sensitive to pure taste stimuli at supra-threshold levels (Bajec and Pickering, 2008; Green and George, 2004; Green et al., 2005), and both retro- and ortho-nasal vanillin simulation (Green and George, 2004) compared with TnT, although an olfactory advantage was not found at detection threshold level in a more recent study (Yang

et al., 2014). However, there is conflicting evidence regarding the impact of thermal taster status on trigeminal stimuli; sensations induced by capsaicin and menthol (burning, stinging and prickling) were not rated differently between TTs and TnTs in a series of experiments by Green et al. (Green et al., 2005). In contrast, the astringency of alum (Bajec and Pickering, 2008), the carbonation and fullness of beer (Pickering et al., 2010a), the astringency of red wine (Pickering et al., 2010b) and the temperature of warm and cold stimuli (Bajec and Pickering, 2008; Yang et al., 2014) have all been rated significantly higher in TTs than TnTs. Current evidence suggests that behavioural differences in TTs may be limited to the oral cavity, as no significant differences have been found for temperature intensity ratings at non-gustatory sites (lip and hand) (Green and George, 2004). The mechanism for this increase in sensitivity in TTs has been hypothesised to be due to a temperature sensitive chemosensory pathway (Cruz and Green, 2000). This hypothesis is supported by the discovery that the TRPM5 cation channel, which responds to sweet, bitter and umami tastes is also heat activated and highly temperature sensitive (Talavera et al., 2008). In TTs, the TRPM5 could depolarise the taste cells through thermal activation. However, the question remains as to whether the phenomenon of thermal taster status is limited to thermal-taste activation, or whether a variety of trigeminal and gustatory stimuli can modulate a different cortical response in TTs compared to TnTs.

Here, the combination of behavioural sensory investigations and brain imaging allows the mechanism behind the thermal taster status phenomenon to be explored. We investigate whether the cortical response to gustatory-trigeminal samples is altered with thermal taster status. Here, carbonation is chosen as a trigeminal stimulus to be modulated and combined with a sweet taste (dextrose) added at fixed levels. Few studies have investigated the effects of carbonation (CO_2) as a somatosensory component of flavour perception and the pathways responsible for its perception in combination with taste stimuli are not fully understood.

Materials and Methods

Participants and Screening. The study was approved by the University of Nottingham Medical School Research Ethics Committee. Recruitment questionnaires screened any volunteers with contraindications to MRI safety or those who had a known taste dysfunction. All subjects gave informed consent before enrolling in the study. 52 subjects (32 female/20 male, age 35 ± 7 yrs) underwent two separate screening session**s** to determine their PROP and thermal taster status. PROP taster status was defined based on the 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. (2008). Intensity was rated on a general Labelled Magnitude Scale (gLMS) (Green et al., 1996), and training on how to use the scale was given prior to data collection in order to increase validity (Bartoshuk et al., 2002). The gLMS scale is a category ratio scale used to measure intensity of sensation with categories of no sensation, barely detectable, weak, moderate, strong, very strong, and strongest imaginable marked at distances of 0, 1.4, 17, 34.7, 52.5, and 100 mm along a continuous line. Only the verbal categories are given, and subjects are instructed to mark anywhere along the continuous line to register their sensation (Green et al., 1996). All subjects were trained on the scale based on an approach by (Bartoshuk et al., 2002) which is described below. A reference sheet with a gLMS presented in exactly the same way as subsequent test sheets was given to each subject. Subjects received verbal and written instructions that the top of the scale corresponded to the strongest imaginable sensation of any kind and were asked to write down what this was at the top of their reference sheet. Subjects were asked to rate a list of 15 remembered or imagined sensations relative to their strongest imaginable sensation of any kind, Table 1.

Thermal taster status was assessed using a Medoc Pathway with intra-oral ATS (advanced thermal stimulator) thermode (Medoc, Israel) on one tongue location only. Subjects were asked with the guidance of researcher to place the intra-oral thermode (6 mm diameter round surface) on the anterior tongue tip, the area which is the most responsive to thermal

taste (Cruz and Green, 2000) and where fungiform papillae are most densely innervated (Shahbake et al., 2005). Subjects were instructed to hold the thermode firmly in place during all temperature trials. Two warming (from 15 °C to 40 °C) and two cooling (from 35 °C to 5 °C) trials were carried out following the procedure of Bajec and Pickering (2008). The warming trial started at 35 °C, cooled to 15 °C and re-warmed to 40 °C and held for 1 s. The cooling trial started at 35 °C was cooled to 5 °C and held for 10 s before rising to baseline (35 °C), as illustrated in Figure 1a and 1b respectively. Warming trials always preceded cooling trials to avoid possible adaptation from the intense, sustained cold stimulation (Green and George 2004). Subjects 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 break. If a thermally induced taste was perceived, subjects were asked to state the taste quality perceived from a selected list ('sweet', 'salty', 'bitter', 'sour', 'umami', and 'other please specify'), and rate its intensity for each trial on a gLMS. Thermal tasters were classified as those who perceived a taste, above weak during both replicates of either the warming or cooling trial. Thermal non-tasters were classified as those who did not perceive a taste on any replicate of any trial.

Oral responsiveness assessments

Subjects were invited to another session on a separate day to rate the intensity of suprathreshold taste and temperature (warming and cooling) stimuli using the gLMS and to check for taste dysfunction. Taste dysfunction was classified by the authors as any subject who rated the stimuli barely detectable or below on the gLMS. Taste samples included 0.32 M sucrose (Tate and Lyle, UK), 0.56 M sodium chloride (NaCl) (Sainsbury, UK), 56 mM citric acid (Sigma Aldrich, UK) 1 mM quinine hydrochloride (QHCl) (Sigma Aldrich, UK), 0.32 mM PROP which were all prepared using deionised water, and presented to subjects in a random order according to the method and concentrations defined by Lim et al., (2008). The ATS was used to deliver temperature stimuli as described for thermal taster screening. For taste response assessment, each subject was instructed to rinse their mouth 3 times with

deionised water before applying the taste. All stimuli were applied to the tongue by rolling a saturated cotton swab across the tip of the tongue for approximately 3 s. The subjects were instructed to actively taste the stimulus between the tongue and the hard palate using a gentle 'smacking' motion and rate the perceived intensity of the taste once it had reached its maximum using the gLMS provided. Separate gLMS were provided for each stimulus. Subjects were presented with their own gLMS reference sheet from the training session and were encouraged to refer to it for guidance on where to rate the intensity of the taste. The four taste stimuli were presented first in a randomised order, PROP was presented last to avoid any cross over effects in PROP sensitive individuals. Subjects were given a 1 min interstimulus-interval (ISI) and instructed to take longer if needed. During the ISI, subjects cleansed their palate with the deionised water and unsalted crackers (Rakusen's, Leeds, UK) provided. After a 5 min break, the procedure was repeated to collect duplicate ratings of each stimulus.

*Samples and Subject's preference**:***

Three sweet samples of differing CO_2 level were prepared for the fMRI scan session: i) a gustatory (sweet + no CO_2) sample "no CO_2", and two gustatory-trigeminal samples ii) a sweet + low CO_2 "low CO_2" and iii) a sweet + high CO_2 "high CO_2" sample. Samples were based on a model beverage system following (Clark et al., 2011a). Samples were prepared by dissolving 70g/L of polydextrose (Litesse® Ultra powder, Danisco, New Century, KS, USA) and 30g/L of dextrose (MyProtein, Manchester, UK) into still mineral water (Danone, Paris, France) and mixed on a roller bed for 6 h to ensure full dispersion. Samples were refrigerated until they reached 5 ± 1 ºC. Polydextrose was added to give 'body' whilst not contributing a taste quality (sub-threshold), dextrose was added to impart suprathreshold sweetness. Samples to be carbonated were aliquoted into 100ml Schott bottles (Fisher Scientific, Loughborough, UK) fitted with modified (Medical Engineering Unit, University of Nottingham, UK) Schott bottle caps (Fisher Scientific, Loughborough, UK) to allow a one-

188 way flow of food grade CO_2 (BOC, Guildford, UK) directly into the vessel ensuring accurate
189 carbonation levels. Once disconnected, the samples maintained pressure and therefore CO_2
190 level. The low CO_2 samples were carbonated to 1 volume and the high CO_2 samples to 2
191 volumes. One volume equates to 1 litre of CO_2 in 1 litre of liquid. Two volumes represent a
192 carbonation level similar to most standard beers. Samples were stored at 5 ± 1 ˚C until
193 required.
194
195 Immediately prior to the fMRI scan, subjects were familiarised with the three samples and
196 presented with three 40 ml random 3 digit coded samples; no CO_2, low CO_2 and high CO_2, in
197 random order, and were asked to evaluate them, using a palate cleanser before each
198 sample (Danone, Paris, France), and place them in order of preference from most to least
199 preferred. The number of subjects who most and least preferred each sample was
200 determined in each group in order to identify any trends in the data. Statistical analysis was
201 not carried out on preference data as the subject numbers were too low for such a
202 behavioural test. After this task, subjects were told that the samples were no, low and high
203 CO_2 and that these same samples would be delivered during the fMRI scanning.

204 ***fMRI paradigm design*:**

205 The samples were delivered to subjects using 60 ml syringes with Luer lock fittings to
206 prevent loss of CO_2 and control the flow of the sample. Thin plastic tubing, (68 cm long, 1.5
207 mm diameter) ran from the Luer stopcock to an individual subjects' bite bar created from
208 dental putty to ensure consistent tube positioning. All samples and a water wash for
209 cleansing between samples (Danone, Paris, France) were delivered at 6 ± 1 °C.

210 Samples were delivered in a pseudo-random order across fMRI cycles, with ten cycles of
211 each sample delivered per fMRI scan. Three runs were acquired in each fMRI session,
212 resulting in a total of 30 replicates of each sample for each subject. New samples were
213 provided for each run in order to maintain sample temperature. The previous samples were

drained from the tubing prior to new samples being connected. New samples were 'washed' through the tubing before the next run commenced to ensure no air bubbles were blocking the flow. In each cycle, 2 mL of sample was manually delivered over a 2 s period (flow rate 1 mL/s). Manual delivery was found to be the most accurate method of delivering carbonated samples, due to the pressurised system, and practice sessions prior to scanning showed that 2 ml could be consistently delivered over a 2 s period. The syringes were situated at a lower level than the subject's mouthpiece to ensure no residue sample was delivered to the subject during the ISI. Presentation software was used to deliver instructions to the researcher delivering the samples to ensure correct delivery to the subjects.

Following sample delivery, subjects were cued to swallow by a visual cue (Presentation Software, Neurobehavioral System, San Francisco, US) and surface electromyography (EMG) was acquired concurrently with the fMRI data acquisition (Eldeghaidy et al., 2011) to determine the exact time of swallow and to determine the duration each sample remained in the mouth. At 4 s after sample delivery, subjects were instructed to press a button to identify the level of carbonation in the sample received: 1 = no CO_2, 2 = low CO_2 and 3 = high CO_2. The responses were collected and analysed to determine the subject's discrimination ability between sample CO_2 level during fMRI scanning. At 12 s following the sample cessation, 1 mL still mineral water (Danone, Paris, France**)** wash was delivered over a 1 s period to clear the oral cavity of any lingering sample. A delay of 7.5 s was allowed before repeating the cycle. Each fMRI scan took ~ 11 minutes to complete.

fMRI data acquisition:

MRI data was acquired on a 3 T Philips Achieva scanner with a 32-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, 240 x 240 mm^2 field of view (FOV), SENSE factor 2 in the right-left (RL) direction, and 34 slices aligned parallel with AC-PC plane. Following fMRI acquisition, 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 x 256 matrix) was collected to aid registration of fMRI data to MNI space.

Data analysis:

Oral responsiveness

Intensity ratings of taste and temperature samples were $\log_{10}$ transformed, with 0 ratings adjusted to 0.4 prior to transformation. **A Multivariate ANOVA (MANOVA) was performed including all oral attributes as independent variables to enable the overall impact of Thermal Taster (TT) group on oral responsiveness to be determined. The effect of each individual variable was also determined from the MANOVA (α = 0.05).** For those subjects classified as TTs, tastes perceived during screening were also $\log_{10}$ transformed, with 0 ratings adjusted to 0.4 prior to transformation, and the intensity of each taste perceived was averaged across the TT group.

Discrimination of CO_2 level

Discrimination of CO_2 level, collected during the fMRI scan, was analysed by calculating the percentage of correctly identified samples for each subject and associated d' value (Ennis, 1993), a measure of sensitivity representing probability of correct responses for that group. A d' value above 1 indicates an ability to discriminate (Lawless and Heymann, 2010). Significant differences (α = 0.05) between groups were evaluated using a student t-test.

fMRI data analysis

fMRI data was processed using SPM5 (Statistical Parametric Mapping, Wellcome Department of Imaging Neuroscience; www.fil.ion.ucl.ac.uk/spm). T_2* maps were formed from the multi-echo data set using a voxel-by-voxel, linear, weighted least squares fit, and used in the weighted summation of the double-echo fMRI data (Posse et al., 1999). The weighted data was slice timing corrected, and realigned. Individual realignment parameters were visually inspected to ensure no subject moved by more than one voxel during the fMRI

scan. Data were then normalised to the MNI template, and spatially smoothed with 8 mm FWHM.

A first level GLM analysis was performed for each subject to generate contrasts for each sample (no CO_2, low CO_2 and high CO_2), using the time each sample remained in the mouth calculated from the EMG trace convolved with a canonical hemodynamic response function (HRF), and temporally filtered with a 135 s high pass filter cut-off**.** The water wash, button press and motion parameters were included as covariates of no interest. To identify areas of the brain which correlated with carbonation level, a linear (1^{st} order) parametric modulation with CO_2 level was performed, and both positive and negative modulations were assessed.

Second level random effects (RFX) group analysis was then performed to determine brain areas active to each sample (no CO_2, low CO_2 and high CO_2) for both the TT and TnT group, with maps threshold at a false discovery rate (FDR) corrected probability of $p < 0.05$. To assess the difference in brain activation between TTs and TnTs for each sample, a two-sample t-test was performed for each CO_2 sample using a binary mask of 'all' samples ($p < 0.05$ uncorrected) and assessed at a threshold level $p < 0.005$ uncorrected, $k > 20$ (Lieberman and Cunningham, 2009). A second level RFX analysis of those areas displaying a linear parametric modulation with CO_2 level was performed for both the TT and TnT group at a threshold level $p < 0.005$ uncorrected, $k > 20$. Finally, to determine whether subjective preference to the CO_2 level of the sample could account for differences in taste activation, we performed a second level RFX analysis of CO_2 level, and compared the inclusion/exclusion of subjective preference rating as a covariate of no interest to the response to CO_2 level.

A region of interest (ROI) analysis based on *a priori* areas was performed on right and left hemispheres for each individual subject's first level maps. The insula was subdivided into anterior (40, 10, -2) and posterior (44, -32, 12) parts, defined as an 8 mm sphere centred at the peak active voxel, as reported in (Eldeghaidy et al. 2011). Thalamus, amygdala, and SII

(BA 43) were anatomically defined by the PickAtlas, and SI as an 8 mm sphere centred at (60, -6, 20). In addition, lateral (26, 32, -10) and medial OFC (-6, 44, -2) ROIs were defined as reported by de Araujo and Rolls (2004), and dorsolateral prefrontal cortex (DLPFC) (44, 32, 12) as reported by (Kringelbach et al., 2004). The ROIs contained a large number of voxels (>250) which encompassed all activated areas of interest, allowing for variability in the location of the activation peak within cortical regions across all subjects. The ROI analysis was performed for 1) TT and TnT contrast maps at each CO_2 level and 2) the linear parametric modulation of CO_2 maps. For each sample, the mean of the top 5 % parameter estimate (β-value) was calculated for each ROI (Fernandez et al., 2003; Mitsis et al., 2008). Since all ROIs were first defined to comprise a large number of voxels, this analysis approach ensured the assessment of the activity in 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 two-factor ANOVA (group and sample) to assess any significant differences ($p < 0.05$) at a global level was performed. Tukey's HSD post-hoc multiple comparison tests determined significant differences between groups for each sample and ROI. For the parametric modulations, significant differences between groups were calculated using Student t-test.

Results

Screening

No subjects were excluded due to taste dysfunction**.** Of the 52 subjects screened, 12 were classified as TTs (23 %) and 40 (77 %) as TnTs. Twenty four subjects were invited to take part in the fMRI scanning, twelve thermal tasters (TT) (8 females, 4 males, 30 ± 7 yrs) and 12 thermal non-tasters (TnT) (7 females, 5 males, 32 ± 5 yrs). Both TT and TnT groups were matched for PROP taster status (4 PROP non-tasters (pNTs), 6 PROP medium-tasters (pMTs) 2 PROP super-tasters (pSTs) in each group). The 12 TnTs were randomly selected

from the group of 24, taking into account availability, for each PROP taster status sub group. During thermal stimulation of the tongue, the intensity of tastes reported by each thermal taster during each replicate was between weak and strong on the gLMS, with an average intensity rating across all tastes and subjects just below moderate. TTs reported perceiving tastes during warming trials, cooling trials or both, with bitter reported as the taste most often perceived during warming trials and metallic most commonly perceived during cooling trails Figure 1c.

Oral responsiveness

The MANOVA revealed a significant effect of TT group according to the Wilks' Lambda test (p = 0.041), with TTs rating oral responsiveness significantly more intense than TnTs. Although this trend was observed across attributes (except Quinine and Cooling) (Figure 2a), it only approached significance for sucrose (p = 0.054) and for warming (p = 0.056) (Table 2), thus these latter two responses drove the overall significance observed.

Sample Preference

Figure 2b shows the preference of each CO_2 sample in percentage values for TTs and TnTs respectively. There is a clear difference in the pattern of response between the TT and TnT group. The TT group most preferred the no CO_2 sample and least preferred the high CO_2 sample. In contrast, TnTs did not show a clear preference for any sample. The 'no CO_2' sample was both most preferred and least preferred by the same number of subjects in the TnT group. For both the TT and TnT group a Spearman rank correlation was performed between the rank of CO_2 level and preference, for both groups a non-significant correlation coefficient was found (TT: ρ = -0.25, p=0.14; TnT: ρ =-0.042, p=0.81).

342

343 *Discrimination of CO_2 level*

344 During the fMRI scan session, both the TT and TnT group had a good level of discrimination
345 ability when the sample was un-carbonated 'no CO_2'. This discrimination ability was reduced
346 for the 'low CO_2' sample and was similar between TT and TnT groups. However, there was a
347 significant difference between groups for the discrimination of the high CO_2 sample. TTs
348 could correctly identify the high CO_2 sample significantly more than the TnTs ($p < 0.05$),
349 Figure 2c. It should be noted that the high CO_2 sample was least preferred by the TT group,
350 Figure 2b.

351 *fMRI Results*

352 The activation maps for TT and TnT revealed brain areas activated in response to each
353 sample including primary taste areas (anterior insula and frontal operculum), oral
354 somatosensory areas (mid and posterior insula, somatosensory cortices (SI and SII), and
355 rolandic operculum), reward areas (including ACC and amygdala), dorsolateral prefrontal
356 cortex (DLPFC) and the thalamus.

357

358 The parameter estimates (β-values) in each ROI were first assessed for each sample in right
359 and left hemispheres. A trend of higher activation in the left hemisphere for both TT and TnT
360 was observed, with a significant increase in left thalamus for “no CO_2” and “high CO_2”
361 samples in TT, whereas the left anterior insula was significantly higher in TnT for the “high
362 CO_2” sample. We then assessed each ROI combined across hemispheres for both groups,
363 Figure 3a. A two-factor ANOVA (group and sample) across all ROI’s revealed a significant
364 main effect at a global level for group ($p < 0.05$) of higher cortical activation across all ROI’s
365 in TTs, but not for sample ($p > 0.05$). Analysis across each ROI revealed significantly higher
366 activation in the secondary somatosensory cortex (SII) for TTs compared to TnTs ($p < 0.05$),
367 with a trend for higher activation in the posterior insula for TTs ($p= 0.067$). The activation

maps for a two-sample t-test between TT and TnT groups for the no CO_2 sample revealed significantly greater BOLD response for the TT group in ,SII, , DLPFC and ACC, as shown in the differential activation maps in Figure 3b. Table 3 gives a summary for those brain areas. When assessing the effect of sample across both groups, a trend of higher activation to the high CO_2 sample compared to the no CO_2 sample was found in the ACC (p= 0.068).

Group ROI analysis on the CO_2 level parametric modulation beta values showed a positive modulation of cortical activation with CO_2 level in all brain areas in the TnT group including somatosensory, taste and reward areas, and a negative correlation in the DLPFC. Combining data across hemispheres in the TT group, a significant negative modulation of cortical activation with CO_2 level was found in the anterior insula, DLPFC, lateral and medial OFC, and a trend in the ACC. A positive modulation in the SI, SII and a trend in the posterior insula (Figure 4a) was also found. A significant difference in the linear parametric modulation with CO_2 level was found between TT and TnT groups in the anterior insula, the DLPFC and the ACC ($p < 0.05$), with a trend in the lateral OFC (p= 0.069). Of note, the DLPFC showed an increase in the left hemisphere compared to the right hemisphere for both TTs and TnTs, whereas the response in left SII was significantly higher than right in TnTs.

Activation maps for the positive modulation of cortical activation with CO_2 level are shown for the TT and TnT group in Figure 4b, and Table 4**.** The RFX maps of the negative modulation with the CO_2 level was found in the DLPFC [(46, 34, 14), z= 3.04, p= 0.001] in the TnT group maps, whereas the TT showed a negative modulation with the CO_2 level in left anterior insula ; [(-34, 24, 0), z= 2.63, p= 0.004] and left amygdala [(-18, 2, -26), z= 2.62, p= 0.004] as shown in Figure 4b.

We assessed whether differences in preference rating could explain the observed differences in taste activation to CO_2 level, but found no difference in the statistically thresholded activation maps when including preference rating as a covariate of no interest compared to when preference was not included as a covariate. Thus we conclude that the

observed differences in taste activation patterns are related to CO_2 level alone and not preference.

Discussion

In this study, thermal tasters perceived a phantom taste during thermal stimulation which was of a similar intensity to the oral response to taste samples themselves, Figure 1c, and 2a. Basic tastes reported during thermal stimulation were bitter, sweet, salty and sour/acidic. The 'other' category was selected by 6 subjects who self-reported metallic or minty tastes. Metallic is purported to have a taste component as well as trigeminal and aroma elements for some divalent salts (Epke et al., 2009; Lawless et al., 2005; Lim and Lawless, 2005). We concluded that minty sensation was important as it may result from the subject experiencing a phantom sweet taste, shown in literature to be an important component of mintiness, (Davidson et al. 1999), in conjunction with the trigeminal temperature stimulation. This may explain why none of the TT's in this study reported sweetness during the warming trial when other studies have reported sweetness on warming (Cruz and Green 2000; Yang et al, 2014). The incidence of tastes reported by thermal tasters has been reported in one other study (Yang et al, 2014) and the most frequent taste reported was metallic. It would be interesting to compare the cortical response in TTs who report basic tastes with those who report other taste sensations in order to understand this further. The ability of thermal stimulation to elicit such a clear taste response in thermal tasters is intriguing. Furthermore, behaviourally, TTs perceived the intensity of oral response (taste and temperature) higher than TnTs (Figure 2a). This suggests that TTs could have a perceptual advantage for some gustatory and trigeminal stimuli when presented in isolation, as previously reported (Bajec and Pickering, 2008; Cruz and Green, 2000). During the fMRI scan session, TTs were significantly more able to discriminate the high CO_2 sample compared to TnTs, and, the high CO_2 sample was clearly the least preferred sample for TTs, supporting a perceptual advantage in this group, Figure 2b and c. Perceptually increasing levels of CO_2 may have reduced perceived sweetness due to cross-modal interactions

(Clark et al., 2011) which could account for the preference trend towards the no CO_2 sample as it may have been perceived sweeter by TTs.

Taste and somatosensory stimuli are usually simultaneously present during food intake. Function convergence between these two modalities has been documented (Cerf-Ducastel et al., 2001; Guest et al., 2007; Rudenga et al., 2010). Cerf-Ducastel et al (2001) showed an overlap in taste and lingual somatosensory representation in the insula, rolandic, frontal and temporal operculum, with superior and inferior parts of the insula being more discriminating to gustatory only samples (sweet, salt, sour and umami) compared with somato-gustatory samples (pungent sour and astringent sweet). In this study, the samples developed for use in the fMRI protocol were designed to elicit a gustatory-trigeminal response (sweet + low CO_2; sweet + high CO_2). Cortical activation to the sweet + no CO_2 sample was significantly higher in the TT group compared to the TnT group in, oral somatosensory (SII, rolandic operculum), and reward areas (ACC), in addition to the DLPFC, an area linked to cognitive evaluation processes, such as evaluation of rewarding taste stimulation (Kringelbach et al., 2004), suggesting that the increase in intensity perception measured behaviourally by the TT group is a result of elevated cortical activation across areas associated with taste perception. This may be due to an elevated perception of sweetness intensity or a modified oral perception due to the sample delivery temperature in TT compared to TnTs.

When the trigeminal component (CO_2 level) of the stimulus increased, the pattern of cortical activation between TT and TnT groups was significantly different. A significant increase in cortical response with increasing CO_2 level ($p < 0.05$, Figure 4) was seen across all *a priori* ROIs (taste, somatosensory and reward areas) in the TnT group. In contrast, only the somatosensory areas (SI, SII) showed a significant positive modulation ($p < 0.05$) with increasing CO_2 level in the TT group. Interestingly, TTs showed a significant negative parametric modulation ($p < 0.05$) with CO_2 level in primary taste (anterior insula) and reward (ACC) areas, in addition to a negative modulation for the DLPFC in both TT and TnT groups. Previous studies have reported DLFPC activation to food-related studies (Small et al., 2001;

448 Tataranni et al., 1999), and Kringelbach et al (2004) showed DLFPC activation to unimodal
449 taste and multimodal flavour stimuli in the human brain.

450 These results suggest that samples containing both gustatory and trigeminal stimulus
451 input are processed differently by the TT and TnT groups. The significantly higher cortical
452 response of TTs to the sweet + no CO_2 sample compared with TnTs, and the limited change
453 in activation in SI and SII with the addition of a trigeminal CO_2 component (sweet + low/ high
454 CO_2 samples), as well as behaviourally the higher intensity perception in TTs, supports the
455 hypothesis that the gustatory and trigeminal nerves are intertwined at the periphery in TTs.
456 These results support previous findings from Essick et al (Essick et al., 2003) that tactile and
457 taste sensitivities covary. The close proximity of gustatory (chorda tympani nerve) and
458 somatosensory (lingual nerve) afferents, particularly at the tongue tip, and small receptive
459 fields at that location, supports coupling between the taste and somatosensory sensations
460 (Whitehead et al., 1985). This hypothesis supports the fact that thermal tasters can
461 experience a phantom taste from temperature activating the gustatory nerve during thermal
462 stimulation, as revealed behaviourally. Here, when gustatory and trigeminal stimuli are
463 presented together (low and high CO_2 level), activation in TTs remains unaltered, likely as
464 both nerves are already highly stimulated, whilst in TnTs increased cortical activation results
465 from the additional stimulation of the trigeminal nerve.

466 We hypothesise that the increase in cortical activation across taste and somatosensory ROIs
467 for the TnT group in response to CO_2 level, results in an increased intensity perception to
468 CO_2 level. However, for TTs cortical activation in taste (anterior insula), DLPFC, and reward
469 (lateral and medial OFC, ACC) areas is negatively modulated with CO_2, suggesting that the
470 sensory advantage of increased intensity perception of simple tastants by TT might be lost
471 when another modality is added, with TTs further rating the high CO_2 sample as least
472 preferred. This could be due to a decrease in sweetness perception with increasing CO_2 as
473 found by others (Clark et al., 2011b; Hewson et al., 2009) which is impacting the cortical

activation patterns differently in each group. We hypothesise that this is due to cross-wiring between gustatory and trigeminal receptors in TTs.

The differences in cortical response observed between TT and TnTs contributes to understanding concerning differences in perception between these two groups. Such differences may impact food choice behaviour and the differences in response to carbonation here could impact on beverage choice and hence could impact on product design considerations in the beverage industry. Current research into the difference between TT and TnT groups for food and drink preferences is very limited. Liking of beer (Pickering et al., 2010a) and wine (Pickering et al., 2010b) was not found to be significantly different between groups**.** It is possible that the preference for uncarbonated samples in TTs found here might result in reduced preference for highly carbonated beers and other soft drinks, however a fully controlled study with more complex beverage systems and a larger sample size is needed to confirm this**.** Differences between thermal taster groups have been found for food liking;. TTs were found to like soft foods significantly less than TnTs, potentially indicating a difference between groups in their oral tactile sensitivity (Bajec and Pickering, 2010). Analogous to our findings of thermal taster status, studies of lexical-gustatory synaesthesia (Jones et al., 2011) - individuals who experience an automatic and highly consistent taste to spoken and written language - have demonstrated increased anterior insula activation related to viewing words that elicited tastes, and it has been shown that genes play a role in such a synaesthesia (Brang and Ramachandran, 2011; Simner and Ward, 2006). Here we show that TTs have different activation patterns compared with TnTs, and it is possible that genotype may also play a role here. Further research now needs to be conducted to understand the mechanism of thermal taster status and this cross modal gustatory and trigeminal interaction.

Conclusion

Few investigations of thermal taster status have been published. This work presents the first study to address changes in the cortical response in thermal tasters. We investigate the difference in cortical activation to trigeminal-gustatory stimuli between thermal and non-thermal taster groups. Behaviourally, thermal tasters respond to taste and temperature stimuli more intensely than TnTs. This is supported by this fMRI data which shows heightened cortical activation in taste, somatosensory and reward areas to gustatory stimuli in TTs compared to TnTs, and that the addition of a trigeminal CO_2 component to stimuli leads to a limited change in cortical response in these areas in TTs. Evidence from this study supports a cross-modal integration mechanism with interaction of stimulation to taste and trigeminal nerves in thermal tasters.

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610

611 Tables

612 **Table 1:** Remembered or imagined sensations used in gLMS scale training.

	Remembered or imagined sensation
1	The brightness of a dimly lit restaurant
2	The brightness of a well-lit room
3	Staring at the sun
4	The loudness of a whisper
5	The loudness of a conversation
6	Hearing a nearby jet-plane take off
7	Warmth of freshly baked bread in your mouth
8	The coldness experienced sucking on an ice-cube
9	The smell of a rose
10	The strongest smell ever experienced
11	The sweetness of candyfloss
12	The bitterness of grapefruit
13	The strongest taste ever experienced
14	The strongest oral burn experienced
15	The strongest oral pain ever experienced

613

614

615 **Table 2:** Mean value of oral responsiveness to individual attributes for thermal tasters (TTs)
616 and thermal-non tasters (TnTs), F-values and associated p-values from the MANOVA[a] are
617 provided.

	Stimulus	Group	Mean	F-value	p-value
Taste	NaCl	TnTs	1.369	0.35	0.558
		TTs	1.420		
	CitricAcid	TnTs	1.392	1.90	0.175
		TTs	1.504		
	Sucrose	TnTs	1.023	3.91	0.054†
		TTs	1.193		
	Quinine	TnTs	1.487	0.93	0.340
		TTs	1.387		
	PROP	TnTs	0.698	0.59	0.810
		TTs	0.741		
Temperature	Warming	TnTs	1.305	3.84	0.056†
		TTs	1.416		
	Cooling	TnTs	1.569	0.01	0.935
		TTs	1.565		

618 [a] Wilks' Lambda test indicated a significant group effect for overall responsiveness (p=0.041)
619 † approaching significance (p= 0.05)
620

Table 3: Brain areas showing higher response to thermal tasters (TTs) compared with thermal non-tasters (TnTs) for gustatory sample "no CO_2".

Area	Side	MNI[1]	Z-score	p-value	Cluster size[2], k
ACC*	L	-6, 2, 48	3.20	0.001	202
Rolanic operculum*	L	-44, -4, 52	3.32	<0.001	106
Secondary somatosensory cortex (SII)*	L	-54, -30, 32	2.99	0.001	132
Precentral gyrus*	R	30, -8, 56	2.93	0.002	59
Middle frontal gyrus/**DLPFC**	R	36, 42, 24	2.73	0.003	22

R, right hemisphere; L, left hemisphere; MNI, Montreal Neurological Institute;

[1] Peak voxel coordinates given in MNI space (x,y,z)

[2] Reported clusters threshold at $p < 0.005$, uncorrected for multiple comparisons, with a cluster extent threshold $k > 20$ voxels, and 'sub' indicates sub-cluster level. Asterisks indicate areas activated with FDR corrected $p < 0.05$**.**

630 **Table 4:** Brain areas showing positive modulation in BOLD amplitude with CO_2 level in

631 thermal tasters (TTs) and thermal non tasters (TnTs).

Area	Side	MNI[1]	Z-score	p-value	Cluster size[2], k
Thermal taster (TT)					
SII					
	L	-62 , -28,16	5.41	<0.001	51
	R	**64, -14, 8**	**3.34**	**0.003**	**26**
Thermal non- taster (TnT)					
Primary Somatosensory Cortex (SI)*	R	62, -12, 42 54, -16, 22 58, -20, 26	3.59 3.25 3.23	<0.001 <0.001 0.001	797
	L	-52, -14, 46	3.52	<0.001	sub
Secondary somatosensory cortex (SII)*	R	62, -26, 18 68, -28, 8	4.55 3.89	<0.001 <0.001	sub
Rolandic operculum*	R	56, 0, 6	4.55	<0.001	sub
	L	-56, 2, 14	3.54	<0.0001	44

ACC*	R	2, 0,50	3.31	<0.0001	312
	L	-6, -10, 48	3.34	<0.0001	sub
Precentral gyrus*	R	60, -2, 42	3.58	<0.0001	sub

R, right hemisphere; L, left hemisphere; MNI, Montreal Neurological Institute.

[1] Peak voxel coordinates given in MNI space (x,y,z).

[2] Reported clusters threshold at $p < 0.005$, uncorrected for multiple comparisons, with a cluster extent threshold $k > 20$ voxels, and 'sub' indicates sub-cluster level, Asterisks indicate areas activated with FDR corrected $p < 0.05$.

Figures

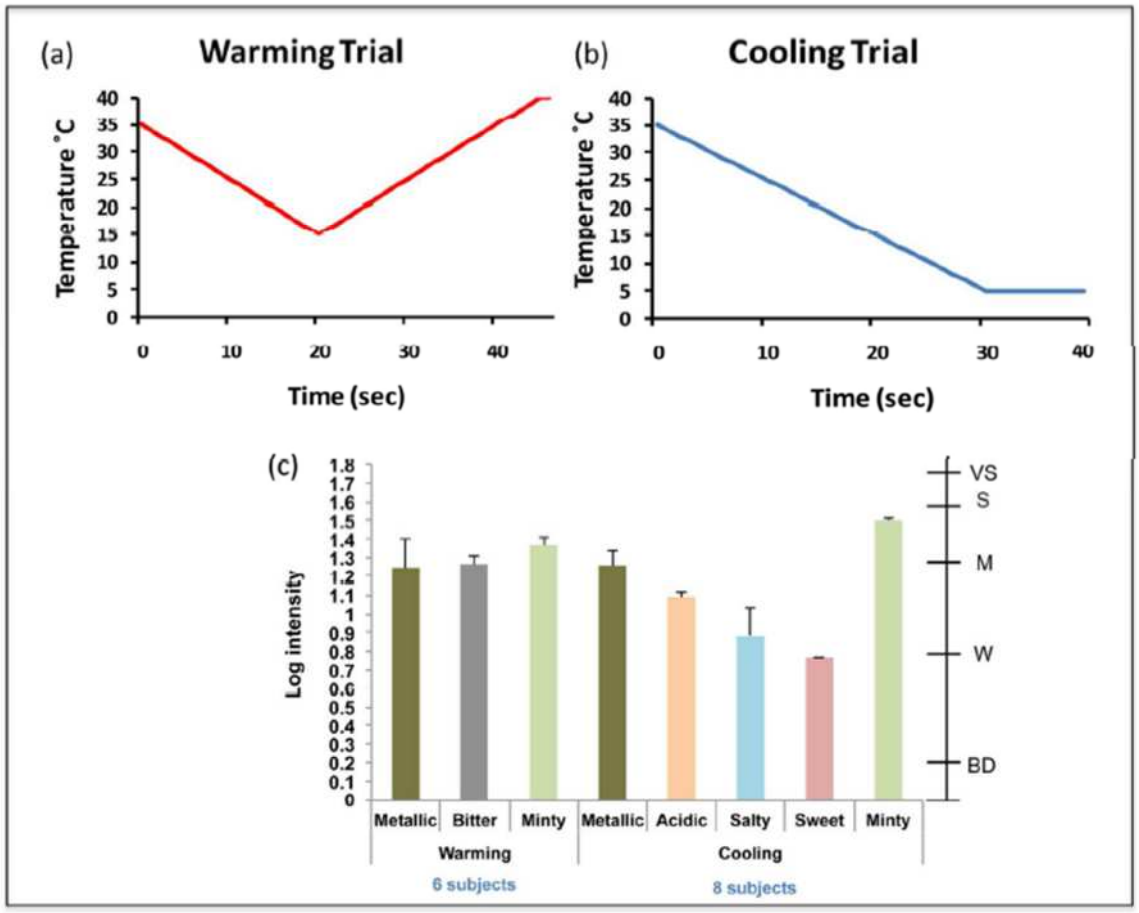
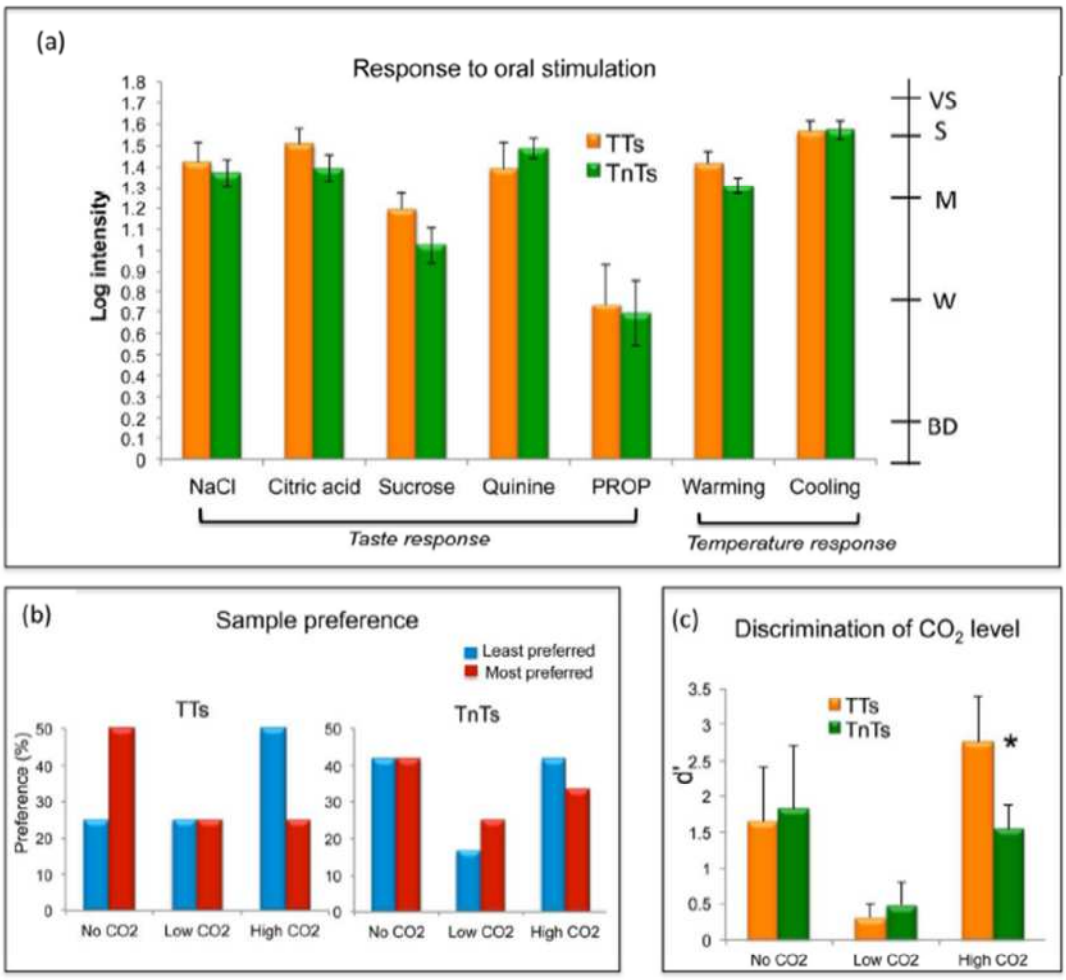


Figure 1: Thermal taster screening protocol. Graphical representation of **(a)** warming trial: cooling to 15 ˚C before warming to 40 ˚C, **(b)** cooling trial: cooling to 5 ˚C where temperature is held for 10 s**. (c)** Taste quality and intensity experienced by thermal tasters to warming (6 subjects) and cooling trials (8 subjects). Note: 2 subjects were both warming and cooling tasters. Secondary scale indicates labels on the gLMS: BD = barely detectable, W = weak, M = moderate, S = strong, VS = very strong



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650 **Figure 2**: Behavioural response: **(a)** Intensity of oral responses perceived by the thermal
651 taster (TT) and thermal non-taster (TnT) group. Secondary scale indicates labels on the
652 gLMS: BD = barely detectable, W = weak, M = moderate, S = strong, VS = very strong. **(b)**
653 Percentage of subject's preference by each group. **(c)** The discrimination ability of subjects
654 to correctly identify the CO_2 level delivered during the fMRI scan session, with the d' value of
655 the TT and TnT group provided. Asterisks indicate a significant difference between groups at
656 $p < 0.05$. Error bars show standard error.

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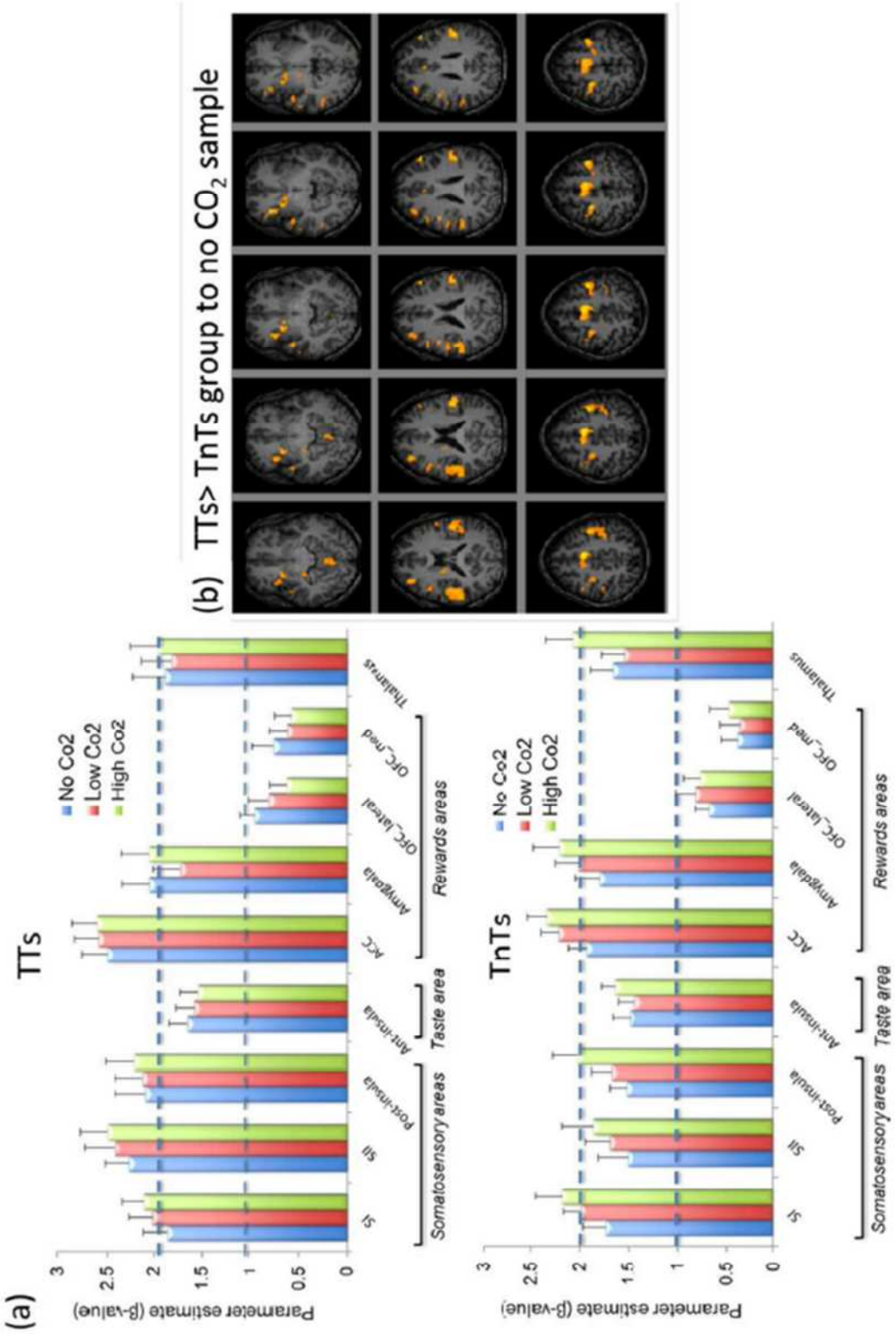

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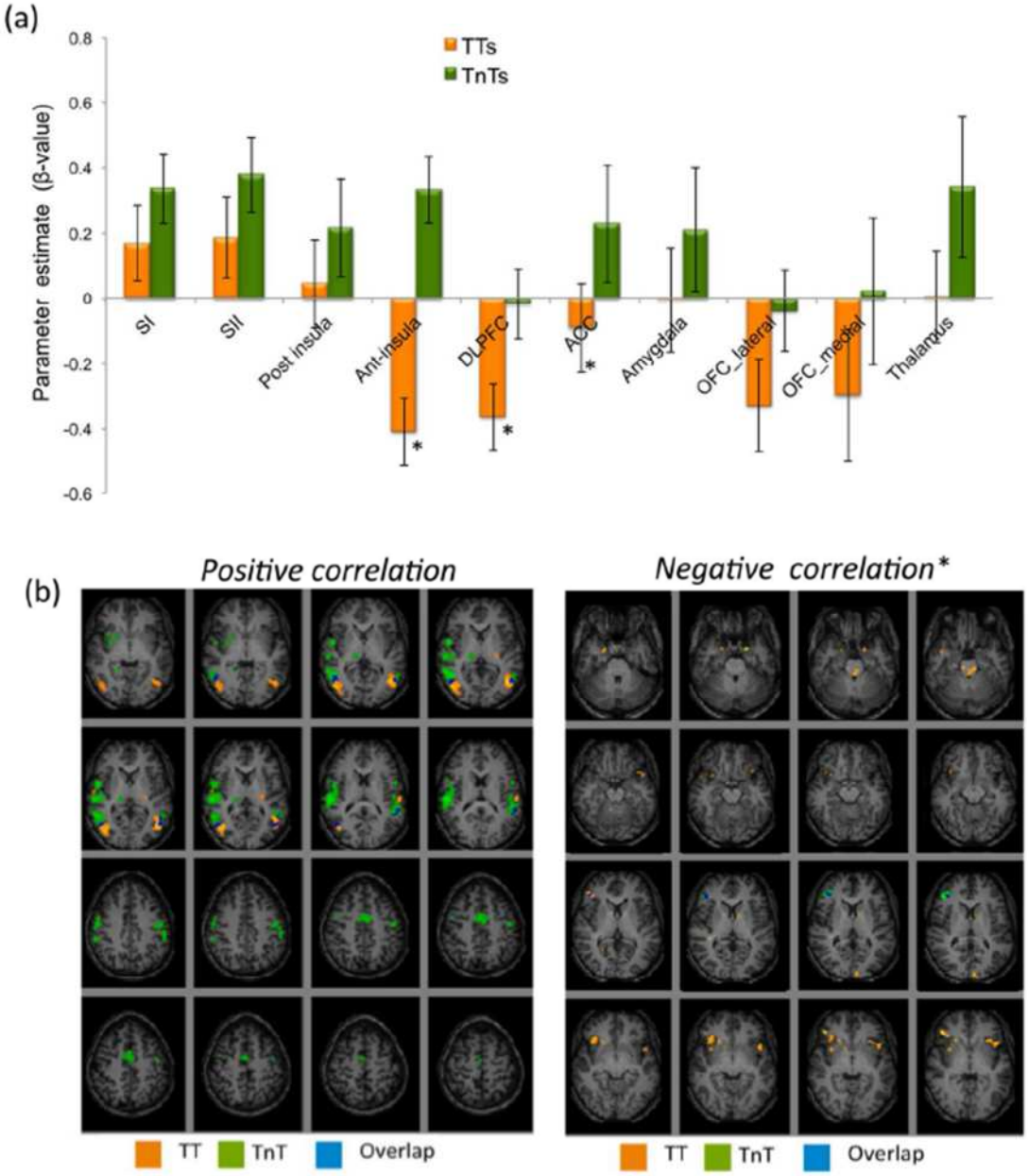
660

661 **Figure 3**: **(a)** Parameter estimate (β-value) for TT and TnT groups highlighting the response

662 in *a priori* cortical areas (error bars indicate the standard error). **(b)** Random effects group

analysis map showing contrast of (TT > TnT group) to the no CO_2 sample (sweet taste alone). Maps overlaid on T_1-weighted images, assessed at threshold $p < 0.005$.



666

667

668 **Figure 4**: **(a)** Positive and negative parameter estimate (β-value) for TT and TnT groups in *a*
669 *priori* cortical areas (error bars indicate the standard error). Asterisks indicate a significant
670 difference between groups at $p < 0.05$. **(b)** Cortical areas showing a positive and negative
671 correlation with CO_2 level. Maps displayed with $p < 0.005$, *$p < 0.05$ uncorrected. In each
672 figure the TT group is shown in orange, the TnT group in green, and the overlap of these
673 groups in blue.