To like or not to like: neural substrates of subjective flavor preferences

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WHAT THIS CHAPTER IS ABOUT

Flavor preferences vary; what one enjoys may be disgusting to another. Previous research has indicated several brain regions associated with flavor preferences. However, by using different stimuli or different internal states to obtain differences in liking, results of these studies may be confounded. Therefore, we used one target stimulus (grapefruit juice) and functional magnetic resonance imaging (fMRI) to compare brain activation patterns between participants that either liked (n=16) or disliked (n=18) this stimulus. Our first aim was to investigate whether differential neural activation exists that accounts for the difference in subjective flavor preference for the target stimulus. Secondly, multivariate analysis was used to investigate whether differences in subjective liking for the target revealed similar activation patterns to differences in general liking for a sweet and bitter solution. A direct comparison of likers and dislikers of the target stimulus revealed only small differences in activations within orbitofrontal cortex (OFC) and dorsal anterior cingulate cortex (dACC). However, when using multivariate analysis, a broader activation pattern (including OFC, dACC, pregenual anterior cingulate, anterior insula and ventral striatum) was identified that discriminated likers from dislikers with an 88% success rate. Interestingly though, little overlap was found between this pattern and the pattern that discriminates liking for the sweet and bitter solutions and lesser voxels contributed to the former compared with the latter. These differences between patterns discerning innate versus learned preferences may suggest that different mechanisms are at work and highlight the importance of elucidating the neural processes of how subjective preferences are learned and acquired.
5.1 INTRODUCTION
Eating is one of the fundamental pleasures in life (Kringelbach and Berridge, 2010). Yet our personal preferences for what we eat and enjoy differ. For example, one person may really like Roquefort cheese whereas another person may have a strong aversion for the same product. Differences in liking for complex tastes vary wildly and appear to depend heavily on how an individual learned to like or dislike a taste (Booth, 1991; Yeomans, 2006). However, humans are predisposed to like or dislike some of the simple basic tastes (Bartoshuk, 1991; Steiner, 1979) as they provide direct information about the presence of nutrients and toxins. Hence, an innate preference for sweet indicating the presence of calories, and an aversion for bitter, signaling that a food may be poisonous, has been essential for human survival. In addition to these intrinsic stimulus characteristics, individual factors also contribute to flavor preference. Most flavor preferences are learned and developed by associations based on subjective experience (Yeomans, 2006). Furthermore, when flavor preferences are acquired, internal states like those of hunger and satiety (Cabanac, 1971; Small et al., 2001) or cognitive/attentional factors such as word or price labels (Grabenhorst and Rolls, 2008; Grabenhorst et al., 2008; Plassmann et al., 2008; Veldhuizen and Small, 2011; Veldhuizen et al., 2007) can still modulate the affective reactions to foods and drinks. Subjective flavor preferences are thus the result of a multitude of both intrinsic and extrinsic factors.

The brain integrates these different signals to produce a subjective flavor perception (Small, 2012). Previous research investigating the neural correlates of taste and flavor preference has already consistently identified several regions, including the insula (Ins) and overlying operculum (Oper) (together, the primary taste cortex), orbitofrontal cortex (OFC), anterior cingulate cortex (ACC), and amygdala (for an overview, see e.g., Small, 2006)). Examination of taste pleasantness by using innate preferences for basic tastes or through modulation of pleasantness by internal state or cognitive and attentional processes has consistently linked activation of the OFC to hedonic experiences (Kringelbach and Radcliffe, 2005; Kringelbach and Rolls, 2004; O’Doherty et al., 2001a). In addition, subjective taste preferences have recently been reported in the ventromedial prefrontal cortex by using participants that liked or disliked the same basic taste (Rudenga and Small, 2013). Valence-specific responses have also frequently been reported in regions of the anterior cingulate cortex (Grabenhorst et al., 2007; Kühn and Gallinat, 2012; O’Doherty et al., 2001b; Small et al., 2003; Zald et al., 2002). In contrast, intensity-specific responses have been localized in middle insula and amygdala (Anderson et al., 2003; Small et al., 2003; Spetter et al., 2010). These findings provide important information regarding flavor preference representation in the brain. However, by using different taste qualities (e.g., sweet and bitter) to elicit opposite affective responses (e.g., O’Doherty et al., 2001b; Zald et al., 2002), taste pleasantness may be confounded with taste quality. Similarly, studies that examined the effect of feeding to satiety on subjective pleasantness of a food product (Kringelbach et al., 2003; Small et al., 2001) cannot dissociate with certainty whether the difference in brain activation is related to the change in levels of satiety or the change in affective value, or both.

Hence, the present study was designed to disentangle intrinsic product properties from...
individual preferences, irrespective of internal or attentional state. Therefore, we used only one target stimulus and compared brain activation patterns between participants that either liked or disliked the taste of this target stimulus. By holding intrinsic product properties (i.e. all participants tasted the same stimulus) and level of satiety constant, we ensured that solely affective reactions towards the product differed. Our first aim was to investigate whether differential neural activation can account for individual differences in flavor preference. We hypothesized that the comparison of likers and dislikers of the target stimulus would reveal differently activated regions, especially within the OFC, in likers versus dislikers. In a recent publication a similar approach was taken by using likers and dislikers of a sweet solution (Rudenga and Small, 2013). In the current study however, the target stimulus is a complex taste that consists of a combination of sweet, sour and bitter tastes. Moreover, the goal of the current study was twofold; our second aim was to compare these brain activation patterns with responses to a sweet and bitter solution that are inherently experienced as pleasant and unpleasant respectively. We expected that the brain activation patterns that underlie individual differences in learned flavor preferences would resemble patterns that underlie differences in innate preferences for basic tastes.

5.2 METHOD

5.2.1 Stimuli
Based on the results of a pilot study, grapefruit juice (GJ) (Albert Heijn, Zaandam, the Netherlands) was chosen as the target stimulus. A sweet solution (Sucr) that was ‘universally pleasant’, consisting of 70 g/l (0.2 M) sucrose dissolved in water (de Graaf and Zandstra, 1999; Small et al., 2003), and a bitter solution (Quin) that was ‘universally unpleasant’, consisting of 0.1622 g/l (5.10^-4 M) quinine (quinine monohydrochloride dihydrate, Sigma Aldrich Chemie B.V., Zwijndrecht, the Netherlands) dissolved in water (Small et al., 2003), were selected that were similar in perceived intensity compared to each other and to GJ. Tap water (W) was used as a neutral (in terms of valence) control stimulus. To clear the palate and remove possible aftertastes, a 10% solution of artificial saliva (Saliva Orthana, Pharmachemie BV, Haarlem, the Netherlands) in water was used as rinsing solution (O’Doherty et al., 2001b).

5.2.2 Subjects
Healthy, normal weight (BMI within 18.5 – 25 kg/m^2), adult (age between 18 and 45), right-handed individuals were recruited. Initial screening was conducted via questionnaires. Exclusion criteria included being hypersensitive to the stimuli used; having a (history of) taste or smell disorder, metabolic or endocrine disease or neurological disorder; following an energy restricted diet or experiencing a change in body weight of > 5 kg during the last 2 months; use of medication (except for aspirin, paracetamol, or oral contraceptive medication); smoking; a history of or current alcohol consumption of > 28 units/week; having a contra-indication to MRI scanning; self-reported neutral liking (i.e. score ≥ -1 or ≤ 1 on a 9-point scale ranging from -4 “dislike extremely” to 4 “like extremely”) or not familiar (i.e. never drank before) with GJ. Of the 348 individuals that responded to requests for participation 125 were eligible.
To ensure that respondents really liked or disliked GJ, these individuals were invited for a tasting session in which they rated their perceived pleasantness of all stimuli used in the experiment. Only those participants that rated GJ as liked or disliked, Sucr as liked, Quin as disliked (i.e. score ≤ -2 or ≥ 2 on a 9-point scale ranging from -4 “extremely unpleasant” to 4 “extremely pleasant”) and W as neutral (i.e. score > -2 and < 2) were considered for inclusion. Recognition thresholds for sweet, salty, sour, and bitter were determined using Taste Strips (Burghart, Wedel, Germany) to confirm that differences in liking for GJ were not due to differences in peripheral taste sensitivity (Drewnowski et al., 1997). A total of 45 participants were included in the experiment. Functional data of 2 participants was excluded due to scanner inconsistencies. In addition, behavioral results during the scanning sessions revealed neutral scores for GJ in 5 participants, disliking for Sucr in 3 participants, and unfamiliarity with GJ in 1 participant, resulting in their exclusion from the analysis.

The final sample consisted of 34 participants (see Figure A1), with 16 likers (10 women, 6 men, age 24.81 ± 7.37, BMI 21.67 ± 1.92, mean ± SD) and 18 dislikers (12 women, 6 men, age 21.61 ± 2.00, BMI 22.00 ± 1.53, mean ± SD). The study protocol was approved by the Medical Ethical Committee of Wageningen University and written informed consent was obtained from all participants according to the Declaration of Helsinki (amendment of Seoul, 2008).

5.2.3 Study design
This study used traditional mass univariate analysis to compare brain responses between likers and dislikers of GJ in order to investigate whether differential neural activation exists that accounts for individual differences in flavor preference. In addition, brain activation patterns in response to GJ were compared with responses to Sucr and Quin and multivariate analysis was used to investigate whether individual differences in learned flavor preferences resembled differences in innate preferences for basic tastes.

5.2.4 Experimental procedures
Before participating in the actual scanning session, participants were invited for an introduction session in which they were familiarized with the experimental procedures and were able to practice tasting and swallowing of the stimuli with minimal head movements while lying in the MR scanner. Introduction and scanning sessions were performed in Hospital de Gelderse Vallei (Ede, the Netherlands) on separate days between 8.30AM and 8PM. Participants were instructed to fast three hours prior to the scanning session (i.e. no foods or caloric- or caffeine-containing drinks), resulting in medium hunger scores (likers 6.1 ± 1.4, dislikers 5.5 ± 1.8, mean ± SD, on a 9-point scale ranging from “not at all” to “extremely”) at the start of the test session.

Upon arrival at the scanning session, recognition thresholds were assessed first by means of the filter-paper taste strips. The subsequent scanning session consisted of four functional runs (± 9 min) with one anatomical scan (± 5 min) in between. During each functional run, 260 gradient echo T2*-weighted echoplanar images (EPI) were acquired with blood-oxygen level-dependent (BOLD) contrast on a 3-Tesla Siemens Magnetom Verio MRI scanner (Sie-
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Whole-brain acquisition (TR=2140ms, TE=40ms, 90° flip angle, FOV=192×192mm, 43 axial slices, descending order, voxel size 3×3×3 mm) was tilted at an oblique angle of 30 degrees to the anterior-posterior commissure line to reduce signal dropout in orbitofrontal and ventral temporal lobes (Deichmann et al., 2003). A high-resolution T1-weighted anatomical MRI scan was made (MPRAGE, TR=1900ms, TE=2.26ms, 9° flip angle, FOV=256×256mm, 192 sagittal slices, voxel size=1×1×1mm). Over the course of the four functional runs, all four stimuli were each delivered 15 times in a pseudo-randomized order. Stimuli were administered with the use of programmable syringe pumps (NewEra Pump Systems Inc, Wantagh, NY) at a rate of 50 mL/min. Participants tasted 2 mL of a stimulus for 11 s, followed by a cue for swallowing (3 s). For each stimulus, in 4 out of the 15 deliveries, participants rated pleasantness on a 9-point scale ranging from “dislike extremely” to “like extremely”. Intensity was rated in 2 out of 15 deliveries and familiarity once out of the 15 deliveries on a 9-point scale ranging from “not at all” to “extremely”. Participants were instructed to mainly focus on the pleasantness (as opposed to intensity) when presented with a taste. Either directly after swallowing or subsequent to rating, participants were cued to rinse (5-6 s), followed again by a cue to swallow (3 s). Rinsing was done twice to fully clear the palate. In between trials, participants were instructed to fixate on a fixation cross (3-5 s). A schematic overview of one such trial is depicted in Figure 1.

5.2.5 Data processing and analysis

5.2.5.1 Univariate analysis (SPM)

Functional MRI data were preprocessed and analyzed using the SPM8 software package (Welcome Department of Imaging Neuroscience, London, UK) running in Matlab (version 7.12.0, The Mathworks, Inc., Natick, MA). The functional volumes of every participant were first realigned and coregistered to the anatomical image. All images were then globally normalized to MNI (Montreal Neurological Institute) space and smoothed with an isotropic Gaussian kernel of 8 mm full width at half maximum. For every participant, a statistical parametric map was generated by fitting a boxcar function to each time series, convolved

![Timeline of events within a trial.](image)

Trials started with delivery of 2 mL of GJ, Sucr, Quin or W. The stimulus was held in the mouth until the end of the 11 s taste period, after which participants were allowed to swallow during a 3 s time window. For each stimulus, in 7 out of the 15 deliveries, participants rated pleasantness (4 times), intensity (2 times) and familiarity. Either directly after swallowing or subsequent to rating, participants were cued to rinse, followed again by a time window of 3 s in which participants were allowed to swallow. To fully clear the palate, rinsing (and swallowing) was performed twice. Trials ended with a rest period of 3-5 s in which participants were instructed to fixate on a crosshair. Total trial time varied between 33 and 43 s.
with the canonical hemodynamic response function (HRF). To remove low-frequency noise, data were high-pass filtered with a cutoff of 128 s. Seven conditions were modeled: tasting of GJ, Sucr, Quin and W (11 s), rinsing (5-6 s), swallowing (3 s) and stimulus rating (6 s). The responses to swallowing, rinsing and rating were not used in further analyses. Realignment parameters were added as regressors to account for motion-related variance.

For every participant, parameters for tasting versus the neutral control (W) were calculated resulting in 3 contrast images ([GJ - W]; [Sucr - W]; [Quin - W]). On group level, these contrast images were entered into a second-level flexible factorial model with factors ‘Subject’, ‘Group’ (2 levels: likers and dislikers), and ‘Condition’ (3 levels, representing the three contrast images) and ‘Group x Condition’ as interaction effect. All contrasts ([GJlikers|Sucr & GJdislikers|Sucr]; [GJlikers|Quin & GJdislikers|Quin]; [GJlikers|Sucr & GJdislikers|Sucr] - [GJlikers|Quin & GJdislikers|Quin]; [GJlikers|GJ]; [GJdislikers|GJ]; [GJlikers|GJ – GJdislikers|GJ]) were thresholded at p<.001 (uncorrected) and a cluster size of k>8 contiguous voxels, similar to (e.g., Seo et al., 2013). Anatomical descriptions of coordinates were obtained combining the Autonomic Anatomical Labeling toolbox (Tzourio-Mazoyer et al., 2002), Harvard-Oxford cortical and subcortical structural atlases (Desikan et al., 2006), and software program MRCron (version 1.4) (Rorden and Brett, 2000).

Subjective ratings were analyzed using SPSS statistical software (version 19; SPSS Inc., Chicago, IL). A repeated measures GLM was used to analyze differences and similarities both between groups and within participants in the subjective ratings of pleasantness and intensity. Ratings for familiarity were compared between groups using a one-way ANOVA. P values <0.05 (two-sided) were considered significant.

5.2.5.2 Multivariate analysis (SVM)

To find the brain areas that best explain the differences in both two-class problems (i.e. Sucr versus Quin and likers versus dislikers of GJ) a linear Support Vector Machine (SVM) (Chang and Lin, 2011) was used. For the SVM classification, beta maps of tasting GJ, Sucr, and Quin contrasted against W (produced by the first level SPM analysis) from all 34 participants were used. These beta images were transformed into row vectors and concatenated in three data matrices with size n * m; where n is the number of subjects and m the number of voxels. In order to only work with grey matter voxels we created a mask comprised of the top 80% voxels of the grey matter group segmentation acquired by using Diffeomorphic Anatomical Registration Through Exponentiated Lie algebra (DARTEL) (Ashburner, 2007). After application of this mask, 45831 voxels were retained for the SVM analysis. Furthermore, to ensure both Sucr-Quin and likers-dislikers of GJ were in the same reference space we removed the mean between Sucr-Quin from all three brain images per subject. Finally we centered the voxel activity by removing the mean per voxel across subjects in the Sucr-Quin and likers-dislikers data separately.

To optimize the parameters of the SVM kernel, we used a grid-search in combination with 5-fold cross validation as proposed by (Hsu et al., 2010). Bootstrapping was applied (1000 cycles) to obtain a confidence interval (CI) on the weights assigned to each voxel by the SVM training (Alonso-Atienza et al., 2012; Duan et al., 2005). During each bootstrap, a

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1 This section has been modified to increase transparency and reproducibility.
random sample was drawn with replacement from the data set. Subsequently, the SVM was trained on this sample and the vector containing the feature weights was stored. After the bootstrap, the 99% CI was calculated for every voxel weight. All voxels with a CI not intersecting 0 were retained.

SVM Selection Algorithm:
1. Start: Selected voxels $C = \{ \}$, All voxels $A = \{1, \ldots, m\}$
2. Bootstrap: repeat 1000 times:
   a. Draw sample $S$ containing $n$ out of $n$ subjects with replacement
   b. Train SVM on sample $S$
   c. Store weight vector $w$ in matrix $W$
3. Calculate 99% confidence interval per voxel weight in $W$
4. Find and remove all voxel weights with 99% CI intersecting 0.
5. Output: select voxels set $C$ and create binary mask containing ones for selected voxels and zeros for discarded voxels.

5.3 RESULTS

5.3.1 Behavioral results
5.3.1.1 Subjective ratings during scanning
All participants rated Sucr as pleasant (on a scale of -4 to 4, mean ± SD, 2.7 ± 0.8), Quin as unpleasant, (-3.5 ± 0.6) and W as neutral (-0.1 ± 0.7). GJ elicited significantly different affective responses in likers compared to dislikers [$F(1,32)=395.3, p<.001$]; likers rated GJ as pleasant (2.5 ± 0.8) and dislikers rated GJ as unpleasant (-2.6 ± 0.8). The hedonic strength (i.e. strength of response irrespective of positive or negative direction) of these responses was similar [$F(1,32)=0.8, p=.38$]. No significant differences in intensity ratings for GJ were found between groups (on a scale of 1 to 9, likers: 7.6 ± 0.7, dislikers: 7.9 ± 0.7, [$F(1,32)=1.78, p=.19$]). Although all subjects were familiar with the stimulus, familiarity ratings were found to differ (on a scale of 1 to 9, likers: 8.2 ± 0.8, dislikers: 7.3 ± 1.0, [$F(1,32)=7.89, p<.01$]) For all subjective ratings see Table 1.

5.3.2 Univariate fMRI results
5.3.2.1 Tasting pleasant sucrose and unpleasant quinine
Whole-brain analysis across all 34 participants revealed clusters of activated voxels related to the pleasant taste of Sucr ((GJlikers|Sucr & GJdislikers|Sucr)) in OFC, bilateral amygdala, striatum and dorsomedial prefrontal cortex (see Table S1 and Figure 2). Activations related to the unpleasant taste of quinine ((GJlikers|Quin & GJdislikers|Quin)) were observed in the OFC, cingulate cortex, dorsomedial prefrontal cortex, striatum, thalamus and operculum (see Table S2 and Figure 2).

When comparing activations in response to Sucr and Quin ((GJlikers|Sucr & GJdislikers|Sucr) - (GJlikers|Quin & GJdislikers|Quin)), tasting Sucr caused greater activations in OFC compared to tasting Quin, whereas tasting Quin resulted in greater activations in cingulate cortex, operculum and precentral gyrus compared to tasting Sucr (see Table 2 and Figure 3).
Figure 2. Main effects of tasting sucrose and quinine
Activations related to the main effects (contrasted against W) of tasting liked Sucr ([likersSucr & dislikersSucr]) depicted in pink and disliked Quin ([likersQuin & dislikersQuin]) depicted in green, thresholded at p<.001 (unc) and k>8. A-C amygdala (Amy) activity in response to Sucr; A overlapping activity (yellow) in left caudate in response to both Sucr and Quin, left striatum activity in response to Quin; B-C medial and left lateral OFC activity in response to both Sucr and Quin; D dorsal anterior cingulate cortex (dACC) and thalamus (Thal) activation in response to Quin.

Table 1. Mean ratings per group (likers/dislikers) of taste stimuli during MRI scanning.

<table>
<thead>
<tr>
<th></th>
<th>Likers</th>
<th>Dislikers</th>
<th>F value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GJ liking</td>
<td>2.5 ± 0.8</td>
<td>−2.6 ± 0.8</td>
<td>395.33</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>GJ intensity</td>
<td>7.6 ± 0.7</td>
<td>7.9 ± 0.7</td>
<td>1.78</td>
<td>.19</td>
</tr>
<tr>
<td>GJ familiarity</td>
<td>8.2 ± 0.8</td>
<td>7.3 ± 1.0</td>
<td>7.89</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Sucr liking</td>
<td>2.6 ± 0.8</td>
<td>2.9 ± 0.8</td>
<td>1.69</td>
<td>.20</td>
</tr>
<tr>
<td>Sucr intensity</td>
<td>6.1 ± 1.4</td>
<td>6.0 ± 1.4</td>
<td>0.02</td>
<td>.89</td>
</tr>
<tr>
<td>Sucr familiarity</td>
<td>6.8 ± 2.4</td>
<td>7.8 ± 0.9</td>
<td>3.33</td>
<td>.08</td>
</tr>
<tr>
<td>Quin liking</td>
<td>−3.4 ± 0.6</td>
<td>−3.5 ± 0.6</td>
<td>0.06</td>
<td>.81</td>
</tr>
<tr>
<td>Quin intensity</td>
<td>7.5 ± 1.1</td>
<td>7.4 ± 1.1</td>
<td>0.05</td>
<td>.83</td>
</tr>
<tr>
<td>Quin familiarity</td>
<td>4.6 ± 2.9</td>
<td>5.2 ± 2.4</td>
<td>0.45</td>
<td>.51</td>
</tr>
<tr>
<td>W liking</td>
<td>−0.1 ± 0.7</td>
<td>−0.1 ± 0.7</td>
<td>0.03</td>
<td>.96</td>
</tr>
<tr>
<td>W intensity</td>
<td>2.5 ± 1.4</td>
<td>2.1 ± 1.4</td>
<td>0.49</td>
<td>.49</td>
</tr>
<tr>
<td>W familiarity</td>
<td>5.1 ± 2.6</td>
<td>5.3 ± 3.0</td>
<td>0.03</td>
<td>.87</td>
</tr>
</tbody>
</table>

a Liking ratings were measured on a 9-point scale ranging from −4 “dislike extremely” to 4 “like extremely”.
b Intensity and familiarity ratings were measured on a 9-point scale ranging from 1 “not at all” to 9 “extremely”.
c Repeated measures GLM was used to analyze differences in liking and intensity ratings between likers and dislikers. Differences in familiarity ratings between groups were analyzed using a one-way ANOVA.
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Figure 3. Valence-specific activations related to tasting sucrose and quinine.
Valence-specific activations related to the comparison of liked Sucr (pink) and disliked Quin (green) \((|\text{likersSucr} & \text{dislikersSucr}) - (|\text{likersQuin} & \text{dislikersQuin})|\), thresholded at \(p<.001\) (unc) and \(k>8\).

A greater dorsal anterior cingulate cortex (dACC) activity when tasting something disliked (i.e. Quin compared to Sucr); B greater bilateral OFC activity when tasting a liked stimulus (i.e. Sucr compared to Quin).

Table 2. Activations* related to the comparison between Sucrose and Quinine

<table>
<thead>
<tr>
<th>Contrast</th>
<th>Area</th>
<th>Cluster size</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>Z-score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose &gt; Quinine</td>
<td>Orbitofrontal cortex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>R Lateral Orbitofrontal</td>
<td>53</td>
<td>33</td>
<td>47</td>
<td>−8</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>L Lateral Orbitofrontal</td>
<td>45</td>
<td>−42</td>
<td>50</td>
<td>−5</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td>L Lateral Orbitofrontal</td>
<td></td>
<td>−30</td>
<td>44</td>
<td>−5</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>Temporal cortex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>L Medial Temporal gyrus</td>
<td>12</td>
<td>−57</td>
<td>−1</td>
<td>−23</td>
<td>3.5</td>
</tr>
<tr>
<td>Quinine &gt; Sucrose</td>
<td>Operculum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>R Frontal Inferior Operculum</td>
<td>23</td>
<td>60</td>
<td>20</td>
<td>7</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td>Cingulate cortex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>L Middle Cingulate</td>
<td>72</td>
<td>−6</td>
<td>26</td>
<td>40</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>Occipital cortex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>R Inferior Occipital gyrus</td>
<td>19</td>
<td>39</td>
<td>−73</td>
<td>−11</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td>R Inferior Occipital gyrus</td>
<td>36</td>
<td>−82</td>
<td>−14</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Precentral gyrus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>L Precentral gyrus</td>
<td>19</td>
<td>−51</td>
<td>8</td>
<td>16</td>
<td>3.4</td>
</tr>
</tbody>
</table>

*a Activations reported are significant at \(p < .001\), uncorrected for multiple comparisons, with a cluster size of \(k > 8\) contiguous voxels. All coordinates are in MNI space.

*b L, left hemisphere; R, right hemisphere
5.3.2.2 Tasting individually liked or disliked grapefruit juice

Whole-brain analysis across likers alone ([G]likers|GJ) revealed significantly activated clusters of voxels related to the subjective pleasant experience of tasting GJ in the OFC, thalamus, striatum, bilateral amygdala, and insula. (Table S3 and Figure 4). For dislikers only ([G]dislikers|GJ), the subjective unpleasant experience of tasting GJ revealed activations in OFC, cingulate cortex, insula, and striatum (Table S4 and Figure 4).

A direct comparison of activations in response to GJ between likers and dislikers ([G]likers|GJ – [G]dislikers|GJ)) revealed significantly more activation for likers compared to dislikers in the OFC. In contrast, tasting GJ resulted in greater activations for dislikers compared to likers in cingulate cortex, insula and operculum, and precentral gyrus (Table 3 and Figure 5).

Table 3. Activations* related to the comparison between likers and dislikers of grapefruit juice

<table>
<thead>
<tr>
<th>Contrast</th>
<th>Area</th>
<th>Cluster size x</th>
<th>y</th>
<th>z</th>
<th>Z-score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Likers &gt; Dislikers</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Orbitofrontal cortex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Precentral gyrus</td>
<td></td>
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<tr>
<td></td>
<td>Temporal cortex</td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Cingulate cortex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Occipital cortex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Insula/Operculum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dislikers &gt; Likers</td>
<td>R Lateral Orbitofrontal gyrus</td>
<td>10</td>
<td>45</td>
<td>47</td>
<td>−11</td>
</tr>
<tr>
<td></td>
<td>L Precentral gyrus</td>
<td></td>
<td>−36</td>
<td>−10</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>L Precentral gyrus</td>
<td></td>
<td>−48</td>
<td>−7</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>L Superior Temporal gyrus</td>
<td></td>
<td>−54</td>
<td>−31</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>L Medial Temporal gyrus</td>
<td></td>
<td>−45</td>
<td>−55</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>L Superior Temporal gyrus</td>
<td></td>
<td>−51</td>
<td>−43</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>R Superior Temporal gyrus</td>
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<td>23</td>
<td>42</td>
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<td>R Medial Temporal gyrus</td>
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<td>39</td>
<td>2</td>
<td>−29</td>
</tr>
<tr>
<td></td>
<td>L Middle Cingulate</td>
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<td>−31</td>
<td>31</td>
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<tr>
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<td>−31</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>L Middle Cingulate</td>
<td></td>
<td>−12</td>
<td>−43</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>L Inferior Occipital</td>
<td></td>
<td>−33</td>
<td>−67</td>
<td>−2</td>
</tr>
<tr>
<td></td>
<td>L Inferior Occipital</td>
<td></td>
<td>−39</td>
<td>−76</td>
<td>−8</td>
</tr>
<tr>
<td></td>
<td>R Rolanid Operculum</td>
<td></td>
<td>35</td>
<td>54</td>
<td>−25</td>
</tr>
<tr>
<td></td>
<td>R Rolanid Operculum</td>
<td></td>
<td>39</td>
<td>−34</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>R Insula</td>
<td></td>
<td>30</td>
<td>−37</td>
<td>25</td>
</tr>
</tbody>
</table>

* Activations reported are significant at p < .001, uncorrected for multiple comparisons, with a cluster size of k > 8 contiguous voxels. All coordinates are in MNI space.

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5.3.2.2 Tasting individually liked or disliked grapefruit juice

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To like or not to like: neural substrates of subjective flavor preferences

5.3.3 Multivariate fMRI results

The SVM selection algorithm selected 7.9% (see Table S5 and Figure 6) and 1.8% (see Table S6 and Figure 7) of the voxels for classification of Sucr versus Quin and likers versus dislikers, respectively. To verify whether the best voxels were selected, we used 5-fold cross validation to predict the classes with the selected voxels. For Sucr and Quin 100% (68/68) of the classifications were correct, for likers and dislikers 88.2% (30/34) of the classifications were correct. To test the overlap of voxels that contribute to the classification of Sucr-Quin and likers-dislikers, we also trained the SVM on Sucr-Quin for predicting likers-dislikers and vice versa. The accuracy scores were 55.9% (19/34) when the SVM trained on Sucr-Quin was used to classify likers-dislikers and 51.5% (35/68) when the SVM trained on likers-dislikers was used to classify Sucr-Quin, indicating a very poor resemblance between both patterns.
Chapter 5

5.4 DISCUSSION

This study investigated whether differential neural activation exists that accounts for individual differences in flavor preference, by using one target stimulus (grapefruit juice: GJ) that was either liked or disliked by participants. In addition, activations related to innate preferences were identified using taste solutions that are inherently experienced as pleasant (sucrose: Sucr) and unpleasant (quinine: Quin). Brain responses to all three stimuli were subsequently used to investigate whether differences in liking for GJ revealed similar activation patterns as differences in liking for Sucr and Quin.

Our results show that the main effects of tasting something liked or disliked (i.e. Sucr, Quin, and GJ) yielded activations in OFC, striatum, cingulate cortex and amygdala. These regions are known to be associated with preference coding and have previously been associated with taste processing and pleasantness. For instance, they have been shown to activate in response to pure tastes that are inherently experienced as pleasant (e.g., sucrose and glucose) or unpleasant (e.g., quinine) (O’Doherty et al., 2001b; Small et al., 2003; Zald et al., 2002). These regions have also been identified when pleasantness was modulated through
consumption of a product from hunger to satiety or when top-down attentional or cognitive processes influenced affective reactions toward taste and flavor stimuli (Grabenhorst et al., 2008; Haase et al., 2009; Kringelbach et al., 2003; Plassmann et al., 2008; Small et al., 2001; Smeets et al., 2006). The current results from both generally and subjectively liked and disliked tastes are thus in accordance with previous findings.

Interestingly, the main effects of the pleasant and unpleasant taste of all three stimuli revealed activations in both medial and lateral parts of the OFC. OFC activation has been consistently linked with hedonic (taste) processing and subjective experiences of displeasure (Berridge and Kringelbach, 2008; Kringelbach and Radcliffe, 2005; Kringelbach, 2010; Small, 2002) and a differentiation in function between medial versus lateral OFC has previously been put forward, with medial OFC activation being more related to monitoring the reward value of reinforcers and lateral OFC activation more related to the evaluation of punishers (Kringelbach and Rolls, 2004; O’Doherty et al., 2001a; Rolls et al., 2003). Based on this distinction, one might have expected that a subjectively pleasant taste would have preferentially activated the medial OFC whereas an unpleasant taste would have preferentially activated the lateral OFC (e.g., similar to (Rolls et al., 2003)). However, we believe that medial and lateral subregions of the OFC both display a graded (linear) scale of activation that positively correlates with subjective pleasantness, but that the change in activation in medial OFC appears to be stronger for pleasant compared to unpleasant stimuli, and vice versa for the lateral parts. Several studies have already demonstrated this graded scale of activation in different subparts of the OFC, with both medial and lateral parts activated by or correlating with subjective experience of pleasure (Grabenhorst and Rolls, 2009; Grabenhorst et al., 2010; Kringelbach et al., 2003; O’Doherty et al., 2001b). The absolute difference in activation in both medial and lateral OFC as a result of contrasting pleasant and unpleasant tastes (i.e. respectively positive and negative changes in BOLD response in OFC) with a neutral taste (i.e. no change), further supports this view.

Contrary to our hypothesis, we did not observe large differences in OFC or other areas related to taste pleasantness between likers and dislikers of GJ. Only a few clusters of activation in posterior middle cingulate cortex (pMCC) extending into precuneus, Ins/Oper, caudate, precentral gyrus, and temporal gyrus differed when comparing dislikers with likers, and one small cluster in right OFC exhibited greater activation in likers compared to dislikers. In contrast, when comparing Sucr and Quin we did observe greater activation in bilateral OFC for Sucr and greater activation in dorsal ACC for Quin. Multivariate analysis revealed similar results. Far less voxels contributed to the classification of likers and dislikers of GJ and showed a lower success rate compared with the classification of Sucr and Quin. It thus seems that the difference between liking and disliking for a learned preference of a complex taste is less pronounced than the difference in liking between innately preferred and unpreferred tastes. Caution should however be exercised when interpreting these results. As mentioned previously, when different taste qualities are used to elicit opposite affective reactions, liking may be confounded with quality. In other words, when comparing Sucr and Quin, we are comparing two different stimuli with different physical properties whereas the stimulus properties are held constant when comparing likers and dislikers of GJ. A greater difference
between Sucr and Quin compared to the difference between likers and dislikers of GJ is thus an expected outcome inherent to the study design. Furthermore, the current difference between Sucr and Quin may be confounded with intensity as these ratings differed significantly for Sucr and Quin (p-values not shown, ratings in Table 1). A recent study that also sought to overcome these possible confounds compared likers and dislikers of a sweet sucrose solution. Their findings show activations in ventromedial prefrontal cortex (vmPFC) associated with individual differences in sweet-taste preference, whereas differential responses were not observed elsewhere in the brain (Rudenga and Small, 2013). Therefore, the authors suggest that preference-specific responses independent of taste quality are not represented in (caudo) lateral OFC but only in vmPFC. Results of the current study do however show that additional as well as different regions are likely to be involved in the representation of individual preferences for a complex taste.

Multivariate analysis did additionally reveal interesting areas that contributed to the classification of likers and dislikers. In addition to the regions identified with univariate analysis, voxels in pregenual anterior cingulate cortex (prACC), anterior insula extending into lateral OFC, ventral striatum, and right amygdala were selected. Activation in prACC has frequently been reported in taste-related studies (e.g., (De Araujo and Rolls, 2004; Rolls and McCabe, 2007; Rolls et al., 2003; Veldhuizen et al., 2011)) and is often reported in co-activation with the OFC (Kringelbach and Radcliffe, 2005). Moreover, it has been shown that prACC activation correlates with pleasantness ratings and preferentially responds when attention is directed to the pleasantness of a taste (Grabenhorst and Rolls, 2008; Grabenhorst et al., 2008). One of these studies (Grabenhorst et al., 2008) also reported activation in ventral striatum when, through cognitive modulation, a flavor stimulus was perceived as more pleasant. More generally, the ventral striatum and in particular the NAcc is widely regarded as one of the key regions in reward processing and is part of a reward-circuitry that includes areas such as OFC, anterior cingulate cortex, amygdala, thalamus, and hippocampus (Berridge, 2009, 2003; Haber and Knutson, 2010; Kringelbach and Berridge, 2010). Interestingly though, more recent research suggests that NAcc is not only implicated in processing rewarding stimuli, but also in the encoding of aversion (Gottfried et al., 2002; McCutcheon et al., 2012). On the other hand, amygdala activation has long been associated with processing threatening, fearful, and highly aversive stimuli (e.g., (Davis, 1992)) though more recent views indicate that it does not preferentially respond to negative stimuli (e.g., (Murray, 2007)). Instead, amygdala activation seems to reflect an interaction between intensity, novelty and intrinsic affective value, thereby proposing an important role for the amygdala in establishing the saliency of sensory stimuli (Liberson et al., 2003; Small, 2006; Small et al., 2003). Finally, the anterior insula, continuous with the primary gustatory cortex, is more commonly known to be involved in the experience of disgust and aversive events (Calder et al., 2000; Wicker et al., 2003). Concurrently, anterior insula activation, often extending into caudolateral OFC, has been found to correlate with subjective ratings of unpleasantness or disgust (Grabenhorst and Rolls, 2009; Small et al., 2003; Zald et al., 1998).

Taken together, these regions are all part of a network known to be involved in the processing of rewarding or aversive stimuli. Yet to our knowledge, current multivariate results
show for the first time that when comparing likers and dislikers of one and the same product, these regions contribute most to the classification of these two groups. In other words, activation in these areas can predict with an 88% success rate whether a given person likes or dislikes the target product. We therefore conclude that, when using multivariate analysis, we were able to identify differential neural patterns that account for individual differences in flavor preference.

A second aim of the present study was to compare responses to innately preferred and unpreferred tastes with responses to a learned flavor preference. As mentioned before, univariate results from the comparison of Sucr and Quin and the comparison of likers and dislikers of GJ revealed different areas. Multivariate SVM classification amplified these results. Very little overlap was found between the activation patterns that contribute to the classification of Sucr and Quin and those contributing to the classification of likers and dislikers. Moreover, when the SVM algorithm trained on the classification of Sucr and Quin was used to predict classification of likers and dislikers or when the SVM trained on the classification of likers and dislikers was used to classify Sucr and Quin, accuracy scores were around chance level. This indicates that the pattern of activation that discriminates Sucr and Quin is not very similar to the pattern that discriminates likers and dislikers and consequently performs poorly when used to discriminate likers from dislikers, and vice versa.

There are a few possible explanations for this difference between the brain activation pattern that discriminates likers from dislikers and the pattern that discriminates Sucr from Quin. It may be that innate taste preferences are mediated by different neural processes than preferences that are learned and developed through subjective experience. As mentioned earlier, innate preferences subserve an evolutionary advantage by providing information about the presence or absence of nutrients and toxins (Mattes, 2003). Innate taste preferences may therefore be regarded as basic or primary rewards as they are essential for human survival by guiding our eating behavior towards edible and nutritional foods. Learned preferences for complex stimuli on the other hand may be regarded as higher order rewards, as they depend more on individual and contextual factors that contribute to a more cognitive evaluation of the flavor. With this in mind, the complex combination of sweet, sour and bitter tastes that are present in the target stimulus GJ do not simply signal either energy or toxic content but rather require a cognitive approach integrating the different taste qualities with personal experience such as previous exposures and acquired preferences, product knowledge, social context, etc. This process of preference formation is thought to occur through associative learning (Hoffmann et al., 2010; Rozin and Zellner, 1985). Both amygdala and striatal activation have been implicated in associative learning processes (Cardinal et al., 2003; Schultz, 1997) and in food preference formation (Galindo et al., 2012; Gottfried et al., 2002; Yamamoto and Ueji, 2011). The presence of amygdala and ventral striatum in the pattern that discerns likers and dislikers of GJ may thus point towards the possibility that learned preferences are mediated by associative learning processes and are consequently represented differently than preferences for innate tastes. However, the neural underpinnings of flavor learning and preference formation are still relatively unexplored and more research in this area is needed before we can attempt to compare brain mechanisms underlying innate versus learned preferences.
Additionally, it may be that the difference in brain activation discriminating likers from dislikers and activation discriminating Sucr from Quin is a result of using on the one hand basic tastes and on the other hand a complex flavor stimulus for the comparison of subjectively liked or disliked stimuli. One way to exclude this possibility would be to replace Sucr and Quin with two different complex flavor stimuli that are perceived as pleasant and unpleasant by all participants. Moreover, the use of only complex flavor stimuli would yield that preferences for these stimuli are all learned. It would be interesting to see whether varying preferences for different learned and complex stimuli would bear more resemblance than the discerning patterns for basic and complex taste stimuli in the current study.

Finally, the difference between the pattern that discriminates likers from dislikers and the pattern that discriminates Sucr from Quin might be accentuated by the difference in comparisons. As mentioned earlier, when different tastes are used to elicit opposite affective reactions, the discriminating pattern may be confounded with sensory quality, whereas this is not the case when opposite affective reactions to one target stimulus are compared. One should therefore exercise some caution when interpreting the differences between the two patterns, or alternatively use an approach similar to Rudenga and Small (2013) to identify differences in basic taste preferences. Another concern that may be thought to influence differences between tastes is the use of a common baseline condition. By contrasting each taste with water at the first level of analysis, it might be possible for differential responses to water to influence the ability to detect differential effects for Sucr, Quin or GJ. However, water (as opposed to for instance an artificial saliva solution) was specifically selected as the control condition because it is most often perceived as neutral in liking. Concurrently, participants’ ratings show that water was neutrally liked and that scores did not differ between likers and dislikers. We therefore do not believe that the resulting differences in brain activations are subject to the use of a common control condition.

5.5 CONCLUSION

In conclusion, the present study showed that both liked and disliked stimuli yielded activation in medial and lateral parts of the OFC, indicating that these subregions do not preferentially respond to one or the other, but more likely display a graded scale of activation that correlates with subjective pleasantness. When directly comparing likers and disliked stimuli of the target stimulus (GJ), we were able to identify differential neural activation that accounts for individual differences in flavor preference. These differences were however less pronounced than expected, whereas the comparison of two differentially appreciated tastes (Sucr and Quin) yielded more robust differences. Interestingly though, both univariate and multivariate analysis show that the neural pattern discriminating learned preferred and unpreferred flavors was different compared with the neural pattern that discerns innately preferred and unpreferred tastes. We posit that this difference may be a result of differential processing, with innate preferences for basic tastes being processed primarily with respect to the nutritional value they signal, whereas learned flavor preferences may be processed more cognitively and depend more on subjectively stored information and experiences. It should be
mentioned though that the greater differences between the basic tastes with different physical properties is an expected outcome inherent to the study design since they differ not only in affective value but also in physical taste quality. Nevertheless, these findings do highlight the importance of elucidating the neural processes of how flavor preferences are learned and acquired, as the poor overlap between patterns discerning innate versus learned preferences may suggest that different mechanisms are at work. Alternatively, the difference could be a result of using simple versus complex taste stimuli, in which case it would be interesting for future research to investigate how different taste aspects become integrated into a complex flavor concept and how this differs from the perception of basic tastes alone.

5.6 APPENDIX A

Figure A1. Consort flow diagram of participant selection.

The figure shows a flow diagram of the recruitment process. Out of 348 initial respondents, 34 were eligible for the fMRI experiment.