Title: The use of 'artificial saliva' as a neutral control condition in gustatory research

Short title: Artificial saliva is not a neutral gustatory stimulus

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Abstract

Distilled water with $NaHCO_3$ and KCl is a solution often referred to as 'artificial saliva' because its chemical composition mimics human saliva. It is often used as a control stimulus in gustatory research, especially in neuroimaging, owing to the claim that it does not produce a response in primary gustatory cortex Yet evidence that human research volunteers perceive this liquid as affectively neutral is lacking. Unpublished data from our lab suggested that this solution might be perceived as aversive. This study set out to systematically test the parameters influencing taste neutrality. We used two different concentrations of distilled water with $NaHCO_3$ and KCl, as well as bottled water as a control stimulus. Healthy adults rated all stimuli on two separate scales to rule out an interpretation based on the specifics of a single scale. Our participants rated artificial saliva as aversive on both scales. The bottled water was rated as neutral in valence on both scales, and as significantly less intense in sensation than both concentrations of the artificial saliva. This is the first study to have directly tested the subjective feelings that accompany the ingestion of these oft-used solutions on a trial-by-trial basis. We found that these stimuli, which were previously assumed to be neutral, may not be perceived as such by research participants. Therefore, future gustatory studies should take care when using this solution as a neutral baseline. It is advised that trial-by-trial ratings are collected. Also, depending on the nature of future studies, bottled water may be considered as a preferable neutral baseline.

Key words: artificial saliva; $NaHCO_3$; KCl; gustatory processing; neutral taste

1. Introduction

Most experiments of gustatory function, as well as those that employ gustatory stimuli to manipulate affective and cognitive function, need a neutral baseline. A prevalent neutral baseline is distilled water with KCl and $NaHCO_3$ (see Franken et al., 2011; Grabenhorst, D'Souza, Parris, Rolls, & Passingham, 2010; Grabenhorst et al., 2008; Hird et al., 2017; Nakamura et al., 2011; Rolls, Kellerhals, & Nichols, 2015). This solution is typically referred to as 'artificial saliva' because its chemical composition mimics that of human saliva. Whereas humans can sense water in their mouths (Bartoshuk et al., 1964), distilled water with $NaHCO_3$ and KCl is designed not to be sensed, and therefore to be less sensorily stimulating than normal water to human research volunteers. Furthermore, artificial saliva solutions do not activate gustatory cortex in the way other nominally neutral stimuli, such as water, do (Frey & Petrides, 1999; Veldhuizen et al., 2011, 2013; Veldhuizen & Small, 2011; Zald & Pardo, 2000). These properties led to its frequent use as a chemosensorily and neurally neutral baseline in neuroimaging research that employs gustatory stimuli (Francis et al., 1999; Frank et al., 2003).

The development of artificial saliva composition comes from several fields of research. Early development was predominantly from the field of dentistry. The first noted use was in 1931 (Souder & Sweeney, 1931) where composition was selected arbitrarily, based on natural human saliva. In subsequent decades many attempts were made to optimise and standardise a formula for artificial saliva composition. Most were based on some attempt to capture the "average" human saliva (e.g. Fusayama et al., 1963). However, to date there is no universally agreed upon formula, with multiple different types of solution employed (for review see Pytko-Polonczyk et al., 2017). This is in part because human saliva composition across individuals is so diverse that an "average" composition does not fit any one individual

(Pytko-Polonczyk et al., 2017). Furthermore, optimal composition depends greatly on the specific aims of the study.

The earliest use of artificial saliva in neuroimaging appears to be a 1999 study by Francis and colleagues (Francis et al., 1999). Here, an "almost tasteless artificial saliva" (pp. 454) was used as a control with an experimental glucose stimulus. Their artificial saliva contained 25mM KCl and 2mM NaHCO3, though the rationale for this choice was not provided. The purpose of a control stimulus in neuroimaging is to serve as a baseline level of activation for the subtraction method. The key to this approach is to utilise a control stimulus that evokes no/limited activation in the regions of interest, whilst being as closely matched to the experimental stimuli as possible in other factors, to cancel out activation not related to the experimental manipulation (Huettel et al., 2004). A control condition that evokes activation too similar to the experimental condition(s) is ineffective, as activation of interest will be subtracted away (Huettel et al., 2004). For gustatory research, a control stimulus would need to produce similar somatosensory and motor processes, for example, without activating primary taste cortex. Francis and colleagues (1999) employed a sample of just six participants, so low statistical power must be considered. They did, however, observe activation of insula and medial orbitofrontal cortices in response to the sweet stimulus above that of the artificial saliva control. This was interpreted as these regions processing taste information, with the underlying assumption presumably that artificial saliva did not.

The composition of artificial saliva solutions in neuroimaging suffers from the same lack of standardisation observed in dentistry. Concentration of solutes differs across laboratories, as does procedure for choosing the solution. Some laboratories use a method of individualised selection for each participant. Several solutions are tasted, with participants asked to select "the one that tastes most like nothing" (Veldhuizen et al., 2007, 2011, 2013; Veldhuizen & Small, 2011). Others use a pre-determined solution for all participants (Francis

et al., 1999; Frank et al., 2003). Given that these solutions do have a taste, as evident in the "tasted most like nothing" instruction in individualised selection (Veldhuizen et al., 2007 pp. 571), solution composition may influence how participants experience these stimuli. This lack of neutrality in participant experience plays an important bearing on interpretation of results. Firstly, researchers must be aware that their comparison is not with a hedonically neutral stimulus, and results should be interpreted accordingly. Secondly, one must be aware that taste experience is much more heterogeneous than in a modality like vision, where experience and neural response are more consistent (Zald & Pardo, 2000). For example, Zald et al. (1998) reported a significant increase in insula activation in response to saline, but only in participants who reported finding the stimulus 'extremely aversive'. Given the heterogeneity in taste experience, and the concomitant effect on neural response, important consideration must be given to selection of control stimuli. Indeed, it is unclear whether 'artificial saliva' is truly perceived as neutral by research participants, as previous studies have not routinely collected ratings of the pleasantness or intensity of its perceived taste on a trial-by-trial basis. The aim of the present study was, therefore, to test the pleasantness and intensity of different concentrations of this solution to establish whether distilled water with $NaHCO_3$ and KCl could be considered affectively neutral. This is important because if it is, this would validate the use of this solution as a control stimulus. In contrast, if the current study shows that this solution is perceived to be pleasant or unpleasant then this would have consequences for its use as a neutral control stimulus in future psychological research where hedonic neutrality was important.

One important factor that needs to be taken into account when collecting gustatory pleasantness ratings is the rating scale that is used. This is because it has been found that the size of measured differences in taste perception between different stimuli depends on the scale that is used (Kalva et al., 2014). Indeed, this may be particularly important for highly

subjective measures such as pleasantness. Despite the influence of scale choice, gustatory research as a field is characterised by use of a wide range of different rating scales (Lim, 2011). In this study, we therefore compared two rating scales that are used frequently in gustatory research to ensure scale choice did not introduce systematic bias in ratings. We used a 9-point rating scale, ranging from one being not pleasant at all, to nine being very pleasant (Kalva et al., 2014b). We included this scale because of its ease of understanding for participants – a simple nine-point scale on which to rate each stimulus. The second scale type we used was a general labelled magnitude scale (hedonic gLMS; Bartoshuk, Catalanotto, Hoffman, Logan, & Snyder, 2012), which is a logarithmic scale. These scales were developed to address the concern that 9-point scales could not appropriately compare across participants. The scale labels assume equivalence of experience across participants, which is not true of gustatory perception. By having participants rate the pleasantness or intensity of stimuli relative to sensory experiences from a different modality, the participant's own subjective experience in terms of taste can be evaluated using a gLMS. These scales have been shown to detect differences in taste perception obscured by standard 9-point scales (Kalva et al., 2014).

The main aim of this study was to investigate the effect of researcher choices on participant taste experience. We sought to test how concentration of artificial saliva solutions of different concentration affect pleasantness and intensity perception on a trial-by-trial basis. As many different concentrations of artificial saliva are used in research, we chose two commonly-used concentrations. This was compared with an alternative neutral stimulus, bottled water. Bottled water is a frequently-consumed liquid that intuitively many might consider hedonically ‘neutral’ in the common sense of the word. Indeed, the familiarity of bottled water might be an influential factor in the subjective experience of “neutrality”. We hypothesised that the artificial saliva would be rated as more unpleasant and more intense

than bottled water. We further sought to test how the scale used to collect ratings of liking and intensity affect the results. We employed two scales common in gustatory research, a 9-point likert scale and a gLMS. We hypothesized that ratings would differ on these two scales.

2. Methods

2.1 Participants

30 participants (seven self-identified as male, no-one self-identified as non binary) were recruited for this study. Participants could take part if they were age 18 or over, non-smokers, with no history of neurological or psychiatric illness (except for binge eating disorder), not currently on a restrictive diet, and with no food allergies that would interfere with the stimuli used in this study. Three participants were excluded from the final data analysis because one or more of their valence ratings for the nominally-neutral stimuli was more than three standard deviations away from the mean, leaving 27 participants in the final sample. Descriptive statistics for the sample are given in Table 1.

INSERT TABLE 1 HERE

2.2 Procedure

Ethical approval for this study was granted by the University of Manchester Research Ethics Committee. All participants gave written consent before commencement. Participants were asked to refrain from eating or drinking anything other than water for at least three hours before the study, in an attempt to ensure that they were experiencing comparable levels of hunger. At the start of the experiment, participants were asked to rate their hunger, fullness, craving and thirst on a scale from one (being the least hungry, full, etc) to ten (being the most hungry, full, etc). Participants were then given careful instructions on

how to use the nine-point and gLMS scales and what the order of experimental tasks would be.

The stimuli were rated on the nine-point scale and the gLMS in two separate blocks. Half the participants first rated all the stimuli using the nine-point valence and intensity scales, and then repeated these ratings in the same order with the hedonic and intensity gLMS in a second block. The order of these blocks was reversed for the other half of participants. Participants always rated the pleasantness of the stimulus first, then the intensity. All stimuli were stored and served at room temperature, to avoid changes in temperature across the time-course of the experiment. Stimuli were served in small disposable plastic cups, with ~50 ml in each cup. Participants were asked to take a sip from the cup, hold it in their mouth for as long as they wished, then swallow. They were instructed that they could spit it out if they could not bear it, but in practise all stimuli were swallowed by all participants. This procedure was selected to be more comparable to neuroimaging studies (Francis et al., 1999; J. O'Doherty et al., 2001). Participants were then asked to rate the stimulus on its pleasantness and intensity. Participants took one sip from each cup. Once participants had rated the stimulus they were prompted to rinse their mouths with water before continuing. This was bottled water in a separate drinking glass, easily distinguishable from the test stimuli. Participants were not informed that this was bottled water identical to one of the test stimuli. Each stimulus was served once in each block. The order of stimuli within the block was randomised individually for each participant, with this same randomised order used for each block. The administration and rating of the stimuli was self-paced. Participants were given a short break in between the two blocks. After the rating of the stimuli was completed participants once again rated their hunger, fullness, craving, and thirst, to determine whether any of these had changed over the course of the experiment. Finally, their height and weight were measured before participants were thanked and dismissed.

2.3 Materials

2.3.1 Stimuli

Three types of nominally-neutral solutions were used. Two of these were two commonly-used 'artificial saliva' control solutions in gustatory research: distilled water with either 2 mM $NaHCO_3$ and 15 mM KCl or with 2.5 mM $NaHCO_3$ and 25 mM KCl (Franken et al., 2011; Hird et al., 2017; van Bloemendaal et al., 2015; Bohon et al., 2009; Grabenhorst, Rolls, Parris, & D'Souza, 2010; Iannilli, Noennig, Hummel, & Schoenfeld, 2014; Kringelbach, O'Doherty, Rolls, & Andrews, 2003; McCabe & Rolls, 2007; Nakamura et al., 2011; O'Doherty, Deichmann, Critchley, & Dolan, 2002; Rolls et al., 2015; Rolls, 2009; Stice, Burger, & Yokum, 2013; Stice et al., 2008; Sun et al., 2014; Wang, Yang, Hajnal, & Rogers, 2015). The third was unflavoured, Tesco own-brand, still bottled water (Per 100ml: Na 0.0018g, $HC0_3$ 0.0200g, K 0.000g, Cl 0.0017g). These three nominally-neutral solutions were compared to two solutions with detectable flavour for comparison. Apple juice (Tesco-own brand, from concentrate) was used as a typically sweet taste (Per 100ml: Na 0g, $HC0_3$ 0g, K 0g, Cl 0g). Bottled water with 5 grams of salt (NaCl) per 100ml (Per 100ml: NaCl 5.0g, $HC0_3$ 0g, K 0g) was used as a salty taste. These comparison stimuli were chosen as they are usually rated as pleasant and unpleasant, respectively. Such comparisons were used to ensure participants use the entire scale and to facilitate interpretation of the participants' ratings of our experimental stimuli (Frank et al., 2003; Zald et al., 1998).They were never referred to as pleasant or unpleasant, or with any other descriptive terms.

2.3.2 Scales

The two scales used in this study were a 9-point rating scale (1=not pleasant at all, to 9=very pleasant) and a gLMS (see Figure 1; adapted from Kalva et al., 2014b from

Bartoshuk et al., 2004; Bartoshuk et al., 2012; Drewnowski et al., 1997). There were two versions of each scale: one to measure valence and one to measure intensity.

The "hedonic 9-point scale", measuring valence, had three labels in this study: 1 ("not pleasant at all"), 5 ("neutral), and 9 ("very pleasant"). The "sensory 9-point scale", measuring intensity had two labels: 1 ("not intense at all") and 9 ("very intense").

The "hedonic gLMS", measuring valence, is a continuous scale that ranges from "strongest imaginable disliking of any kind" to "strongest imaginable liking of any kind" (Kalva et al., 2014b). It requires people to mark an X on the scale based on where they think the preceding stimulus falls on this continuum. Participants are first informed that the "strongest imaginable (dis)liking of any kind" can be any sensory feeling and does not need to be related to taste. They are instructed that they should rate the taste stimuli against this feeling. The precise instructions given to participants were *"Use this scale to rate how much you liked the taste. You can place an X anywhere on the scale, ranging from the 'strongest imaginable dislike' to 'strongest imaginable like,' and 'neutral' in the middle. These do not refer to flavours only; rather, they refer to the worst sensation you can imagine, and the best sensation you can imagine. The same is true for the next scale, where you rate the intensity of the taste. This ranges from experiencing no sensation at all, to the strongest possible sensation you can imagine, which is not exclusive to taste. Again, you can place an X anywhere on the scale."* This scale was scored by measuring the distance (in millimetres (mm)) between the X put on the scale by the participant and the 'neutral' marker. In the current study, the hedonic gLMS ranged from -79mm, indicating "strongest imaginable dislike" to 79mm, indicating "strongest imaginable like", with 0mm indicating "neutral". The other markers on the scale were at -42mm ("very strong dislike"), -29mm ("strong dislike"), -13mm ("moderate dislike"), -5mm ("weak dislike"), 5mm ("weak like"), 13mm ("moderate like"), 29mm ("strong like"), and 42mm ("very strong like").

The "intensity gLMS" ranged from "no sensation" to "strongest imaginable sensation of any kind". The intensity gLMS was scored by measuring the distance in mm between the X put on the scale by the participant and "no sensation" maker of the scale, which was scored as 0mm. The other markers on this scale were at 5mm ("weak sensation"), 23mm ("moderate sensation"), 53mm ("strong sensation"), 80mm ("very strong sensation") and 155mm "strongest imaginable sensation of any kind").

INSERT FIGURE 1 HERE

2.4 Statistical analysis

The data were analysed in SPSS 22 (IBM Corp, 2013). Most of the data were not normally distributed (Kurtosis > 1), so non-parametric tests were used to analyse the data. One-sample Wilcoxon signed rank tests were carried out to compare the valence and intensity ratings of the five stimuli to the neutral point on the hedonic scales and the least intense point on the intensity scales. To compare the three nominally-neutral stimuli we conducted four one-way Friedman related-samples ANOVAs, a non-parametric equivalent to repeated-measured ANOVAs. Where significant, Friedman ANOVAs were followed up with related-samples Wilcoxon signed rank post-hoc comparisons. As each post-hoc analysis would involve three Wilcoxon signed rank tests, a Bonferroni correction of alpha =.017 was applied.

3. Results

Pleasantness ratings are given in Figure 2. The apple juice was rated as significantly more pleasant than neutral on both scales (gLMS: median = 25, inter-quartile range = 15.5-28; 9-point: median = 8, inter-quartile range = 7-8.5; both $p<.001$). The salty water was rated as significantly more unpleasant than neutral on both scales (gLMS: median = -34, inter-quartile range = -43—26.5; 9-point: median = 2, inter-quartile range = 1-2; both $p<.001$).

The distilled water with 2 mM $NaHCO_3$ and 15 mM KCl was rated as significantly less pleasant than neutral on the hedonic gLMS (median = 0, inter-quartile range = -12-0.5; p=.041) , and on the 9-point scale (median = 4, inter-quartile range = 3.5-5; p=.001). The distilled water with 2.5 mM $NaHCO_3$ and 25 mM KCl was also rated as significantly less pleasant than neutral on both the hedonic gLMS (median = -3, inter-quartile range = -14.5-0; p=.001) , and on the 9-point scale (median = 4, inter-quartile range = 3-5; p<.001). Bottled water was not rated as significantly different from neutral on either the hedonic gLMS (median = 0, inter-quartile range = 0-2.5; p=.080)[1] or the 9-point scale (median = 5, inter-quartile range = 5-5; p=.269) . In summary, participants' ratings suggested only the bottled water was perceived as hedonically neutral.

INSERT FIGURE 2 HERE

Intensity ratings are given in Figure 3. The apple juice was rated as significantly more intense than the 'no sensation' mark on both scales (gLMS: median = 36, inter-quartile range = 19.5-45.5; 9-point: median = 6, inter-quartile range = 5-7; both p<.001). The salty bottled water was also rated as significantly more intense than 'no sensation' on both scales (gLMS: median = 68, inter-quartile range = 48.5-85.5; 9-point: median = 8, inter-quartile range = 7-9; both p<.001). The distilled water with 2 mM $NaHCO_3$ and 15 mM KCl was rated as more intense than 'no sensation' on both scales (gLMS: median = 10, inter-quartile range = 3-16.5; 9-point: median = 2, inter-quartile range = 1-4.5; both p<.001). This was also the case for the distilled water with 2.5 mM $NaHCO_3$ and 25 mM KCl (gLMS: median = 9, inter-quartile range = 5-18.5; 9-point: median = 3, inter-quartile range = 2-4; both p<.001), and the bottled water (gLMS: median = 4, inter-quartile range = 2-7.5, p=.003; 9-point: median = 1, inter-quartile range = 1-2,

[1] When analysing this data without excluding outliers (i.e. with all 30 participants), bottled water was rated as significantly more pleasant than the neutral point on the hedonic gLMS (p=.025). None of the other Wilcoxon signed-rank results changed as a result of including the outliers.

p<.001). In summary, participants perceived all of the stimuli to be more intense than neutral. None of the three neutral solutions mimicked saliva's characteristic of not being noticeable in the mouth.

INSERT FIGURE 3 HERE

The Friedman ANOVAs showed that the three nominally-neutral solutions differed in their pleasantness ratings on both the hedonic gLMS (χ^2 (2, N=27)=10.18, p=.006, Kendall's W=.188) and 9-point valence scale (χ^2 (2, N=27)=20.69, p<.001, Kendall's W=.383). Post-hoc tests showed that bottled water was rated as significantly more pleasant than the distilled water with 2 mM $NaHCO_3$ and 15 mM KCl both on the hedonic gLMS (Z=2.50, p=.013) and the 9-point scale (Z=3.21, p=.001). Bottled water was also rated as significantly more pleasant than the distilled water with 2.5 mM $NaHCO_3$ and 25 mM KCl on both the hedonic gLMS (Z=3.33, p=.001) and the 9-point valence scale (Z=3.67, p<.001). The two distilled-water solutions did not differ significantly from one another on the hedonic gLMS (Z=1.07, p=.283) or the 9-point valence scale (Z=1.47, p=.142).

The Friedman ANOVA testing the intensity ratings showed significant differences between the nominally-neutral solutions on both the intensity gLMS (χ^2 (2, N=27)=14.69, p=.001, Kendall's W=.272) and intensity 9-point scale (χ^2 (2, N=27)=8.58, p=.014, Kendall's W=.159). Post-hocs tests revealed that bottled water was rated as less intense than the distilled water with 2 mM $NaHCO_3$ and 15 mM KCl liquids on both the intensity gLMS (Z=3.33, p=.001) and 9-point intensity scale (Z=3.21, p=.001). Bottled water was also rated as less intense than the distilled water with 2.5 mM $NaHCO_3$ and 25 mM KCl on both the intensity gLMS (Z=3.57, p<.001) and 9-point scale (Z=3.09, =.002). There was no difference in intensity between the two distilled water solutions on the intensity gLMS (Z=1.14, p=.253) or 9-point scale (Z=0.36, p=.719).

4. Discussion

This study compared the pleasantness and intensity ratings for bottled water, distilled water with 2 mM $NaHCO_3$ and 15 mM KCl, and distilled water with 2.5 mM $NaHCO_3$ and 25 mM KCl, on hedonic and intensity 9-point rating scales and gLMS'. Across the board, bottled water was rated as being the most neutral of the three solutions. Distilled water with 2 mM $NaHCO_3$ and 15 mM KCl was rated as mildly aversive on both scales, as was distilled water with 2.5 mM $NaHCO_3$ and 25 mM. Bottled water was rated as the least intense of the three, significantly less intense than both artificial saliva solutions. Using two separate scales, it was shown that both concentrations of 'artificial saliva' were not actually rated as neutral but as mildly aversive, and that bottled water may be considered more neutral in both valence and intensity.

The observation that artificial saliva solutions were rated as aversive casts doubt on their use as control stimuli in experiments where hedonic neutrality is vital (e.g. Franken et al., 2011; Grabenhorst et al., 2010; Grabenhorst et al., 2008; Rolls et al., 2015). In the current study, we were not able to establish why these solutions were rated as aversive. Although these stimuli do not activate primary gustatory cortex, and have no strong flavour (Bartoshuk et al., 1964), any solution can be detected in the mouth, with neurons representing qualities such as texture and viscosity (Kadohisa et al., 2005). Our participants clearly experienced these solutions in the sense that they produced an aversive phenomenological experience. It is possible the amount of liquid is an influential factor. The usual experience with bottled water is through drinking, thus people are familiar with a relatively large volume in the mouth. Saliva, in contrast, is usually present in much lower volume (Pytko-Polonczyk et al., 2017). The amount of liquid in the mouth in each trial of this experiment was more similar to

everyday drinking, than to the amount of saliva in the mouth. This could emphasise the unfamiliarity of the two artificial saliva stimuli and provoke an aversive rating. Indeed, the initial neuroimaging study to use artificial saliva as a control makes no mention of testing activation in response to the artificial saliva solution itself (Francis et al., 1999). When later studies did test such effects, it was observed that some taste-related activation is elicted by stimuli such as sweet solutions that is not produced by artificial saliva, including insula activation (Zald & Pardo 2000). Artificial saliva might therefore be a good choice of control when the experimental aim is to maximise signal in gustatory cortex (Veldhuizen et al., 2011, 2013; Veldhuizen & Small, 2011). The majority of activation produced by artificial saliva, however, was due to non-gustatory processes related to swallowing. This demonstrated acceptable signal can still be obtained when using a different control stimulus, such as water (Zald & Pardo 2000).

It is possible the amount of liquid is an influential factor. The usual experience with bottled water is through drinking, thus people are familiar with a relatively large volume in the mouth. Saliva, in contrast, is usually present in much lower volume (Pytko-Polonczyk et al., 2017). The amount of liquid in the mouth in each trial of this experiment was more similar to everyday drinking than to the amount of saliva in the mouth. This could emphasise the unfamiliarity of the two artificial saliva stimuli in a drinking context, and provoke an aversive rating.

If artificial saliva is indeed rated as aversive because of unfamiliarity, experimenters who use this as a control condition could familiarise participants with the solution prior to testing, in order for the solution to really be perceived as neutral. Another alternative for researchers could be to consider using neutral solutions participants are familiar with prior to experiments, such as the bottled water used in this study. While this solution does not mimic the composition of saliva (chemical neutrality), our results suggest that it is hedonically

neutral. Furthermore, acceptable BOLD signal can still be achieved with its use as a baseline for the subtractive method (Zald & Pardo, 2000).Therefore, it would make a good control condition for studies in which hedonic, rather than chemical, neutrality it most important. Alternatively, it might be worthwhile to create a solution that mimics the properties of saliva more closely than distilled water with added $NaHCO_3$ and KCl. The composition of saliva is complex, and in addition to electrolytes such as $NaHCO_3$ and KCl, it also contains a range of enzymes and amino acids, among other compounds (Carpenter, 2013; Humphrey & Williamson, 2001). It might be the case that a solution that mimics the properties of saliva more closely would be rated as hedonically neutral, and could therefore be used as a control condition in studies investigating gustatory processing. This might be of particular value in neuroimaging, where lack of activation of gustatory cortex is important (Veldhuizen et al., 2011, 2013; Veldhuizen & Small, 2011). Alternatively, determining the composition that most closely matches an individual's own saliva, rather than an average, might be more effective (Pytko-Polonczyk et al., 2017). Indeed, some neuroimaging studies using artificial saliva concentrations matched to the individual participant's ratings of maximal tastelessness have observed pleasantness ratings with error bars overlapping with neutral (see Figure 2, Veldhuizen & Small, 2011). This demonstrates the importance of taking into account the heterogeneity of taste perception when choosing the control stimulus. Our results demonstrate a generic artificial saliva solution will not provide a neutral baseline for all. Only when a very small volume of the most neutral concentration is selected at the individual participant level, as in Veldhuizen & Small (2011), does the hedonic rating approach neutral. We argue that our results suggest that in studies where the participant's experience is vital, such as those examining hedonic effects, individual selection of artificial saliva concentration or bottled water would make a more appropriate control stimulus than artificial saliva solutions of the concentrations we tested.

One potential influence on our participants' subjective experience of taste is adaptation. Taste adaptation refers to the reduction in receptor stimulation and consequently experienced taste following repeated stimulation of the gustatory receptors by a given stimulus (McBurney & Bartoshuk, 1973). Of particular relevance to this study is the observation that with adaptation to one stimulus, the taste qualities of other, non-adapted, stimuli are enhanced (McBurney & Bartoshuk, 1973). In this study the use of bottled water as both test stimulus and rinse meant this stimulus was presented more frequently and consequently with less time between each encounter than the other stimuli. This procedure was chosen in accordance with prior literature (e.g. Veldhuizen et al., 2007), where the neutral stimulus is frequently also used as the rinse. We selected the lowest concentration solution of our three "neutral" stimuli as the rinse, but in many cases the rinse is an artificial saliva solution (e.g Hird et al. 2017, Veldhuizen & Small, 2011). It is possible that our participants adapted to the bottled water as a result of these methodological choices. Consequently presenting the artificial saliva solutions in the context of adaptation to bottled water may have enhanced the perceived taste of these stimuli. If these stimuli were liked less than the bottled water, Fechner's law of hedonic contrast suggests that if a stimulus is perceived as aversive, the contrast with adapted stimuli may render its perception to become even more aversive (1898, cited in Lim, 2011). It would be useful in future work to specifically test this possibility by purposely adapting participants separately to artificial saliva and bottled water, and comparing ratings in the two cases.

Despite this possible influence, there are several reasons why adaptation may not have been the primary factor in the aversiveness of the artificial saliva solutions. This study was motivated by observations from our imaging work (Baines, Hensels and Talmi, in preparation) that participants rated artificial saliva as aversive. In that study, artificial saliva was used as both the neutral stimulus and the rinse. Therefore if adaptation does occur to the

rinse, in that case the artificial saliva would have been the adapted stimulus, yet it was still rated as significantly more aversive than neutral. In addition, the bottled water was still rated as significantly more intense than neutral in our study, suggesting adaptation would have been incomplete at best.

The stimulus delivery method employed may also have affected adaptation. We utilised a variant of the sip-and-spit technique, rather than the gravitational flow method often utilised in neuroimaging work. We believed that without the constraints of neuroimaging that require gravitational flow presentation, sipping as the mode of delivery provided the best balance of experimental control and ecological validity. The sip-and-spit technique has the additional benefit of being more resistant to adaptation. Rather than passive presentation, the participant must take an active role to receive the stimulus. This increases arousal level (Schifferstein & Frijters, 1992). It also results in greater movement of the tongue, leading to stimulation of different regions of the tongue for each stimulus, thus stimulation of different receptors, slowing or eliminating adaptation (Schifferstein & Frijters, 1992). Sipping also results in stimulation of a larger area of the tongue. This leads to enhanced perceived intensity of stimuli. High-intensity stimuli take longer to induce adaptation than low-intensity stimuli (Schifferstein & Frijters, 1992). All of our stimuli were rated as significantly more intense than zero, consistent with this. Though it may still occur in time (Fisher & Fisher, 1969) all of these factors decrease the likelihood or extent of adaptation and/or slow its onset. Kroeze (1979) also argued that temporal consistency was required for adaptation. Sip-and-spit delivery, in contrast, results in "pulastile" stimuli that resist adaptation. Although we did not control the ISI, Kroeze (1979) argued that 50s ISIs were sufficient to restore normal sensitivity. In practise, our participants took longer than this to make their rating and pick up the next stimulus for tasting. Taken together, these factors suggest adaptation is unlikely to be the primary reason for the dislike of our artificial saliva stimuli.

In this study, we chose to use stimuli at room temperature. This was to avoid changes in stimulus temperature across the time-course of the experiment that we were not controlling or able to measure. We also selected this temperature to be consistent with prior literature, where for neuroimaging in particular, room temperature stimuli are most common (see Lemon, 2017 for review; Zald et al., 1998). Minimising changes in stimulus temperature was particularly important given that our neutral stimuli contained relatively low concentrations of the solutions of interest. Though we were not aware of any systematic tests of temperature-concentration effects using artificial saliva, both sodium chloride and sweet tastants, which have received the most attention, show temperature effects that are concentration dependant. Greater temperature modulation is observed at lower concentrations. Presenting stimuli at room temperature therefore allowed us to keep stimulus temperature consistent across stimuli and trials, to minimise any effects on taste experience.

The larger volume of our stimuli compared with the small bolus delivered in neuroimaging studies (5-10mL on average, e.g. Lemon, 2017; Zald et al., 1998) may have made tongue and stimulus temperatures more problematic in this case. Interactions between tongue and solution temperature can influence taste (Green & Frankmann, 1987). We did not control the temperature of the tongue in this study. However, temperature of the solution at the time of delivery shows greater influence over ratings (Green & Frankmann, 1988), and discrepancies in ratings of solution intensity with and without tongue temperature controlled appear to manifest at higher temperatures than used in our study (Green & Frankmann, 1987; Moskowitz, 1973). Thus whilst such interactions might have affected our results, they are likely to have been minimal in this case, and consistent across trials, stimuli, and with neuroimaging literature.

Of particular interest to this study is the observation that detection thresholds of sodium chloride are lowest at room temperature (McBurney et al., 1973; Pangborn et al.,

1970). This is consistent with mouse neural responses to peri-threshold sodium chloride, which were maximal at 22 degrees Celcius (Li & Lemon, 2015). If the solutes in our artificial saliva solutions are similarly most readily detected at room temperature, this could have resulted in elevated intensity ratings. If intensity and pleasantness are associated, then this may have influenced (un)pleasantness ratings. However, the intensity of bottled water was also rated more intense than zero, and contained the same solutes as the artificial salivas, but was not rated as aversive. A straightforward relationship between temperature, intensity and (un)pleasantness is therefore unlikely in this case. Furthermore, Lemon (2017) argues the primary effect of temperature on stimuli presented at room temperature might be on signal strength, rather than taste quality (Lemon, 2017). Thus whilst our high intensity ratings might be a consequence of temperature, the aversive ratings of artificial saliva are unlikely to be primarily due to this.

The comparatively large volume of our stimuli may have elevated intensity ratings. We presented approximately 50mL per stimulus, but participants only took one sip. This was comparable to the usual 5-20mL (De Graaf & Zandstra, 1999; Haase et al., 2011) in sip-and-spit experiments, and considerably higher than the approximately 0.5mL bolus size of fMRI studies (Francis et al., 1999; O'Doherty et al., 2002; Veldhuizen et al., 2007, 2011; Veldhuizen & Small, 2011). Larger volumes tend to increase perceived intensity (Haase et al., 2009). This may be due to stimulation of a larger area of the tongue. There is a positive correlation between intensity rating and the number of taste buds stimulated (Miller Jr & Reedy Jr, 1990; Zuniga et al., 1993). In addition, fMRI stimulus delivery is typically restricted to the anterior portion of the tongue, where taste bud density is lower (Miller Jr, 1986). The high volume of our stimuli may therefore have produced particularly high intensity ratings. Whilst this may be responsible for greater intensity ratings than zero for all

of our stimuli, it is unlikely to systematically affect the artificial saliva solutions. There is no evidence relating volume to pleasantness ratings.

The flow rate at which stimuli are presented also affects how they are experienced. Meiselmann, Bose and Nykvist (1972) tested this effect with sucrose and salt, demonstrating as flow rate increased (from 2 to 5 to 8cc per second), perceived intensity of stimuli increased (Meiselman et al., 1972). Whilst we did not control flow rate of our stimuli, sipping arguably results in a flow rate at the upper end of the scale. This may have led to greater perceived intensity. Our stimuli were rated as significantly more intense than zero, but this was the case for all our stimuli, not just the artificial saliva solutions. Furthermore, Meiselmann et al., (1972) noted the upper end of their function appeared to be approaching asymptote. They argued there may be an upper limit, beyond which flow rate there were no further intensity increases. Alternatively, higher flow rate may cause stimuli to wash over the tongue so fast as to interfere with receptor processes, decreasing perceived intensity. Such a relationship has been demonstrated with quinine hydrochloride (Feallock, 1965, cited in Meiselman et al., 1972), a stimulus judged aversive like our artificial saliva solutions. A systematic test of the effect of flow rate on perceived intensity or comparison of these response functions across stimuli was beyond the scope of this study but would be an interesting avenue for future research. Whilst it is unlikely flow rate is primarily responsible for our results, it would be advisable for future studies to present stimuli at a fixed flow rate to prevent any undue influence.

In conclusion, this was the first study to formally test the pleasantness and intensity of two supposedly neutral solutions often used in gustatory research as a control condition. We found that these stimuli might be experienced as sensorily meaningful, as they were perceived as moderately intense and mildly unpleasant. To circumvent this issue in future gustatory research, we have recommended that researchers use still, room temperature bottled

water as their control stimulus, or a familiarisation procedure to acquaint participants with the artificial saliva. More broadly, researchers are advised not to assume that any experimental stimulus is emotionally neutral without empirical evidence.

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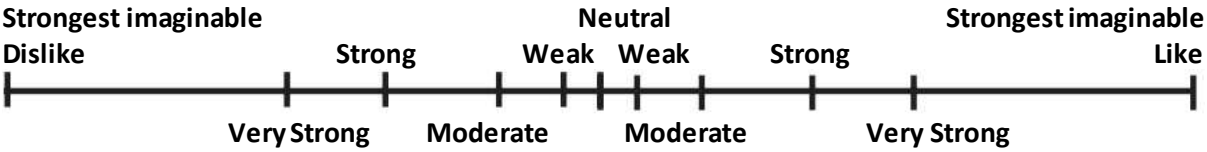
How much did you **like** this stimulus? Please mark it on the spectrum below.



How **intense** did you find this stimulus? Please mark it on the spectrum below.

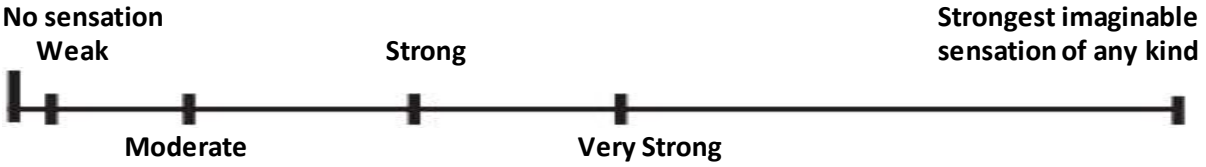


Figure 1. Example of the gLMS rating scale, showing the hedonic gLMS above and the sensory gLMS below.

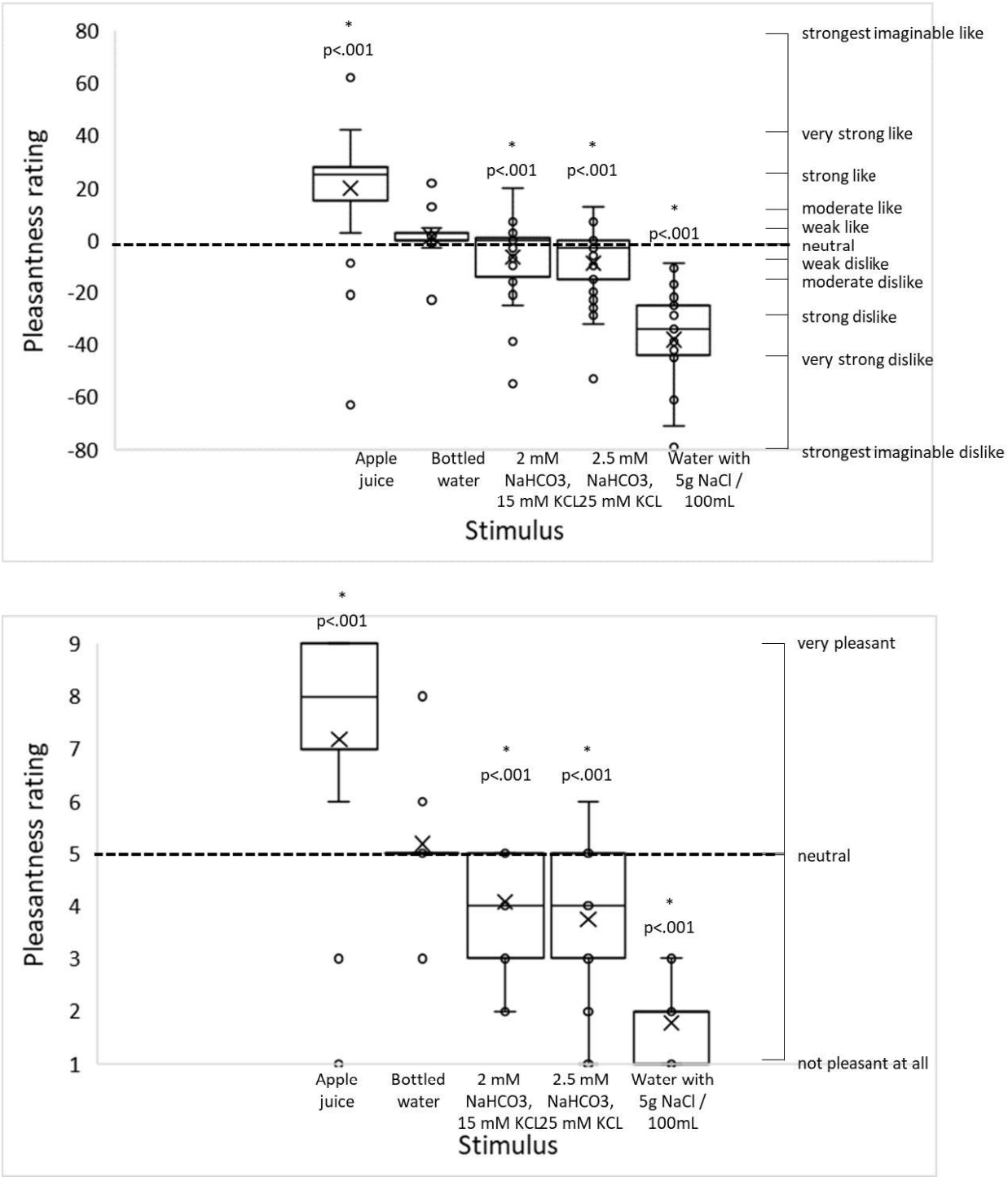


Figure 2: Box-and-whisker plots showing pleasantness ratings as a function of stimulus. Plots show ratings for the gLMS (top panel) and the 9-point scale (lower panel). Dashed lines indicate neutral point. * denotes significant difference from neutral, with p value shown.

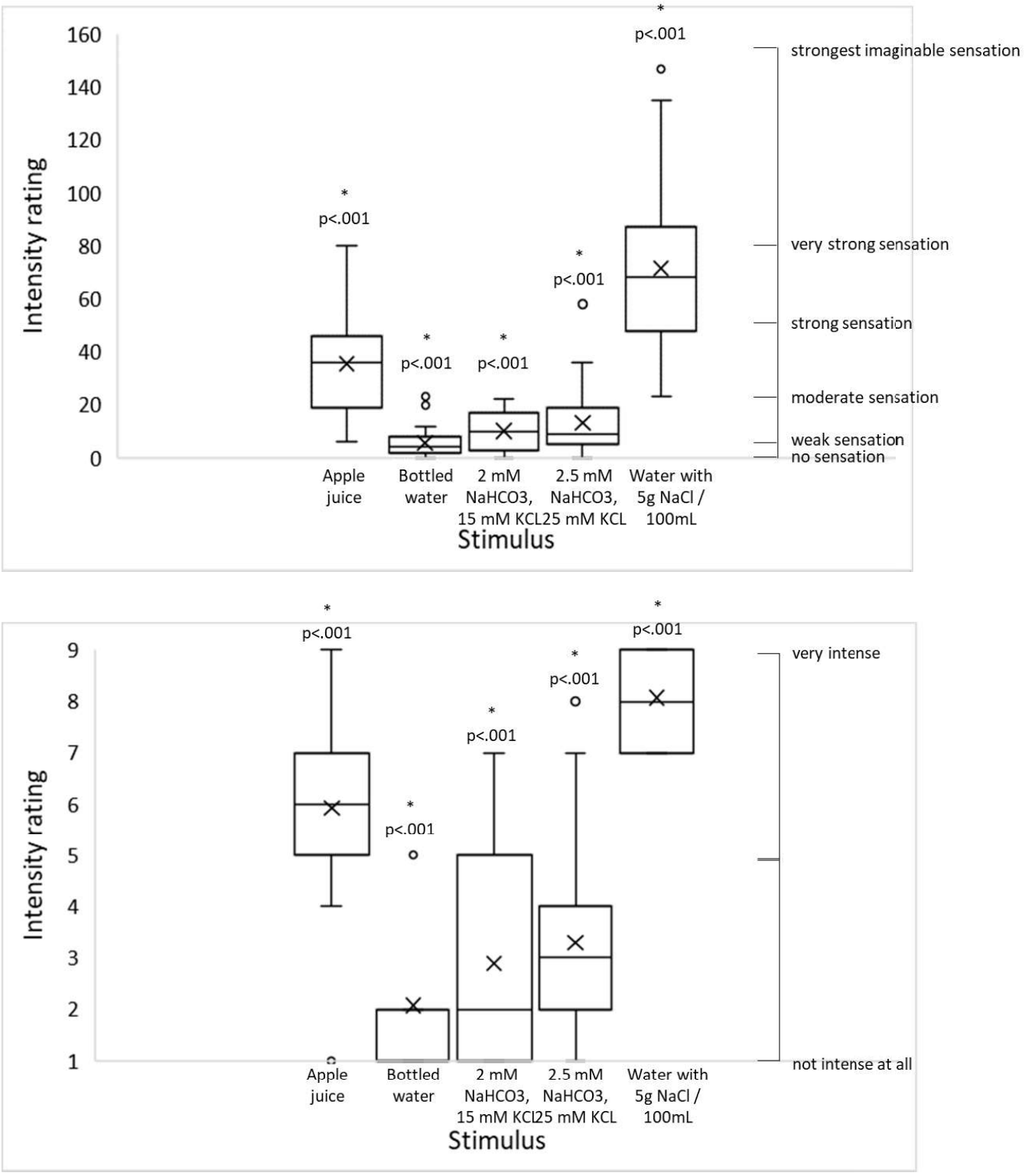


Figure 3: Box-and-whisker plots showing intensity ratings as a function of stimulus. Plots show ratings for the gLMS (top panel) and the 9-point scale (lower panel). * denotes significant difference from neutral, with p value shown.

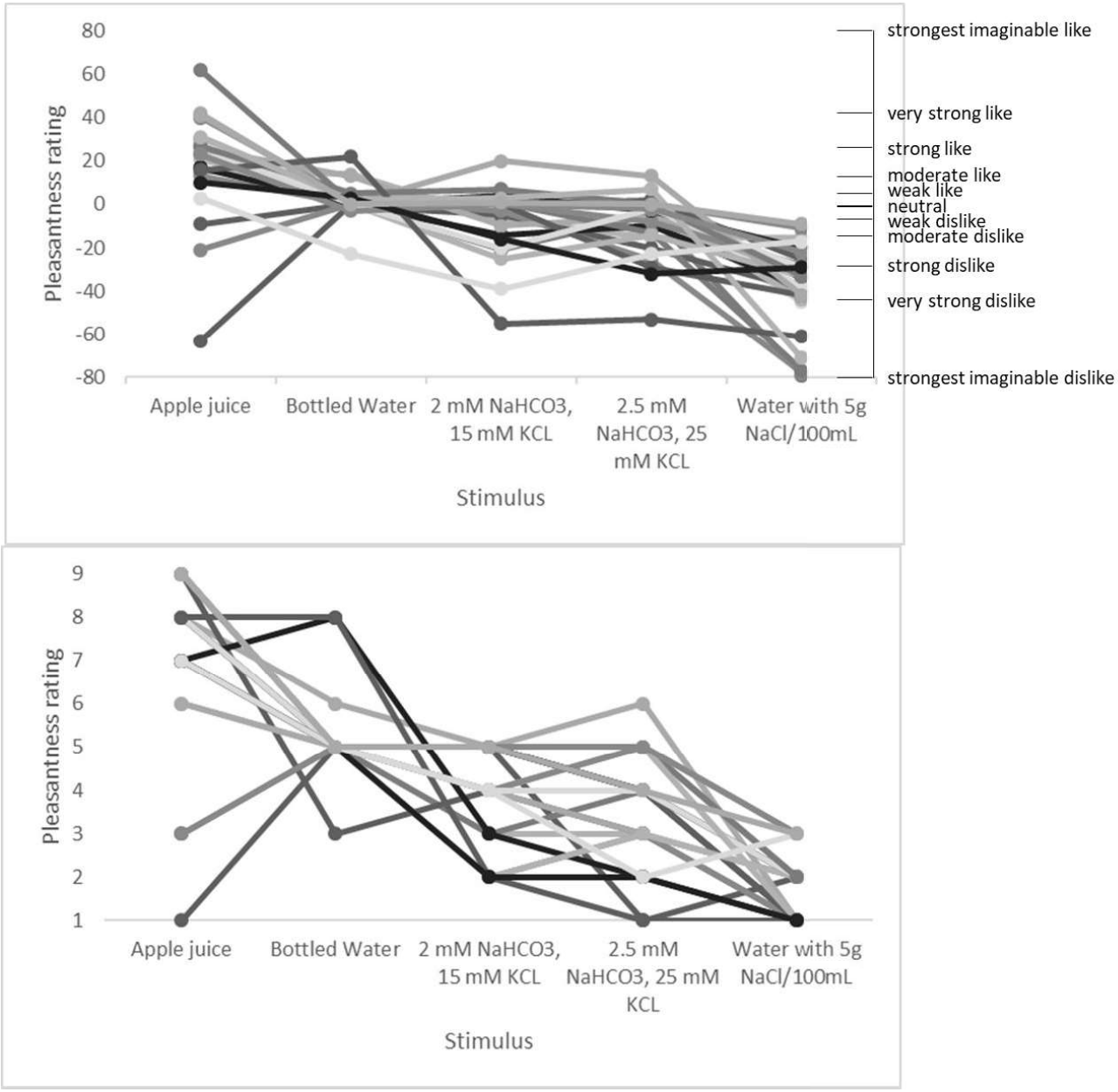


Figure 4: Pleasantness ratings by stimulus for the gLMS (top panel) and 9-point (lower panel) rating scales. Plots show each participant's data as a separate line. The labels for the gLMS as given to the participants is shown on the right.

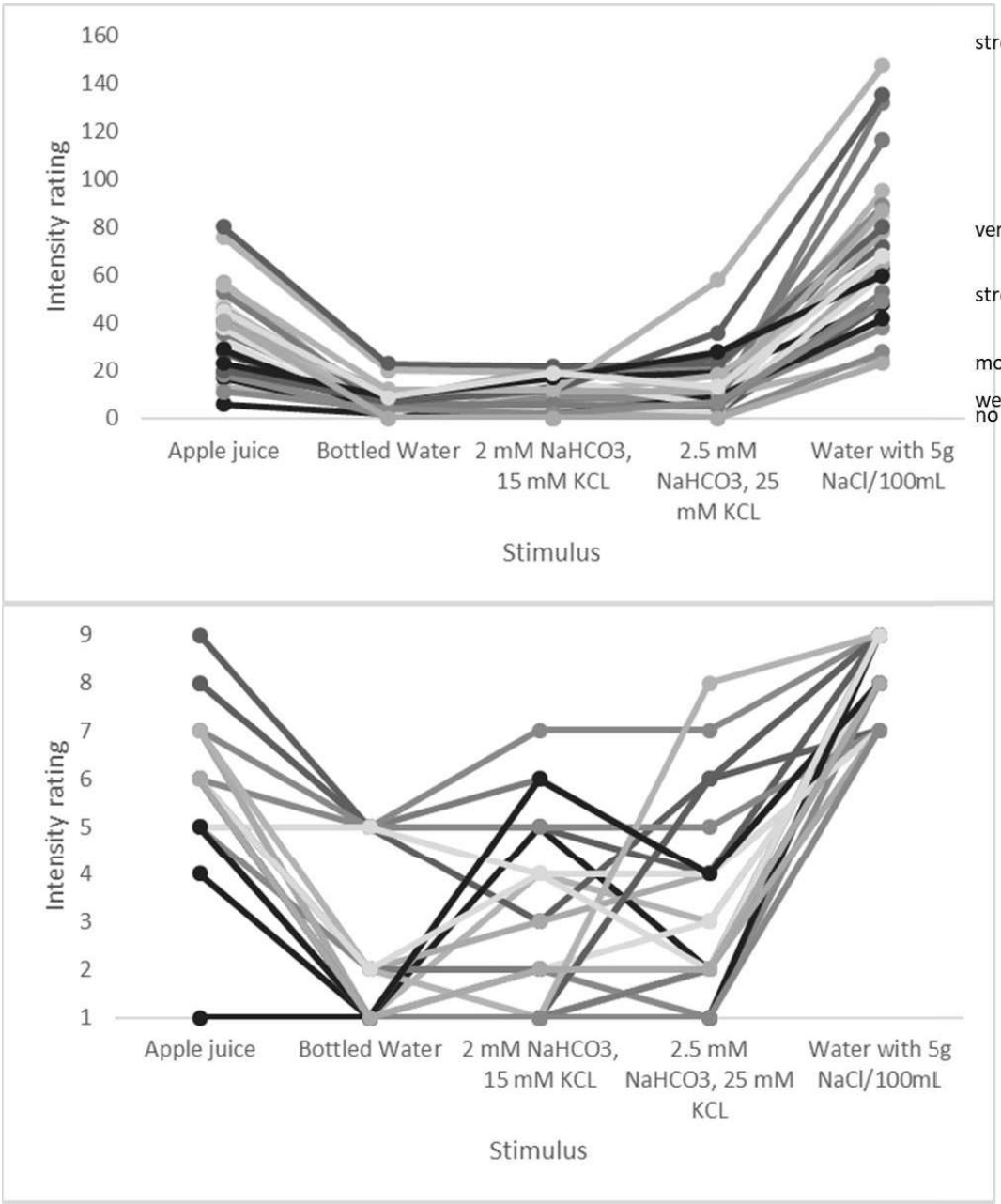


Figure 5: Intensity ratings by stimulus for the gLMS (top panel) and 9-point (lower panel) rating scales. Plots show each participant's data as a separate line. The labels for the gLMS as given to the participants is shown on the right.

Appendix A: Hunger, fullness, thirst, and craving ratings

Participant number: _____

"On a scale from 1 to 10, how **hungry** do you feel right now?" _____

"On a scale from 1 to 10, how **full** do you feel right now?" _____

"On a scale from 1 to 10, how much do you **crave food** right now?" _____

"On a scale from 1 to 10, how **thirsty** do you feel right now?" _____

Table