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Original Article

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Assessment of the triangle test methodology for determining umami discrimination status

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Abstract

The prototypical stimuli for umami taste is monosodium glutamate (MSG), which is the sodium salt form of glutamic acid. A proportion of the population has a reduced or complete inability to taste l-glutamate independent to the sodium ion. To determine individuals' umami discrimination status, many studies use a series of triangle tests containing isomolar (29 mM) sodium chloride (NaCl) and MSG, requiring participants to correctly identify the odd sample. Across studies, inconsistent categorization criteria have been applied. The aim of this study was to determine the optimal classification criterion based on the number of tests assessed to ascertain an individual's ability to discriminate between MSG and NaCl. Thirty-eight participants attended 3 taste assessment sessions, each involving 24 triangle tests (2 blocks of 12 tests) containing 29 mM NaCl and 29 mM MSG, detection and recognition threshold were measured for MSG, monopotassium glutamate (MPG), and sweet (sucrose) tastes. There was no learning, or fatigue trend over $n = 24$ ($P = 0.228$), and $n = 12$ ($P = 0.940$) triangle tests across each testing session. Twenty-four triangle tests produced the most consistent categorization of tasters across sessions (68.4%). The test–retest correlation across each testing session was highest for $n = 24$ triangle tests (ICC = 0.50), in comparison to 12 (ICC = 0.37). Overall, conducting $n = 24$ compared with $n = 12$ triangle tests provided the optimal classification to determine an individual's ability to discriminate l-glutamate from NaCl and thus their umami discrimination status, based on the number of tests assessed in this study.

Key words: MSG, salt taste, taste, umami

Introduction

Umami taste, described as savory, delicious, and having mouthful qualities, is elicited by certain l-glutamates (predominately monosodium glutamate [MSG]) and is synergistically enhanced when combined with 5′-ribonucleotides such as inosine monophosphate (IMP) (Yamaguchi 1967; Yamaguchi 1991; Shigemura et al. 2009). Glutamate in isolation is in the form of glutamic acid and produces a sour taste (Kurihara 2015), but when in sodium salt form (MSG) we experience umami taste, MSG is widely used in psychophysical research. The discovery of umami taste receptors (T1R1/T1R3 and mGluRs) was key evidence supporting umami as the fifth basic taste (Nelson et al. 2002; Li et al. 2002; Zhao et al. 2003). However, due to a lack of perceptual independence from other basic tastes and associations with satiation and satiety, it has been suggested that umami should not be a basic taste, rather form part of a new category of tastes classified as an "alimentary" (Hartley et al. 2019; Keast et al. 2021). Alimentary tastes have importance postingestion and umami stimuli regulate the release of certain digestive hormones throughout the gastrointestinal tract via the umami taste receptors present in the gastrointestinal tract (San Gabriel and Uneyama 2013; Keast et al. 2021).

An individual's ability to taste umami stimuli has been linked with both food preference and intake, suprathreshold intensity perception, and intake of umami containing stimuli (Pepino et al. 2010; Kubota et al. 2018). Umami tasters have been shown to have a higher preference toward foods such as mushrooms, cheese, milk, and some vegetables, and consumption of higher amounts of seaweed and less sugar than umami hypotasters (Kubota et al. 2018). When consuming sodium reduced vegetable broths with added MSG, umami discriminators experienced a higher taste intensity from these broths than nondiscriminators (Hartley et al. 2020), indicating better sensory outcomes for sodium reduced food using MSG in those individuals with an ability to discriminate between NaCl and MSG. Aside from potential implications of umami discriminator status on preference, intake, and intensity perception of sodium reduced foods, there is research suggesting consumption of umami stimuli plays a role in controlling food intake (Miyaki et al. 2016; Noel et al. 2018), enhancing energy compensation (Masic and Yeomans 2014), and increasing postingestive satiety (Masic and Yeomans 2014). Additionally, modulation of digestive hormones is observed when MSG is consumed with protein (Anderson et al. 2018) or in a liquid form (Hosaka et al. 2012). However, the role of individual variation in umami taste perception in intake,

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satiety, and postconsumption gastrointestinal hormone release requires further research. All in, research suggests physiological and behavioral outcomes of umami taste stimulation, with participant's ability to perceive umami stimuli being associated with food preference and intake, leading to a number of studies focusing on participants' ability to detect l-glutamate.

As umami psychophysical research has developed, a subgroup of the population that appears to have either a reduced ability or complete ageusia to umami quality has been identified, and methodology to isolate this subgroup was documented (Lugaz et al. 2002). This subpopulation has a reduced or complete inability to discriminate isomolar MSG from NaCl, and detection thresholds (DT) for both MSG and NaCl were correlated. This indicates that they are predominately tasting the sodium ion in MSG (Lugaz et al. 2002). Multiple studies have subsequently reported a reduced or complete inability in some individuals to discriminate isomolar MSG and NaCl varying across the study populations and methodology used (Lugaz et al. 2002; Chen et al. 2009; Pepino et al. 2010; Singh et al. 2010; Hartley et al. 2020). Further studies into this subpopulation will develop our understanding of the importance of taste detection of l-glutamate and the detection throughout the entire alimentary canal on driving liking, and regulation of digestive hormone release to mediate consumption.

The previously mentioned studies all utilized the same triangle test methodology to ascertain whether their participants could discriminate between MSG and NaCl. Triangle test set up is simple for a participant, they must discriminate the odd sample from a set of 3 samples. So, for example a triangle test may comprise two 29 mM NaCl and one 29 mM MSG sample presented in random order. The task for the participant is to correctly identify the odd sample, in this example 29 mM MSG. This was first outlined by Lugaz and colleagues, who conducted 24 repeats of the same triangle test, which contained isomolar concentrations of 29 mM MSG and NaCl, participants were considered to have discriminated between these 2 solutions if they met the β risk criterion in these triangle tests (P = 0.10) (Lugaz et al. 2002). Following on from this, a number of studies used this same triangle test methodology to discriminate those who were discriminators, semidiscriminators, and nondiscriminators, using varying numbers of repetitions of this triangle test including; 24 repetitions (Chen et al. 2009); 10 repetitions (Singh et al. 2010); 2 sets of 12 repetitions (Pepino et al. 2010); and 6 repetitions (Hartley et al. 2020). As the number of triangle tests conducted varied between studies, so did the number of correct assessments participants were required to achieve to be considered a discriminator, semidiscriminator, or nondiscriminator, see Table 1. Understanding how umami taste sensitivity relates to consumption and anthropometry requires consistency across studies and application of an umami taster tool.

Classification criteria outlined from previous studies followed binomial probability distribution, that is, there is a set probability of success (or correct guess) for each trial, and in the case of a triangle test, there is a 1/3 probability that the participant will select the odd (correct) sample by chance. Following the binomial distribution for discrimination testing, it is assumed that each triangle test, which is presented to the participant, is independent to all other triangle tests completed for each participant. In this case, the

Table 1. Studies that have utilized the triangle test methodology, number of repetitions used, and proportion of discriminators, semidiscriminator (where applicable), and nondiscriminators identified.

Author	Number of triangle tests	Number correct for discriminator and %	Number correct for semidiscriminator and %	Number correct for nondiscriminators
Lugaz et al. (2002)	24	N/A*	8–12 correct: 10.3%	<8 correct: 3.5%
Chen et al. (2009)	24	13–24 correct: 78.9%	8–12 correct: 17.3%	6–8 correct: 2.9%
Singh et al. (2010)	10	N/A*	7–10 correct: 2–12%	0–7 correct: 3–4.6%
Pepino et al. (2010)	2 × 12	8–12 correct each day: 41–52%	8–12 correct one day. <8 correct one day. 22–29.5%	<8 each day 26–29.5%
Hartley et al. (2020)	6	5–6 correct: 32%	N/A	0–4 correct: 68%

*These papers classified discriminators based on other psychophysical measures, and only conducted the triangle tests for those participants who were classified as semidiscriminators or nondiscriminators based on the initial psychophysical measures.

binary response is the only possibility, so a 1 (yes/correct response) or 0 (no/incorrect response). This assumes that over the course of each participant repeating the triangle test 24 times, there is no learning effect occurring, no sensory fatigue occurring, and each triangle test completed by an individual is a completely independent triangle test to the previously and subsequently completed test. By following the binomial probability distribution, we can ascertain the minimum number of correct responses required to be significantly different from chance at the 5% level, for different numbers of triangle test repetitions. For example, if we conduct 24 triangle tests, participants need to correctly guess the different (correct) sample ≥13/24 to be considered to have successfully discriminated between the 2 samples above chance ($P < 0.05$); for 12 triangle test repetitions, a correct guess of ≥8/12 is significantly different from chance at the 5% ($P < 0.05$) level (Roessler et al. 1978; Bi 2006).

Interestingly, when different numbers of repetitions (triangle tests) are performed based on the same binomial probability distribution, different proportions of discriminators and nondiscriminators are seen across the various studies (see Table 1). The trend shows the fewer repetitions of the triangle test required from the participants, the higher the proportion of nondiscriminators compared with discriminators. For example, n = 24 repetitions, approximately 80% of participants can successfully discriminate between the 2 solutions at a level significantly different from chance (Chen et al. 2009). When n = 12 repetitions are conducted, this reduces to 47–70% of participants who can successfully discriminate (Pepino et al. 2010), and this further reduces to 32% of participants with 6 repetitions (Hartley et al. 2020). This indicates that the number of repetitions conducted dictates the proportion of discriminators and nondiscriminators classified from the study population. This may also suggest that the repeated triangle tests are not independent, and that learning may occur with a higher the number of repetitions completed. To date, no studies have investigated whether a learning effect occurs as the number of triangle tests increase, nor have any studies investigated how many triangle tests are required for assessing an individual's ability to discriminate between isomolar MSG and NaCl. Establishing a number of triangle tests required per participant to determine their ability to discriminate between the MSG and NaCl would result in consistent categorization across studies.

Moreover, previous research has conducted this methodology at the one timepoint (i.e. has not repeated this measure to determine consistency) (Lugaz et al. 2002; Singh et al. 2010; Hartley et al. 2020). Chen et al. investigated the test–retest reliability of this method, finding a test–retest correlation of r = 0.92, indicating a strong correlation, but this was only conducted in 5 highly sensitive (20–24/24 correct), and 5 highly insensitive (6-8/24 correct) participants out of a total of n = 242 participants (Chen et al. 2009). This does not allow for a generalization of the test–retest reliability to the entire population, as it only accounts for participants at the extremes of sensitivity, not the majority of participants who are moderately sensitive (9–19/24 correct). Pepino et al. tested across 2 days, separated by a minimum of 5 days and repeated the same 12 triangle tests on each day, they found a significant relationship between how participants performed on day 1 and day 2 (χ^2(df = 1) = 10.35; $P < 0.005$), nevertheless, 26% of women who discriminated on day one, did not discriminate on day 2, and 30% of women who did not discriminate MSG from NaCl on day one, did on day 2, indicating that individual variation across days is occurring (Pepino et al. 2010).

Therefore, based on the previous literature, the different number of triangle tests used, and variability in discrimination status based on the number of repetitions conducted, it is not clear how many triangle test repetitions are required to determine whether a participant can consistently discriminate between MSG and NaCl. Moreover, as these approaches have been used at a single point in time, it is not clear whether this measure of taste sensation is a static measure (i.e. will not change over time) or whether this measure can change for each subject across days or weeks. By understanding this, a consistent number of triangle tests required for screening umami discrimination status can be established to help ensure consistency in future studies.

Aim

Overall, the aim is to assess the triangle test methodology for determining umami discrimination status. The objectives of this study are to determine (i) whether individual's discrimination capacity changes over the repetitions of triangle tests (i.e. there is a learning or fatigue trend as the number of triangle tests increases); (ii) consistency in categorization of discrimination status based on the number of tests outlined in prior studies; and (iii) further confirm the consistency of individuals' ability to perform the test, when utilizing the results as a continuous variable and to determine the smallest number of triangle tests required to obtain reliable information about an individual's capacity to discriminate between isomolar 29 mM NaCl and MSG.

Materials and methods

Overview

This study is an exploratory study of secondary data from a larger study. As part of the larger study, participants attended the CASS Food Research Centre, Deakin University, Melbourne, for 3 taste assessments, separated by a minimum 5-week period. Ethics was approved by the Deakin University Human Research Ethics Committee (HEAG-H 094-2019), and subject's consent was obtained according to the Declaration of Helsinki. Participants were eligible if they were 18–50 years old, nonsmokers, had no allergies to any of the stimuli used in the psychophysical assessments, were not following any specific diet, and were not currently pregnant. All taste assessment sessions occurred at 8.30 am, with participants instructed to refrain from eating, drinking (except water), or brushing their teeth for a minimum of 1 h prior to attending the session.

Triangle test (3-alternative forced-choice test)

To ascertain whether participants could discriminate between NaCl (SAXA, Australia) and MSG (Ajinomoto, Japan), 24 triangle tests were completed by each participant during each taste assessment session (total of 3 sessions of 24 triangle tests). Each triangle test contained MSG and NaCl at 29 mM to ensure they were sodium matched and followed the methodology outlined by previous papers (Lugaz et al. 2002).

For each triangle test presentation, participants were provided with 3 solutions, 2 containing a 15-ml aliquot of the

same solution (either 29 mM MSG or 29 mM NaCl) and the third cup contained the other stimulus (either 29 mM MSG or 29 mM NaCl). All solutions were presented with unique 3-digit blinding codes. The 24 triangle tests in a taste assessment session were split into 2 blocks of 12 tests, separated by a 5-min break to give participants a rest from the task, both mentally and sensorially. By the end of each taste assessment session, participants had completed 24 triangle tests, 12 containing 2 solutions of 29 mM MSG and one 29 mM NaCl, and 12 containing 2 solutions of 29 mM NaCl and one 29 mM MSG.

The presentation order of the 3 solutions was randomized, and within each block of 12 tests, there were 6 tests that contained 2 solutions of 29 mM MSG, and 6 tests that contained 2 solutions of 29 mM NaCl. Participants were required to taste all 3 cups from left to right and select the solution that they perceived to taste different from the other 2 solutions, participants could not go back and retaste solutions, participants were also instructed to rinse their mouths between each triangle test presented. All tasting was conducted under red light to prevent any visual differences influencing the selection of the odd solution, and with participants wearing nose-clips to ensure taste was isolated.

Detection and recognition threshold

DT, the point at which participants can detect that the solution contains something other than water, but they are not sure what taste it is, and recognition threshold (RT) the concentration at which participants can identify the taste correctly were measured for MSG, MPG, and sucrose. To determine DT and RT, a method outlined by Webb et al. (2015) was adopted, this is a modified version of the International Standards Organisation method (ISO3972 1991), see Table 2 for concentrations (ISO 2011; Webb et al. 2015). DT and RT measures were taken in duplicate, on each testing session, for a total of 6 assessments over the 3 testing sessions. Before testing commenced, participants were familiarized to each taste (umami; MSG, sweet; sucrose), following methods outlined by ISO3972, if participants could not detect the specific taste at the familiarization concentration provided, they could request a stronger solution until they were able to detect the taste. Once participants were familiarized with the presented tastes, they began completing the test solutions.

For each of the stimuli assessed, participants were provided with a tray containing 10 solutions, these solutions were presented in 15-ml aliquots, with randomized 3-digit codes and were tasted in ascending concentrations. The first 8 solutions were following concentrations outlined by ISO3972 and the final 2 highest concentrations followed those outlined by Webb et al. (2015). As MPG concentrations are not outlined in ISO3972, these concentrations were isomolar to the MSG to ensure they were glutamate matched.

Participants were instructed to put the 15-ml aliquot in their mouth, swirl it around for 5 s and then expectorate the sample. Subjects were asked to record their taste perception from the following options: "the solution tastes like water," "the solution tastes something like water, but I am not sure what," "sweet," "sour," "salty," "bitter," or "umami." Participants then completed this for all 10 samples presented to them, in ascending concentrations. Upon completion of the taste assessment, DT was defined as the concentration participants selected "the solution tastes something like water, but I am not sure what," and RT was defined as the first concentration where the correct taste quality was identified twice in a row (i.e. the lowest concentration of the 2 consecutive samples), as outlined by previous research (Webb et al. 2015).

Statistical analysis

This study involved secondary analysis of data collected as part of a larger study. To analyze whether any differences in body mass index (BMI) and age existed between the discrimination categories, Kruskal–Wallis test was conducted and to determine whether there were any differences in sex between discrimination groups. Chi-squared test for independence was conducted, and significance was set at $P < 0.05$.

For objective 1, we aimed to investigate whether there were any trends to suggest learning or fatigue effects were occurring over the course of either each session (24 individual triangle test repetitions), and block of tests within each session (2 blocks of 12 individual triangle test repetitions), if a "learning effect" was identified, then this would provide justification for a block of training triangle tests prior to commencing the methodology. In the same way, a "fatigue" effect might call for a shorter sequence of test repetitions. A generalized estimated equations (GEE) approach for the binary outcome (correct/incorrect responses) was used to account for the multilevel (session, block within session) and repeated measure structure of the observations (individual triangle test result within blocks) within individual participants. The first model included the session (1, 2, or 3) as a categorical variable and triangle test sequence as a continuous variable (1–24). A second model included session (1, 2, or 3) and block within each session (1, 2) as categorical and triangle test sequence (1–12) as a continuous variable. Slope estimates, 95% confidence interval are presented for the trends in slopes, and *P*-values are presented for interactions estimated from the GEE model.

Objective 2 looked to investigate the consistency in categorization of participant's discrimination status based on the number of triangle tests and classification criteria outlined in prior studies. For each testing session, participants were classified into discriminator or nondiscriminator, based on different number of tests as used previously in other

Table 2. Taste quality, reference chemical, and concentrations evaluated by participants for DT and RT tasks.

Taste quality	Reference chemical	Sample concentrations (mM)									
		1	2	3	4	5	6	7	8	9	10
Sweet	Sucrose	1	1.6	2.7	4.5	7.5	12.6	21.0	35.0	70.0	140
Umami	MSG	0.5	0.7	1.0	1.4	2.0	2.9	4.1	5.9	11.8	23.6
Umami	MPG	0.5	0.7	1.0	1.4	2.0	2.9	4.1	5.9	11.8	23.6

studies (Lugaz et al. 2002; Chen et al. 2009; Pepino et al. 2010; Hartley et al. 2020). We consider sequences of 6, 12, and 24 repetitions with threshold ≥5/6, ≥8/12, ≥13/24, respectively, which correspond to a 1.8%, 1.8%, and 2.8% probability that a person that is selecting the correct sample at random for each independent test would be classified as a discriminator, i.e., under a binomial distribution with success probability 1/3. We additionally consider those participants consistently correctly identifying the odd sample at less than <1% probability in 24 repetitions to identify whether a consistent high-discriminator group exists, this corresponds with ≥18/24 correct triangle tests.

Consistency within sessions for the discriminator/nondiscriminator classification based on the sequence of pairs of 12 tests (i.e. first block of 12 on session 1 compared with second block of 12 session 1) and pairs of 6 tests (4 blocks of 6 tests for each testing session, first block of 6 session 1 compared with second block of 6 session 1) was assessed. Consistency across sessions for the discriminator/nondiscriminator classification was also assessed based on pairs of 24 tests (i.e. session 1 compared with session 2), pairs of 12 tests (i.e. first block of 12 on session 1 compared with first block of 12 on session 2), and pairs of 6 tests (i.e. first block of 6 on session 1 compared with first block of 6 on session 2). We report the consistency rate, defined as the proportion (%) of individuals who maintained their discrimination status classification across each paired comparison.

Objective 3, to further confirm whether results within and across sessions are consistent within each participant, intraclass correlations (ICCs) were performed to determine the test–retest reliability of the triangle tests for both within and across sessions. The model selected included a 2-way random-effects model to allow for the results to be generalized to the broader population, with absolute agreement chosen for the test–retest measure, and as the use of this methodology is to determine the reliability of this measure for each single participant, the single measurement ICC was selected. ICCs above 0.5 were accepted as this indicates moderate reliability, and the ICCs, 95% confidence intervals, and *P*-values were reported (Koo and Li 2016).

Finally, whether the discriminators and nondiscriminators had different DT/RT characteristics for glutamate (in the form of both MSG and MPG), Spearman rank correlation coefficients were conducted for each discrimination status, i.e., correlations were separately carried out for discriminators and nondiscriminators. The correlation analysis included participants average number of correct triangle tests correlated with their average dilution step for DT and RT, separate correlations were carried out for each tastant (MSG, MPG, and sweet). Sweet taste DT and RT was used as a control taste measure. Correlations were considered weak at r_s < 0.30, moderate r_s 0.4–0.6, or strong r_s 0.7–0.9, the criterion for statistical significance was set at $P < 0.05$ (Akoglu 2018). To analyze whether any differences in DT and RT existed between the discrimination categories for MSG, MPG, and sweet tastes, Kruskal–Wallis test was conducted with significance set at $P < 0.05$.

Results

Demographics

A total of $n = 38$ participants completed all 3 tasting sessions, 24 females, mean BMI of 24.5 ± 5.6, and mean age of 29.2 ± 8.0 years, and 14 males, mean BMI of 27.1 ± 5.7 and mean age of 31.0 ± 8.9 years. There were no significant differences in BMI, sex, or age between all discrimination status categories (all $P > 0.05$).

Trends in slopes results from GEE analysis

To explore whether there were trends to suggest a learning (improvement) or fatigue (declining) effect occurring within each session, trends in probability of success were estimated under a GEE model for $n = 38$ participants. For a sequence of 24 triangle tests, there was no triangle test sequence (0–24), response (correct or incorrect), and session (1, 2, or 3) interactions found ($P = 0.228$) (Table 3). For 12 triangle tests, no triangle test sequence (0–12), response (correct or incorrect), and block (first or second) interaction ($P = 0.568$), finally no session (1, 2, or 3), and block (first or second) interaction found ($P = 0.998$), or test sequence (0–12), session (1, 2, or 3), and block (first or second) interaction found ($P = 0.940$) (Table 3). There may be a small learning effect occurring across session 1 for the sequence of 24 triangle tests, with a slope estimate and 95% confidence interval of 0.446 (0.055, 0.837); however, no significant interactions for triangle test response, sequence, and session were identified. Therefore, we cannot reject the hypothesis that the slopes are parallel and that no learning or fatigue trends are occurring.

Analysis of discriminator/nondiscriminator classification based on different sequences of triangle tests

Participants classified as discriminators and nondiscriminators for each testing day, for 6, 12, and 24 triangle tests are summarized in Table 4. The mean proportion of discriminators based on 24 triangle tests was 86% ($n = 32.7$), for 12 triangle test sequence mean proportion of discriminators was

Table 3. Trends in slope results from the GEE for 24 triangle test results overall in each session, and the 2 blocks of 12 triangle test results in each session, slope estimates, and 95% confidence interval are presented, and *P*-values for interactions are also presented.

	Block 1 (12 triangle tests)	Block 2 (12 triangle tests)	Overall (24 triangle tests)
Session 1	0.009 (–0.004, 0.021)	0.014 (0.002, 0.025)	0.446 (0.055, 0.837)
Session 2	0.002 (–0.009, 0.012)	0.002 (–0.01, 0.014)	–0.024 (–0.405, 0.357)
Session 3	0.002 (–0.011, 0.015)	0.005 (–0.006, 0.015)	0.098 (–0.237, 0.433)
Interaction block × triangle test response (0–12)			$P = 0.568$
Interaction session × block × triangle test response (0–12)			$P = 0.940$
Interaction triangle test response (0–24) × session			$P = 0.228$

73.3% (n = 27.8), see Table 4, and for 6 triangle test sequence was 59.7% (n = 22.7). The remainder of the analysis for 6 triangle test repeats is shown in Supplementary Data. These data suggest that categorizing based on 6 triangle tests clearly provides inconsistent categorization data (see Supplementary Data 1).

Table 4. Proportion of participants classified as discriminators (*N*, %), for each session and block for 24 and 12 triangle test sequence, remaining participants were classified as nondiscriminators.

Session	Block	N (discriminator %)
24 triangle test sequence		
1	n/a	34 (89.5%)
2	n/a	30 (78.9%)
3	n/a	34 (89.5%)
Mean		32.7 (86.0%)
12 triangle test sequence		
1	1	27 (71.1%)
1	2	27 (71.1%)
2	1	29 (76.3%)
2	2	27 (71.1%)
3	1	29 (76.3%)
3	2	28 (73.7%)
Mean		27.8 (73.3%)

For 24 triangle test sequence, participants were classified as discriminators if they achieved ≥13/24 correct and nondiscriminators otherwise. For 12 triangle test sequence, participants were classified as discriminators if they achieved ≥8/12 correct, and nondiscriminators otherwise.

Consistency of discriminator status within and across sessions, for all 3 sessions was investigated to ascertain the number of triangle tests that is required to achieve the most consistent categorization. Different sequences of triangle tests (6, 12, and 24 triangle test sequence) followed in previous studies were employed for discriminator status categorizations (Lugaz et al. 2002; Chen et al. 2009; Pepino et al. 2010; Hartley et al. 2020). Consistency rate of discriminator status classification for groupings based on 24 triangle tests, across sessions, is presented in Table 5. Within-session consistency rate was lowest for categorization based on 6 triangle tests, ranging from 55.3 to 89.5%, mean 73.4% (see Supplementary Data 2), compared with 12 triangle test sequence ranging from 73.7 to 86.8%, mean 79.8% (see Table 5), due to this low consistency rate seen for 6 triangle test sequence, the following statistical analysis will be conducted only on 12 and 24 triangle test groupings. When comparing consistency rates for 24 triangle tests across the 3 sessions, and across sessions for 12 triangle tests, the consistency rate was higher for 24 triangle tests (73.7–84.2%, mean consistency 79.8% for 24; and 60.5–100%, mean consistency 72.8% for 12). Moreover, based on 24 triangle tests, 68.4% of participants were consistently categorized as discriminators for all 3 sessions, compared with 47.4% of participants consistently categorized as discriminators for all blocks of 12 tests.

For 24 triangle tests, of the 68.4% discriminators, there were n = 15 (39.5%) who consistently correctly identified the odd sample ≥18/24, on each testing session, these participants

Table 5. Consistency rate (percentage of participants consistently categorized into the same discrimination status classification) based on 24 individual triangle tests, across sessions (i.e. first session vs. second session) and within and across sessions for 12 triangle tests (i.e. first block vs. second block).

24 triangle test sequence		Consistency rate, N (%)
Session 1—24 tests	Session 2—24 tests	32 (84.2%)
Session 1—24 tests	Session 3—24 tests	28 (73.7%)
Session 2—24 tests	Session 3—24 tests	32 (84.2%)
Mean consistency rate		30.7 (80.7%)
Discriminators (≥13/24) on all testing sessions		26 (68.4%)
Nondiscriminators on all testing sessions (ageusic)		1 (2.6%)
12 triangle test sequence, within-session consistency rate		
Session 1—first block (12)	Session 1—second block (12)	28 (73.7%)
Session 2—first block (12)	Session 2—second block (12)	30 (78.9%)
Session 3—first block (12)	Session 3—second block (12)	33 (86.8%)
Mean		30.3 (79.8%)
12 triangle test sequence, across-session consistency rate		
Session 1—first block (12)	Session 2—first block (12)	26 (68.4%)
Session 1—first block (12)	Session 3—first block (12)	26 (68.4%)
Session 2—first block (12)	Session 3—first block (12)	30 (78.9%)
Session 1—second block (12)	Session 2—second block (12)	38 (100%)
Session 1—second block (12)	Session 3—second block (12)	23 (60.5%)
Session 2—second block (12)	Session 3—second block (12)	23 (60.5%)
Mean consistency rate		27.7 (72.8%)
Discriminators (≥8/12) on all testing sessions		18 (47.4%)
Nondiscriminator on all testing sessions (ageusic)		1 (2.6%)

For 24 triangle test sequence, participants were classified as discriminators if they achieved ≥13/24 correct and nondiscriminators otherwise. For 12 triangle test sequence, participants were classified as discriminators if they achieved ≥8/12 correct and nondiscriminators otherwise.

were the high discriminators, and n = 11 (28.9%) of participants were classified as discriminators but did not correctly identify the odd sample below the 1% probability (≥18/24) on every testing session and were considered semidiscriminators. Nondiscriminators were participants who were on some sessions categorized as discriminators and some sessions nondiscriminators, for 24 triangle tests nondiscriminators made up n = 11 (28.9%), see Supplementary Data 3.

Overall, conducting 24 triangle tests produced a higher consistency rate than both 12 and 6 triangle tests, across sessions. Nevertheless, inconsistent categorization rates of up to 20% for 24 triangle test sequence (across session) is still high, so it is worthwhile investigating whether this methodology is reliable in continuous variable form and whether the results from the triangle test methodology could be used as a continuous variable to avoid imposing specific cutoffs.

Intraclass correlation analysis

To further determine the consistency of the data in continuous variable form (i.e. no cutoffs imposed for categorization), and to determine the lowest number of triangle tests required to obtain reliable information about a participant's capacity to discriminate between NaCl and MSG at 29 mM, ICC's were conducted. First, we ascertained whether less than 24 triangle tests would render a moderate and thus accepted ICC value (≥0.5). ICCs between sequences of different number of tests (6 [see Supplementary Data 3], 12, and 24) were estimated within and across sessions.

The 12 triangle test sequence results indicate that an accepted ICC (≥0.5) was met for within-session ICCs when 2 blocks of 12 triangle tests were completed (all ICC's >0.57). All ICCs for 6 triangle test sequences within sessions were <0.50, excepting session 3, third block vs. fourth block of 6 sequence (ICC = 0.63, 95% CI = 0.40–0.79), see Supplementary Data 4.

Further ICC analysis to determine whether 12 triangle tests can be used to assess participant's ability to discriminate between MSG and NaCl across sessions. The ICC for the same block (i.e. first block of 12 triangle tests) across sessions (session 1 vs. session 2 vs. session 3, first blocks), the ICC was considered poor (ICC = 0.37, 95% CI = 0.17–0.57), ICC's for across sessions for 6 triangle test sequence is similarly <0.5, see Supplementary Data 3. Therefore, for across sessions, the only acceptable ICC is observed when we assess 24 triangle tests across sessions 1, 2, and 3 (ICC = 0.50, 95% CI = 0.31–0.68).

Spearman rank correlation coefficient results

Spearman rank correlation coefficients were performed for participants consistently classified as discriminators based on 24 triangle tests, discriminators were participants who were classified as discriminators on each 3 sessions, nondiscriminators were participants who varied between discriminator and nondiscriminator across the 3 sessions, and the one participant who could not discriminate on any 3 of the sessions was considered ageusic (analysis not conducted on one participant) (Table 6). The correlations were conducted based on the 24 triangle tests sequence categorization, as results from the GEE, consistency rate (%), and ICC analysis showed the categorization based on 24 triangle tests was the most consistent of the groupings assessed overall.

Moderate and strong negative correlations were identified for MSG RT (r_s = −0.680, P < 0.000) and MPG RT (r_s = −0.775, P < 0.000), respectively, indicating that for those who were classified as umami discriminators, the higher number of correct triangle test responses the lower mM their RT. The same effect was not seen in those who varied between discriminator and nondiscriminator across the 3 sessions (nondiscriminators).

Further spearman rank correlation analysis was conducted on the group of discriminators, to assess whether any differences existed for those high discriminators (consistently achieved ≥18/24) and semidiscriminators (consistently categorized as discriminators but did not consistently achieve ≥18/24). It was found that for both high discriminators and semidiscriminators, there were moderate–strong negative correlations with MSG RT (r_s = −0.629, r_s = −0.746, P < 0.05, respectively), and strong negative correlations for MPG RT (r_s = −0.760, r_s = −0.795, P < 0.01, respectively), see Table 7.

There were no significant differences in mean DT and RT for MSG, MPG, and sweet tastes across the different discrimination status groups, all P > 0.05, see Table 8.

Table 6. Spearman rank correlations between mean number of correct triangle test responses and DT and RT of MSG, MPG, and sweet, r_s and *P*-values are reported.

	Nondiscriminators (r_s)	Sig	Discriminators (r_s)	Sig
MSG DT avg	0.135	0.693	−0.238	0.242
MSG RT avg	0.009	0.979	−0.680**	0.000
MPG DT avg	0.055	0.871	−0.32	0.111
MPG RT avg	0.189	0.579	−0.775**	0.000
Sweet DT avg	0.429	0.189	−0.040	0.845
Sweet RT avg	−0.280	0.404	−0.362	0.069

**Significance <0.01 level.

Table 7. Spearman rank correlations between mean number of correct triangle test responses and DT and RT of MSG, MPG, and sweet, r_s and *P*-values are reported.

	Semidiscriminator (r_s)	Sig	High discriminators (r_s)	Sig
MSG DT avg	0.000	1.000	−0.205	0.463
MSG RT avg	−0.749**	0.008	−0.629*	0.012
MPG DT avg	−0.279	0.406	0.096	0.734
MPG RT avg	−0.795**	0.003	−0.760**	0.001
Sweet DT avg	0.128	0.708	−0.066	0.815
Sweet RT avg	0.329	0.324	−0.191	0.495

*Significance at <0.05 level. **Significance at <0.01 level.

Table 8. DT and RT mean ± SD for MSG, MPG, and sucrose across the discrimination groups (discriminator, nondiscriminator, and ageusic).

	MSG	MPG	Sucrose
DT (mM)			
Discriminator (n = 26)	1.06 ± 1.44	1.02 ± 0.95	4.59 ± 6.26
High discriminators (n = 15)	0.73 ± 0.36	0.81 ± 0.73	3.87 ± 4.28
Semidiscriminators (n = 11)	1.50 ± 2.14	1.3 ± 1.16	5.57 ± 8.41
Nondiscriminator (n = 11)	0.72 ± 0.37	0.76 ± 0.37	2.57 ± 2.77
Ageusic (n = 1)	0.57	0.57	1.20
RT (mM)			
Discriminator (n = 26)	13.01 ± 13.45	11.32 ± 12.71	24.51 ± 16.94
High discriminators (n = 15)	8.27 ± 10.12	6.92 ± 8.60	20.83 ± 14.06
Semidiscriminators (n = 11)	18.86 ± 15.61	17.33 ± 15.22	29.54 ± 19.82
Nondiscriminator (n = 11)	9.37 ± 9.17	9.30 ± 11.02	28.85 ± 22.74
Ageusic (n = 1)	4.42	3.73	35.75

Discussion

Our data illustrate that conducting 24 triangle tests with 29 mM isomolar NaCl and MSG on any given testing session provides a consistent evaluation of umami discriminator status. First, from the results of the GEE analysis, there was no statistical indication of learning trends, nor fatigue trends occurring across each session of testing, this was true for both 12, and 24 sequence of triangle tests. Second, categorization for 24 triangle tests provided the most consistent categorization across the 3 sessions (68.4% categorized as discriminators on all 3 sessions), in comparison to 12 (47.4% categorized as discriminators on each of the 3 sessions and blocks), and 6 triangle tests (18.4% categorized on each session and blocks). Finally, the ICC for 12 triangle tests within session (but not across session), and 24 triangle tests across session were both ≥0.5, indicating that 12 triangle tests may be sufficient for ascertaining a participant's ability to discriminate between 29 mM MSG and NaCl, within session, but when comparing across-session results, 24 triangle tests provide more reliable results.

It was found that 24 triangle tests produced the most consistent categorization of umami discriminator status, in comparison to 12 and 6 sequences of triangle tests. However, a higher number of tests were not performed to determine whether completing a higher number of triangle tests, i.e., 30 or 36 triangle tests would further improve consistency or whether a ceiling effect would occur. Nevertheless, the aim of this study was to determine the consistency of umami discrimination status categorization based on methodology previously outlined and to determine based on previous methods used, the number of triangle tests required to obtain consistent categorization across sessions. Additionally, conducting a higher number of triangle tests may become problematic from a practical aspect, as it increases the testing time for the participant and may become impractical to complete in conjunction with other taste measures from a researcher perspective.

The proportion of individuals with an ability to discriminate between 29 mM MSG and NaCl was higher in this study than previous studies based on categorization from 24 triangle tests. Chen et al. identified 79% of participants as umami discriminators, while Lugaz et al. identified 81% of participants as umami discriminators (based on a host of psychophysical testing), whereas the current study found an average of 86% of participants were umami discriminators on any of the 3 sessions (Lugaz et al. 2002; Chen et al. 2009). Over the 3 sessions, 68% of participants were consistently categorized as umami discriminators in the current study based on 24 triangle tests; however, a comparison cannot be made to previous studies as they measured at one time point only (Lugaz et al. 2002; Chen et al. 2009). Based on categorization from the 12 triangle test sequences, we found that on average, 47.5% of participants were categorized as umami discriminators for all the 3 testing sessions and blocks (a total of 6 repeats of 12 triangle tests), which is consistent with Pepino et al. who classified 52% of participants as umami discriminators across the 2 blocks of 12 triangle tests over 2 testing days (Pepino et al. 2010). Pepino et al. (2010) also found a similar consistency rate across 2 blocks of 12 triangle test sequences showing that 74% of their participants who were classified as a discriminator on one day, were similarly classified as a discriminator on the second day. This is consistent with the current study showing that on average, 72.8% of participants were classified as an umami discriminator across any 2 blocks of 12 triangle tests. When we look at the proportion of participants who were consistently categorized as an umami nondiscriminator across all sessions and blocks, categorized from both 12 or 24 triangle test sequences, we found one ageusic participant (2.6%) unable to discriminate between MSG and NaCl, which is consistent with findings from Lugaz et al. (3.5%), Chen et al. (2.9%), and Singh et al. (4.6%). From our data, and previous research it appears that across categorization based on 12 and 24 triangle tests, and various population groups there is a consistent proportion of discriminators, nondiscriminators, and participants who have an ageusia to umami quality. This suggests that the isomolar triangle test methodology is a useful tool for assessing individual's ability to discriminate umami from salt taste as it provides consistent proportions of discriminators and nondiscriminators across various studies and population groups.

In terms of the consistency and reliability of our findings, there is limited literature to provide a comparison as most previous studies investigated discrimination status at one time point only. Pepino et al. (2010) found a consistency rate of 74% between their 2 blocks of 12 triangle tests (separated by 5 days), which is consistent with our findings that showed an average of 72.8% of participants were classified as discriminators for any 2 blocks of 12 tests. Chen et al. (2009) found a test–retest value of r_s = 0.92 for a repetition of 24 triangle tests; however, they only conducted a test–retest on participants from the extreme ends of sensitivity (5 highly sensitive and 5 highly insensitive participants). From this study, we can see that those who are highly insensitive (the 2.6%) appear to be consistently unable to detect MSG on each given testing session, and those who are highly sensitive (≥18/24) similarly

appear to consistently be categorized as discriminators across sessions. In this study there was a test–retest ICC of $r_s = 0.50$, but it was for all participants, across the 3 sessions of 24 triangle tests, ensuring the data provide information about the entire sample population, rather than those at the extremities of sensitivity. In addition, as there were 5-week intervals between each testing session, variation in individual's ability to complete the triangle tests and thus a lower test–retest ICC value than Chen *et al*. may have occurred due to changes in dietary intake, modulation in taste perception due to dietary intake has been previously demonstrated in tastes including fat (Costanzo et al. 2018), salt (Bertino et al. 1982; Bertino et al. 1986; Riis et al. 2021), carbohydrate (Low et al. 2017), and umami (Noel et al. 2018). Overall, this study adds to the findings from Chen et al. to display an acceptable test–retest ICC of $r_s = 0.50$ for participants across all sensitivities, when conducting 24 triangle tests.

As it was identified that there was a group of participants who moved from discriminator to nondiscriminator across different testing sessions, we aimed to ascertain whether this was due to differences in sensitivity in other taste measures. No significant correlations were found with the group of individuals who varied from discriminator to nondiscriminator across sessions. Previous studies have found that NaCl and MSG DT are correlated in nondiscriminators (Lugaz et al. 2002), and at suprathreshold umami nondiscriminators experience less savouriness in MSG (Pepino et al. 2010) and experience a reduced intensity in broths containing MSG than tasters (Hartley et al. 2020). However, we found no associations between these nondiscriminators' average triangle test responses and their MSG DT or RT. However, umami discriminators (participants classified as discriminators, on all testing sessions) had moderate–strong negative correlations with MSG and MPG ($r_s = -0.680$, $r_s = -0.775$, $P < 0.001$, respectively) perceived RT, while there was no similar association with sweet taste ($r_s = -0.362$, $P = 0.069$). Moreover, when we conducted further analysis on the high discriminators (participants consistently achieved ≥18/24) and semidiscriminators (consistently categorized as discriminators but did not consistently achieve ≥18/24), it was found that for both high discriminators and semidiscriminators, there were moderate-strong negative correlations with MSG RT ($r_s = -0.629$, $r_s = -0.746$, $P < 0.05$, respectively), and strong negative correlations for MPG RT ($r_s = -0.760$, $r_s = -0.795$, $P < 0.01$, respectively), no correlations were observed for the sweet control taste. Therefore, in participants who are umami discriminators (both semidiscriminators and high discriminators), the greater their sensitivity to recognize glutamate (i.e. reach RT at lower concentrations), the greater the number of successes they will achieve in the triangle test task. This result is interesting as umami discrimination status as determined by an isomolar NaCl/MSG triangle test classification of umami discriminators (and both further classification of semidiscriminators and high discriminators) is consistent with recognition of umami as determined by both MSG and MPG, providing weight of evidence for this classification. However, although correlations were found for discriminators total correct triangle test responses with their MSG and MPG RT, there was no significant difference found in mean DT and RT for all tastes (MSG, MPG, and sweet) between the different discrimination groups. This finding is consistent with Pepino et al., who found no significant difference in threshold measures across the discrimination groups, for both umami and sweet taste (Pepino et al. 2010). This conceivably could be due to a function of the concentrations used, as we know that different taste dimensions are not necessarily associated (Webb et al. 2015), and the 29 mM used in the discrimination task is well above MSG and MPG DT and RT in this study and previous research (Pepino et al. 2010). Now that the umami discrimination tool has been evaluated as reproducible and reliable, and the number of repetitions of this method has been established, future studies could utilize this tool to further the understanding of umami discrimination status on other behavioral and physiological outcomes.

As this study was part of a larger trial, some limitations should be acknowledged. First, $n = 38$ is a small sample size; however, the repeated nature of the observations with all participants contributing to data on each testing session strengthens the results. Moreover, previous similar studies have used similar sample sizes or fewer to establish test–retest values for the same triangle test methodology (Chen et al. 2009), and other discrimination testing (Newman and Keast 2013). Second, as we did not measure more than 24 triangle tests in one testing session, we cannot ascertain whether conducting further triangle tests in a session would increase the proportion of discriminators, or whether a ceiling effect would occur. However, as previously mentioned, the aim of the study was to ascertain whether the previously outlined triangle test methodology produces reliable and repeatable categorization of discrimination status. Third, although we could measure statistical trends in correct/incorrect responses across the 24 triangle tests through the GEE analysis, we did not obtain further qualitative measures from the participants regarding perceived difficulty/or perceived fatigue across the 24 tests. Therefore, we cannot establish whether the lack of a statistical trends in incorrect/correct responses was due to an absence of fatigue or learning effects. Finally, the threshold testing (DT and RT) did not use forced-choice testing, therefore response bias cannot be ruled out, however, as the modified ISO3972 methodology that has been extensively utilized in prior psychophysical testing was followed (Webb et al. 2015; Low et al. 2016; Low et al. 2018), and DT/ RT were measured a total of 6 times across the 3 testing sessions the results provide good estimates of participants individual DT and RT.

Conclusion, an umami discrimination tool

Based on the current study's findings, and previous literature, this study found an absence of a learning or fatigue trends across 24 triangle tests and demonstrates that discriminator/nondiscriminator classification consistency is greatest for 24 triangle tests, relative to 12 and 6 triangle tests. Finally, 24 triangle tests produced an acceptable test–retest ICC value across multiple testing sessions. This indicates that the tool for assessing an individual's ability to discriminate between MSG and NaCl, and thus determine their umami discrimination categorization is to conduct 24 triangle tests, on any given testing session, with an additional categorization option for further categorizing discriminators (≥13/24) into those who are high discriminators (≥18/24) and semidiscriminators (13–17/24).

All in, we suggest for determination of umami discrimination status, studies should conduct 24 triangle tests to

ensure they are obtaining reliable and consistent information regarding an individual's ability to discriminate l-glutamate from the sodium ion.

Supplementary material

Supplementary data are available at *Chemical Senses* online.
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